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Randomized, Double-Blind Placebo-Controlled Trial To Evaluate the Safety and Immunogenicity of Combined *Salmonella typhi* Ty21a and *Vibrio cholerae* CVD 103-HgR Live Oral Vaccines

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Healthy adults ($n = 330$) were randomized to receive either a bivalent vaccine composed of *Vibrio cholerae* CVD 103-HgR and *Salmonella typhi* Ty21a or a placebo. The combined vaccine was well tolerated. Approximately 80% of vaccinees manifested a significant rise in anti-*S. typhi* immunoglobulin G or immunoglobulin A lipopolysaccharide antibody levels. Significant (fourfold or greater) rises in anti-Inaba or anti-Ogawa vibriocidal antibody titer were achieved by 94 and 80% of vaccine recipients, respectively. Elevated baseline vibriocidal antibody titers showed a modest suppressive effect on the rate of seroconversion.

Enteric diseases continue to cause significant morbidity and mortality among individuals residing in undeveloped areas of the world (3, 28, 30). Many enteric pathogens, such as *Salmonella typhi* and *Vibrio cholerae*, are coendemic in such areas. While monovalent vaccines against cholera and typhoid fever have been available for some time, combined vaccines would offer a distinct public health benefit by simplifying immunization programs, expanding disease coverage, and reducing costs. The introduction of vaccination programs may prove to be necessary in areas where antibiotic resistance to first-line drugs used to treat such infections is widespread or where conditions are ripe for epidemic outbreaks (13, 22, 29).

Presently, two live oral attenuated bacterial enteric vaccines have been licensed for human use: *V. cholerae* CVD 103-HgR (18, 19) and *S. typhi* Ty21a (6). The fact that protection is afforded after only a single dose of CVD 103-HgR, versus three doses for Ty21a, had led us to investigate various vaccination regimens to optimize the immune response to both vaccines (9). Coadministration of both vaccine strains on day 1 followed by two additional doses of monovalent Ty21a vaccine on days 3 and 5 engendered the optimal rates of immune response to both vaccine components. Combining these two vaccines did not appear to increase the frequency or severity of adverse reactions above what was observed for the monovalent vaccines. However, since no placebo was included in our previous study, it was not possible to determine if there was an absolute excess rate of reactions with the bivalent formulation.

In the present study, healthy adult volunteers were randomized to receive either three doses of a heat-inactivated *Escherichia coli* placebo or the combined Ty21a and CVD 103-HgR vaccine strains followed by two doses of monovalent Ty21a given on alternate days.

S. typhi Ty21a and *V. cholerae* CVD 103-HgR were grown, harvested, and processed as previously described (9). Each dose of combined vaccine contained 2×10^9 to 6×10^9 CFU of Ty21a and 2×10^8 to 6×10^8 CFU of CVD 103-HgR. Each dose of the monovalent Ty21a vaccine also contained 2×10^9 to 6×10^9 CFU. Each dose of placebo contained approximately 5×10^8 heat-killed *E. coli* K-12 organisms. The vaccines (bivalent vaccine or monovalent Ty21a vaccine) or placebo were used to fill identically appearing aluminum foil sachets, which were labeled with an identifier. Buffer sachets contained 2.5 g of $NaHCO_3$ and 1.65 g of ascorbic acid. All sachets were tested for microbial purity and freedom from abnormal toxicity by standard tests.

A total of 330 healthy adult volunteers of both sexes were recruited from the student body and faculty of the University of Vienna, Vienna, Austria. Potential participants were given a physical examination, and a brief medical history was taken. Exclusion criteria included previous immunization with the trial vaccines, known hypersensitivity to the contents of the vaccine or placebo, acute febrile or gastrointestinal illness, an underlying disease which could adversely affect the immune response, and current receipt of immunosuppressive therapy. After written informed consent was obtained, each subject was randomized to receive either vaccine or placebo, at a ratio of approximately 4:1 for vaccine and placebo groups, respectively. Food intake was restricted for approximately 1 h before and after immunization. Vaccination was performed as described previously (9). The bivalent vaccine was given on day 1 and was followed by two doses of *S. typhi* Ty21a on days 3 and 5. The control group received three doses of *E. coli* placebo on days 1, 3, and 5. Subjects were observed for approximately 30 min after each immunization to detect immediate-type reactions. Subjects were requested to complete an adverse-reaction report form following each immunization (9). Each adverse event noted was to be scored as follows: none, mild, moderate (symptoms were found to affect normal daily activities), or severe (symptoms prevented normal daily activities). Diarrhea was classified as follows: absent, mild (slight deviation from normal stool movements, three or fewer loose stools in 24 h), moderate (four to six loose stools in 24 h), or severe (more than six loose stools in 24 h). Venous blood samples were collected before immunization (baseline) and at 10 and 14 days after immunization.

Anti-*S. typhi* immunoglobulin A (IgA) and IgG serum antibody levels were determined by enzyme-linked immunosorbent assay (ELISA) with *S. typhi* Ty2 lipopolysaccharide (LPS) as described elsewhere (9, 17). A significant response was defined as an increase of ≥ 0.15 optical density units (>3 standard deviations) over the baseline value at the same serum dilution.

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TABLE 1. Adverse reactions reported after immunization

Symptom	% (No.) reporting symptom[a]	
	Placebo group (n = 65)	Vaccine group (n = 256)
Nausea	9.2 (6)	14.8 (38)
Vomiting	1.5 (1)	2.3 (6)
Diarrhea	29.2 (19)	30.1 (77)
Abdominal cramps	9.2 (6)	15.6 (40)
Fever	1.5 (1)	2.7 (7)
Headache	43.1 (28)	37.5 (96)
Fatigue	56.9 (37)	49.6 (127)
Rash	4.6 (3)	3.1 (8)

[a] Combined rate for all three doses of vaccine or placebo administered. In all cases the differences between the vaccine and placebo groups were not significant ($P > 0.05$) as determined by chi-square analysis.

Inaba and Ogawa vibriocidal antibody titers were determined by a microtiter plate assay as previously described (5). A significant response was defined as a fourfold or greater rise in titer over the baseline value. Serum samples were analyzed in a blinded fashion.

Of the total of 330 subjects enrolled, there were 6 dropouts, none of which was due to an adverse event associated with immunization. Completed adverse-reaction report forms were returned by 321 subjects (256 vaccine recipients and 65 placebo recipients). The rates of adverse reactions reported for the two groups are shown in Table 1. The percentages of subjects who reported no reaction after ingesting all three doses were comparable among placebo and vaccine recipients (~25%). Approximately 75% of subjects from either group reported one or more reactions subsequent to immunization. The vast majority of symptoms reported were classified as mild and resolved spontaneously. There was no statistically significant difference in reaction rates between the two groups for any type of adverse event.

A complete set of serum samples was obtained from 260 vaccine and 65 placebo recipients. Immunization with CVD 103-HgR and Ty21a on day 1 followed by monovalent doses of Ty21a on days 3 and 5 induced a vigorous humoral immune response to both vaccine components (Tables 2 and 3). The anti-*S. typhi* IgA and IgG response rates were similar, with 80 and 83% of subjects responding, respectively (Table 2). Over 90% of the subjects responded with a significant rise in either IgG or IgA antibody levels. No placebo recipient showed a significant rise in either antibody class.

The serum Inaba and Ogawa vibriocidal antibody responses are shown in Table 3. There was a >40-fold rise in the Inaba geometric mean titer (GMT) 14 days after receipt of the bivalent vaccine, with 94% of the subjects achieving a ≥4-fold rise in titer. The increase in the Ogawa GMT was more modest (12-fold). This may be related to the fact that the baseline Ogawa mean titers were higher than the Inaba titers (see below). Eighty percent of vaccine recipients manifested a fourfold or greater rise in Ogawa vibriocidal titers after vaccination. Only five placebo recipients (6%) showed a significant rise; three of them did so against both test strains.

TABLE 2. Anti-*S. typhi* LPS antibody response following immunization with bivalent vaccine

Group[a]	GMT						% Seroconversion		
	IgG			IgA			IgG	IgA	IgG or IgA
	Day 1	Day 11	Day 15	Day 1	Day 11	Day 15			
Placebo	0.122	0.131	0.142	0.031	0.033	0.030	0	0	0
Vaccine	0.137	0.550[b]	0.571[b]	0.033	0.433[b]	0.367[b]	83	80	91

[a] Subjects were vaccinated on days 1, 3, and 5, and serum samples were obtained on days 1, 11, and 15.
[b] $P < 0.01$ versus baseline value.

There was a trend towards a lower rate of seroconversion with increasing baseline titers for both test strains (data not shown). Subjects with a baseline titer of ≥80 were significantly ($P < 0.05$) less likely to seroconvert after immunization than those with lower titers.

Combined vaccines have long formed the cornerstone of routine childhood vaccination programs. The ability to simultaneously confer protection against multiple pathogens offers many advantages to both the patient and the health care provider, offering expanded disease coverage, convenience, and reduced costs. The fact that many enteric pathogens are coendemic in developing nations indicates that multivalent vaccines would offer a distinct public health benefit to residents and to travellers visiting such areas (15, 20). In the present study we have confirmed and extended results from a previous preliminary clinical trial in which the Ty21a typhoid and CVD 103-HgR cholera vaccines were combined and administered to volunteers by various regimens (9).

The excellent safety previously demonstrated for monovalent Ty21a and CVD 103-HgR vaccines (19, 21, 23, 24) was not compromised when the vaccines were combined. While certain adverse reactions, such as nausea and abdominal cramps, occurred at a somewhat higher rate among subjects who received the vaccine than among those who received the placebo, the difference was not statistically significant. Such reactions were predominantly mild and transient. The overall rate for all types of adverse events in both groups was higher than would be expected on the basis of prior studies with these vaccines and an *E. coli* K-12 placebo (19, 21, 23, 24). This may be due in large part to intense monitoring for any potential reaction by a highly motivated study group. For example, nearly 40% of subjects complained of headache and malaise, which would not be expected to be caused by an oral vaccine. The fact that nearly 30% of placebo recipients noted abnormal stool movements, while 10% reported nausea or abdominal cramps, suggests that many reactions may be caused by sodium bicarbonate buffer even though it was neutralized with ascorbic acid to make it more palatable. Comparable elevated adverse reactions rates have been observed in two previous studies involving European subjects (2, 7).

Serum vibriocidal antibody rises following immunization have proven to be an excellent correlate of immunity to cholera in field studies (4, 11, 19, 27). The Inaba vibriocidal antibody seroconversion rate currently found (94%) was nearly identical to that in a previous study (92.5%) with this bivalent vaccine formulation and the same immunization regimen. These seroconversion rates are comparable to those obtained previously among North Americans and Europeans after the administration of a single dose of monovalent CVD 103-HgR (7, 14, 19, 27). As in prior studies with CVD 103-HgR, elevated baseline levels of vibriocidal antibodies suppressed the immune response to vaccination (12, 25, 26). This most likely reflects preexisting local gut immunity which acts to clear the vaccine strain before a degree of multiplication sufficient to engender an immune response is achieved (8, 26).

Rises in serum anti-*S. typhi* LPS antibodies engendered by vaccination with Ty21a have been shown to correlate with protection against disease in the field and may serve as a surrogate marker of immunity (15). The currently employed liquid Ty21a vaccine formulation was found to confer a higher

TABLE 3. Inaba and Ogawa vibriocidal antibody responses following immunization

Group[a]	GMT (range)						% Seroconversion[b]	
	Inaba			Ogawa			Inaba	Ogawa
	Day 1	Day 11	Day 15	Day 1	Day 11	Day 15		
Placebo	29.2 (<10–2,560)	32.9 (<10–1,280)	38.3 (<10–1,280)	75.8 (<10–5,120)	85.4 (<10–5,120)	99.4 (<10–5,120)	6	0
Vaccine	18.5 (<10–1,280)	5,120 (<10–20,480)	808 (<10–20,480)	62.4 (<10–10,240)	525 (<10–20,480)	778 (<10–20,480)	94	80

[a] Subjects were vaccinated on days 1, 3, and 5, and serum samples were obtained on days 1, 11, and 15.
[b] Seroconversion was defined as a fourfold or greater rise in titer over the baseline value.

level of protection than enteric coated vaccine in field trials conducted in Chile and Indonesia (16, 24). Among school-aged Chilean children, three doses of Ty21a presented as enteric coated capsules resulted in a 67% seroconversion rate (15). A similar rate (69%) was observed among young Thai children who were vaccinated with three doses of the liquid formulation (10, 21). Among adults residing in Europe or North America, the seroconversion rate ranged from 51 to 55% following the ingestion of three or four doses of enteric coated Ty21a vaccine with a test system identical to that used in the present study. In an independent study using a different ELISA system, the rate of response to three doses of enteric coated Ty21a was 86% (1; our unpublished observation). These data suggest that the liquid formulation possesses enhanced immunogenicity in individuals residing in regions where typhoid is not endemic which is not compromised by simultaneous administration of CVD 103-HgR. Also encouraging is the fact that the magnitude of the immune response to both vaccine strains was comparable to that observed among residents where these diseases are endemic. This would argue against the need to prime the immune system via natural exposure to obtain an optimal response rate (1, 10, 15, 21).

The present findings confirm that it is indeed possible to combine different live oral attenuated vaccine strains without compromising either safety or immunogenicity, even when the vaccines are used in a multiple dosing scheme. This has important practical implications with regard to how such vaccines can be formulated. For example, the protection conferred by CVD 103-HgR, and by its parent strain, CVD 103, is far superior if the challenge strain of *V. cholerae* expresses the homologous biotype and serotype compared with a cross-biotype, cross-serotype challenge (19, 27; our unpublished observations). Therefore, studies are now under way to evaluate the safety and immunogenicity of a bivalent cholera vaccine composed of CVD 103-HgR combined with an El Tor Ogawa variant.

REFERENCES

1. **Ambrosch, F., A. Hirschl, P. Kremsner, M. Kundi, C. Kunz, E. Rappold, and G. Wiedermann.** 1985. Orale Typhus-Lebendimpfung. Neuere Aspekte. Muench. Med. Wochenzeitschr. **127:**775–778.
2. **Barret, P., P. Clarke, and S. J. Cryz, Jr.** 1993. Oral cholera vaccine well tolerated. Br. Med. J. **307:**1425.
3. **Centers for Disease Control.** 1992. Cholera associated with international travel, 1992. Morbid. Mortal. Weekly Rep. **41:**664–667.
4. **Clements, J. D., F. van Loon, D. A. Sack, J. Chakraborty, M. R. Rao, F. Ahmed, J. R. Harris, M. R. Khan, M. Yunus, S. Huda, B. A. Kay, A.-M. Svennerholm, and J. Holmgren.** 1991. Field trial of oral cholera vaccines in Bangladesh: serum vibriocidal and antitoxic antibodies as markers of the risk of cholera. J. Infect. Dis. **163:**1235–1242.
5. **Clements, M. L., M. M. Levine, C. R. Young, R. E. Black, Y.-L. Lim, and R. M. Robins-Browne.** 1982. Magnitude, kinetics and duration of vibriocidal antibody response in North Americans after ingestion of *Vibrio cholerae*. J. Infect. Dis. **145:**465–473.
6. **Cryz, S. J., Jr.** 1990. Post-marketing experience with live oral Ty21a. Lancet **341:**49–50.
7. **Cryz, S. J., Jr., M. M. Levine, J. B. Kaper, E. Fürer, and B. Althaus.** 1990. Randomized, double-blind, placebo-controlled trial to evaluate the safety and immunogenicity of the live oral cholera vaccine strain CVD 103-HgR in Swiss adults. Vaccine **8:**577–580.
8. **Cryz, S. J., Jr., M. M. Levine, G. A. Losonsky, J. B. Kaper, and B. Althaus.** 1992. Safety and immunogenicity of a booster dose of *Vibrio cholerae* CVD 103-HgR live oral cholera vaccine in Swiss adults. Infect. Immun. **60:**3916–3917.
9. **Cryz, S. J., Jr., J. U. Que, M. M. Levine, G. Wiedermann, and H. Kollaritsch.** 1995. Safety and immunogenicity of a live oral bivalent typhoid fever (*Salmonella typhi* Ty21a)-cholera (*Vibrio cholerae* CVD 103-HgR) vaccine in healthy adults. Infect. Immun. **63:**1336–1339.
10. **Cryz, S. J., Jr., N. Vanprapaz, U. Thisyakorn, T. Olanratmanee, G. Losonsky, M. M. Levine, and S. Chearskul.** 1993. Safety and immunogenicity of *Salmonella typhi* Ty21a vaccine in young Thai children. Infect. Immun. **61:**1149–1151.
11. **Glass, R. I., A.-M. Svennerholm, M. R. Khan, S. Huda, M. I. Huq, and J. Holmgren.** 1985. Seroepidemiological studies of El Tor cholera in Bangladesh: association of serum antibody levels with protection. J. Infect. Dis. **151:**236–246.
12. **Gotuzzo, E., B. Butron, C. Seas, M. Penny, R. Ruiz, G. Losonsky, C. F. Lanata, S. S. Wasserman, E. Salazar, J. B. Kaper, S. J. Cryz, Jr., and M. M. Levine.** 1993. Safety, immunogenicity, and excretion pattern of single-dose live oral cholera vaccine CVD 103-HgR in Peruvian adults of high and low socioeconomic levels. Infect. Immun. **61:**3994–3997.
13. **Gupta, B. L., R. A. Bhujwala, and Shriniwas.** 1990. Multiresistant *Salmonella typhi* in India. Lancet **336:**252.
14. **Kotloff, K. L., S. S. Wasserman, S. O'Donnell, G. A. Losonsky, S. J. Cryz, Jr., and M. M. Levine.** 1992. Safety and immunogenicity in North Americans of a single dose of live oral cholera vaccine CVD 103-HgR: results of a randomized, placebo-controlled, double-blind, crossover trial. Infect. Immun. **60:**4430–4432.
15. **Levine, M. M., C. Ferreccio, R. E. Black, C. O. Tacket, R. Germanier, and The Chilean Typhoid Committee.** 1989. Progress in vaccines against typhoid fever. Rev. Infect. Dis. **11**(Suppl. 3)**:**S552–S567.
16. **Levine, M. M., C. Ferreccio, S. J. Cryz, Jr., and E. Ortiz.** 1990. Comparison of enteric-coated capsules and liquid formulation of Ty21a typhoid vaccine in a randomized controlled field trial. Lancet **336:**891–894.
17. **Levine, M. M., D. Herrington, J. R. Murphy, J. G. Morris, G. Losonsky, B. Tall, A. Lindberg, S. Svenson, S. Baqar, M. F. Edwards, and B. Stocker.** 1987. Safety, infectivity, immunogenicity and in vivo stability of two attenuated auxotrophic mutant strains of *Salmonella typhi*, 541 Ty and 543 Ty, as live oral vaccines in man. J. Clin. Invest. **79:**888–902.
18. **Levine, M. M., and J. Kaper.** 1993. Live vaccines against cholera: an update. Vaccine **11:**207–212.
19. **Levine, M. M., J. Kaper, D. Herrington, J. Ketley, G. Losonsky, C. O. Tacket, B. Tall, and S. J. Cryz, Jr.** 1988. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD 103 and CVD 103-HgR. Lancet **ii:**467–470.
20. **Nossal, G.** 1993. Prospects for new vaccines. World Health **46:**7–9.
21. **Olanratmanee, T., M. Levine, G. Losonsky, U. Thisyakorn, and S. J. Cryz, Jr.** 1992. Safety and immunogenicity of *Salmonella typhi* Ty21a liquid formulation vaccine in 4 to 6-year-old Thai children. J. Infect. Dis. **166:**451–452.
22. **Siddique, A. K., A. Salam, M. S. Islam, K. Akram, R. N. Majumdar, K. Zaman, N. Fronczak, and S. Laston.** 1995. Why treatment centres failed to prevent cholera deaths among Rwandan refugees in Goma, Zaire. Lancet **345:**359–361.
23. **Simanjuntak, C. H., P. O'Hanley, N. H. Punjabi, F. Noriego, G. Pazzaglia, P. Dykstra, B. Kay, C. Suharyono, A. Budiarso, A. R. Rifai, S. S. Wasserman, G. Losonsky, J. Kaper, S. J. Cryz, Jr., and M. M. Levine.** 1993. Safety, immunogenicity, and transmissibility of single-dose CVD 103-HgR in 24- to 59-month-old Indonesian children. J. Infect. Dis. **168:**1169–1176.
24. **Simanjuntak, C. H., F. P. Paleologo, N. H. Punjabi, R. Darmowigoto, S. H. Totosudirjo, P. Haryanto, E. Suprijanto, N. D. Witham, and S. L. Hoffman.** 1991. Oral immunization against typhoid fever in Indonesia with Ty21a vaccine. Lancet **338:**1055–1059.
25. **Su-Arehawatana, P., P. Singharaj, D. Taylor, et al.** 1992. Safety and immu-

nogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. J. Infect. Dis. **165:**1042–1048.

26. **Suharyono, C., C. Simanjuntak, N. Witham, N. Punjabi, D. Gray Heppner, G. Losonsky, H. Totosudirjo, A. R. Rifai, J. Clemens, Y. L. Lim, D. Burr, S. S. Wasserman, J. Kaper, K. Sorenson, S. J. Cryz, Jr., and M. M. Levine.** 1992. Safety and immunogenicity of single-dose live oral cholera vaccine CVD 103-HgR in 5-9 year-old Indonesian children. Lancet **340:**689–694.
27. **Tacket, C. O., G. Losonsky, J. P. Nataro, S. J. Cryz, Jr., R. Edelman, J. B. Kaper, and M. M. Levine.** 1992. Onset and duration of protective immunity in challenged volunteers after vaccination with live oral cholera vaccine CVD 103-HgR. J. Infect. Dis. **166:**837–841.
28. **Taylor, D. N., R. A. Pollard, and P. A. Blake.** 1983. Typhoid in the United States and the risk to the international traveller. J. Infect. Dis. **148:**599–602.
29. **Threefall, E. J., B. Said, B. Rowe, and A. Dávalos-Pérez.** 1993. Emergence of multiple drug resistance in *Vibrio cholerae* 01 El Tor from Ecuador. Lancet **342:**1173.
30. **World Health Organization.** 1992. Infected areas as of 28 November, 1991. Epidemiol. Rec. W. H. O. **48:**358–360.

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