

CONCISE COMMUNICATIONS

Effect of Small Bowel Bacterial Overgrowth on the Immunogenicity of Single-Dose Live Oral Cholera Vaccine CVD 103-HgR

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Several live oral vaccines (polio, bovine rotavirus, CVD 103-HgR cholera) are less immunogenic in developing than in industrialized countries. It was hypothesized that proximal small bowel bacterial overgrowth (common in children in less developed countries but rare in industrialized settings) diminishes the vibriocidal antibody response to CVD 103-HgR. In total, 202 fasting Santiago schoolchildren aged 5–9 years had lactulose breath H₂ tests to detect proximal small bowel bacteria 1 day before ingesting CVD 103-HgR. Florid small bowel overgrowth was observed in 10 (5.6%) of 178 analyzable children. In children with florid overgrowth, vibriocidal seroconversion differed little from other children (60% vs. 67%), but the geometric mean titer was lower (160 vs. 368; $P = .25$). By logistic regression, increased peak breath H₂ at small bowel time points was associated with diminished seroconversion ($P = .04$), as was the interaction of H₂ value and weight (children >25 kg had lower seroconversion rates among subjects with heaviest overgrowth).

Oral or intranasal vaccines offer several advantages over parenteral immunization by providing both mucosal and systemic immune barriers and by their increased acceptability, thereby increasing subject compliance. Certain formulations of oral vaccines (e.g., Sabin polio) are extraordinarily practical and allow rapid mass immunization. Mucosal immunization is attractive for developing countries, where occasional use of improperly sterilized needles and syringes inadvertently transmits hepatitis B and C and human immunodeficiency virus. A future when all vaccines can be administered nonparenterally is a goal of the World Health Organization. Regrettably, in less developed countries, several oral vaccines have been less immunogenic than in industrialized countries. In the 1960s and 1970s, the Sabin type 1 and 3 polioviruses were less immunogenic in

infants in India and Africa [1, 2]; it was proposed that some unknown host factors were responsible [2]. Similarly, RIT 4237 attenuated bovine rotavirus strain was quite immunogenic and protective in Finnish infants [3] but less so in African and Latin American infants [4]. Proposed explanations for this diminished immunogenicity include SIgA antibodies in breast milk, interference from enteroviruses, and cold chain problems.

We noted this phenomenon in phase 2 studies of live oral cholera vaccine CVD 103-HgR in adults and children aged 2–9 years in Asia and Latin America [5–7]. High seroconversion rates in Indonesian children living in poor conditions required a 10-fold higher dose (5×10^9 cfu) of CVD 103-HgR [5] than the dose (5×10^8 cfu) that is consistently immunogenic in North Americans and Europeans. This was partly explained by the effect of prior immunity [6] and by socioeconomic level [6]. Thus, there is a poorly understood “barrier” to successful intestinal immunization of children in less developed countries by live oral vaccines.

The proximal small intestine of healthy children and adults in industrialized countries is virtually free of Enterobacteriaceae, and bacterial counts are quite low (usually $<10^4$ organisms/mL of aspirated intestinal fluid) [8]. However, disadvantaged children in less developed countries commonly have excessive bacterial colonization of their proximal small intestine [9, 10], accompanied by architectural changes in the intestinal mucosa (environmental enteropathy) [9] after repeated ingestion of food and water containing high bacterial counts.

Bacterial overgrowth flora anaerobically ferment certain sugars, leading to H₂ production, much of which is excreted as

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Informed consent was obtained from parents of participating children. This field research followed the guidelines on human experimentation of the US Department of Health and Human Services, and the protocol was approved by the Servicio de Salud Metropolitano Norte ethics committee and the University of Maryland institutional review board.

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breath H_2 . The breath hydrogen excretion test has been extensively used in developing countries as a practical field method for detecting small bowel bacterial overgrowth (SBBO) [11, 12]. To test whether SBBO diminishes the immune response to CVD 103-HgR, 202 Chilean schoolchildren were enrolled in a trial in which breath H_2 tests were performed after an overnight fast, followed the next day by immunization with a single vaccine dose. The serum vibriocidal response was analyzed in relation to breath H_2 test results and other factors, such as blood group.

Materials and Methods

Target population and recruitment. The study was aimed at healthy schoolchildren aged 5–9 years in Area Norte, Santiago. An informational brochure and consent form were sent to parents of children aged 5–9 years in schools serving low and low–middle socioeconomic level neighborhoods, requesting consent for their child to participate. Consenting parents were informed of the study date and asked that on that day their child come to school without breakfast (which was provided as part of the study).

Exclusion criteria. Children who received antibiotics within the previous 2 weeks or who had dental caries or periodontal disease detected by a study nurse were excluded, because oral anaerobic bacteria can ferment the test sugar, leading to a false-positive test.

Breath H_2 tests. The breath H_2 tests were performed 1 day prior to vaccination. On arrival at school, the fasting participants immediately had a baseline alveolar air sample collected by gas chromatograph (Microlyzer SC; Quintron, Menomonee, WI) and were weighed and given oral lactulose (0.3 g/kg, maximum 10 g) in 100 mL of water. Additional breath samples were collected at 15, 30, 40, 60, 90, and 120 min after ingestion of the lactulose challenge. Lactulose is not digested by human intestinal enzymes but is metabolized by most intestinal bacteria. Trace gas concentrations were automatically normalized to a constant CO_2 of 5.5% to account for differences in sample collection among subjects of the same age but different body size.

In healthy children, H_2 values at baseline and 15 and 30 min after ingesting lactulose are very low. When lactulose reaches the terminal ileum and colon in normal children (40–120 min after ingestion), it is metabolized by resident bacteria, leading to an elevation (≥ 10 ppm over baseline) of breath H_2 . Children who exhibited elevated breath H_2 values in the baseline specimen were excluded. Nonproducing children who failed to show the normal expected late colonic peak were excluded from the analysis. Florid overgrowth was defined as an increase of 20 ppm of H_2 in specimens collected at 15 or 30 min (reflecting small bowel), compared with baseline. After the breath H_2 test, the children were given breakfast.

Vaccination. The morning after the breath H_2 tests, participants received a vaccine cocktail containing 5×10^9 cfu CVD 103-HgR in buffer [7]. The lyophilized vaccine (Swiss Serum and Vaccine Institute, Berne, Switzerland) was in aluminum foil packets also containing 18.8 mg of aspartame. Contents of the vaccine packet and a packet containing buffer (1.88 g of $NaHCO_3$ and 1.24 g of ascorbic acid) were added to 100 mL of H_2O to create the cocktail.

Blood specimens. Blood specimens were obtained before vaccination and 10 days thereafter. Blood group was identified by

baseline blood. Serum Inaba vibriocidal antibodies were measured, and a 4-fold rise was considered seroconversion [5–7].

Water, sanitation, and socioeconomic level information. Parents provided information about living conditions known to influence the level of fecal contamination in the domiciliary environment in Santiago [13].

Data analysis. Factors influencing seroconversion were analyzed by Fisher's exact (dichotomous variables) or Wilcoxon tests (continuous variables). Factors influencing postimmunization vibriocidal antibody titers and the relationship between preimmunization titers and vibriocidal seroconversion were assessed by Spearman's test. Multivariate analysis of factors influencing the seroconversion was performed by use of logistic regression. The initial model included factors independently associated with seroconversion ($P < .3$; i.e., gender, refrigerator in home, O blood group, weight, days between vaccination and postimmunization blood specimen, baseline vibriocidal titer, and bacterial overgrowth [peak breath H_2 at 15 or 30 min]) and two-way interaction terms among gender, weight, peak breath H_2 , days between vaccination and postimmunization blood specimen, and O blood group. Excluded from the multivariate analysis were "age" (replaced by "weight") and "sewer system in home," because the variable "refrigerator in home" was more strongly correlated with seroconversion. Independent factors that had nonsignificant relationships with the dependent variable and whose removal did not affect the relationship between remaining factors and the dependent variable were successively removed. Significance was assessed at $P \leq .05$.

Sample sizes. We estimated that the prevalence of bacterial overgrowth in Chilean children aged 5–9 years would be 20% [11, 12] and that the postimmunization vibriocidal antibody geometric mean titer (GMT) in children with SBBO would be 40% of the GMT among children with normal breath H_2 values (330 vs. 800, respectively). If 200 children were enrolled and 20% had SBBO, we could detect a significant difference in GMT between the two groups of children ($\alpha = 0.05$, $\beta = 0.19$, single-tail hypothesis).

Results

Breath H_2 tests. In total, 202 fasting schoolchildren aged 5–9 years had breath H_2 tests performed after ingestion of lactulose. Some 94% of the children were aged 6–8 years; 53% were boys. Twenty-three of the 202 children were excluded from analyses: 11 had an elevated baseline breath H_2 specimen, 7 were breath test nonproducers (i.e., did not show a normal colonic peak of H_2), 1 had a missing 30-min breath H_2 specimen (precluding detection of a rise in H_2 at that time), and 4 imbibed $< 70\%$ of the vaccine suspension. Florid SBBO (rise of 20 ppm or more over baseline at 15 or 30 min after lactulose ingestion) was observed in 10 (5.6%) of the 179 children.

Serum vibriocidal antibody. Vibriocidal seroconversions were observed in 119 (67%) of the 178 children who provided pre- and postimmunization sera. The GMT of vibriocidal antibody rose from 14 at baseline to 351 after vaccination. Children with lower preimmunization antibody titers were more likely to seroconvert than children with higher titers (Spearman's $r = -.22$; $P = .002$).

Relationships between breath H₂ tests and vibriocidal antibody responses. The seroconversion rate was similar in the 10 children with florid SBBO (60%) and in the other 168 children (67%); none of the 10 children with florid overgrowth had elevated baseline vibriocidal titers. Postvaccination GMT of the children with florid overgrowth (160) was not significantly lower than in the remaining children (GMT = 368; *P* = .25).

By logistic regression, weight was significantly associated with vibriocidal response (table 1). Peak breath H₂ level 15 or 30 min after ingestion of lactulose was inversely correlated with seroconversion (*P* = .02), as were interaction terms between weight and peak H₂ excretion, weight and date of postimmunization specimen, and preimmunization vibriocidal antibody titer. The interaction between weight and peak H₂ excretion was manifested as a difference in seroconversion among subjects with the highest peak H₂ excretion (>17): children weighing >25 kg were less likely to seroconvert (33% [3/9]) than were lighter children (100% [9/9]; *P* < .001). These weight groups had similar rates of seroconversion among subjects with lower peak H₂ excretion (59% and 71%, respectively; *P* = .11).

We applied this final logistic model separately to subjects of blood group O and non-O. In contrast to the non-O group (*n* = 83), where none of the independent factors reached significance, there was a significant effect of all factors on vibriocidal seroconversion (*P* ≤ .02), except for refrigerator in home (*P* = .28), among subjects of blood group O (*n* = 112).

Discussion

CVD 103-HgR is less immunogenic in adults and children in Asia and Latin America than in US and Swiss subjects [5–7]. Vibriocidal seroconversion and GMT were diminished even in developing country populations where cholera (and therefore preexisting contact with *Vibrio cholerae* O1) was rare [7] and

was correlated with socioeconomic level [6]. Because persons living in poverty in developing countries endure fecally contaminated environments and many children have SBBO [9–11], we hypothesized that this might account for the diminished serologic response to CVD 103-HgR. Results of this trial support the hypothesis, even though the prevalence of florid SBBO encountered (5.6%) was lower than anticipated (20%). These children were from an urban area of Santiago with a low and low–middle socioeconomic level population; a higher prevalence of florid SBBO might have been found in a population of lower socioeconomic level. Nevertheless, we observed a clear inverse relationship between H₂ production in the small bowel and the propensity for vibriocidal antibody seroconversion. Because seroconversion was significantly correlated with lighter weight (table 1), we hypothesize that in these nonmalnourished children, lighter weight was associated with smaller intestinal surface, fewer total bacteria, and lower peak breath H₂ values, resulting in less inhibition of vaccine organisms.

A direct effect of SBBO on blunting the immune response to CVD 103-HgR would derive from intestinal flora producing short-chain fatty acids that inhibit *V. cholerae* O1 [14]. Alternatively, the effect may be indirect. Persons with SBBO typically have abnormal intestinal architecture [9, 10] and increased lymphocytes in the mucosa [9, 10], indicating an immunologically “tolerant” gut with impaired antigen uptake by microfold cells overlying organized lymphoid tissue and suppressed immune responses.

Our data support the hypothesis that proximal SBBO interferes with vibriocidal seroconversion after ingestion of CVD 103-HgR. Study limitations include the selection of a pediatric population in which the SBBO prevalence was lower than anticipated and reliance on breath H₂ tests as a proxy for SBBO. Whereas some investigators report a good correlation using this test [12], others report low sensitivity and specificity [15]. Fur-

Table 1. Relationship between demographic, socioeconomic, and biologic factors and seroconversion of vibriocidal antibody after administration of a single 5 × 10⁹ cfu dose of CVD 103-HgR live oral cholera vaccine.

Factor	<i>P</i> in univariate analysis ^a	Multivariate (logistic regression) analysis		
		<i>P</i>	Odds ratio ^b (95% CL)	Relationship of factor to seroconversion
Sex	.09	N/I ^c	N/I	Females > males
Refrigerator in home	.10	.09	2.52 (0.86, 7.32)	Yes > no
Peak breath H ₂ ^d	.27	.02	1.76 (1.08, 2.86)	Low H ₂ > high H ₂
Weight	.004	.03	0.36 (0.14, 0.92)	Lighter > heavier
Days after vaccination ^e	.02	.09	0.15 (0.02, 1.33)	Later > earlier specimen
Day 0 titer ^f	.002	.004	0.47 (0.28, 0.78)	Low before > high before
Weight × peak breath H ₂		.02	0.98 (0.96, 0.99)	
Weight × days after vaccination		.045	1.09 (1.00, 1.19)	

NOTE. CL, confidence limit; N/I, not included.
^a Fisher’s exact test for dichotomous variables, Wilcoxon tests for continuous variables.
^b Odds ratio for a 1-U change in variable.
^c Although included in initial model, this factor was not included in final model.
^d Peak breath H₂ value at 15 or 30 min.
^e Days elapsed between vaccination and collection of postimmunization blood specimen.
^f Day 0 vibriocidal antibody titer.

ther trials in pediatric populations living in disadvantaged conditions should investigate the role of SBBO in modulating immune responses to live oral vaccines and seek practical ways to overcome this barrier.

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