

The New England Journal of Medicine

Copyright © 2001 by the Massachusetts Medical Society

VOLUME 344

APRIL 26, 2001

NUMBER 17



THE EFFICACY OF A *SALMONELLA TYPHI* Vi CONJUGATE VACCINE IN TWO-TO-FIVE-YEAR-OLD CHILDREN

FENG YING C. LIN, M.D., M.P.H., VO ANH HO, M.D., HA BA KHIEM, M.D., DANG DUC TRACH, M.D., PH.D.,
PHAN VAN BAY, M.D., TRAN CONG THANH, M.D., ZUZANA KOSSACZKA, PH.D., DOLORES A. BRYLA, M.P.H.,
JOSEPH SHILOACH, PH.D., JOHN B. ROBBINS, M.D., RACHEL SCHNEERSON, M.D., AND SHOUSUN C. SZU, PH.D.

ABSTRACT

Background Typhoid fever is common in developing countries. The licensed typhoid vaccines confer only about 70 percent immunity, do not protect young children, and are not used for routine vaccination. A newly devised conjugate of the capsular polysaccharide of *Salmonella typhi*, Vi, bound to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A (rEPA), has enhanced immunogenicity in adults and in children 5 to 14 years old and has elicited a booster response in children 2 to 4 years old.

Methods In a double-blind, randomized trial, we evaluated the safety, immunogenicity, and efficacy of the Vi-rEPA vaccine in children two to five years old in 16 communes in Dong Thap Province, Vietnam. Each of the 11,091 children received two injections six weeks apart of either Vi-rEPA or a saline placebo. Cases of typhoid, diagnosed by the isolation of *S. typhi* from blood cultures after 3 or more days of fever (a temperature of 37.5°C or higher), were identified by active surveillance over a period of 27 months. We estimated efficacy by comparing the attack rate of typhoid in the vaccine group with that in the placebo group.

Results *S. typhi* was isolated from 4 of the 5525 children who were fully vaccinated with Vi-rEPA and from 47 of the 5566 children who received both injections of placebo (efficacy, 91.5 percent; 95 percent confidence interval, 77.1 to 96.6 percent; $P < 0.001$). Among the 771 children who received only one injection, there was 1 case of typhoid in the vaccine group and 8 cases in the placebo group. Cases were distributed evenly among all age groups and throughout the study period. No serious adverse reactions were observed. In all 36 children studied four weeks after the second injection of the vaccine, levels of serum IgG Vi antibodies had increased by a factor of 10 or more.

Conclusions The Vi-rEPA conjugate typhoid vaccine is safe and immunogenic and has more than 90 percent efficacy in children two to five years old. The antibody responses and the efficacy suggest that this vaccine should be at least as protective in persons who are more than five years old. (N Engl J Med 2001;344:1263-9.)

Copyright © 2001 Massachusetts Medical Society.

IN developing countries, typhoid fever is common, serious, and increasingly difficult to treat because of the increasing resistance of *Salmonella typhi* to antibiotics.¹⁻⁵ Typhoid fever was once thought to occur primarily in older children and young adults. In children younger than five years of age, typhoid fever was often unrecognized, because of atypical clinical symptoms, difficulties in drawing blood, and the use of culture mediums that were less than optimal.⁶⁻⁸ In a population-based study of three communes in Dong Thap Province in Vietnam, the annual attack rate for typhoid fever was highest among children younger than 15 years of age: it was 413 cases per 100,000 in this age group, and 358 per 100,000 among children 2 to 4 years old.⁹ Similar findings have been reported in other parts of Southeast Asia.¹⁰⁻¹²

Unfortunately, it is unlikely that the drinking water and foodstuffs in many developing countries, especially in rural areas, will become safe in the near future.^{1,5,13} No program to control the spread of typhoid fever by routine vaccination has been adopted, because of the limitations of the three licensed vaccines (parenteral inactivated whole-cell vaccines, oral attenuated *S. typhi* Ty21a vaccine, and parenteral Vi polysaccharide vaccine). These vaccines confer only about 70 percent protection in older children and adults and do not protect young children.^{1,14-17}

The capsular polysaccharide of *S. typhi*, Vi, is both an essential virulence factor and a protective anti-

From the National Institute of Child Health and Human Development (E.Y.C.L., Z.K., D.A.B., J.B.R., R.S., S.C.S.), and the National Institute of Diabetes and Digestive and Kidney Diseases (J.S.), National Institutes of Health, Bethesda, Md.; and Dong Thap Provincial Hospital, Cao Lanh (V.A.H., P.V.B.), the Pasteur Institute, Ho Chi Minh City (H.B.K., T.C.T.), and the National Institute of Hygiene and Epidemiology, Hanoi (D.D.T.) — all in Vietnam. Address reprint requests to Dr. Lin at the National Institute of Child Health and Human Development, Rm. 7B03, 6100 Executive Blvd., Bethesda, MD 20892-7510, or at link@exchange.nih.gov.

Other authors were Mai Ngoc Lanh, M.D., Steven Hunt, B.S., and Loc Trinh, B.S., National Institute of Child Health and Human Development; and Jeanne B. Kaufman, B.S., National Institute of Diabetes and Digestive and Kidney Diseases.

gen.^{18,19} Like most polysaccharide vaccines, the Vi vaccine does not induce either protective levels of antibodies in young children or a booster response.¹⁹⁻²¹ To overcome the limitations of the age-related and T-cell-independent immunogenicity of the vaccine, the same strategy was used as for the *Haemophilus influenzae* type b polysaccharide vaccine.^{22,23} Vi was bound to a nontoxic recombinant protein that is antigenically identical to *Pseudomonas aeruginosa* exotoxin A (rEPA).²⁴⁻²⁸ In initial trials, the resultant conjugate (Vi-rEPA) both enhanced the immunogenicity of Vi and gave it T-cell-dependent properties.^{27,28} Vi-rEPA elicited a booster response in children 2 to 4 years old, whose levels of IgG Vi antibody were approximately three times as high as those elicited by Vi in children 5 to 14 years old.²⁸ None of the vaccinated children had a temperature higher than 38.5°C or an area of swelling that was more than 2.5 cm in diameter after receiving an injection. On the basis of these results, we initiated a double-blind, randomized trial to determine the efficacy of Vi-rEPA in Vietnamese children two to five years old — an age group for which there is not yet an effective typhoid vaccine.

METHODS

Study Design

The study protocol was approved by the institutional review boards of the U.S. National Institute of Child Health and Human Development, National Institutes of Health, the Ministry of Health in Vietnam, and the Center for Biologics Evaluation and Research of the U.S. Food and Drug Administration, and the study was assigned a Single Project Assurance Number by the Office for Human Research Protections of the U.S. Department of Health and Human Services.

In November 1997, a census of 16 communes in the Cao Lanh District of Dong Thap Province in the Mekong Delta of Vietnam identified 14,285 children who were two to five years old. Almost all the households in the district rely on rivers and rain as their sources of water. Approximately 95 percent of the population of the district is engaged in agricultural activities. Cao Lanh District is served by the Dong Thap Provincial Hospital, and each commune (with a population of 5000 to 20,000) has a health center with a physician, assistant physicians, nurses, and approximately 20 community health workers.

Enrollment of Children

Informed consent for the enrollment of 13,776 children (96.4 percent of the two-to-five-year-old children in the district) was obtained from their parents or guardians during group meetings or home visits conducted by the health workers. Children with illnesses that required ongoing medical care were excluded. A unique seven-digit identification number was assigned to each enrolled child.

Vaccine

Vi-rEPA was prepared with Vi (lot 126A, Aventis Pasteur, Lyons, France) and rEPA and characterized as described previously.²⁷⁻²⁹ Each dose of Vi-rEPA conjugate contained 22.5 µg of Vi and 22 µg of rEPA in 0.5 ml of phosphate-buffered saline containing 0.01 percent thimerosal; its appearance was indistinguishable from that of the saline placebo. The five-dose vials, each containing 2.8 ml of Vi-rEPA or placebo, were labeled on a random basis with a number from 0 through 9, packaged 10 per box, and stored at 4°C. The code, kept by the Department of Pharmacy of the National Institutes of Health Clinical Center and by the chair-

man of the safety monitoring committee in Ho Chi Minh City, was broken on June 23, 2000.

Injection Protocol

Two rounds of injections were conducted in 1998, the first from February 21 through March 8 and the second from April 4 through April 20. The injections were administered by 64 teams. Each child was injected twice approximately six weeks apart, each time with 0.5 ml from a vial with a number identical to the last digit of his or her identification number. Before receiving the injection, the children were examined by the health staff, and their axillary temperatures were measured. Those with no fever (a temperature of less than 37.5°C) received injections in the left deltoid muscle, and the vial number was recorded. The children were observed for 20 minutes; 6, 24, and 48 hours after the injection, a community health worker measured body temperature and inspected the injection site.

Detection of Cases

The diagnosis of typhoid fever was made only when *S. typhi* was isolated from a blood culture. Children were visited weekly by a community health worker, at which time their history was taken and their axillary temperature was measured. Children who had a fever (a temperature of 37.5°C or higher) for at least three days were referred to the health station, and 6 ml of blood was drawn; 5 ml of this blood was transferred to a blood-culture bottle (DIFCO, Detroit), and 1 ml was used for serologic testing. Blood cultures were maintained at 37°C, and the clotted blood was maintained at refrigerator temperature (4° to 8°C); the samples were brought to the provincial hospital on the day they were obtained. Cultures were checked after one, two, and seven days, and *S. typhi* was identified by established biochemical and serologic assays at the microbiology laboratory of the provincial hospital and at the Clinical Microbiology Department of the National Institutes of Health. All isolates were also verified for Vi by the antiserum agar technique at the National Institute of Child Health and Human Development. There were no discrepancies in the results from the three laboratories.³⁰

To detect any additional cases of typhoid fever, we reviewed the bacteriologic records of the provincial hospital and the district hospital, which is located just south of the Cao Lanh District; no additional cases were found. The study ended on May 31, 2000, 27 months after the first injection had been administered.

Immunogenicity and Persistence of Vaccine-Induced IgG Vi Antibodies

Paired serum samples were obtained from 76 children before the first injection and four weeks after the second injection. To evaluate the duration of vaccine-induced Vi antibodies, a 2-ml blood sample was obtained from four randomly selected vaccinated children in each commune each month after the second injection. Serum IgG Vi antibodies were assayed by enzyme-linked immunosorbent assay (ELISA) and expressed in ELISA units relative to a standard arbitrarily assigned a value of 100 units.²⁸

Statistical Analysis

The efficacy of the vaccine was calculated with the following equation: $(1 - [\text{attack rate in the vaccine group} \div \text{attack rate in the placebo group}]) \times 100$ percent. Confidence limits were calculated by the method of Miettinen and Nurminen.³¹ The chi-square test or, when appropriate, Fisher's exact test was used for the comparison of categorical variables. Logarithms of the antibody concentrations were used in all calculations. Antibody levels were expressed as geometric means, with 25th and 75th percentiles. Comparisons of geometric means were performed with the unpaired or paired t-test.

RESULTS

Characteristics of the Study Participants

A total of 12,008 children received at least one injection (Table 1); 11,091 (92.4 percent) received two

TABLE 1. CHARACTERISTICS OF THE CHILDREN IN THE VACCINE AND PLACEBO GROUPS.

VARIABLE	VACCINE GROUP (N=5991)	PLACEBO GROUP (N=6017)
Male sex — no. of children (%)	3033 (50.6)	3120 (51.9)
Households — no.	5076	5082
Mean no. of persons/household		
≥18 Yr	2.89	2.94
<18 Yr	2.74	2.76
Injections received — no. of children		
2	5525	5566
1	388	383
Vial did not match child's ID no. — no. of children	78	68
Age at vaccination — no. of children (%)		
2 Yr	1338 (22.3)	1356 (22.5)
3 Yr	1422 (23.7)	1418 (23.6)
4 Yr	1628 (27.2)	1597 (26.5)
5 Yr	1603 (26.8)	1646 (27.4)
Days between the 2 injections		
Median	42	42
Range	29–57	28–56

injections from vials that were correctly labeled (5525 in the vaccine group and 5566 in the placebo group); 771 (6.4 percent) received one injection (388 in the vaccine group and 383 in the placebo group); and 146 (1.2 percent) were injected from a vial with an incorrect code (78 in the vaccine group and 68 in the placebo group). The sex, age at vaccination, household composition and size, and interval between the two injections were similar in the vaccine group and the placebo group. The interval between the two injections ranged from 28 to 57 days (median, 42).

Adverse Reactions

No serious adverse reactions were noted (Table 2). The highest temperature and maximal local reaction

recorded for each participant (from the values recorded at 6, 24, and 48 hours) were used for analysis. After the first injection, 113 children had a temperature of 37.5°C or higher — 81 in the vaccine group and 32 in the placebo group (P<0.001). Of the children with a temperature of 39.0°C or higher, 17 were in the vaccine group and 5 were in the placebo group (P=0.01). None of the children had erythema or swelling at the infection site of 5 cm or more in diameter.

After the second injection, a temperature of 37.5°C or higher was recorded for 109 children in the vaccine group and 25 children in the placebo group (P<0.001). One child in the vaccine group and one in the placebo group had a temperature of 39.0°C or higher. Swelling of more than 5 cm in diameter was noted in 20 children in the vaccine group, as compared with 1 in the placebo group (P<0.001). Erythema of 5 cm or more without swelling was noted in two children in the vaccine group and none in the placebo group. None of these reactions persisted for more than 48 hours after the injection.

Vaccine Efficacy

During the surveillance period (March 8, 1998, through May 31, 2000), 2335 blood cultures were obtained — 1121 from children in the vaccine group and 1214 from children in the placebo group. *S. typhi* was isolated from 61 children (34 boys and 27 girls) representing all the communes (Table 3). The number of cases of typhoid fever in each commune ranged from one to nine (median, four).

Among the 11,091 children who received two injections from vials with correct codes, there were 4 cases of typhoid fever in children in the vaccine group and 47 in children in the placebo group (efficacy, 91.5 percent; 95 percent confidence interval, 77.1 to 96.6). In the placebo group, one additional child had typhoid fever after receiving two injections of placebo from incorrectly labeled vials.

TABLE 2. LOCAL REACTIONS AND FEVER AFTER THE INJECTIONS OF Vi-rEPA CONJUGATE VACCINE AND SALINE PLACEBO.*

VARIABLE	FIRST INJECTION			SECOND INJECTION		
	Vi-rEPA (N=5991)	PLACEBO (N=6017)	P VALUE	Vi-rEPA (N=5525)	PLACEBO (N=5566)	P VALUE
	no. (%)			no. (%)		
Fever						
Temperature ≥37.5°C	81 (1.35)	32 (0.53)	<0.001	109 (1.97)	25 (0.45)	<0.001
Temperature ≥39.0°C	17 (0.28)	5 (0.08)	0.01	1 (0.02)	1 (0.02)	0.99
Swelling ≥5 cm	0	0		20 (0.36)	1 (0.02)	<0.001
Erythema ≥5 cm	0	0		2 (0.04)	0	0.24

*After injection, a community health worker measured the axillary temperatures of the children and inspected the injection sites for redness and swelling at 6, 24, and 48 hours. The highest temperature and maximal local reaction of those recorded at each time point for each participant were used to derive the numbers in this table.

TABLE 3. EFFICACY OF Vi-rEPA CONJUGATE VACCINE.

VARIABLE	VACCINE GROUP	PLACEBO GROUP	VACCINE EFFICACY (95% CI)*	P VALUE†
			%	
Children who received two correctly labeled injections — no.	5525	5566		—
Children with typhoid fever — no.	4	47	91.5 (77.1–96.6)	
Attack rate (cases/1000 children)	0.72	8.44		
All children — no.‡	5991	6017		—
Children with typhoid fever — no.	5	56§	91.1 (78.6–96.5)	
Attack rate (cases/1000 children)	0.83	9.31		
Children with typhoid fever				
Sex — no. (%)			—	0.05
Male	5 (100)	29 (52)		
Female	0	27 (48)		
Age at vaccination — no. (%)			—	0.58
2 Yr	2 (40)	16 (29)		
3 Yr	1 (20)	7 (12)		
4 Yr	0	16 (29)		
5 Yr	2 (40)	17 (30)		
Date of isolation of <i>S. typhi</i> — no. (%)			—	0.36
3/98–2/99 (12 mo)	2 (40)	33 (59)		
3/99–5/00 (15 mo)	3 (60)	23 (41)		

*CI denotes confidence interval.

†P values were calculated by Fisher's exact test.

‡The numbers of children include those who received two injections from vials with correct codes and those who received one or two injections from vials with incorrect codes.

§One child with typhoid fever who received two injections of placebo from a vial with an incorrect code is included.

Of the 771 children who received only one correctly labeled injection each, 1 child had typhoid fever 42 days after injection with Vi-rEPA and 8 children in the placebo group had typhoid fever at various times during the study period (vaccine efficacy, 87.7 percent). In total, there were 5 cases of typhoid fever in the vaccine group and 56 in the placebo group (efficacy, 91.1 percent; 95 percent confidence interval, 78.6 to 96.5). The cases in both groups were distributed evenly across the range of ages. Among the children in the vaccine group, there were two cases of typhoid fever during the first year after the second injection and three cases during the second year.

Among the 61 children with typhoid fever, 21 children (34.4 percent) were hospitalized for an average of 13 days (median, 12; range, 7 to 24); all of the hospitalized children were in the placebo group (21 of 56, vs. 0 of 5 in the vaccine group; P=0.22).

A total of 339 children (165 in the vaccine group and 174 in the placebo group) were lost to follow-up (3.1 percent): 308 moved out of the study area, 2 withdrew from the study, and 29 (12 in the vaccine group and 17 in the placebo group) died from drowning (19 children), dengue fever (3), pneumonia (2), the Stevens–Johnson syndrome (2), burns (1), a foreign body in the airway (1), or leukemia (1). No death was attributed to the vaccine or to typhoid fe-

ver. There were four isolates of *Salmonella paratyphi* A — one from a child in the vaccine group and three from children in the placebo group.

Serum IgG Vi Antibodies before the First Injection and Four Weeks after the Second Injection

A total of 76 paired serum samples were obtained from children before their first injection and four weeks after their second injection. There was no significant difference between the levels of IgG Vi antibodies before the first injection in the two groups, nor was there a significant difference between the level before the first injection and the level four weeks after the second injection in the placebo group (Table 4). In the group that received Vi-rEPA, in contrast, the level of IgG Vi antibodies increased by a factor of more than 575 (P<0.001); in 100 percent of the children in the vaccine group the level increased by a factor of at least 10.

Persistence of Vaccine-Induced IgG Vi Antibodies

Blood samples were obtained each month from four randomly selected children in each commune; only those obtained before the first injection and six months, one year, and two years after the second injection are represented in Table 5. The levels of IgG Vi antibodies before the first injection were similar

TABLE 4. SERUM IgG Vi ANTIBODY LEVELS BEFORE THE FIRST INJECTION AND FOUR WEEKS AFTER THE SECOND INJECTION.*

GROUP	NO. OF CHILDREN	IgG Vi ANTIBODY LEVELS	
		BEFORE 1ST INJECTION	4 WK AFTER 2ND INJECTION
		geometric mean ELISA units (25th–75th percentile)	
Vaccine group	36	0.11 (0.06–0.18)	72.9 (50.7–124)†
2–3 Yr old	13	0.10 (0.06–0.12)	69.0 (56.0–186)‡
4–5 Yr old	23	0.13 (0.07–0.26)	75.2 (46.9–124)‡
Placebo group	40	0.15 (0.06–0.19)	0.27 (0.08–0.55)†
2–3 Yr old	13	0.13 (0.07–0.20)	0.13 (0.07–0.14)
4–5 Yr old	27	0.16 (0.07–0.20)	0.40 (0.09–1.39)

*Serum IgG Vi antibodies were assayed by ELISA and expressed in ELISA units relative to a standard arbitrarily assigned a value of 100 units.²⁸ The serum IgG Vi antibody level rose by a factor of 10 or more in all children in the vaccine group.

†P<0.001 for the comparison of Vi-rEPA four weeks after the second injection (72.9) with placebo four weeks after the second injection (0.27).

‡P=0.08 for the comparison of Vi-rEPA four weeks after the second injection for children four to five years old at the time of injection (75.2) to Vi-rEPA four weeks after the second injection for children two to three years old at the time of injection (69.0).

in the vaccine group and the placebo group. Six months after the second injection, the IgG Vi antibody level in the children in the vaccine group was 22.5 ELISA units, or approximately 35 times as high as that in the children in the placebo group (P<0.001). The level of vaccine-induced IgG Vi antibodies decreased from 22.5 ELISA units at six months to 10.7 ELISA units at two years; this level was approximately 19 times as high as that among the children in the placebo group (10.7 vs. 0.57, P<0.001).

Over the two-year period, the IgG Vi antibody level in the placebo group increased from 0.15 to 0.57 ELISA unit (P<0.001).

A tendency toward age-related immunogenicity and the persistence of IgG Vi antibodies was observed when the vaccinated children were stratified into two age groups — two to three years old and four to five years old. The children four to five years old had a higher level of IgG Vi antibodies at all three time points after immunization, but these differences were not statistically significant. There was a smaller decline in the level of IgG Vi antibodies between six months and two years among the children four to five years old (26.7 percent) than among the children two to three years old (59.1 percent) (P=0.32).

Levels of Serum IgG Vi Antibodies in Vaccinated Patients

Only three serum samples collected at the time of blood culture were available from the four fully vaccinated children with typhoid; the levels of IgG Vi antibodies in the three samples were 4.76, 14.6, and 40.3 ELISA units. There were 37 serum samples from children with typhoid in the placebo group; in these samples, the levels of IgG Vi antibodies ranged from 0.05 ELISA unit to 3.7 ELISA units in 36 samples (geometric mean, 0.41 ELISA unit); one child had a level of 85.8 ELISA units.

DISCUSSION

In this trial the efficacy of Vi-rEPA (91.5 percent) was the highest reported for any typhoid vaccine. The results of this trial also show the efficacy of a typhoid vaccine in young children, for whom no effective vaccine was previously available. The high degree of efficacy was predicted by the immunogenicity of Vi-rEPA in children 2 to 4 years old as compared

TABLE 5. PERSISTENCE OF SERUM IgG Vi ANTIBODIES IN CHILDREN WHO RECEIVED TWO INJECTIONS, ACCORDING TO AGE AT THE TIME OF INJECTIONS.*

GROUP	SERUM IgG Vi ANTIBODY LEVELS			
	BEFORE INJECTIONS	6 MO AFTER 2ND INJECTION	1 YR AFTER 2ND INJECTION	2 YR AFTER 2ND INJECTION
	geometric mean ELISA units (25th–75th percentile)			
Vaccine group	0.12 (0.06–0.20)	22.5 (13.8–47.3)	18.7 (10.3–32.6)	10.7 (6.4–24.8)†
2–3 Yr old	0.12 (0.05–0.23)	18.6 (13.1–47.3)	14.3 (7.1–18.6)	7.6 (6.2–17.4)‡
4–5 Yr old	0.13 (0.07–0.20)	25.1 (13.8–49.0)	21.4 (14.0–37.5)	18.4 (8.2–41.4)‡
Placebo group	0.15 (0.06–0.25)	0.65 (0.28–1.03)	0.31 (0.17–0.52)	0.57 (0.15–2.50)†
2–3 Yr old	0.10 (0.05–0.18)	0.50 (0.27–0.51)	0.30 (0.15–0.38)	0.70 (0.26–1.13)
4–5 Yr old	0.20 (0.07–0.59)	1.00 (0.33–1.72)	0.30 (0.17–0.59)	0.50 (0.15–2.50)

*A blood sample was taken from four randomly chosen children in each of the 16 communes every month for 24 months. Only data for serum IgG Vi antibody levels before the injections and six months, one year, and two years after the injections are shown.

†P<0.001 for the comparison of Vi-rEPA at two years (10.7) with placebo at two years (0.57).

‡P=0.79 for the comparison of Vi-rEPA at two years for children four to five years old at the time of vaccination (18.4) with Vi-rEPA at two years for children two to three years old at the time of vaccination (7.6).

with that of Vi in adults and in children 5 to 14 years old.²⁸ Since it is the level of serum IgG Vi antibodies induced by a Vi-based vaccine that determines its efficacy, we predict that Vi-rEPA will be at least as protective in older children and adults.³² We are conducting passive surveillance of the participants for another two years to evaluate the duration of protection and the persistence of IgG Vi antibodies induced by Vi-rEPA.

Vi-rEPA was safe. For each type of adverse reaction, we subtracted the number of reactions among children in the placebo group from that in the vaccine group to estimate the rate of adverse reactions attributable to the vaccine. After the first injection, 0.8 percent of the children had a temperature of 37.5°C or higher, and 0.2 percent had a temperature of 39.0°C or higher; these high temperatures were attributable to the vaccine. After the second injection, fever attributable to Vi-rEPA occurred in 1.5 percent of the children, with a temperature of 37.5°C or higher, but none had a temperature of 39.0°C or higher. An area of swelling of 5 cm or more in diameter at the injection site was observed after the second injection in 20 children in the vaccine group, as compared with 1 child in the placebo group. This high degree of safety was also observed in our phase 1 and phase 2 studies of another lot of Vi-rEPA. Both lots of conjugates met the safety requirements of the World Health Organization for Vi polysaccharide vaccine.^{28,29} These specifications should therefore serve as minimal requirements for future lots of Vi-rEPA.

Our results suggest that the course of illness in the 5 children who received one or two injections of Vi-rEPA and in whom typhoid developed was milder than that in the 56 children with typhoid in the placebo group, because 21 of the latter (37.5 percent) were hospitalized, as compared with none of the former. The higher levels of IgG Vi antibody in the children with typhoid who had been vaccinated with Vi-rEPA, as compared with those in the children who had been injected with placebo, may explain this milder course of disease.

Similar numbers of blood cultures were obtained from children in the vaccine group (1121) and children in the placebo group (1214). Children in the placebo group from whom *S. typhi* was isolated (from 56 of the 1214 cultures) represent 4.6 percent of the children who had fever for three or more days. But the diagnosis of typhoid on the basis of the results of a single blood culture is not efficient.³³⁻³⁵ Only about 50 percent of typhoid cases identified by culture of the bone marrow are identified by blood culture. The yield of *S. typhi* from blood cultures is related to the duration of fever: the proportion of children with positive cultures of blood samples obtained after three days of fever was 4.6 percent, as compared with 17.1 percent after seven or more days of fever.⁹

The unusual structure, molecular size, and physicochemical properties of Vi made the development

of Vi-rEPA difficult.^{24-28,32,36,37} Both the safety and the immunogenicity of Vi-rEPA in this trial were similar to those of a similar product evaluated in phase 1 and phase 2 studies in Vietnam, indicating a consistency in the production of this new vaccine.²⁸ Vi-rEPA-induced levels of IgG Vi antibody declined by a factor of approximately two over two years, but there was no change in the efficacy of the vaccine. On the basis of the antibody level in children two to three years of age two years after vaccination, we estimate that the protective level of Vi-rEPA-induced IgG Vi antibodies is 7 ELISA units or lower. It is likely that Vi-rEPA will be at least 92 percent protective for persons older than five years of age, including military personnel and travelers to areas in which typhoid is endemic.⁴ Should Vi-rEPA elicit levels of Vi antibody in infants similar to those in children two to five years of age, this typhoid vaccine could be administered as part of the WHO Expanded Program on Immunization.¹

Supported in part by a contract with the National Institute of Child Health and Human Development (N01-HD-3269) and by Aventis Pasteur, Lyons, France.

We are indebted to the parents and local leaders of Cao Lanh District, Dong Thap Province, for their support of the program; to Robert Austrian, Robert Chanock, Joan D. Robbins, and Robert Tauxe for their helpful suggestions and comments on the manuscript; to Vee Gill, M.D., of the Clinical Microbiology Department of the National Institutes of Health for help with microbiologic testing; and to the following contributors to this study: Arthur Karpas, Ph.D., Jian Ping Li, Ph.D., Patricia Moyer, B.S., James Trundle, Ph.D., Mya Hlaing, A.B., National Institute of Child Health and Human Development; Luisa Gravelin, George Grimes, Pharmacy Development Branch, Clinical Center, National Institutes of Health; Martha C. Anderson, Center for Biologics Evaluation and Research, Food and Drug Administration; Doan Van Hong, M.D., Director, Health Services of Dong Thap Province; Dong Phi, M.D., Cao Lanh District Health Center; the health staff of the 16 commune health centers, Cao Lanh District Health Center, and Dong Thap Provincial Hospital; and Nguyen Thi Minh Phuong, M.D., and Nguyen Thi My Thanh, Pasteur Institute, Ho Chi Minh City, Vietnam.

REFERENCES

- Ivanoff B, Levine MM, Lambert PH. Vaccination against typhoid fever: present status. *Bull World Health Organ* 1994;72:957-71.
- Bhutta ZA, Naqvi SH, Razzaq RA, Farooqui BJ. Multidrug-resistant typhoid in children: presentation and clinical features. *Rev Infect Dis* 1991; 13:832-6.
- Wain J, Ho NT, Chinh NT, et al. Quinolone-resistant *Salmonella typhi* in Viet Nam: molecular basis of resistance and clinical response to treatment. *Clin Infect Dis* 1997;25:1404-10.
- Mermin JH, Townes JM, Gerber M, Dolan N, Mintz ED, Tauxe RV. Typhoid fever in the United States, 1985-1994: changing risks of international travel and increasing antimicrobial resistance. *Arch Intern Med* 1998; 158:633-8.
- Mermin JH, Villar R, Carpenter J, et al. A massive epidemic of multi-drug-resistant typhoid fever in Tajikistan associated with consumption of municipal water. *J Infect Dis* 1999;179:1416-22.
- Rao PT, Rao KVK. Typhoid fever in children. *Indian J Pediatr* 1959; 26:258-64.
- Scragg J, Rubidge C, Wallace HL. Typhoid fever in African and Indian children in Durban. *Arch Dis Child* 1969;44:18-28.
- Galloway H, Clark NS, Blackhall M. Paediatric aspects of the Aberdeen typhoid outbreak. *Arch Dis Child* 1966;41:63-8.
- Lin FY, Ho VA, Bay PV, et al. The epidemiology of typhoid fever in the Dong Thap Province, Mekong Delta region of Vietnam. *Am J Trop Med Hyg* 2000;62:642-6.

10. Thisyakorn U, Mansuwan P, Taylor DN. Typhoid and paratyphoid fever in 192 hospitalized children in Thailand. *Am J Dis Child* 1987;141:862-5.
11. Simanjuntak CH, Paleologo FP, Punjabi NH, et al. Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine. *Lancet* 1991;338:1055-9.
12. Sinha A, Sazawal S, Kumar R, et al. Typhoid fever in children aged less than 5 years. *Lancet* 1999;354:734-7.
13. Tarr PE, Kuppens L, Jones TC, Ivanoff B, Aparin PG, Heymann DL. Considerations regarding mass vaccination against typhoid fever as an adjunct to sanitation and public health measures: potential use in an epidemic in Tajikistan. *Am J Trop Med Hyg* 1999;61:163-70.
14. Acharya IL, Lowe CU, Thapa R, et al. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi*: a preliminary report. *N Engl J Med* 1987;317:1101-4.
15. Klugman KP, Gilbertson IT, Koornhof HJ, et al. Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet* 1987;2:1165-9.
16. Levine MM, Ferreccio C, Abrego P, Martin OS, Ortiz E, Cryz S. Duration of efficacy of Ty21a, attenuated *Salmonella typhi* live oral vaccine. *Vaccine* 1999;17:Suppl 2:S22-S27.
17. Typhoid immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1994;43(RR-14):1-7.
18. Felix A, Pitt RM. A new antigen of *B. typhosus*. *Lancet* 1934;2:186-91.
19. Robbins JD, Robbins JB. Re-examination of the protective role of the capsular polysaccharide (Vi antigen) of *Salmonella typhi*. *J Infect Dis* 1984;150:436-49.
20. Landy M. Studies on Vi antigen. VI. Immunization of human beings with purified Vi antigen. *Am J Hyg* 1954;60:52-62.
21. Keitel WA, Bond NL, Zahradnik JM, Cramton TA, Robbins JB. Clinical and serological responses following primary and booster immunization with *Salmonella typhi* Vi capsular polysaccharide vaccines. *Vaccine* 1994;12:195-9.
22. Schneerson R, Barrera O, Sutton A, Robbins JB. Preparation, characterization, and immunogenicity of *Haemophilus influenzae* type b polysaccharide-protein conjugates. *J Exp Med* 1980;152:361-76.
23. Robbins JB, Schneerson R, Anderson P, Smith DH. Prevention of systemic infections, especially meningitis, caused by *Haemophilus influenzae* type b: impact on public health and implications for other polysaccharide-based vaccines. *JAMA* 1996;276:1181-5.
24. Szu SC, Stone AL, Robbins JD, Schneerson R, Robbins JB. Vi capsular polysaccharide-protein conjugates for prevention of typhoid fever: preparation, characterization, and immunogenicity in laboratory animals. *J Exp Med* 1987;166:1510-24.
25. Szu SC, Li XR, Schneerson R, Vickers JH, Bryla DA, Robbins JB. Comparative immunogenicities of Vi polysaccharide-protein conjugates composed of cholera toxin or its B subunit as a carrier bound to high- or lower-molecular-weight Vi. *Infect Immun* 1989;57:3823-7.
26. Szu SC, Taylor DN, Trofa AC, et al. Laboratory and preliminary clinical characterization of Vi capsular polysaccharide-protein conjugate vaccines. *Infect Immun* 1994;62:4440-4.
27. Kossaczka Z, Bystricky S, Bryla DA, Shiloach J, Robbins JB, Szu SC. Synthesis and immunological properties of Vi and di-O-acetyl pectin protein conjugates with adipic acid dihydrazide as the linker. *Infect Immun* 1997;65:2088-93.
28. Kossaczka Z, Lin FY, Ho AV, et al. Safety and immunogenicity of Vi conjugate vaccines for typhoid fever in adults, teenagers, and 2- to 4-year-old children in Vietnam. *Infect Immun* 1999;67:5806-10.
29. Requirements for Vi polysaccharide typhoid vaccine (requirements for biological substances no. 48). WHO Tech Rep Ser 1994;840:15-29.
30. Nolan CM, LaBorde EA, Howell RT, Robbins JB. Identification of *Salmonella typhi* in faecal specimens by an antiserum-agar method. *J Med Microbiol* 1980;13:373-7.
31. Miettinen O, Nurminen M. Comparative analysis of two rates. *Stat Med* 1985;4:213-26.
32. Robbins JB, Schneerson R, Szu SC. Hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. *J Infect Dis* 1995;171:1387-98.
33. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet* 1975;1:1211-3.
34. Escamilla J, Santiago LT, Uylangco CV, Cross JH. Evaluation of sodium polyacrylate sulfonate as a blood culture additive for recovery of *Salmonella typhi* and *Salmonella paratyphi* A. *J Clin Microbiol* 1983;18:380-3.
35. Farooqui BJ, Khurshid M, Ashfaq MK, Khan MA. Comparative yield of *Salmonella typhi* from blood and bone marrow cultures in patients with fever of unknown origin. *J Clin Pathol* 1991;44:258-9.
36. Szu SC, Li XR, Stone AL, Robbins JB. Relation between structure and immunologic properties of the Vi capsular polysaccharide. *Infect Immun* 1991;59:4555-61.
37. Bystricky S, Szu SC. O-acetylation affects the binding properties of the carboxyl groups on the Vi bacterial polysaccharide. *Biophys Chem* 1994;51:1-7.

Copyright © 2001 Massachusetts Medical Society.