INFECTION AND IMMUNITY, Feb. 1995, p. 707–709 Vol. 63, No. 2
0019-9567/95/$04.00+0

Attenuated Live Cholera Vaccine Strain CVD 103-HgR Elicits Significantly Higher Serum Vibriocidal Antibody Titers in Persons of Blood Group O

ROSANNA LAGOS,[1,2,3] ALFREDO AVENDAÑO,[2] VALERIA PRADO,[4] ISIDORO HORWITZ,[2] STEVEN WASSERMAN,[3] GENEVIEVE LOSONSKY,[3] STANLEY CRYZ, JR.,[5] JAMES B. KAPER,[3] AND MYRON M. LEVINE[1,3]*

Centro para Vacunas en Desarrollo—Chile,[1] Servicio de Salud Metropolitano Norte,[2] and Unidad de Microbiologia, Universidad de Chile Campus Oriente,[4] Santiago, Chile; Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland[3]; and Swiss Serum and Vaccine Institute, Berne, Switzerland[5]

Received 14 July 1994/Returned for modification 28 September 1994/Accepted 7 November 1994

Persons of blood group O are at increased risk of developing cholera gravis. In a randomized, placebo-controlled, double-blind safety-immunogenicity trial of live oral cholera vaccine CVD 103-HgR in 5- to 9-year-old Chilean children, vibriocidal antibody seroconversion (74% overall) did not differ by blood group. However, the reciprocal geometric mean titer (GMT) in blood group O vaccinees (GMT = 486) was higher than that in non-O vaccines (GMT = 179) ($P < 0.02$).

When infected with *Vibrio cholerae* O1 of the El Tor biotype, persons of blood group O are at significantly higher risk of developing severe cholera (cholera gravis) than individuals of other blood groups (1, 10, 22, 25). Whether such a correlation exists with classical biotype *V. cholerae* O1 is less clear (5). That the population living in the Ganges delta of Bangladesh and West Bengal, India, has one of the lowest prevalences of blood group O in the world (10) presupposes that this may be the consequence of selective genetic pressure in the ancestral home of cholera. The biologic basis for the relationship between O blood group and cholera gravis has not been elucidated, but it has been hypothesized that *V. cholerae* O1 may adhere better to mucosa of persons of this phenotype. Because of the increased susceptibility of individuals of O blood group to develop severe cholera, the immunogenicity and protective efficacy of cholera vaccines in persons of this blood group should be examined with particular attention. Such studies may also provide insights into blood group O as a risk factor. In the course of a large, randomized, placebo-controlled, double-blind trial designed to evaluate the clinical acceptability and immunogenicity of live attenuated oral cholera vaccine CVD 103-HgR among 5- to 9-year-old children in Santiago, Chile, we had the opportunity to assess the relationship between blood group and vibriocidal antibody titer.

The clinical protocol was approved by an ethics committee of the Servicio de Salud Metropolitano Norte in Santiago, Chile, and by the Institutional Review Board of the University of Maryland at Baltimore. Informed parental consent was obtained for age-eligible children recruited in public schools in a low-socioeconomic-level community. Excluded were children with fever or chronic diseases or those on antibiotic therapy.

Lyophilized vaccine and placebo were contained in randomized coded aluminum foil sachets. The code remained unbroken until the clinical study, including serology, was completed. Each vaccine packet contained 5×10^9 CFU of lyophilized CVD 103-HgR (recombinant vaccine strain derived from classical Inaba 569B) (15, 20, 21). Placebo packets contained heat-inactivated *Escherichia coli* K-12 (11, 16, 17, 28–30). Vaccine or placebo was mixed with buffer and water and administered as an oral cocktail (11, 16, 17, 28–30). Double-blind clinical follow-up to detect adverse reactions was maintained daily for 8 days after the single oral immunization. Diarrhea was defined as the passage of three or more liquid stools in 24 h; vomiting was defined as one or more episodes of emesis; an axillary temperature of ≥37.5°C was considered to be fever.

In total, 349 5- to 9-year-old children were enrolled, of whom 178 received the vaccine and 171 imbibed the placebo formulation. Gender, age strata, and blood groups were distributed similarly among the vaccine and placebo groups. Adverse events during the follow-up period were observed no more frequently in the vaccine than in the control group (Table 1). Diarrhea occurred in 2 of 178 vaccinees (1.1%) and in 1 of 176 controls (0.6%). Neither nausea, vomiting, nor malaise occurred more frequently in vaccinees than in placebo recipients (Table 1).

Serum samples collected on the day of vaccination and 8 days thereafter were assayed for vibriocidal antibody by microtiter method (2), and a fourfold or greater rise in reciprocal titer was considered significant. Serum Inaba vibriocidal antibodies were transformed to logarithms to better approximate normality. Rates of seroconversion were compared by chi-square test or by Fisher's exact test when appropriate. Paired sera were available from 97% of the participants (171 vaccinees and 168 placebo recipients). Overall, a fourfold or greater rise in serum vibriocidal antibodies was seen in 127 of 171 (74.3%) vaccinees, representing a mean 16-fold rise in vibriocidal reciprocal titer (Table 2). No vibriocidal seroconversions occurred among the 168 placebo recipients (prevaccination geometric mean titer [GMT] = 15; postvaccination GMT = 15).

Blood group was determined in 168 of the 171 vaccinees for whom paired sera were available. The relationship between blood groups (O and non-O) and the postimmunization reciprocal GMT of vibriocidal and antitoxic antibodies (vide infra) was examined by analysis of covariance (the independent variable was the blood group, the dependent variable was the day 8 titer, and the covariate was the day 0 titer). The rate of seroconversion among individuals of blood group O (78.2%) was not significantly different from that of the subjects with a

* Corresponding author. Phone: (410) 706-7588. Fax: (410) 706-6205.

TABLE 1. Adverse reactions among 5- to 9-year-old Chilean children receiving a single 5×10^9 CFU dose of CVD 103-HgR or placebo during 9 days of follow-up after immunization

Group	Children with[a]:		
	Diarrhea	Vomiting	Malaise
Vaccine	2/178 (1.1)	12/178 (6.7)	34/178 (19.1)
Placebo	1/171 (0.6)	16/171 (9.3)	34/171 (19.8)

[a] Each value is the number of children with the reaction per total number of children vaccinated. Percentages are shown in parentheses.

non-O phenotype (67.2%) ($P = 0.11$) (Table 2). However, the postimmunization vibriocidal reciprocal GMT in persons of blood group O (GMT = 486) was significantly higher than the GMT in persons of non-O blood groups (GMT = 179) ($P < 0.002$). The mean rise in vibriocidal titer was 23-fold in subjects of O blood group and 9-fold in vaccinees of other blood groups ($P = 0.004$).

Stool cultures were obtained on the first and seventh days following oral immunization and processed as described previously to detect excretion of the vaccine strain (16, 28, 30). The occurrence of positive stool cultures was not significantly greater in vaccinated children of blood group O (10.2%) than in vaccinees of other blood groups (6.7%) ($P = 0.37$). This study corroborates results of earlier studies that demonstrate that the CVD 103-HgR vaccine strain is highly immunogenic and yet minimally shed, thereby minimizing the chance of transmission to contacts or of introduction into environmental niches (20, 28, 30).

Immunoglobulin G antitoxin was measured by an enzyme-linked immunosorbent assay (24) wherein a rise in net optical density of ≥ 0.20, from comparing the baseline with the postimmunization specimen, was considered significant (11, 16, 17, 28–30). Rises in serum immunoglobulin G antitoxin occurred at similar rates in children of blood group O (37%) and those of other blood groups (31%). However, the postvaccination GMT of children of blood group O (i.e., 269) was slightly higher than that of children of other blood groups (i.e., 231) ($P = 0.16$). Children of blood group O manifested a mean 2.3-fold rise in GMT over baseline, whereas subjects of non-O blood group had a 1.7-fold rise in GMT.

Natural infection with *V. cholerae* O1, particularly of the classical biotype, elicits an immune response that confers long-lived protection against cholera (7, 9, 19). While intestinal secretory antibodies are believed to be the basis for this protection, serum vibriocidal response is generally accepted as the best correlate of protection (23). Antitoxin titers do not correlate with protection (23). For this reason, quantitating the rate of vibriocidal seroconversion and the mean fold rise and GMT of vibriocidal antibodies has been the main measure to evaluate the immunogenicity of live and inactivated oral cholera vaccines (6, 11, 16, 17, 20, 21, 27–30). The overall seroconversion rate (74%) and the mean vibriocidal rise (16-fold) observed in this study appear quite satisfactory, considering that only a single oral dose was administered. As a point of comparison, three doses of oral inactivated antigen vaccines stimulated much lower rates of vibriocidal antibody seroconversion among children of similar ages in a clinical trial in Bangladesh, and there was no difference in the immune responses of subjects of blood group O and those of non-O blood groups (6, 27).

TABLE 2. Blood group and vibriocidal antibody response to CVD 103-HgR live oral cholera vaccine in 5- to 9-year-old Chilean children

Blood group of children	*N*	Seroconversion (%)	GMT		Fold rise in titer
			Prevaccination	Postvaccination	
Non-O	67	67	19	179	9
O	101	78	21	486	23
P		NS	NS	0.002	0.004

This clinical trial in Santiago, Chile, offered an unusual opportunity to examine the vibriocidal antibody response in relation to blood group in recipients of a live oral cholera vaccine. The population of metropolitan Santiago, which has a high prevalence of individuals of blood group O (circa 60% of the population), is at risk of cholera since the disease is now endemic in neighboring Peru and Bolivia (26). However, to date, this population has had little contact with cholera as there have been only two small outbreaks of cholera in Santiago (18). The lack of prior antigenic contact with *V. cholerae* O1 means that immunogenicity could be examined in this population in relation to blood group in the absence of possible confounding effects stemming from prior immunity. The serological response recorded in this trial suggests that attenuated vaccine strain CVD 103-HgR may be more immunogenic in persons of blood group O (Table 2). What possible biological explanation can be evoked to explain this finding, and what are its implications?

The predisposition of blood group O individuals for cholera gravis does not derive from an increased susceptibility to infection with *V. cholerae* O1 (22) but rather from a more severe host clinical response to infection. One of the steps in the pathogenesis of cholera involves attachment of vibrios to the intestinal mucosa and then uptake of cholera enterotoxin into the enterocytes. Fimbrial colonization factors including toxin coregulated pili (31) and mannose-sensitive hemagglutinin fimbriae (14) mediate vibrio attachment to the mucosa. Furthermore, volunteer studies with recombinant strains lacking toxin-coregulated pili have shown that these fimbriae must be expressed by classical biotype strains for a vigorous vibriocidal response to ensue (13).

One possible explanation for the increased severity of cholera in persons of blood group O is that an enhanced adherence of vibrios to the intestinal mucosa occurs in such individuals. The human small intestinal epithelium is rich in blood group antigens; indeed, they should more appropriately be referred to as histo-blood group antigens (4, 8). An analysis of the structure of these antigens reveals that they consist of a backbone of L-fucose $\alpha 1 \rightarrow 2$D-gal$\beta 1 \rightarrow$R (the H antigen of O blood group) from which may emanate *N*-acetylgalactosamine or D-galactose residues, giving rise to A and B antigens, respectively (4). It is conceivable that the H antigen of blood group O serves as a better receptor for vibrio adhesins than the A or B antigens. The observation that both biotypes of *V. cholerae* O1 exhibit hemagglutination of human O erythrocytes that is inhibited by L-fucose provides some support to this hypothesis (12). An analogous interaction between gastrointestinal pathogen and histo-blood group of the host has been reported recently (3). *Helicobacter pylori* adheres more avidly to gastric mucosa of persons who express histo-blood group antigen Le^b (which has a terminal L-fucose in its structure, like H antigen) (3). Thus, if attenuated CVD 103-HgR, like wild-type *V. cholerae* O1, adheres more avidly to the intestinal mucosa of persons of blood group O, one might expect to encounter a heightened vibriocidal response in individuals of this blood group, as was observed.

Inactivated oral vaccines protect persons of blood group O less well than they protect persons of other blood groups (5). Since persons of blood group O who are immunized with CVD 103-HgR appear to exhibit enhanced vibriocidal antibody responses, this raises the intriguing question of whether this may translate to somewhat improved protection for this high-risk group. This question of the protective efficacy of CVD 103-HgR in relation to blood group must be answered in the course of large-scale, randomized, placebo-controlled, double-blind field trials. One such trial is currently being carried out with 67,000 subjects in North Jakarta, Indonesia, by the Indonesian National Institute of Health Research and Development (Cyrus Simanjuntak, principal investigator) and collaborating institutions under sponsorship of the World Health Organization.

This work was supported by a grant from the World Health Organization and by research contract NO1 AI 15096 and cooperative agreement UO1AI35948 from NIAID.

REFERENCES

1. **Barua, D.** 1977. ABO blood groups and cholera. Ann. Hum. Biol. **4:**489–492.
2. **Benenson, A. S., A. Saad, and W. H. Mosley.** 1968. Serological studies in cholera. The vibriocidal antibody response of cholera patients determined by a microtechnique. Bull. W.H.O. **38:**277–285.
3. **Boren, T., P. Falk, K. A. Roth, G. Larson, and S. Normark.** 1991. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science **262:**1892–1895.
4. **Clausen, H., and S. Hakamori.** 1989. ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang. **56:**1–20.
5. **Clemens, J. D., D. A. Sack, J. R. Harris, J. Chakraborty, M. R. Khan, S. Huda, F. Ahmed, J. Gomes, M. R. Rao, and A. M. Svennerholm.** 1989. ABO blood groups and cholera: new observations on specificity of risk and modifications of vaccine efficacy. J. Infect. Dis. **159:**770–773.
6. **Clemens, J. D., B. F. Stanton, J. Chakraborty et al.** 1987. B subunit-whole cell and whole cell-only oral vaccines against cholera: studies on reactogenicity and immunogenicity. J. Infect. Dis. **155:**79–85.
7. **Clemens, J. D., F. van Loon, D. A. Sack, M. R. Rao, F. Ahmed, J. Chakraborty, B. A. Kay, M. R. Khan, M. Yunus, J. R. Harris, A.-M. Svennerholm, and J. Holmgren.** 1991. Biotype as determinant of natural immunising effect of cholera. Lancet **337:**883–884.
8. **Finne, J., M. E. Breimer, G. C. Hansson, K. A. Karlsson, H. Leffler, J. F. G. Vliegenthart, and H. van Halbeek.** 1989. Novel polyfucosylated N-linked glycopeptides with blood group A,H,X,Y determinants from human small intestinal epithelial cells. J. Biol. Chem. **264:**5720–5735.
9. **Glass, R. I., S. Becker, I. Huq, B. J. Stoll, M. U. Khan, M. H. Merson, J. V. Lee, and R. E. Black.** 1982. Endemic cholera in rural Bangladesh, 1966–1980. Am. J. Epidemiol. **116:**959–970.
10. **Glass, R. I., J. Holmgren, C. E. Haley, M. R. Khan, A. M. Svennerholm, B. J. Stoll, K. M. Belayet Hossain, R. E. Black, M. Yunus, and D. Barua.** 1985. Predisposition for cholera of individuals with O blood group. Possible evolutionary significance. Am. J. Epidemiol. **121:**791–796.
11. **Gotuzzo, E., B. Butron, C. Seas, M. Penny, R. Ruiz, G. Losonsky, C. F. Lanata, S. S. Wasserman, E. Salazar, J. B. Kaper et al.** 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.
12. **Hanne, L. F., and R. A. Finkelstein.** 1982. Characterization and distribution of the hemagglutinins produced by *Vibrio cholerae*. Infect. Immun. **36:**209–214.
13. **Herrington, D. A., R. H. Hall, G. Losonsky, J. J. Mekalanos, R. K. Taylor, and M. M. Levine.** 1988. Toxin, toxin-coregulated pili, and the toxR regulon are essential for *Vibrio cholerae* pathogenesis in humans. J. Exp. Med. **168:** 1487–1492.
14. **Jonson, G., J. Sanchez, and A. M. Svennerholm.** 1989. Expression and detection of different biotype-associated cell-bound hemagglutinins of *Vibrio cholerae* O1. J. Gen. Microbiol. **135:**111–120.
15. **Kaper, J. B., and M. M. Levine.** 1990. Recombinant attenuated *Vibrio cholerae* strains used as live oral vaccines. Res. Microbiol. **141:**901–906.
16. **Kotloff, K. L., S. S. Wasserman, S. O'Donnell, G. A. Losonsky, S. J. Cryz, 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.
17. **Lagos, R., A. Avendano, I. Horwitz, V. Prado, C. Ferreccio, G. Losonsky, S. S. Wasserman, S. Cryz, J. B. Kaper, and M. M. Levine.** 1993. Tolerancia e inmunogenicidad de una dosis oral de la cepa de *Vibrio cholerae* O1, viva-atenuada, CVD 103-HgR: estudio de doble ciego en adultos Chilenos. Rev. Med. Chile **121:**857–863.
18. **Levine, M. M.** 1991. South America: the return of cholera. Lancet **338:**45–46.
19. **Levine, M. M., R. E. Black, M. L. Clements, L. Cisneros, D. R. Nalin, and C. R. Young.** 1981. Duration of infection-derived immunity to cholera. J. Infect. Dis. **143:**818–820.
20. **Levine, M. M., and J. B. Kaper.** 1993. Live oral vaccines against cholera: an update. Vaccine **11:**207–212.
21. **Levine, M. M., J. B. Kaper, D. Herrington, J. Ketley, G. Losonsky, C. O. Tacket, B. Tall, and S. Cryz.** 1988. Safety, immunogenicity, and efficacy of recombinant live oral cholera vaccines, CVD 103 and CVD 103-HgR. Lancet **2:**467–470.
22. **Levine, M. M., D. R. Nalin, M. B. Rennels, R. B. Hornick, S. Sotman, G. Van Blerk, T. P. Hughes, S. O'Donnell, and D. Barua.** 1979. Genetic susceptibility to cholera. Ann. Hum. Biol. **6:**369–374.
23. **Levine, M. M., and N. F. Pierce.** 1992. Immunity and vaccine development, p. 285–327. *In* D. Barua and W. B. Greenough III (ed.), Cholera. Plenum Medical Book Co., New York.
24. **Levine, M. M., C. R. Young, R. E. Black, Y. Takeda, and R. A. Finkelstein.** 1985. Enzyme-linked immunosorbent assay to measure antibodies to purified heat-labile enterotoxins from human and porcine strains of *Escherichia coli* and to cholera toxin: application in serodiagnosis and seroepidemiology. J. Clin. Microbiol. **21:**174–179.
25. **Motoky, T., and S. Murao.** 1977. Cholera and blood groups. Lancet **2:**404.
26. **Ries, A. A., D. J. Vugia, L. Beingolea, A. M. Palacios, E. Vasquez, J. G. Wells, N. G. Baca, D. L. Swerdlow, M. Pollack, N. H. Bean, L. Seminario, and R. V. Tauxe.** 1992. Cholera in Piura, Peru: a modern urban epidemic. J. Infect. Dis. **166:**1429–1433.
27. **Sack, D. A., J. D. Clemens, S. Huda et al.** 1991. Antibody response after immunization with killed oral cholera vaccines during the 1985 vaccine field trial in Bangladesh. J. Infect. Dis. **164:**407–411.
28. **Simanjuntak, C. H., P. O'Hanley, N. H. Punjabi, F. Noriega, G. Pazzaglia, P. Dykstra, B. Kay, Suharyono, A. Budiarso, A. Rifai, S. S. Wasserman, G. A. Losonsky, J. Kaper, S. Cryz, and M. M. Levine.** 1993. The safety, immunogenicity, and transmissibility of single-dose live oral cholera vaccine CVD 103-HgR in 24 to 59 month old Indonesian children. J. Infect. Dis. **168:**1169–1176.
29. **Su-Arehawatana, P., P. Singharaj, D. N. Taylor, C. Hoge, A. Trofa, K. Kuvanont, S. Migasena, P. Pitisuttitham, Y. L. Lim, G. Losonsky, J. B. Kaper, S. S. Wasserman, S. Cryz, P. Echeverria, and M. M. Levine.** 1992. Safety and immunogenicity of different immunization regimens of CVD 103-HgR live oral cholera vaccine in soldiers and civilians in Thailand. J. Infect. Dis. **165:**1042–1048.
30. **Suharyono, X., C. Simanjuntak, N. Witham, N. Punjabi, D. G. Heppner, G. Losonsky, H. Totosudirjo, A. R. Rifai, J. Clemens, Y. L. Lim, D. Burr, S. S. Wasserman, J. Kaper, K. Sorenson, S. Cryz, 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.
31. **Taylor, R. K., V. L. Miller, D. B. Furlong, and J. J. Mekalanos.** 1987. Use of phoA gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. Proc. Natl. Acad. Sci. USA **84:**2833.