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Safety and Immunogenicity of Vi Conjugate Vaccines for Typhoid Fever in Adults, Teenagers, and 2- to 4-Year-Old Children in Vietnam

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The capsular polysaccharide of *Salmonella typhi*, Vi, is an essential virulence factor and a protective vaccine for people older than 5 years. The safety and immunogenicity of two investigational Vi conjugate vaccines were evaluated in adults, 5- to 14-year-old children, and 2- to 4-year-old children in Vietnam. The conjugates were prepared with *Pseudomonas aeruginosa* recombinant exoprotein A (*r*EPA) as the carrier, using either *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP; Vi-*r*EPA$_1$) or adipic acid dihydrazide (ADH; Vi-*r*EPA$_2$) as linkers. None of the recipients experienced a temperature of >38.5°C or significant local reactions. One injection of Vi-*r*EPA$_2$ into adults elicited a geometric mean (GM) increase in anti-Vi immunoglobulin G (IgG) from 9.62 enzyme-linked immunosorbent assay units/ml (EU) to 465 EU at 6 weeks; this level fell to 119 EU after 26 weeks. In the 5- to 14-year-old children, anti-Vi IgG levels at 6 weeks elicited by Vi-*r*EPA$_2$, Vi-*r*EPA$_1$, and Vi were 169, 22.8, and 18.9 EU, respectively ($P = 0.0001$ for Vi-*r*EPA$_1$ and Vi with respect to Vi-*r*EPA$_2$). At 26 weeks, the anti-Vi IgG levels for recipients of Vi-*r*EPA$_2$, Vi-*r*EPA$_1$, and Vi were 30.0, 10.8, and 13.4 EU, respectively ($P < 0.001$ for Vi-*r*EPA$_1$ and Vi with respect to Vi-*r*EPA$_2$); all were higher than the preinjection levels ($P = 0.0001$). Vi-*r*EPA$_2$ also elicited the highest anti-Vi IgM and IgA levels of the three vaccines. In the 2- to 4-year-old children at 6 weeks following the first injection, Vi-*r*EPA$_2$ elicited an anti-Vi IgG level of 69.9 EU compared to 28.9 EU for Vi-*r*EPA$_1$ ($P = 0.0001$). Reinjection increased Vi antibody levels from 69.9 to 95.4 EU for Vi-*r*EPA$_2$ and from 28.9 to 83.0 EU for Vi-*r*EPA$_1$. At 26 weeks, anti-Vi IgG levels remained higher than those at preinjection (30.6 versus 0.18 for Vi-*r*EPA$_2$ and 12.8 versus 0.33 for Vi-*r*EPA$_1$; $P = 0.0001$ for both). Vi vaccine is recommended for individuals of 5 years of age or older. In the present study, the GM level of anti-Vi IgG elicited by two injections of Vi-*r*EPA$_2$ in the 2- to 4-year-old children was higher than that elicited by Vi in the 5- to 14-year-old children (30.6 versus 13.4; $P = 0.0001$). The safety and immunogenicity of the Vi-*r*EPA$_2$ conjugate warrant further investigation.

Typhoid fever remains a common and serious disease that is increasingly difficult to treat because of resistance to multiple antibiotics (10, 23, 25, 31). More than 80% of *Salmonella typhi* strains from the Mekong Delta of Vietnam are now resistant to ampicillin, chloramphenicol, nalidixic acid, or ciprofloxacin (10, 25).

Typhoid fever in children younger than 5 years old was often unrecognized due to atypical clinical symptoms, difficulties in the number and volume of blood drawings, and less-than-optimal culture media (4, 9, 22, 27, 34). Similar to findings in other parts of Southeast Asia, a recent study in the Mekong Delta showed that the attack rate of typhoid fever was 198/100,000 population annually, with the highest incidence occurring among children under 15 years of age; 478/100,000 annually for school-age children; and 358/100,000 for 2- to 4-year-old children (22, 33). The three licensed typhoid vaccines are not suitable for routine immunization of infants (5, 12). Orally administered attenuated *S. typhi* Ty21a requires at least three doses and had a low rate of efficacy in an area with a high incidence rate of typhoid fever, and its efficacy has not been demonstrated in young children (24, 33). Failure to identify the protective antigen(s) or the vaccine-induced immune response has hindered improvement of the Ty21a vaccine. Parenterally administered inactivated cellular vaccines elicit a high rate of adverse reactions and have not been shown to be effective in young children (2, 11). In two randomized double-blinded vaccine-controlled clinical trials in Nepal and the Republic of South Africa, one injection of Vi induced about 70% efficacy in children 5 years old or older (1, 17, 18). Recently, similar results were obtained by the Lanzhou Institute of Biologic Products in the People's Republic of China (reference 38 and unpublished data). Vi is easily standardized and is licensed in more than 60 countries including the United States (37). However, Vi induces only short-lived antibody responses in children 2 to 5 years of age (unpublished data) and does not elicit protective levels in children younger than 2 years; in adults, reinjection after 2 years restores the level of vaccine-induced Vi antibody but does not elicit a booster response (16, 20). These age-related and T-independent immunologic properties are similar to those of most polysaccharide vaccines (28).

To improve its immunogenicity, Vi was conjugated to proteins with *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) (35, 36). Recently, we used another method, in which carrier proteins were treated with adipic acid dihydrazide (ADH) and bound to Vi in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (19, 32). Vi conjugates synthesized with ADH proved to be more immunogenic in mice and guinea pigs than those prepared with SPDP (19). In this study, the safety and immunogenicity of Vi conjugates prepared by these

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methods were compared in adults, 5- to 14-year-old children, and 2- to 4-year-old children in Vietnam.

MATERIALS AND METHODS

Vi polysaccharide. Vi, manufactured by Pasteur Mérieux Connaught, Serums et Vaccins, Lyon, France, complied with the requirements of the World Health Organization (37).

Protein. Recombinant exoprotein A (*r*EPA), a genetically reconstructed, nontoxic, fully antigenic derivative of *Pseudomonas aeruginosa* exotoxin A (ETA) that was used as the carrier protein, was isolated from *Escherichia coli* BL21 as described previously (6, 13, 19). The endotoxin content of *r*EPA was <50 endotoxin units/mg. *r*EPA showed no toxicity in mice at 500 times the lethal dose of ETA.

Conjugates Vi-*r*EPA$_1$ and Vi-*r*EPA$_2$. Vi-*r*EPA$_1$ was prepared with SPDP as the linker (35, 36). Briefly, 360 mg of cystamine, dissolved in 20 ml of pyrogen-free saline (PFS), was mixed with 120 mg of the Vi, and the pH was brought to 5.0 with 0.1 M NaOH. EDC was added to a final concentration of 0.1 M, and the pH was maintained at 5.0 for 3 h with 0.1 N HCl. The reaction mixture was dialyzed against pyrogen-free water at 4°C and freeze-dried. The sulfhydryl content was 1.3% (wt/wt). SPDP, 14 mg in 1.6 ml of ethanol, was added to 7 ml of *r*EPA (10 mg/mL) and mixed for 2 h at room temperature and then overnight at 4°C. The reaction mixture was passed through a Bio-Gel P-6 column in phosphate-buffered saline (pH 7.4) (PBS)–1 mM EDTA (pH 7.2), and the void-volume fractions were pooled, concentrated, sterile-filtered, and stored at 4°C. The SPDP-to-*r*EPA ratio was 10.6 mol/mol. Dithiothreitol (37.3 mg) was added to 3 ml of Vi-cystamine (10 mg/ml in PBS) with stirring for 2 h at room temperature. The reaction mixture was passed through a 2.5- by 30-cm column of Bio-Gel P-6 in PFS, and the void-volume fractions were sterile-filtered and added to 4.0 ml of *r*EPA-SPDP (31.5 mg). After being mixed for 2 h at room temperature, the mixture was passed through a 2.5- by 90-cm column of Sephacryl S-1000 in PBS at 4°C. The conjugate-containing fractions were pooled and denoted Vi-*r*EPA$_1$.

Vi-*r*EPA$_2$ was synthesized with ADH as the linker (19, 32). Briefly, 4.6 ml of 0.5 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (pH 5.6) was added to 300 mg of *r*EPA in 24.6 ml of PFS: the resultant mixture had a pH of 5.7. ADH (1.05 g) and then EDC (60.8 mg) were added with stirring for 1 h at room temperature. The mixture was dialyzed overnight at 4°C against 6 liters of PFS (pH 6.8) containing 0.25 mM sodium phosphate (PFS+P). Then the mixture was passed through a 1.5- by 90-cm column of Sephadex G-50 in PFS+P. The void-volume fractions were concentrated on an Amicon membrane (YM10) and sterile-filtered. The adipic acid hydrazide-to-protein ratio of *r*EPA-AH was 0.023 (wt/wt).

Vi, 100 mg in 10 ml of PFS, was mixed with 2.4 ml of 0.5 M MES buffer (pH 5.6) at room temperature. While the mixture was being stirred, 63 mg of EDC followed by 100 mg of *r*EPA-AH (10.1 mg/ml) were added. The volume of the reaction mixture was brought to 33.3 ml with PFS so that the final concentration of Vi and *r*EPA was 3 mg/ml each and that of EDC was 10 mM. The reaction mixture (pH 5.6) was stirred for 3 h at room temperature. After 3 h, the pH of the mixture was adjusted to 7.0 with 1 M sodium phosphate buffer (pH 7.2) and the mixture was stored overnight at 4°C. The mixture was passed through a 2.5- by 90-cm Sephacryl S-1000 column in PFS–0.1% thimerosal–0.005 M sodium phosphate buffer (pH 7.0). The void-volume fractions were pooled and denoted Vi-*r*EPA$_2$.

The final containers were assayed in accordance with Code of Federal Regulations item 610.11. The final containers of Vi-*r*EPA$_1$ (75 μg of Vi/ml and 71 μg of protein/ml) and Vi-*r*EPA$_2$ (48 μg of Vi/ml and 43 μg of protein/ml) were stored at 4°C.

The Vi vaccine, a U.S.-licensed vaccine, was lot K1140 manufactured by Pasteur Mérieux Connaught, Swiftwater, Pa., and contained 50 μg of Vi/ml.

Clinical protocol. The investigation was approved by the Ministry of Health of Vietnam, the Institutional Review Board of the National Institute of Child Health and Human Development (OH-96-CH-NO44 for Vi-*r*EPA$_1$ and OH-95-CH-NO45 for Vi-*r*EPA$_2$) and the Food and Drug Administration (IND 4334, SPAS-11089-01 for Vi-*r*EPA$_1$; IND 6990, SPAS-13609-01 for Vi-*r*EPA$_2$).

Informed consent was obtained from adults and from parents or guardians of vaccinees younger than 18 years. All studies were carried out in Cao Lânh District, Dong Thap Province, Vietnam. A 0.5-ml dose of Vi, Vi-*r*EPA$_1$, or Vi-*r*EPA$_2$ was administered intramuscularly into the deltoid muscle. The temperature and the condition of the injection site of the vaccinees were determined 6, 24, and 48 h following vaccination.

The safety and immunogenicity of Vi-*r*EPA$_1$ had been evaluated in U.S. adults (36). In the present study, only Vi-*r*EPA$_2$ was evaluated in adults. After the administration of Vi-*r*EPA$_2$ to 22 adults proved safe, 157 5- to 14-year-old children, recruited from the elementary, middle, and high schools in the district, were randomized to receive one injection of a conjugate or Vi. After no serious side reactions were observed, 203 2- to 4-year-old children, recruited from the Bong Sen Nursery, were randomized to receive either one or two injections of the same conjugate 6 weeks apart. Of these children, 103 received Vi-*r*EPA$_1$ (58 received one dose, and 45 received two doses) and 100 received Vi-*r*EPA$_2$ (48 received one dose, and 52 received two doses). Children who were absent from school on the ensuing 2 days were visited at home by the District Health medical staff.

TABLE 1. Vi antibody levels in serum elicited by one injection of Vi-*r*EPA$_2$ in adults[a]

Antibody	Vi antibody level (EU) in serum[b]		
	Preinjection	6 wk postinjection	26 wk postinjection
IgG[c]	9.62 (5.0–20.8)	465 (293–894)	119 (52.8–277)
IgM[d]	4.76 (2.68–7.48)	19.0 (6.27–36.2)	9.34 (4.78–18.2)
IgA[e]	0.20 (0.10–0.30)	8.85 (1.92–18.2)	4.99 (1.22–10.7)

[a] A total of 22 adults, 18 to 35 years old, were injected intramuscularly with 0.5 ml of Vi-*r*EPA$_2$, and blood samples drawn 6 and 26 weeks later.

[b] Levels are given as GM and 25–75 centiles.

[c] For IgG antibody levels, 465 and 119 versus 9.62 EU, $P = 0.0001$; 465 versus 119 EU, $P = 0.0001$.

[d] For IgM antibody levels, 19.0 and 9.34 versus 4.76 EU, $P < 0.01$; 19.0 versus 9.34 EU, not significant.

[e] For IgA antibody levels, 8.85 and 4.99 versus 0.20 EU, $P = 0.0001$; 8.85 versus 4.99 EU, $P = 0.0001$.

Blood samples were taken from all volunteers before and 6 and 26 weeks after the first injection. An additional blood sample was taken from all 2- to 4-year-old children 10 weeks after the first injection.

Serologic testing. Vi antibody was assayed by an enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with Vi (0.2 μg/well) from *Citrobacter freundii* WR7011; this Vi is structurally and serologically identical to the Vi from *S. typhi* (19).

Sera were assayed for immunoglobulin G (IgG) and anti-Vi IgM by using goat anti-human IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) or IgM (Sigma, St. Louis, Mo.) conjugated to alkaline phosphatase. The Anti-Vi IgG standard consisted of a plasma sample from an adult vaccinated with Vi polysaccharide typhoid vaccine (provided by Wendy Keitel, Baylor University, Houston, Tex.) (16). The Vi antibody content of this serum and of 12 additional samples, taken at random from adult vaccinees, was also assayed by a radioimmunoassay (RIA) by Pasteur Mérieux Connaught. Consistent with a previous finding (3), the levels of total anti-Vi antibody determined by RIA and of anti-Vi IgG determined by ELISA of these 12 serum samples showed a correlation at $r = 0.964$ ($P = 0.0001$). Serum from a typhoid carrier with high titer of anti-Vi IgM was used as the reference. The correlation between RIA results and IgM was low ($r = 0.084$). The lowest detectable level of the assay for anti-Vi IgG is 0.1 ELISA unit/ml (EU) and that for IgM is 1 EU.

The anti-Vi IgA level was measured by ELISA with a murine monoclonal anti-human IgA (HP6107; provided by George Carlone, Centers for Disease Control and Prevention) and rat alkaline phosphatase-labeled anti-murine IgG (H+L; Jackson ImmunoResearch Laboratories). The anti-Vi IgA standard was a high-titer serum sample from this study. The correlation coefficient between RIA and anti-VI IgA level measured by ELISA was 0.0045. The lowest detectable level of the assay for anti-Vi IgA is 0.01 EU.

The anti-*r*EPA IpG level was measured by ELISA with *r*EPA-coated plates (0.4 μg/well). Murine monoclonal anti-human IgG (HP6045) and rat alkaline phosphatase-labeled anti-mouse IgG (H+L) were used. The correlation coefficient of ELISA results when *r*EPA or *P. aeruginosa* ETA was used as the coating antigen was 0.99. The *r*EPA antibody titers were expressed as the geometric mean (GM) with respect to a reference human serum assigned a value of 100 EU.

Results were computed with an ELISA data-processing program (provided by the Biostatistics and Information Management Branch, Centers for Disease Control and Prevention) based on a four-parameter logistic-log function with a Taylor series linearization algorithm (26). Antibody titers are expressed as the GM and 25th to 75th centiles.

Statistical analysis. GM were calculated by using log transformation data and compared by paired and unpaired *t* tests as appropriate.

RESULTS

Clinical reactions. None of the volunteers had a temperature of >38.5°C or erythema or swelling of >2.5 cm following the first or second injection. Local reactions were confined to mild transient pain in a small fraction of the vaccinees of any age.

Vi antibody levels in adults. Because a conjugate prepared by the same method as Vi-*r*EPA$_1$ had been evaluated previously (36), only Vi-*r*EPA$_2$ was evaluated in adults in this study (Table 1). All adults had preinjection levels of anti-Vi IpG that

TABLE 2. Vi antibody levels in serum elicited by one injection of Vi, Vi-*r*EPA$_1$, or Vi-*r*EPA$_2$ in 5- to 14-year-old children[a]

Antibody and time	Antibody level (EU) in serum[b]		
	Vi (n = 50)	Vi-*r*EPA$_1$ (n = 52)	Vi-*r*EPA$_2$ (n = 55)
IgG			
Preinjection	0.44 (0.28–0.59)	0.42 (0.24–0.53)	0.67 (0.24–1.81)
6 wk	18.9 (7.84–44.1)	22.8 (7.86–58.9)	169.0 (80.8–290)
26 wk	13.4a (6.00–29.4)	10.8a (3.64–28.8)	30.0b (14.1–45.5)
IgM			
Preinjection	6.47 (4.02–9.50)	6.75 (4.16–10.2)	5.79 (3.33–8.25)
6 wk	25.2 (17.4–40.3)	48.0 (21.0–81.1)	92.1 (51.5–154)
26 wk	12.3 (6.64–21.2)c	26.2 (13.0–49.0)d	31.3 (17.9–56.7)e
IgA			
Preinjection	0.05 (0.03–0.07)	0.03 (0.02–0.04)	0.05 (0.02–0.10)
6 wk	2.64 (0.81–7.59)	1.99 (0.73–5.13)	16.5 (9.19–43.5)
26 wk	2.04 (0.81–6.72)f	0.99 (0.35–2.77)g	4.99 (3.34–18.9)h

[a] Children, 5 to 14 years old, were injected intramuscularly with 0.5 ml of Vi-*r*EPA$_2$, and blood samples were drawn 6 and 26 weeks later.
[b] Levels are given as GM and 25–75 centiles. b versus a, $P < 0.001$; d versus c, $P = 0.0002$; e versus d, not significant; h versus f, not significant; h versus g, $P = 0.02$.

were higher than those of the 5- to 14- and 2- to 4-year-old children (9.62 versus 0.51 or 0.26 EU [$P = 0.0001$]). Six weeks after injection, there was a 48-fold rise in the IgG level (465 versus 9.62 EU [$P = 0.0001$]), a 4-fold rise in the IgM level (19.0 versus 4.76 EU [$P = 0.0001$]), and a 44-fold rise in the IgA level (8.85 versus 0.20 EU [$P = 0.0001$]). At 26 weeks, the IgG level declined to 119 EU, the IgM level declined to 9.34 EU, and the IgA level declined to 4.99 EU; all three immunoglobulin Vi antibody levels were significantly higher than the preinjection levels.

Vi antibody levels in 5- to 14-year-old children. Preinjection levels of anti-Vi IgG, but not IgM or IgA, were significantly lower than those in adults (Table 2).

(i) IgG. At 6 weeks, all volunteers responded with greater than fourfold rises of the Vi antibody levels. Vi-*r*EPA$_2$ elicited higher levels of anti-Vi IgG than Vi-*r*EPA$_1$ or Vi (169 versus 22.8 or 18.9 EU [$P = 0.0001$]). At 26 weeks, the Vi antibody levels in all groups declined but remained more than fourfold higher than the preinjection levels: Vi-*r*EPA$_2$ > Vi > Vi-*r*EPA$_1$ (30.0 versus 13.4 or 10.8 EU [$P < 0.001$]). Of interest is that similar levels of anti-Vi antibody were elicited by Vi-*r*EPA$_1$ and Vi at both 6 and 26 weeks following vaccination.

(ii) IgM. At 6 weeks, all three vaccines elicited significant rises in the anti-Vi IgM levels: Vi-*r*EPA$_2$ > Vi-*r*EPA$_1$ > Vi (92.1, 48.0, and 25.2 EU, respectively). Vi-rEPA$_1$ induced a higher Vi antibody level than did Vi alone at both postvaccination intervals ($P \leq 0.0002$). At 26 weeks, the Vi antibody levels in the three groups were higher than those at preinjection: the levels in the recipients of the conjugates were higher than those in the recipients of Vi (31.3 or 26.2 versus 12.3 EU [$P \leq 0.0002$]).

(iii) IgA. At 6 weeks, Vi-*r*EPA$_2$ elicited the highest level of anti-Vi IgA among the three vaccines: Vi-*r*EPA$_2$ > Vi > Vi-*r*EPA$_1$ (16.5 versus 2.64 or 1.99 EU [$P = 0.002$]). The levels in each group declined at 26 weeks, but the rank order of anti-Vi IgA levels remained the same and all were higher than those at preinjection ($P = 0.0001$).

Vi antibody levels elicited by one or two injections of Vi conjugates in 2- to 4-year-old children. The preinjection levels of Vi antibodies of all isotypes were slightly lower than those in the 5- to 14-year-old children (Table 3).

(i) IgG. At 6 weeks after the first injection, 202 of 203 vaccinees responded with greater than an eightfold rise in the Vi antibody level, and there was no significant difference for each conjugate between the groups receiving one or two injections. At 6 weeks after one injection, Vi-*r*EPA$_2$ elicited higher levels of Vi antibody than did Vi-*r*EPA$_1$ (77.2 or 69.9 EU versus 30.2 or 28.9 EU [$P = 0.0001$]). Four weeks after the second injection, both conjugates elicited a rise in the anti-Vi IgG level (from 28.9 to 83.0 EU, a 2.87-fold rise, for Vi-*r*EPA$_1$ and from 69.9 to 95.4 EU, a 1.36-fold rise, for Vi-*r*EPA$_2$) (95.4 versus 83.0 EU [not significant]). At the 26-week interval, the

TABLE 3. Vi antibody levels in serum of 2- to 4-year-old children injected once or twice, 6 weeks apart, with Vi-*r*EPA$_1$ or Vi-*r*EPA$_2$[a]

Antibody and time	Antibody level (EU) in serum[b]			
	Vi-*r*EPA$_1$ (n = 58), one injection	Vi-*r*EPA$_1$ (n = 45), two injections	Vi-*r*EPA$_2$ (n = 48), one injection	Vi-*r*EPA$_2$ (n = 52), two injections
IgG				
Preinjection	0.32 (0.23–0.40)	0.33 (0.23–0.43)	0.19 (0.10–0.27)	0.18 (0.11–0.23)
6 wk	30.2a (15.2–53.5)	28.9a (18.0–53.0)	77.2b (41.3–165)	69.9b (36.5–126)
10 wk	21.4c (10.9–39.8)	83.0d (46.3–185)	54.3e (34.5–165)	95.4f (60.0–126)
26 wk	5.50 (2.90–9.80)	12.8g (9.66–25.1)	20.4 (9.82–40.9)	30.6h (22.4–51.6)
IgM				
Preinjection	4.72 (2.67–7.91)	5.00 (3.06–7.48)	3.61 (2.50–4.80)	3.93 (2.84–5.18)
6 wk	37.7 (24.1–55.2)	41.8 (26.0–62.7)	47.5 (27.8–81.5)	39.8 (22.9–57.5)
10 wk	35.7 (20.3–65.2)	82.5 (51.2–155)	34.8 (20.1–58.6)	31.8 (19.3–48.6)
26 wk	19.5 (12.2–29.4)	36.2 (21.8–62.1)	20.1 (13.1–32.3)	19.5 (12.8–30.6)
IgA				
Preinjection	0.02 (0.01–0.02)	0.02 (0.01–0.02)	0.02 (0.01–0.02)	0.02 (0.01–0.02)
6 wk	1.76 (1.30–2.54)	1.32 (0.71–3.34)	6.23 (2.79–18.1)	5.68 (2.22–12.9)
10 wk	1.48 (1.03–2.68)	2.00 (0.74–3.69)	4.21 (1.86–9.90)	4.99 (2.24–11.8)
26 wk	0.70 (0.50–1.12)	0.85 (0.50–2.02)	3.00 (1.37–8.49)	2.62 (1.09–7.29)

[a] Children, 2 to 4 years old, were injected once or twice, 6 weeks apart, with 0.5 ml of Vi-*r*EPA$_1$ or Vi-*r*EPA$_2$. All vaccinees had blood drawn before each injection and 4 and 20 weeks after the second injection.
[b] Levels are given as GM and 25–75 centiles. b versus a, $P < 0.001$; e and f versus c, $P = 0.0001$; f versus e, $P = 0.004$; h versus g, $P = 0.0001$; f versus d, not significant.

TABLE 4. anti-*r*EPA IgG levels in serum in the volunteers[a]

Age of volunteer (yr)	Vaccine	Anti-*r*EPA IgG level (EU) in serum[b]			
		0 wk	6 wk	10 wk	26 wk
15–44	Vi-*r*EPA$_2$	0.70 (0.5–0.8)	3.28 (1.6–9.5)	NA[c]	1.94 (1.1–5.9)
5–14	Vi	0.27 (0.1–0.6)	0.28 (0.1–0.6)	NA	0.29 (0.1–0.6)
	Vi-*r*EPA$_1$	0.32 (0.2–0.4)	1.97 (0.7–3.6)	NA	0.99 (0.4–1.8)
	Vi-*r*EPA$_2$	0.27 (0.2–0.6)	0.96 (0.3–2.5)	NA	0.93 (0.6–1.5)
2–4	Vi-*r*EPA$_1$	0.17 (0.1–0.3)	1.38 (0.8–1.9)	1.63 (1.1–2.1)	0.89 (0.5–1.5)
	Vi-*r*EPA$_1$[d]	0.17 (0.1–0.4)	1.29 (0.7–1.5)	5.94 (4.1–9.6)	1.88 (1.5–2.6)
	Vi-*r*EPA$_2$	0.22 (0.1–0.3)	0.57 (0.3–1.5)	0.73 (0.4–1.2)	0.77 (0.4–1.3)
	Vi-*r*EPA$_2$[d]	0.11 (0.1–0.2)	0.50 (0.2–1.0)	2.18 (1.4–3.9)	1.61 (1.0–2.8)

[a] The schedule of immunization for each age group has been described.
[b] Levels are given as GM and 25–75 centiles.
[c] NA, not applicable.
[d] Received second injection at 6 weeks.

anti-Vi IgG levels in recipients of two injections of Vi-*r*EPA$_2$ were the highest (30.6 EU). Although the numbers of children were small, the anti-Vi IgG levels in the recipients of two injections of Vi-*r*EPA$_2$, stratified for ages 2 years (20.7 EU [$n = 6$]), 3 years (35.6 EU [$n = 12$]), and 4 years (31.5 EU [$n = 19$]), were not statistically different.

At 26 weeks, two injections of Vi-rEPA$_2$ elicited a higher antibody level than did one injection of the Vi in the 5- to 14-year-old children (30.6 versus 13.4 EU [$P = 0.0001$]).

(ii) IgM. The preinjection anti-Vi IgM levels were slightly lower than those in the 5- to 14-year-old children. All the 2- to 4-year-old children responded with at least fourfold rises in antibody levels after the first injection. Reinjection of Vi-*r*EPA$_1$ elicited a rise in the anti-Vi IgM level (82.5 versus 41.8 EU [$P = 0.0003$]). Two injections of Vi-*r*EPA$_1$ elicited higher levels of anti-Vi IgM at 10 and 26 weeks than did two injections of Vi-*r*EPA$_2$ (82.5 versus 31.8 EU and 36.2 versus 19.5 EU [$P \leq 0.001$]).

(iii) IgA. At 6 weeks after one injection, both conjugates elicited rises in the levels of anti-Vi IgA (Vi-*r*EPA$_2$ > Vi-*r*EPA$_1$). Only a slight rise in the level of anti-Vi IgA was elicited by Vi-*r*EPA$_1$ and none was elicited by Vi-*r*EPA$_2$ after the second injection. The levels declined at the 26-week interval in all groups but remained significantly higher than those prior to injection.

anti-*r*EPA IgG. Both conjugates induced *r*EPA antibody in all age groups (Table 4). At 6 weeks after one injection, Vi-*r*EPA$_1$ elicited higher levels of anti-*r*EPA IgG than did Vi-*r*EPA$_2$ in both the 5- to 14-year-old and 2- to 4-year-old children (1.97 versus 0.96 EU in the first age group [$P = 0.02$]; 1.38 versus 0.57 EU in the second age group [$P = 0.003$]). Four weeks following the second injection, children receiving Vi-*r*EPA$_1$ had 5.94 EU of anti-*r*EPA IgG whereas the recipients of Vi-*r*EPA$_2$ had 2.18 EU ($P = 0.0004$). At 26 weeks, recipients of either conjugate had significantly higher levels of anti-*r*EPA IgG than those found preinjection.

DISCUSSION

One injection of Vi-*r*EPA$_2$ in children elicited higher anti-Vi IgG levels than did one injection of Vi-*r*EPA$_1$ in both age groups at all intervals after immunization. Two injections of Vi-*r*EPA$_2$ in the 2- to 4-year-old children elicited significantly higher anti-Vi IgG levels than did one injection of Vi in the 5- to 14-year-old children ($P = 0.0001$). Reinjection of either conjugate induced rises in antibody levels in the 2- to 4-year-old children (T-cell dependence). It can be predicted, therefore, that Vi-*r*EPA$_2$ will be more effective than Vi in individuals older than 5 years and will also protect children down to 2 years of age from typhoid fever (29).

Serum antibodies are the major response elicited by Vi (28). In passive-immunization experiments with sera taken from mice and sera from humans injected with cellular vaccines, anti-Vi IgG accounted for the protection conferred by the sera against challenge of mice with *S. typhi* (8, 14). Further, it is IgG, not IgM or IgA, that exudes onto the epithelial surface and accounts for most of the serum antibodies in the intestine (28, 29). On the basis of these data and by analogy to other encapsulated pathogens, we proposed that a critical level of anti-Vi IgG in serum is sufficient to confer immunity to typhoid fever and that its measurement will be essential to standardize Vi conjugates for licensure (30).

The greater immunogenicity of Vi-*r*EPA$_2$ than of Vi-*r*EPA$_1$ in animals and humans is consistent with the immunogenicity in mice of conjugates of *Staphylococcus aureus* capsular polysaccharide with ADH or SPDP as the linker (7). A Vi conjugate prepared by the same method as used for Vi-*r*EPA$_1$ injected in U.S. adults elicited an ~13-fold rise in the total anti-Vi IgG level 26 weeks after injection, as measured by RIA (0.21 to 2.69 μg of antibody/ml) (36). Based on our results with 5- to 14-year-old children, the increased immunogenicity of the Vi-*r*EPA$_1$-like conjugate (36) over Vi in adults is probably due mostly to increased IgM levels (unpublished data).

In areas of endemic infection with typhoid fever, including Vietnam, children and adolescents usually have a higher incidence of typhoid than do adults (2, 5, 15, 21). Our study shows that the preinjection levels of anti-Vi IgG in adults were significantly higher than those in individuals younger than 15 years. The elevated levels of anti-Vi IgG in adults could be attributed to multiple exposures to *S. typhi*. A 10-year follow-up study of a Vi efficacy trial in school-age children in South Africa showed that the Vi antibody levels had risen significantly following immunization but were similar in recipients of Vi and the control individuals (given groups A and C meningococcal polysaccharide vaccine) (15). This suggests that Vi antibodies are continually being stimulated in areas of endemic typhoid infection and explains the comparative resistance of adults to this disease.

With an increasing burden from multiple-antibiotic-resistant strains, the most effective measure to prevent the spread of typhoid fever is vaccination of all age groups. Accordingly, an efficacy trial of Vi-*r*EPA$_2$ in 2- to 5-year-old children is ongoing

in southern Vietnam, and an evaluation of its safety and immunogenicity in infants as part of their routine immunization is planned.

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