
A single dose of live oral cholera vaccine CVD 103-HgR is safe and immunogenic in HIV-infected and HIV-noninfected adults in Mali

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Despite considerable experience with single-dose, live, oral cholera vaccine CVD 103-HgR in Asia, Europe, and the Americas, the vaccine had not been evaluated in sub-Saharan Africa or on individuals infected with human immunodeficiency virus (HIV). We therefore conducted a randomized, placebo-controlled, double-blind, cross-over clinical trial in 38 HIV-seropositive (without clinical acquired immunodeficiency syndrome (AIDS)) and 387 HIV-seronegative adults in Mali to assess its safety and immunogenicity. Adverse reactions (fever, diarrhoea and vomiting) were observed with similar frequency among vaccine and placebo recipients. The vaccine strain was not isolated from the coprocultures of any subject. The baseline geometric mean titre (GMT) of serum vibriocidal antibody was significantly lower in HIV-seropositives (1:23) than in HIV-seronegatives (1:65) (P = 0.002). Significant rises in vibriocidal antibody were observed in 71% of HIV-seronegatives and 58% of HIV-seropositives, and in 40% of HIV-seropositives with CD4⁺ counts below 500 per μ l. Following immunization, the peak vibriocidal GMT in HIV-seronegatives was 1:584 versus 1:124 in HIV-seropositives (P = 0.0006); in HIV-seropositives with CD4⁺ counts <500 per μ l, the peak vibriocidal GMT was 1:40 (P = 0.03 versus other HIV-seropositives).

CVD 103-HgR was safe in HIV-infected Malian adults, although serological responses were significantly attenuated among HIV-seropositives (particularly in those with CD4⁺ counts < 500 per μ l) relative to HIV-seronegatives. These results encourage further evaluations of this single-dose, oral cholera vaccine in high-risk populations such as refugees in sub-Saharan Africa.

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Introduction

The seventh cholera pandemic, caused by *Vibrio cholerae* O1 biotype El Tor, began in Celebes (Sulawesi), Indonesia, in 1961, reached Africa by 1970 (1, 2) and persisted thereafter, causing both endemic and epidemic disease (3–5). One of the most dramatic epidemiological events in this pandemic was the explosive outbreak of cholera that swept through Rwandan refugee camps near Goma, Zaire, during several weeks in 1994, when an estimated 70 000 cases and 12 000 deaths occurred (6). Several months after this tragedy, public health experts convened at WHO to consider the possible role that the new generation of oral cholera vaccines (7) might play in helping to control cholera in refugee camps and other emergency situations. It is unlikely that any vaccine could have had a marked impact on the outbreak in Goma because of the unusual circumstances in that specific setting (8). Nevertheless, it is reasonable to expect that the new vaccines may prove beneficial in future refugee situations where cholera poses a public health threat (9–11). In less acute contexts, vaccinating against cholera could

serve a useful adjunct role, particularly with an easily administered oral vaccine that rapidly confers protection after a single dose.

CVD 103-HgR is a genetically engineered, attenuated, *V. cholerae* O1 vaccine strain that has shown considerable promise as a single-dose, live, oral vaccine against cholera (12–21). Phase II clinical trials involving more than 6000 subjects, from 7 months to 65 years of age, have unequivocally established the safety and immunogenicity of single-dose CVD 103-HgR. Moreover, the live vaccine is minimally excreted and only rarely transmitted to contacts and has not been recovered from the environment (12–21). A single dose of CVD 103-HgR confers complete protection against moderate or severe cholera diarrhoea in volunteers experimentally challenged with wild-type *V. cholerae* O1 of either El Tor or classic biotype and Inaba or Ogawa serotype (12–14, 22). Moreover, protection is fully evident as early as the eighth day post vaccination (14). Despite the considerable experience with CVD 103-HgR in developing countries in Asia and Latin America (as well as in Europe and North America), it had not been evaluated in sub-Saharan Africa or on individuals infected with human immunodeficiency virus (HIV). Serological surveys of refugees in sub-Saharan Africa reveal an HIV prevalence of 1.2–10.8% (23–26). Before this live oral vaccine can be considered to be an adjunct control measure in regions where HIV and cholera pandemics coexist, its safety and immunogenicity have to be established in HIV-infected individuals in Africa. To provide these data, we performed a randomized, double-blind, cross-over trial in Mali, a country repeatedly struck by cholera during the past decade (1, 4, 5).

Materials and methods

Subjects, eligibility, and vaccination

The study protocol was approved by the Institutional Review Board of the University of Maryland, the Comité d'Ethiques of the Ecole Nationale de Médecine et de Pharmacie, Mali, and the WHO Secretariat Committee on Research Involving Human Subjects. The protocol also received technical review by the Food and Drug Administration (FDA), Bethesda, MD, USA.

This clinical trial required a population group with a high prevalence of HIV infection. In the initial step to identify such a population, post-secondary school students in Sikasso, Mali, were screened for antibodies to HIV, but proved to have a low seroprevalence (0.7%). Therefore, after consulting

with local health and civil authorities, a second stage of the study was initiated in Bamako in which commercial sex workers, a group known to have high prevalence of HIV infection, were invited to participate.

During both steps of the study, clinically healthy adults aged 18–50 years were screened using the HIV spot test (see below) to detect serological evidence of HIV-1 or HIV-2 infection. For each HIV-positive participant, an HIV-negative control was chosen, matched by age (± 5 years) and of the same sex. The screening and selection of the HIV-positive and HIV-negative subjects for the study were done by an investigator not directly involved with the clinical follow-up and evaluation. Excluded were individuals who had previously been vaccinated against cholera, who had a prior history of cholera infection, who had taken antibiotics within 4 days of enrolment, or who had diarrhoea or other acute illnesses on the day of enrolment. Women were screened for pregnancy using a rapid β -hCG test on serum or urine (Abbott TestPack Plus hCG-Combo, Abbott Park, IL, USA) and any individual testing positive was excluded. HIV-infected persons with thrush, wasting, or a history of chronic diarrhoea, weight loss, or repetitive pneumonias (i.e., with overt clinical acquired immunodeficiency syndrome (AIDS)) were also specifically excluded.

After the enrolled subjects had given their informed consent one half were randomly allocated to receive a dose of vaccine on day 0, while the other half received placebo. On day 12, a second oral inoculation was given, whereupon the participants who had ingested vaccine on day 0 now received placebo and vice versa. Packets of vaccine contained 5×10^9 colony forming units (CFU) of lyophilized CVD 103-HgR (15–20) together with a lactose filler (1.8g) and an aspartame sweetener (25mg). Identically appearing placebo packets contained only the lactose filler (1.8g) and aspartame (25mg). Each vaccine or placebo packet was accompanied by another packet containing an effervescent buffer made up of 2.5g sodium bicarbonate and 1.65g ascorbic acid. The buffer was mixed with water, the contents of the vaccine (or placebo) packet were added, and the resulting suspension was administered as an oral vaccine cocktail (16–21). The identity of the contents of the packets was unknown to both the clinical investigators performing the study and to the participants.

Medical supervision

After each dose of vaccine, the study participants were visited daily by physicians for the first 6 days and every other day for six more days to document

complaints, perform physical examinations, and measure axillary temperatures. All responses were recorded on standardized forms. Diarrhoea was defined as passage of at least three loose stools within a 24-hour period, vomiting as one or more episodes of emesis, and an axillary temperature $\geq 37.5^{\circ}\text{C}$ signified fever. Any subject who passed three or more loose stools in a 24-hour period was given oral rehydration with glucose and electrolytes.

Cholera serology

Venous blood was collected immediately prior to each oral inoculation (on days 0 and 12) and on day 24 (12 days after ingestion of the second dose) (27). Coded frozen serum specimens were sent to the Center for Vaccine Development, Baltimore, MD, USA, where Inaba vibriocidal antibody titres were determined (28, 29). Serological assays for all time points were run simultaneously in a blinded fashion. Since serum vibriocidal antibodies remain the best correlate of protection against O1 cholera (30–33), serum vibriocidal antibody response was used as the primary measure of immunogenicity, with a four-fold or greater rise between baseline and the maximum post-vaccination titre defining seroconversion. IgG cholera antitoxin was determined by enzyme-linked immunosorbent assay (ELISA) in serum specimens diluted 1:50 (34); a 0.15 or greater rise in net absorbance of the peak post-vaccination specimen over the pre-vaccination specimen was considered significant.

Bacteriology

Participants were asked to provide a rectal swab on the day of each oral inoculation, then daily for the first 4 days and again on the 6th and 12th days after dosing. The swabs were placed in Cary-Blair transport medium (Difco Laboratories, Detroit, MI, USA) and brought to the bacteriology laboratory at the Institut National de Recherche en Santé Publique, Bamako, where the samples were enriched for 5–6 hours in alkaline peptone water before being inoculated onto thiosulfate citrate bile salts sucrose (TCBS) and GNAB media. *V. cholerae* O1 was identified by standard techniques (35). The TCBS medium came from a single lot (BBL, Cockeysville, MD, USA) sent to Mali after quality testing in Baltimore. To document the sensitivity of the bacteriological methods, we concomitantly processed clinical samples from several patients hospitalized with cholera and a sample from a suspension of the vaccine strain (as positive con-

trols) using the same bacteriological techniques and media.

HIV serology and CD4⁺ lymphocyte determination

Preliminary HIV serology in the field was determined using the HIV Spot test (Genelabs Diagnostics, Geneva, Switzerland). These results were confirmed in Baltimore by ELISA (Abbott HIV-1/2, Abbott Laboratories, North Chicago, IL, USA); specimens positive by ELISA were tested with specific HIV-1 and HIV-2 Western blots (Bio-Rad HIV-1 WB, Bio-Rad Laboratories, Hercules, CA, USA; and Diagnostic Biotech HIV-2 WB, Genelabs Diagnostics, Redwood City, CA, USA). The results of the confirmatory assays determined each subject's HIV serological status for the data analysis. CD4⁺ lymphocyte counts were measured on all HIV-seropositive individuals at the time of the screening blood draw using the Coulter manual CD4⁺ count kit (Coulter Diagnostics, Miami, FL, USA). This result did not influence enrolment in the trial. Following the protocols of the Malian National AIDS Control Programme, individuals who were confirmed to be HIV-positive were offered the opportunity to be informed of their serostatus at the end of the study period and were counselled about the natural history of the disease and precautions that they must take with sexual partners.

Statistical tests and analysis

Reactogenicity was analysed separately for HIV-positive and HIV-negative participants. McNemar's matched-pair statistic was used to compare the frequencies of adverse reactions after vaccination and placebo. The intergroup comparisons of seroconversion rate (HIV-positive versus HIV-negative participants) were statistically appraised with continuity-corrected χ^2 tests or Fisher's exact test. Continuous outcomes such as vibriocidal titres were compared between groups using the Wilcoxon's rank-sum test. The relationship between CD4⁺ count and post-immunization titre among HIV-seropositives was evaluated using Spearman's correlation coefficient. Differences in vibriocidal antibody responses between HIV-seropositive and HIV-seronegative subjects were assessed by analysis of covariance (dependent variable = peak titre; independent variables = HIV status and pre-immunization vibriocidal titre). Analyses were performed using Statistical Analysis System (SAS) software. Two-tailed tests were used throughout and *P* values below 0.05 were considered statistically significant.

Results

Study population

Seven HIV-positive subjects were detected among the 1033 students screened for HIV antibodies in Sikasso; these seven HIV-seropositives (6 females and 1 male) and seven matched seronegatives (6 females and 1 male) were enrolled and all completed the trial. In Bamako, 124 female commercial sex workers were screened for HIV antibodies. Of these, 43 were directly excluded, including 29 who had evidence of previous vaccination against cholera, 13 with AIDS-like symptoms, and one who reported that she had had cholera. On the day of enrolment, one woman complained of acute diarrhoea, two were found to have a positive urine β -hCG test, two declined to participate, three were taking antibiotics under the direction of a physician, three failed to appear, and four had left Bamako. Thus, 66 were enrolled, and 62 (31 HIV-seropositive, 31 HIV-seronegative) took vaccine or placebo on the two oral inoculation days and provided at least two serum samples. These 62 women and the 14 students from Sikasso formed the analysis group (overall, 74 females and 2 males). The subjects had a median age of 26.5 years (range, 18–47 years). HIV-seropositives had a median age of 28 years (range, 18–46 years); the HIV-seronegatives had a median age of 27 years (range, 18–47 years). Losses of patients at follow-up before the second serum collection were due to migration out of the city ($n = 2$) or refusal to give a serum sample ($n = 2$). Of the 38 HIV-seropositive subjects available for analysis, 34 were infected with HIV-1 and four with HIV-2.

Reactogenicity

During the pilot stage of the trial in Sikasso, the report forms were found not to have been translated

properly into French. As a result, although none of these 14 subjects complained of diarrhoea, information on the number of daily stools was not recorded. This error in the questionnaire was rectified before the main stage of the study was undertaken in Bamako.

Since cholera has an incubation period of 24–72 hours and previous studies on early generations of reactogenic live oral cholera vaccines have shown that most reactions appear within the first few days after vaccination (36), the rate of reactions was calculated separately for the first four days after administration of vaccine or placebo and for the entire 12-day follow-up period. In HIV-seropositive individuals, as well as in HIV seronegatives, the rates of reactions after taking vaccine and after taking placebo were similar (Table 1). HIV-seronegative individuals had slightly lower (but not significantly different) rates of reactions than HIV-seropositive subjects. The most common untoward event was fever, occurring within the first four days of follow-up in 11% of HIV-seronegatives after ingesting vaccine and in 8% after receiving placebo. In the entire follow-up period, diarrhoea occurred in three HIV-seropositive subjects (in two after vaccine and one after placebo) and in three HIV-seronegative persons (one after ingesting vaccine and two after receiving placebo).

Bacteriology

V. cholerae did not grow in the stool cultures from the six subjects who met the definition of diarrhoea or from the others without diarrhoea. The clinical specimens from the hospitalized cholera patients and the aliquot from a suspension of the vaccine strain grew *V. cholerae* O1, indicating the sensitivity of the bacteriological methods.

Table 1: Reactogenicity after ingestion of CVD 103-HgR live oral cholera vaccine or placebo^a

HIV Status	Treatment	No. of cases with symptoms after administration:					
		During first 4 days of follow-up			During entire 12 days of follow-up		
		Diarrhoea	Fever	Emesis	Diarrhoea	Fever	Emesis
Seronegative	Vaccine	1/31 ^b (3) ^c	4/37 (11)	0/38 (0)	1/27 (4)	5/34 (15)	1/34 (3)
	Placebo	1/31 (3)	3/37 (8)	0/38 (0)	2/27 (7)	6/34 (18)	1/34 (3)
Seropositive	Vaccine	2/30 (7)	4/37 (11)	0/27 (0)	2/27 (7)	6/36 (17)	1/34 (3)
	Placebo	1/30 (3)	6/37 (16)	1/27 (3)	1/27 (4)	6/36 (17)	1/34 (3)

^a None of the differences between vaccine and placebo (McNemar's test) or between HIV-seropositive and HIV-seronegative groups (Fisher's exact test) were statistically significant.

^b Denominator is the total number of subjects for which there were evaluable data.

^c Figures in parentheses are percentages.

Serological responses

The geometric mean titre (GMT) of vibriocidal antibody at baseline was significantly lower in the HIV-seropositive than in the HIV-seronegative individuals (1:23 versus 1:65, $P = 0.002$ by Wilcoxon's test) (Table 2). After receiving vaccine, the GMT of vibriocidal antibody of the HIV-positive subjects increased over the baseline value but the peak GMT was significantly lower than that of the HIV-seronegative subjects (1:124 versus 1:584, $P < 0.001$). After we made adjustments for differences in pre-immunization vibriocidal titres, HIV-seropositive subjects still showed lower peak titres than HIV-seronegative individuals (analysis of covariance, $P = 0.02$).

The rate of vibriocidal seroconversion was slightly but not significantly higher among the HIV-seronegative participants than among the seropositives (71% versus 58%, $P = 0.41$, χ^2 test). The seroconversion rate for serum IgG cholera antitoxin in HIV-seropositive subjects (17/35, 49%) was significantly lower than in HIV-seronegatives (27/34, 79%) ($P = 0.01$, Fisher's exact test). Among the 31 HIV-seropositive subjects for whom CD4⁺ counts were available, rises in IgG cholera antitoxin were observed in 13 of 23 subjects (57%) whose counts were ≥ 500 per μl but in 0 of 5 individuals with counts < 500 per μl ($P = 0.04$, Fisher's exact test). The CD4⁺ count and post-immunization titre among HIV-seropositives were not significantly correlated (Spearman's test, $r = 0.21$, $P = 0.20$).

Discussion

Potential target populations for the new generation of cholera vaccines include groups of refugees who are assembled, often on short notice, into makeshift camps without adequate access to treated water supplies or sanitary means to dispose of human faecal wastes. Under such circumstances in Asia (37) and Africa (6), cholera has spread in an explosive fashion, resulting in extraordinarily high attack rates. Even though aggressive rehydration can diminish case fatality to about 1% in well-equipped treatment facilities staffed by experienced personnel (37), such resources are not always available and much higher case fatalities have been recorded, particularly in Africa (38). In the past, there was considerable aversion to the use of cholera vaccine as a potential control measure in refugee camps where cholera posed a threat. In large part this was based on the inadequacies of the parenteral, killed whole-cell cholera vaccine, which conferred only moderate protection for short periods of time (39), did not adequately protect young children, was fairly reactogenic, and suffered from the drawbacks of any parenteral vaccines (local discomfort and potential for inadvertent transmission of other agents via contaminated needles).

The most important characteristics of an ideal cholera vaccine include the following: provision of a high level of long-term protection following administration of a single dose; onset of protection within a few days of vaccination; oral administration for prac-

Table 2: Seroconversion following immunization with a single dose of CVD 103-HgR live oral cholera vaccine

HIV status	<i>n</i>	Vibriocidal antibody		Rate of seroconversion ^b	IgG cholera antitoxin seroconversion rate
		Geometric mean titre:			
		Pre-immunization	Peak ^a		
Seronegative	38	1:65 ^c <i>1:37–1:117^g</i>	1:584 ^d <i>1:288–1:1184</i>	22/31 (71) ^e	27/34 (79) ^f
Seropositive	38 ^h	1:23 ^c <i>1:15–1:35</i>	1:124 ^d <i>1:73–1:210</i>	21/36 (58)	17/35 (49) ^f
CD4 ⁺ $\geq 500/\mu\text{l}$ ⁱ	26	1:15	1:178 ^j	17/24 (71)	13/23 (57) ^k
CD4 ⁺ $< 500/\mu\text{l}$ ⁱ	5	1:22	1:40 ^j	2/5 (40)	0/5 (0) ^k

^a Peak post-vaccination titre.

^b Seroconversion was defined as a fourfold or greater rise in titre. Participants showing no evidence of seroconversion but for whom only two serum specimens were available were excluded from the analysis.

^c $P = 0.002$ by Wilcoxon's rank-sum test for seronegative versus seropositive.

^d $P = 0.0006$ by Wilcoxon's rank-sum test for seronegative versus seropositive.

^e Figures in parentheses are percentages.

^f $P = 0.01$ by Fisher's exact test for seronegative versus seropositive.

^g Figures in italics are 95% confidence intervals.

^h CD4⁺ counts were unavailable for seven subjects.

ⁱ Range of CD4⁺ counts, 528–1789.

^j $P = 0.03$ by Wilcoxon's rank-sum test for CD4⁺ $\geq 500/\mu\text{l}$ versus CD4⁺ $< 500/\mu\text{l}$.

^k $P = 0.04$ by Fisher's exact test for CD4⁺ $\geq 500/\mu\text{l}$ versus CD4⁺ $< 500/\mu\text{l}$.

^l Range of CD4⁺ counts, 345–462.

ticality and for optimal stimulation of the mucosal immune system of the intestine; protection of young children and individuals of blood group O (hosts that have not been consistently protected by earlier cholera vaccines (39, 40)); packaging in a practical formulation that facilitates mass vaccination, including young children; and relatively low cost. Two oral vaccines, B subunit/inactivated whole vibrios combination vaccine (BS/WCV) (40–42) and live recombinant vaccine strain CVD 103-HgR (12, 22), exhibit many (but not all) of the characteristics of an ideal cholera vaccine.

BS/WCV offers virtually complete safety and acceptable efficacy: three spaced doses conferred 50% protection for 3 years upon a population living in Bangladesh, an endemic area (40). In the short-term, i.e., 6 months after vaccination, BS/WCV conferred a higher level of protection in trials in Bangladesh and Peru (85–86%) (41, 42). The main drawback to BS/WCV is the need for at least two doses to confer protection; in the Bangladesh field trial the doses were administered 6 weeks apart, and in the Peru trial 2 weeks apart.

A single dose of CVD 103-HgR was well-tolerated in randomized, placebo-controlled, double-blind trials in adults, young children and toddlers, and was remarkably immunogenic after administration of a 10^9 -CFU dose. In multiple, experimental challenge studies, a single dose of CVD 103-HgR was completely protective against moderate and severe cholera (the severity of diarrhoea that makes cholera a public health problem) caused by El Tor and classical biotype vibrios. Moreover, the protection was fully evident as early as 8 days following vaccination (14).

Despite these highly encouraging results from clinical trials with CVD 103-HgR in many countries, before the Malian trial the vaccine had not been evaluated in sub-Saharan Africa, nor had it been specifically tested in HIV-positive subjects. A live cholera vaccine must be safe in such subjects: this is an additional, necessary hurdle to overcome that is irrelevant for non-live oral cholera vaccines (43). The double-blind, crossover design of the present trial enabled the comparison, for each subject, of the clinical response following ingestion of vaccine to that following ingestion of placebo. No important adverse events occurred, such as severe diarrhoea or suspected sepsis, and the vaccine was as well-tolerated as placebo by both HIV-positive and HIV-negative subjects. Moreover, the results of the clinical bacteriological studies showed that the live vaccine behaved no differently in the intestine of HIV-positive subjects: faecal excretion of the vaccine was not detected in any subjects, irrespective of HIV status. Clearly, the live vaccine did not act as an

opportunistic organism in the HIV-infected African subjects. Indeed, these data corroborate an attractive feature of CVD 103-HgR observed in previous studies: although it is quite capable of stimulating robust vibriocidal antibody responses, it is only minimally excreted and only a small proportion of vaccinees (0.6–17%) manifest positive coprocultures, usually clustering within 2–3 days after vaccination (16, 17, 19–21).

As expected for a population living in an endemic area, the baseline vibriocidal antibody titres were elevated in the vast majority of HIV-negative subjects (Table 2), with 60% having a titre $> 1:40$ and almost one-quarter having titres $> 1:320$ (17–19, 30–33). The distribution of titres for HIV-seropositive individuals, however, was similar to that of a nonendemic region, with three quarters of the group having a baseline vibriocidal titre $< 1:40$ (29). Most of the subjects in the HIV-negative group who did not exhibit significant increases in vibriocidal antibody had elevated baseline titres, which would not be expected to be boosted (18, 19). The significantly lower post-vaccination vibriocidal GMT in the HIV-positive subjects was almost entirely the result of poor (or absent) responses in HIV-positive subjects with $CD4^+$ counts < 500 per μ l. Moreover, no subjects with $CD4^+$ counts < 500 per μ l exhibited rises in serum IgG cholera antitoxin. These findings with an oral vaccine corroborate earlier reports of diminished serological responses of HIV-positive subjects compared with HIV-negative subjects following parenteral immunization with pneumococcal polysaccharide, *Haemophilus influenzae* type b conjugate, influenza, tetanus toxoid, and hepatitis B vaccines (44–49).

Although immunization of HIV-infected individuals with a variety of parenteral vaccines may provoke a transient burst of viral replication (50–52), recent studies (53, 54) show that this is not a consistent finding. Studies are planned to investigate whether oral immunization with CVD 103-HgR induces a temporary increase in virus replication, since little is known about the effect of mucosal immunization on viral replication and load. Nevertheless, the use of oral vaccine to protect refugees and other high-risk populations against cholera in the face of a potentially explosive outbreak should take precedence over theoretical risks stemming from viral replication. The argument in favour of vaccination is strengthened by recent data indicating that the case fatality from cholera among refugee children in Goma was higher among HIV-positives than HIV-negatives (23).

The promising results in this initial study demonstrating the safety, immunogenicity and lack of excretion of live oral cholera vaccine CVD 103-HgR

in HIV-positive subjects in Mali encourage further, larger-scale evaluations of this single-dose oral vaccine in sub-Saharan populations. The potent protective efficacy of this live oral vaccine has been repeatedly demonstrated in experimental challenge studies (12–14, 22). A large-scale, randomized, placebo-controlled field trial to assess the efficacy of CVD 103-HgR in preventing endemic El Tor cholera under conditions of natural challenge is currently under way in North Jakarta, Indonesia. CVD 103-HgR has been licensed in a number of countries (most recently Canada) for the prevention of cholera in travellers from industrialized countries who visit developing countries where cholera is present. However, based on its single-dose regimen and early onset of protective effect, a particularly rational use for CVD 103-HgR vaccine would be the protection of refugee populations in areas of the world where cholera is prevalent. In this respect, it would be useful to initiate demonstration projects in stable refugee populations in sub-Saharan Africa to gain practical field experience with this vaccine in such settings.

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Résumé

Innocuité et immunogénicité d'une dose unique de vaccin anticholérique buccal vivant CVD 103-HgR chez l'adulte au Mali

La souche CVD 103-HgR est une souche vaccinale de *Vibrio cholerae* O1 génétiquement modifiée et atténuée, qui ouvre des perspectives considérables en tant que vaccin anticholérique buccal vivant administrable en une seule dose. La protection est apparente dès le huitième jour suivant la vaccination, ce qui permet d'envisager un emploi à titre de mesure de lutte supplémentaire pendant les flambées de choléra comme il s'en produit dans les

campes de réfugiés. Bien qu'on dispose déjà d'une vaste expérience de ce vaccin dans les pays en développement d'Asie et d'Amérique latine, de même qu'en Europe et en Amérique du Nord, il n'a pas été évalué en Afrique subsaharienne. Avant de pouvoir envisager d'utiliser ce vaccin buccal vivant dans des régions touchées à la fois par l'infection à virus de l'immunodéficience humaine (VIH) et par des épidémies de choléra, il est nécessaire d'établir son innocuité chez les sujets infectés par le VIH.

Nous avons en conséquence effectué au Mali un essai clinique randomisé croisé en double aveugle contre placebo sur des adultes VIH-séropositifs (ne présentant pas de SIDA (syndrome d'immunodéficience acquise) clinique) et VIH-séronégatifs (n = 76) afin d'évaluer l'innocuité et l'immunogénicité d'une dose unique du vaccin anticholérique buccal vivant CVD 103-HgR. Le jour 0, la moitié des sujets ont reçu le vaccin et l'autre moitié un placebo d'aspect identique; 12 jours plus tard, les sujets ayant reçu le vaccin ont reçu le placebo et vice versa. Les participants ont été vus les jours 1–6, 8, 10 et 12 après chaque prise de vaccin ou de placebo et les réactions indésirables ont été notées. Des prélèvements de sang ont été réalisés juste avant chaque prise buccale et 12 jours après la deuxième prise, pour rechercher les anticorps vibriocides dans le sérum (qui constituent le meilleur corrélat de la protection contre *V. cholerae* O1) et les IgG anti-toxine cholérique.

Des réactions indésirables consistant en fièvre, diarrhée et vomissements ont été observées avec la même fréquence après l'administration du vaccin et du placebo. La souche vaccinale n'a été isolée dans aucune coproculture. Le titre moyen géométrique (GMT) de référence des anticorps vibriocides sériques était significativement plus faible chez les sujets VIH-séropositifs (1:23) que chez les sujets VIH-séronégatifs (1:65) (p = 0,02). Une augmentation significative du titre a été observée chez 71% des sujets VIH-séronégatifs (la plupart des sujets non répondeurs avaient déjà des titres élevés), chez 58% de l'ensemble des sujets VIH-séropositifs et chez 40% des sujets VIH-séropositifs ayant un nombre de CD4+ inférieur à 500 par μ l. Après la vaccination, le GMT maximal des anticorps vibriocides chez les sujets VIH-séronégatifs était de 1:584 contre 1:124 chez les sujets VIH-séropositifs (p = 0,0006); chez les sujets VIH-séropositifs ayant moins de 500 CD4+ par μ l, le GMT maximal des anticorps vibriocides était de 1:40 (p = 0,03 par rapport aux autres sujets VIH-séropositifs). De même, une conversion des IgG anti-toxine cholérique sériques a été observée chez 79% des sujets VIH-séronégatifs, chez 49% de l'ensemble des sujets VIH-séropositifs et chez

aucun des 5 sujets VIH-séropositifs ayant moins de 500 CD4+ par μ l.

Ces résultats montrent que le vaccin anticholérique buccal vivant CVD 103-HgR est sans danger chez les Maliens adultes infectés par le VIH, bien que la réponse sérologique soit significativement plus faible chez ces sujets (en particulier ceux qui ont un nombre de CD4+ inférieur à 500 par μ l) que chez les sujets VIH-séronégatifs. Ils plaident en faveur de la poursuite de l'évaluation de ce vaccin anticholérique buccal administrable en une seule dose chez des populations à haut risque comme les réfugiés d'Afrique subsaharienne.

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