Safety and Immunogenicity of *Escherichia coli* O157 O-Specific Polysaccharide Conjugate Vaccine in 2–5-Year-Old Children

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**Background.** *Escherichia coli* O157:H7 causes severe enteritis and hemolytic-uremic syndrome, mostly in young children and older adults. Similar to the case with *Shigella*, serum IgG against the O-specific polysaccharide of *E. coli* O157:H7 may confer immunity by lysing the inoculum in the intestine. A phase 1 trial in adults showed that a vaccine of *E. coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) was safe and immunogenic.

**Methods.** A phase 2 trial of the O157-rEPA vaccine was conducted in 49 children 2–5 years old who were divided randomly into groups receiving 1 or 2 doses of vaccine. Adverse reactions were monitored. Serum IgG lipopolysaccharide (LPS) antibodies were determined.

**Results.** No significant adverse reactions were observed. At 1 week after the first dose was administered, most children (81%) responded with a >4-fold increase in serum IgG LPS antibodies. At 6 weeks after the first dose was administered, all children responded with a >8-fold increase; a second dose did not elicit a booster response. At 26 weeks after the first dose was administered, the geometric mean titer of serum IgG LPS antibodies was ~20-fold higher than was the prevaccination titer. These serum samples had high titers of bactericidal activity that were correlated roughly with serum IgG LPS antibody titers (r = .78).

**Conclusions.** The O157-rEPA vaccine was safe and immunogenic in young children. A phase 3 trial of the administration of this conjugate vaccine concurrently with routine immunization in infants is planned.
ride (LPS) of E. coli O157:H7 are detectable after symptomatic infection [9, 27]. The protective role that these antibodies play is suggested in populations with frequent contact with cattle, such as dairy farm workers, who have higher serum titers of antibodies to E. coli O157:H7 and a lower risk of symptomatic infection than does the general population [28, 29]. That children <5 years old have the highest risk of acquiring E. coli O157:H7 infection suggests that natural immunity is acquired with age [28]. Adults probably have low serum IgG LPS antibody titers induced by either E. coli O157:H7 or cross-reactive organisms in the environment, including Citrobacter species (authors’ unpublished data and [30]).

E. coli and Shigella can be considered a single genus, because their genomic sequences show extensive similarities and share a phylogenetic tree [31, 32]. Both E. coli O157:H7 and Shigella dysenteriae produce Shigella toxin and cause bloody diarrhea [32−34]. On the basis of our study of Shigella and Salmonella species, we propose that to protect young children against E. coli O157:H7 infection, a polysaccharide conjugate vaccine eliciting IgG antibodies to the LPS could lyse the inoculum on contact and confer immunity [28, 29, 34−40].

In a phase 1 trial, we showed that such conjugate vaccines administered to healthy adults were safe and elicited high serum IgG LPS antibody titers with bactericidal activity [41]. In the present study, we report the safety and immunogenicity of an E. coli O157:H7 investigational conjugate vaccine in 2−5-year-old children.

SUBJECTS, MATERIALS, AND METHODS

Investigational vaccine. E. coli O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of Pseudomonas aeruginosa (O157-REPA; lot 011094) was produced at the National Institute of Child Health and Human Development, National Institutes of Health (NIH), and bottled by the Pharmaceutical Development Section, Pharmacy Department, Clinical Center, NIH. The investigational vaccine was approved by the US Food and Drug Administration (investigational new drug no. 5528). Of the 3 conjugates studied in our phase 1 trial, the conjugate using acid-detoxified LPS (O-specific polysaccharide) as the polysaccharide antigen elicited the highest serum IgG LPS antibody titers, although the differences between the titers were not statistically significantly different, and it was used in the present phase 2 trial [41]. Briefly, LPS was purified from E. coli O157:H7 that was fermented in a medium containing no bovine components [41]. LPS was detoxified in 1 N acetic acid and covalently linked to the recombinant exoprotein A from P. aeruginosa, which was purified at the NIH [41, 42]. The endotoxin levels of O-specific polysaccharide in the final vaccine were <20 endotoxin units/dose. The vaccine met the safety requirements of US Code of Federal Regulations 610. Each 0.5-mL dose contained 26 μg of polysaccharide and 77 μg of protein.

Study design. The human experimentation guidelines of the US Department of Health and Human Services and those of the Carolinas Medical Center were followed in the conduct of the clinical research. The clinical protocol was approved by the institutional review boards of the National Institute of Child Health and Human Development and of Carolinas Medical Centers and by the Office for Human Research Protection. Healthy children, 2−5 years old, were recruited from April 2002 to July 2003. Children who had a chronic disease warranting daily systemic medications, were immunocompromised, or had a history of vaccine-associated reactions were excluded. Informed consent was obtained from parents or guardians, and baseline laboratory measurements (serum transaminase levels, HIV status, and prevaccination serum E. coli O157:H7 LPS antibody titers) were obtained.

Children returned 7−14 days after the enrollment visit for vaccination. Children were randomized using computer-generated numbers to receive 1 or 2 doses of vaccine. They were examined by a physician, and their axillary temperature was measured. Children with a temperature ≤37.5°C received an intramuscular injection of 0.5 mL of vaccine in the deltoid muscle and then were monitored for local and systemic reactions for 30 min by the vaccination team. Parents/guardians were provided with diaries, thermometers, and measuring tapes to record temperatures and reactions at 6, 24, 48, and 72 h after each dose was administered. Redness or swelling at the injection site was recorded in millimeters. Signs and symptoms (pain at injection site, myalgias or arthralgias, headache, nausea, vomiting, diarrhea, abdominal pain, and appetite or activity changes) were categorized as mild, moderate, or severe. Other signs that were elicited included rash and increased somnolence. To revew observations by parents/guardians, the study coordinator conducted telephone interviews at 6, 24, and 48 h after each dose was administered. Serum transaminase levels were measured in all children at 1 and 10 weeks after vaccination. Rectal swab samples for culture of E. coli O157:H7 were collected at 6, 10, and 26 weeks after vaccination.

Serum E. coli O157 LPS antibodies. Serum antibodies to E. coli O157:H7 LPS were measured by ELISA [41, 42]. A serum sample (from subject no. ECO 161) from our phase 1 trial was used as a reference for IgG antibody titers and was arbitrarily assigned a value of 100.00 ELISA units (EU) of IgG antibodies. A second serum sample (from subject no. ECO 110) from our phase 1 trial was selected as a reference for IgM antibody titers and was arbitrarily assigned a value of 100.00 EU of IgM antibodies [41]. Serum antibody titers were expressed as geometric means, with 25th−75th percentiles. Comparisons of geometric means were made with the unpaired or paired t test. The χ2 test or, when appropriate, Fisher’s exact test for paired
or unpaired t test was used for comparison of categorical variables.

Bactericidal activity was measured in representative serum samples before and 26 weeks after the first dose was administered [41, 43]. An isolate of *E. coli* O157:H7 from a patient was used as the test strain. Guinea pig serum (MP Biomedicals) was used as the source of complement [41, 44]. Serum samples were analyzed before and after treatment with 2-mercaptoethanol (50 mmol/L at 37°C for 30 min).

**Cross-reaction with Vibrio cholerae O1 LPS antibodies.** Perosamine, a 3,6-di-deoxy-α-mannosamine, is present on the O-specific polysaccharide of *E. coli* O157:H7 and *V. cholerae* O1 [45, 46]. Serum samples from children who received 1 or 2 doses of the O157-rEPA vaccine were assayed by ELISA for antibodies to LPS from *V. cholerae* O1 serotypes Inaba and Ogawa. A serum sample (from subject no. CHO 24) from our cholera vaccine clinical trial was used as a reference for IgG antibody titers and was arbitrarily assigned a value of 100.00 EU of IgG antibodies [44]. The correlation between IgG antibodies to *E. coli* O157:H7 LPS and IgG antibodies to *V. cholerae* O1 LPS was evaluated.

### RESULTS

**Subjects.** Fifty-five children were enrolled. Six children completed ≤3 visits and were excluded from the analysis. Of the remaining 49 children, 25 received 1 dose of vaccine, and 24 received 2 doses of vaccine. The mean age for each group was 3.5 years.

**Safety.** No serious adverse reactions were reported after either dose was administered. There were no hospitalizations that were considered to be vaccine related. For the group receiving 1 dose, 24 (96%) of 25 diaries were completed. For the group receiving 2 doses, all diaries were completed after the first dose was administered, and 19 (79%) of 24 were completed after the second dose was administered. Compliance was >99% for all visits. One child had a fever (temperature, 38.2°C) at 72 h after the second dose was administered; otherwise, no fevers (temperature, >38.0°C) were reported within 72 h of the administration of either dose. Mild erythema (3–17 mm), with spontaneous resolution, was reported by 5 subjects at 6 h and by 2 subjects at 24 h after the first dose was administered. There were no reports of erythema after the second dose was administered. One subject reported mild pain at 6 h after the second dose was administered, and 1 subject reported swelling of >3 cm at 24 h and resolution by 48 h after the second dose was administered. Rashes were reported by 2 children in the 2-dose group. One child developed palmar erythema 24–48 h after the first dose was administered, and it resolved spontaneously. The same child developed a maculopapular rash 72 h after the second dose was administered. This rash was attributed by her primary care physician to an antibiotic she received to treat otitis media, and it resolved with discontinuation of the antibiotic. There were no significant differences between prevaccination and postvaccination serum transaminase levels for any of the children. *E. coli* O157:H7 was not isolated from any of the rectal swab samples.

**Serum antibody response to E. coli O157 LPS.** All children had low serum IgG LPS antibody titers before vaccination, and there was no statistically significant difference between the groups (table 1). At 1 week after the first dose was administered, there was a significant increase in serum IgG LPS antibody titers (for all children, 3.32 vs. 0.27 EU; *P* < .0001): 81% of the children had a >4-fold increase, compared with prevaccination titers. There was an additional increase at 6 weeks after the first dose was administered (for all children, 11.36 vs. 3.32 EU; *P* < .001); only 1 of 49 children had a <10-fold increase (this child had a 6.45-fold increase).

Children who received a second dose of vaccine (given at 6 weeks after the first dose was administered) had an increase in serum IgG LPS antibody titers 4 weeks later, at week 10 of the study (13.75 vs. 10.28 EU; *P* = .01). Children who received a second dose had higher serum IgG LPS antibody titers at 10 weeks.

### Table 1. Serum IgG and IgM lipopolysaccharide antibodies in 2-5-year-old children, as assayed by ELISA.

<table>
<thead>
<tr>
<th>Antibody, no. of vaccine doses</th>
<th>Time after vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 weeks</td>
</tr>
<tr>
<td>IgG (n = 25)</td>
<td>0.27 (0.19–0.50)</td>
</tr>
<tr>
<td>2 (n = 24)</td>
<td>0.27 (0.15–0.63)</td>
</tr>
<tr>
<td>IgM (n = 25)</td>
<td>2.48 (1.43–3.45)</td>
</tr>
<tr>
<td>2 (n = 24)</td>
<td>2.07 (1.50–3.36)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are geometric mean ELISA units (25th–75th percentiles). Comparisons of means were made using Student’s t test. For IgG: all postvaccination vs. prevaccination comparisons, *P* < .001; 2 doses vs. 1 dose at 10 weeks, *P* = .01; 2 doses vs. 1 dose at 0 weeks and 2 doses at 10 weeks vs. 2 doses at 6 weeks, not significant. For IgM: 1 dose at 26 weeks vs. 1 dose at 0 weeks, *P* = .02; all other postvaccination vs. prevaccination comparisons, *P* < .01; 1 dose vs. 2 doses at 0 weeks, 2 doses at 10 weeks vs. 2 doses at 6 weeks, 2 doses vs. 1 dose at 10 weeks, and 2 doses vs. 1 dose at 26 weeks, not significant.

* a The second dose was given to 1 group of children at this time point.
in our phase 1 trial. The bactericidal titers were approximately posite to the 0157-rEPA vaccine was age related (table 2). Compared with those in adults in our phase 1 trial, children had lower prevaccination titers, and the differences were statistically significant (in the 1-dose group, 3.66 vs. 5.29 EU), but the increase was not statistically significant. At 26 weeks after the first dose was administered, the geometric mean titers of serum IgM LPS antibodies in this group of children (6.97 vs. 5.29 EU), remained higher than were the prevaccination titers, and the increase was not statistically significant.

To a lesser degree than it induced increases in serum IgG LPS antibody titers, the 0157-rEPA vaccine induced increases in serum IgM LPS antibody titers (table 1). An increase was observed as early as 1 week after the first dose was administered (in all children, 7.09 vs. 2.28 EU; P < .005). However, unlike serum IgG LPS antibody titers, serum IgM LPS antibody titers declined at 6 weeks after the first dose was administered. Four weeks after the second dose was administered (at week 10 of the study), there was a small increase in geometric mean titers of serum IgM LPS antibodies in this group of children (6.97 vs. 5.29 EU), but the increase was not statistically significant. At 26 weeks after the first dose was administered, the geometric mean titers of serum IgM LPS antibodies in both groups remained higher than were the prevaccination titers, and the differences were statistically significant (in the 1-dose group, 3.66 vs. 2.48 EU [P = .02]; in the 2-dose group, 4.61 vs. 2.07 EU [P < .0001]). There was no correlation between the serum IgG and IgM LPS antibody titers.

**Age-related serum IgG LPS antibody responses elicited by the 0157-rEPA vaccine.** The antibody response to the 0157-rEPA vaccine was age related (table 2). Compared with those in adults in our phase 1 trial, children had lower prevaccination and postvaccination titers (prevaccination titers, 0.27 vs. 0.47 EU; postvaccination titers, 5.63 vs. 32.82 EU). At 26 weeks after the first dose was administered, the increase in serum IgG LPS antibody titers was ~20-fold in children and 70-fold in adults.

**Bactericidal assay.** Neither the prevaccination serum samples nor the samples treated with complement showed bactericidal activity (table 3). Serum samples from children receiving 1 or 2 doses of vaccine showed high bactericidal titers against E. coli O157:H7 LPS, and these were similar to those observed in our phase 1 trial. The bactericidal titers were approximately correlated with the serum IgG LPS antibody titers (before 2-mercaptoethanol treatment, r = .75; after 2-mercaptoethanol treatment, r = .78). There was no correlation between bactericidal titers and IgM LPS antibody titers (r < .10).

**Cross-reaction with V. cholerae O1.** Fourteen postvaccination serum samples were chosen randomly for analysis of serum IgG antibodies to LPS of V. cholerae O1 serotypes Inaba and Ogawa. None showed significant increases in geometric mean titers of serum IgG antibodies to serotype Ogawa LPS (table 4). There was, however, an ~3-fold increase in the geometric mean titers of serum IgG antibodies to serotype Inaba LPS (21.38 vs. 7.22 EU; P < .001). There was no correlation between the titers of serum IgG antibodies to LPS of E. coli O157:H7 LPS and those to LPS of V. cholerae O1 serotype Inaba (r < .1).

**DISCUSSION**

The investigational E. coli O157:H7 conjugate vaccine was safe and immunogenic in 2-5-year-old children. None of the children had a fever or a significant skin reaction. Nearly all of the children had a >10-fold increase in serum IgG LPS antibody titers at 6 weeks after the first dose was administered. At 26 weeks after the first dose was administered, the serum IgG LPS antibody titers remained significantly higher than were the prevaccination titers (an ~20-fold increase). A second dose of the vaccine did not induce a booster response, as has been observed in this age group in response to most conjugate vaccines [40, 47-49]. The vaccine also elicited high bactericidal activity that

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>IgG antibody titer</th>
<th>IgM antibody titer</th>
<th>Bactericidal titer in Serum treated with 50 mmol/L 2-mercaptoethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECO 161</td>
<td>100.00</td>
<td>75.60</td>
<td>1:1280</td>
</tr>
<tr>
<td>023</td>
<td>33.83</td>
<td>6.81</td>
<td>1:2560</td>
</tr>
<tr>
<td>033</td>
<td>24.44</td>
<td>7.82</td>
<td>1:320</td>
</tr>
<tr>
<td>035</td>
<td>17.21</td>
<td>7.72</td>
<td>1:320</td>
</tr>
<tr>
<td>041</td>
<td>14.23</td>
<td>3.76</td>
<td>1:320</td>
</tr>
<tr>
<td>043</td>
<td>11.28</td>
<td>3.12</td>
<td>1:320</td>
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<tr>
<td>047</td>
<td>12.98</td>
<td>18.94</td>
<td>1:320</td>
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<tr>
<td>048</td>
<td>21.87</td>
<td>12.25</td>
<td>1:320</td>
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<tr>
<td>054</td>
<td>18.65</td>
<td>16.00</td>
<td>1:320</td>
</tr>
<tr>
<td>055</td>
<td>21.60</td>
<td>4.61</td>
<td>1:320</td>
</tr>
</tbody>
</table>

**Table 3. Reciprocal bactericidal activity of serum lipopolysaccharide (LPS) antibodies elicited in 2-5-year-old children.**

**NOTE.** Serum samples were collected from children 42 days after the first dose of vaccine was administered. The reference serum samples (from subject nos. ECO 161 and ECO 110) were from a phase 1 trial in adults who received the same vaccine. Titers are expressed as the reciprocal of the dilution that killed 50% of the organisms. Antibody titers were calculated in comparison with those of the reference serum sample, which was arbitrarily assigned a value of 100.00 ELISA units of IgG (ECO 161) or IgM (ECO 110) antibodies. Correlation coefficient for IgG antibody titers vs. serum, r = .75; correlation coefficient for IgG antibody titers vs. serum treated with 2-mercaptoethanol, r = .78. All bactericidal titers vs. IgM antibody titers, no correlation (r < .10).
animal-to-person or person-to-person spread is described in the infection of humans may prevent the establishment of infection. The findings of the present study suggest that vaccination should prevent the establishment of the inoculum through vaccination should prevent the establishment of the infection. Because of the low infectious dose of the organism, lysis of the erythrocytes can occur. This is particularly relevant in outbreaks [1-6,12-13,18-24,52]. Although the findings demonstrate that vaccination elicits high bactericidal antibody response was age related; adults responded with a 4-fold higher serum IgG LPS antibody titers than did children. The postvaccination serum IgG LPS antibody titers in 2-5-year-old children were higher than were the prevaccination titers in adults (6.08 vs. 0.47 EU; P<0.001), implying that the children had immunity to E. coli O157:H7 after vaccination.

In the past 2 decades, E. coli O157:H7 infection has evolved from a clinical novelty to a major public health concern. Hundreds of outbreaks have been reported worldwide, with hospitalization required in up to 47% of symptomatic patients [7-9, 11, 50]. Extrarenal complications also occur in substantial numbers [11, 50, 51]. Complications following hemolytic-uremic syndrome include stroke, seizures, and myocardial dysfunction [51, 52].

Because there is no effective treatment for E. coli O157:H7 infection, efforts have been directed toward prevention. Our findings demonstrate that vaccination elicits high bactericidal activity that is correlated with serum IgG LPS antibody titers. Because of the low infectious dose of the organism, lysis of the inoculum through vaccination should prevent the establishment of infection. The findings of the present study suggest that vaccination of humans may prevent the establishment of infection.

Most E. coli O157:H7 infections occur either sporadically or in outbreaks [1-6, 9, 12-13, 18-24, 52]. Although E. coli O157: H7 infection is generally considered to be a foodborne disease, animal-to-person or person-to-person spread is described in petting zoos, households, day care centers, and chronic care facilities [53-56]. In day care centers, where shedding among children may last 2-4 weeks, a high potential for secondary transmission is combined with a population at risk for severe outcomes. Because the O157-rEPA vaccine elicited high serum LPS antibody titers as early as 7 days after the first dose was administered, and on the basis of our experience with a Shigella sonnei-rEPA conjugate vaccine trial during an outbreak, this vaccine may be used to prevent primary or secondary transmission of E. coli O157:H7 during outbreaks [37,41]. Alternatively, E. coli O157:H7 conjugates could be used to prepare high-titer IgG LPS antibody globulin for prophylaxis and treatment of case contacts during an outbreak.

The O-specific polysaccharide of E. coli O157 LPS is a linear copolymer composed of the tetrasaccharide repeating unit (1-3)-α-D-GalpNAC-(1-2)-α-D-PerpNAC-(1-3)-α-L-Fucp-(1-4)-β-D-Glccp-(1-2) [45]. Although perosamine is found in the O-specific polysaccharide of both the Inaba and Ogawa serotypes of V. cholerae 01, only a weak cross-reaction with serotype Inaba was found in children who received the E. coli O157:H7 conjugate vaccine. This cross-reaction, however, was not observed in our phase 1 trial [41]. In a previous study, antibody cross-reactions with LPS from E. coli O157:H7 were found after cholera vaccinations [57]. Numerous other E. coli serotypes—as well as other gram-negative organisms containing LPS, such as Citrobacter species, Yersinia enterocolitica, Salmonella urbana, Pseudomonas maltophilia, and Brucella melitensis—cross-react.

### Table 4. IgG antibodies to Vibrio cholerae 01 serotype Inaba lipopolysaccharide (LPS) or Escherichia coli O157:H7 LPS.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Prevacuation serum sample</th>
<th>Postvaccination serum sample</th>
<th>Prevacuation serum sample</th>
<th>Postvaccination serum sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>005</td>
<td>0.26</td>
<td>96.06</td>
<td>3.71</td>
<td>15.32</td>
</tr>
<tr>
<td>006</td>
<td>1.17</td>
<td>78.58</td>
<td>9.10</td>
<td>21.69</td>
</tr>
<tr>
<td>019</td>
<td>0.51</td>
<td>58.71</td>
<td>5.47</td>
<td>29.45</td>
</tr>
<tr>
<td>021</td>
<td>2.25</td>
<td>32.26</td>
<td>13.03</td>
<td>32.57</td>
</tr>
<tr>
<td>024</td>
<td>0.99</td>
<td>22.52</td>
<td>21.59</td>
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</tr>
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<td>030</td>
<td>0.12</td>
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<td>1.75</td>
<td>23.72</td>
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<td>0.39</td>
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<td>036</td>
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<td>13.94</td>
<td>4.55</td>
<td>5.67</td>
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<td>15.54</td>
<td>16.42</td>
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<td>039</td>
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<td>15.18</td>
<td>1.59</td>
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</tr>
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<td>043</td>
<td>0.27</td>
<td>16.12</td>
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<td>26.85</td>
</tr>
<tr>
<td>047</td>
<td>0.39</td>
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<td>28.13</td>
<td>35.59</td>
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<tr>
<td>055</td>
<td>0.31</td>
<td>30.85</td>
<td>4.87</td>
<td>13.80</td>
</tr>
<tr>
<td>Geometric mean titer</td>
<td>0.47</td>
<td>26.73</td>
<td>7.22</td>
<td>21.38</td>
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</table>

**NOTE.** Data are ELISA units (EU). Antibodies were measured by ELISA and compared with those in a reference serum sample (from subject no. ECO 161 in a phase 1 trial in adults who received the same vaccine), which was arbitrarily assigned 100.00 EU of E. coli O157:H7 LPS antibodies, and a reference serum sample (from subject no. CHO 24 in a cholera vaccine clinical trial), which was arbitrarily assigned 100.00 EU of V. cholerae O1 serotype Inaba LPS antibodies.
with \textit{E. coli} O157:H7 [30, 58–62]. Prolonged exposure to these antigens in the environment probably contributes to the high serum IgG LPS antibody titers found in US adults and, thus, may explain the age-related attack rate of \textit{E. coli} O157:H7.

Shigella toxin (Stx1) and Stx2 are important virulence factors for causing hemolytic-uremic syndrome [2, 7, 10, 33]. We have prepared a conjugate of O-specific polysaccharide with the B-subunit of Stx1, and this conjugate elicited high serum titers of neutralizing antitoxin in mice [63]. Because Stx2 is the most common Stx found in patients with hemolytic-uremic syndrome, we plan to prepare an O-specific polysaccharide conjugate with the B-subunit of Stx2 [64].

It is apparent that \textit{E. coli} O157:H7 infections impose a substantial clinical and public health burden. Given the seriousness of the disease, the lack of therapeutic options, and the vastness of transmission sources, prevention of infection should focus on immunization. Efforts should be directed toward those at highest risk, especially those in community settings. Although outbreaks of infection have gained the most attention, sporadic disease accounts for the majority of the disease burden, and this situation underscores the importance of universal immunization to prevent disease. Because hemolytic-uremic syndrome occurs most often in children <5 years old, we plan to evaluate the efficacy of the \textit{E. coli} O157:H7 conjugate vaccine in infants, with the objective of administering it as part of routine vaccination [65, 66].

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