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Comparison of Immune Responses in Patients Infected with *Vibrio cholerae* O139 and O1

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***Vibrio cholerae* O139 has recently emerged as the second etiologic agent of cholera in Asia. A study was carried out to evaluate the induction of specific immune responses to the organism in *V. cholerae* O139-infected patients. The immune responses to *V. cholerae* O139 Bengal were studied in patients by measuring antibody-secreting cells (ASC), as well as vibriocidal and antitoxic antibodies in the circulation. These responses were compared with those in patients with *V. cholerae* O1 disease. Strong immunoglobulin A (IgA) and IgM ASC responses were seen against the homologous lipopolysaccharide or serogroup of *V. cholerae*. The magnitude and isotype of the responses were similar in O139- and O1-infected patients. Vibriocidal antibody responses were seen against bacteria of the homologous but not heterologous serogroup, and these responses reflect the lack of cross-protection between the infections caused by the two serogroups. The two groups of patients showed comparable cholera toxin-specific ASC responses, with the IgG isotype dominating over the IgA isotype, as well as comparable antitoxic immune responses in plasma. These results suggest that despite having a polysaccharide capsule, *V. cholerae* O139 induces systemic and intestine-derived ASC responses in peripheral blood comparable to those seen in patients with *V. cholerae* O1 disease.**

Vibrio cholerae O139 Bengal is the second etiologic agent of cholera, and the disease due to this organism has now become endemic in the Indian subcontinent and the neighboring countries (1). Prior infection with *V. cholerae* O1, the traditional causative agent of cholera, does not cross-protect against infection with *V. cholerae* O139 (2, 4), since the lipopolysaccharide (LPS) antigens of the two vibrios are different (13). In addition, unlike *V. cholerae* O1, *V. cholerae* O139 possesses a capsular polysaccharide (CPS) (16, 17, 37, 38). By analogy with other capsulated *V. cholerae* strains, it is likely that the CPS can potentially mask certain critical surface antigens, with a resulting decrease in the host immune response (26). Therefore, it was of interest to compare the immune responses in patients with cholera due to both vibrio serogroups. We did this by comparing the whole-cell (WC) and LPS- and cholera toxin (CT)-specific antibody-secreting cell (ASC) responses in *V. cholerae* O139-infected patients with the corresponding responses in patients with *V. cholerae* O1 disease as a proxy indicator of the mucosal immune response (22). In addition, conventional serological markers of cholera, i.e., the vibriocidal antibody and the CT-specific antibody responses, have also been evaluated in both categories of patients (12, 14).

MATERIALS AND METHODS

Study group. Twenty-three patients with cholera caused by *V. cholerae* O139 and 28 patients with *V. cholerae* O1 El Tor (26 with *V. cholerae* O1 Ogawa and 2 with *V. cholerae* O1 Inaba) cholera were recruited for the study from November 1993 to April 1995. The degree of dehydration in the patients (mild to severe) was assessed by a physician according to the Denver system (42). Twenty adult males in the same age group (18 to 40 years, with a median age of 24.5 years) with no history of diarrhea during the previous 6 months were also studied as controls.

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Confirmation of bacterial strains. The stools of patients suffering from acute watery diarrhea were screened by dark-field microscopy for the darting movement of vibrios (5) and its inhibition by antibodies specific for *V. cholerae* O1 or O139 (27). Stools were then plated on taurocholate-tellurite-gelatin agar (23) and gelatin agar (Difco, Detroit, Mich.); after overnight incubation of plates, serological confirmation of suspected vibrio colonies was carried out by slide agglutination (27, 32). Stools were also cultured to detect other enteric pathogens, including enterotoxigenic *Escherichia coli* (35, 36) and *Salmonella*, *Shigella*, and *Campylobacter* spp. (41) and, in addition, were tested by direct microscopy for cyst and vegetative forms of parasites and ova of helminths. The stools of the healthy controls were also screened for these pathogens.

Sample collection. After microbiological confirmation of cholera, venous blood was collected from patients at the acute stage. This occurred on the second day of hospitalization and was considered to be approximately 2 days after the onset of diarrhea (day 2) for the purpose of this study. Blood was also collected 5, 9, and 20 days later, during convalescence (that is, 7, 11, and 22 days, respectively, after the onset of the disease). Single blood samples were collected from the controls.

MNC, plasma, and serum. Peripheral blood mononuclear cells (MNC) were isolated from heparinized venous blood by gradient centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden). Plasma collected from the top of the Ficoll gradient and serum separated from another aliquot of blood were stored in aliquots at −20°C.

Detection of ASC. The MNC were assayed for ASC, using a lower number of cells (10^5 to 10^4) for total cells and a higher number of cells (10^6 to 10^5) for specific cells, by a two-color micromodification of the ELISPOT (enzyme-linked immunospot) technique (8), with samples collected on days 2, 7, and 11 postinfection. The ASC response was also studied in controls. Assays were carried out in plates coated with WC, LPS, or purified recombinant B subunit of CT (CTB) (34).

For ASC response to WC antigens, whole live cells of *V. cholerae* O1 El Tor Ogawa (strain 25049), *V. cholerae* O1 El Tor Inaba (strain T-19479), or *V. cholerae* O139 (strain 4260B) isolated from patients with cholera were used as the solid-phase antigens. Bacteria from blood agar plates were grown in Trypticase soy broth (without glucose; Difco) at 37°C for 3 to 4 h with shaking (120 rpm). After centrifugation at 4°C for 20 min, bacteria were washed once in phosphate-buffered saline (10 mM, pH 7.2) and adjusted to 10^{10} CFU per ml, which was confirmed by colony counts. Wells of nitrocellulose-bottom 96-well plates (Millititer HA; Millipore Corp., Bedford, Mass.) were coated with WC as described previously (15). Plates were also coated with purified LPS (28, 40) isolated from strains of *V. cholerae* O1 and O139 (used for coating as WC antigens) by using similar procedures (15). Both the WC- and LPS-coated plates prepared in this way were stored at −20°C and used for up to 4 weeks. For the detection of a CT-specific ASC response, the GM1-CTB ELISPOT procedure

(39) was used with purified recombinant CTB as the antigen (34). Before use, the plates (coated with WC, LPS, and CTB) were washed with phosphate-buffered saline three to four times, and remaining binding sites were blocked with Iscove's complete medium (Gibco, Gaithersburg, Md.) containing 5% (vol/vol) fetal calf serum and 50 μg of gentamicin per ml for 30 min at room temperature. Suspensions of 0.1 ml of MNC were added to wells (10^5 to 10^6 cells), and plates were incubated at 37°C for 3 to 4 h in the presence of 5% CO_2. Assays were carried out for all three isotypes, immunoglobulin A (IgA), IgM, and IgG, as described previously (39). A positive response was defined as greater than 3 standard deviations (SD) above the geometric mean (GM) of the ASC of the controls for that antigen and antibody class. Total numbers of IgA-, IgG-, and IgM-secreting cells were similarly detected in wells previously coated with affinity-purified goat antibodies to the $F(ab')_2$ fragment of IgG, which recognizes all three isotypes (Jackson Immunoresearch Laboratories Inc., West Grove, Pa.).

Detection of plasma antibodies. Plasma samples from patients were tested for CT-specific antibodies of the IgA and IgG isotypes (33), and endpoint titers were determined by ELISA (29). A twofold or greater increase in titer between the acute- and convalescent-phase samples on day 7, 11, or 22 was used to signify seroconversion and a positive response.

Vibriocidal antibodies. Serum samples collected at early (day 2) and convalescent (day 7 and 11) phases of cholera infection were tested for vibriocidal antibody response, using *V. cholerae* O1 El Tor Ogawa (strain 25049) (14) or *V. cholerae* O139 (strain 4260B) (17) as the target organism in procedures optimized previously (15, 30). A fourfold or greater increase in titer between the early- and convalescent-phase samples on day 7 or 11 was used to signify seroconversion. A reference pooled serum specimen from O1- and O139-infected patients (30) was included in each test to control for variation between analyses on different occasions.

Analyses. The Wilcoxon signed rank test and the Mann-Whitney *U* test were used where applicable for statistical analysis. A *P* value of <0.05 was the criterion for a significant difference. Analyses were carried out with the statistical software SigmaStat (Jandel Scientific, San Rafael, Calif.). The GM and standard errors (SE) of the mean values were calculated for all samples. A comparison of the number of positive antibody responses in vibriocidal assay with LPS-specific IgA ASC response was made by using four-field table analyses. The sensitivity and predictive accuracy for antibody measurements using the ASC responses were determined by using the vibriocidal antibody response as a reference since it has previously been used as a reasonably accurate indirect measure of the intestinal immune response in O1 cholera (14). A similar comparison was made between the CT-specific IgG ASC and the antitoxic IgG response in plasma.

RESULTS

Clinical history of the study groups. The patients with *V. cholerae* O139 disease were 21 to 45 (median, 30) years of age who had severe (61% cases) and moderate (39% cases) dehydration with a history of 4 to 15 (median, 8) h of watery diarrhea prior to hospitalization. The patients with O1 cholera were 18 to 42 (median, 25) years of age who had severe (82% cases) or moderate (18% cases) dehydration with a history of 4 to 14 (median, 9) h of diarrhea before hospitalization. The two groups of patients were similar in all respects except that *V. cholerae* O139 patients had a higher median age ($P = 0.006$).

Examination of stools from patients showed that *Campylobacter jejuni* was isolated from one O1 cholera patient. Stool microscopy revealed the presence of a few ova of *Ascaris lumbricoides* in one patient with O1 and three patients with O139 cholera. No bacterial pathogens were isolated from stools of the healthy controls. However, *A. lumbricoides* was detected in two individuals, and hookworm was observed in one.

Specific ASC responses in healthy controls. In 16 of 20 apparently healthy controls, the ASC response to the LPS of *V. cholerae* O1 or O139 was absent or poor (range of GM from 1.1 to 1.8 in the three isotypes). Four controls exhibited ASC responses to the O1 Ogawa, Inaba, and O139 LPS antigens (maximum response in a control, about 30 ASC/10^6 MNC). The responses in the control group, however, did not differ significantly from the responses seen at the acute stage in *V. cholerae* O1- or O139-infected patients (P = not significant [NS] for all comparisons). A response in patients was considered positive when it exceeded >3 SD of the GM of the ASC response of the controls for each antigen and isotype.

ASC responses to WC antigens. All *V. cholerae* O139 patients showed a response to O139 WC antigen which peaked on day 7. On average, a greater than 60-fold increase in ASC numbers specific for O139 WC antigen (Table 1) was seen by day 7 of the onset of the disease in both IgA and IgM isotypes ($P < 0.0001$), but only a 7-fold increase ($P < 0.001$) was seen in the IgG isotype. The response decreased significantly in all isotypes by day 11. A response to heterologous Inaba or Ogawa WC antigen was seen mainly in the IgA isotype and was observed in 34 to 48% of the patients; these increases, though very modest, were significant ($P = 0.006$).

The response to the homologous and heterologous WC bacteria was also studied in 18 patients with *V. cholerae* O1 infection (Table 2). *V. cholerae* O1 patients showed a peak ASC response to Ogawa WC antigens around 7 days after the onset of cholera which declined by day 11 ($P < 0.001$ for all three isotypes). Numbers of Ogawa-specific IgA ASC increased on average 52-fold ($P < 0.001$), and 83% of the patients showed a response. Corresponding increases in the IgM and IgG isotypes were 24- and 12-fold, respectively, and significant differences were observed only in the peak ASC response between the IgA ASC and IgG ASC responses ($P = 0.0001$). The patients also responded to Inaba WC antigen, with peak ASC increases by day 7 after the onset of disease in the IgA (52-fold), IgM (22-fold; $P < 0.0021$), and IgG (14-fold; $P < 0.005$) isotypes. Responses to both the O1 Ogawa and Inaba WC antigens decreased significantly by day 11 ($P < 0.05$ for the different isotypes). The magnitudes of the ASC responses against the Ogawa and Inaba WC antigens were comparable.

Some *V. cholerae* O1-infected patients also showed a response to WC antigens of *V. cholerae* O139 in the different isotypes at the acute stage and at convalescence (Table 2). These responses were modest and not statistically significant.

LPS-specific ASC responses. The *V. cholerae* O139-infected patients showed a strong response to the homologous LPS (Table 1). All patients showed a response in the IgA isotype, and 83% showed a response in the IgM isotype. A 94-fold-higher IgA ASC response was observed by day 7 of the onset of the disease; this response decreased later. The IgM response also was high. An increase in the LPS-specific IgG response was observed ($P < 0.0001$). The *V. cholerae* O139-infected patients showed a weak response to the heterologous Ogawa or Inaba LPS antigen which was not statistically significant for any isotype.

V. cholerae O1-infected patients showed strong Ogawa LPS-specific IgA and IgM ASC responses and a moderate response in the IgG isotype (Table 2). About 93% of these patients showed an IgA ASC response to the homologous LPS antigen. Two patients also showed a weak response to the O139 LPS antigen mainly in the IgA isotype. An Inaba LPS-specific ASC response was also seen in all three isotypes, with an increase in the IgA response and the IgM response dominating over IgG response. The magnitude and isotype distribution of the ASC response to the homologous or heterologous LPS in *V. cholerae* O139- or *V. cholerae* O1-infected patients mirrored the response to the WC antigen (P = NS for all comparisons).

CT-specific ASC responses. All *V. cholerae* O139-infected patients showed CT-specific responses peaking on day 7 in the IgG isotype ($P < 0.0001$), followed by a response in the IgA isotype ($P < 0.0001$). The response in the IgM isotype was poor (P = NS) (Table 1). *V. cholerae* O1 patients showed a comparable CT-specific response (Table 2). No differences were observed with respect to the magnitude of the antitoxin ASC responses between O1- and O139-infected patients.

Total ASC response. In *V. cholerae* O139-infected patients, the total numbers of IgA ASC increased by day 7 of the onset

of cholera ($P = 0.01$) and decreased to the acute levels by day 11 (P = NS) (GM on days 2, 7, and 11 = 1,413, 2,754, and 1,862 ASC/10^6 MNC) and to the levels seen in healthy controls (GM = 1,820 ASC/10^6 MNC). In *V. cholerae* O1-infected patients, the total IgA ASC numbers were already elevated in the acute stage, and further elevation at day 7 was modest (P = NS) (GM on days 2 and 7 = 2,194 and 2,637 ASC/10^6 MNC) and then decreased by day 11 (1,950 ASC/10^6 MNC to control levels). In both groups of patients, total numbers of IgG ASC on day 7 (GM on day 7 for O139- and O1-infected patients = 2,235 and 1,445 ASC/10^6 MNC) were significantly higher than those on day 2 (GM for O139- and O1-infected patients = 777 and 1,185 ASC/10^6 MNC; $P < 0.002$) or day 11 (GM for O139- and O1-infected patients = 1,380 and 1,230 ASC/10^6 MNC; $P < 0.04$) and higher than the values for healthy controls (GM = 1,318 ASC/10^6 MNC). The levels of total IgM ASC remained unchanged (GM ranging from 263 to over 660 ASC/10^6 MNC for O1- or O139-infected patients) and were not significantly different from those in controls (GM = 490 ASC/10^6 MNC; P = NS in all cases).

CT-specific antibody response in plasma. The majority of *V. cholerae* O139-infected patients seroconverted, with CT-specific IgA and IgG antibodies in plasma by day 22 of convalescence (Fig. 1). On average, an eightfold increase in titer in the IgA isotypes was observed by day 7 ($P < 0.0001$); this level declined by day 22 ($P = 0.0018$). The CT-specific IgG plasma response was greater than that seen in the IgA isotype at all time points ($P = 0.0001$).

As expected, the majority of the *V. cholerae* O1-infected patients showed highly significant increases in toxin-specific antibodies in the IgG and IgA isotypes ($P < 0.0001$ for responses in both IgA and IgG isotypes) (Fig. 1). Again the plasma IgG response was significantly higher than the IgA response at all stages of the infection ($P < 0.0001$). The maximal antibody titer increase was 12-fold for IgA on day 11 and 9-fold for IgG on day 22 ($P = 0.0001$ for both isotypes).

Among 23 O139-infected patients showing a CT-specific IgG ASC response, 17 responded with the plasma antibody on day 7, whereas by day 22 all patients that could be studied showed seroconversion. The ASC response on day 7 thus showed a sensitivity and an accuracy of 100% compared with the IgG response in plasma at late convalescence. In O1 cholera, of the 27 of 28 patients responding with ASC on day 7, 26 responded with IgG antibodies. The ASC response thus showed a sensitivity and accuracy of 96% for comparisons with the plasma IgG response.

Vibriocidal antibody responses. As shown in Table 3, both the *V. cholerae* O139- and *V. cholerae* O1-infected patients responded with vibriocidal antibodies to the homologous serogroup of bacteria at the convalescent stage of illness. The response to the homologous organism was higher in *V. cholerae* O1-infected than in *V. cholerae* O139-infected patients ($P <$ 0.0001). both groups of patients did not respond to the heterologous serogroup O1 or O139.

When the vibriocidal antibody response in O139-infected patients measured on day 7 was compared with the LPS-specific IgA ASC response on day 7, the ASC response showed a sensitivity of 100% and a predictive accuracy of 91%. When we compared the ASC response on day 7 with the vibriocidal response on day 11, both the sensitivity and predictive accuracy were 100%. In O1 patients, a sensitivity of 93% and predictive accuracy of 100% were found for the ASC response when comparisons were made with vibriocidal responses measured on day 7 or 11.

TABLE 1. ASC responses in *V. cholerae* O139-infected patients

V. cholerae antigen[a]	GM and range[b]							
	IgA			IgM			IgG	
	Day 2	Day 7	Day 11	Day 2	Day 7	Day 11	Day 2	Day 7	Day 11
WC									
O1 Ogawa	2.0 (1.7–2.7)	7.0 (5.0–9.8)	3.0 (2.3–4.1)	1.0 (1)	2.3 (1.9–2.9)	1.3 (1.1–1.5)	1.0 (1)	1.0 (1)	1.46 (1.3–1.7)
%PR	13	34	17	0	4	0	0	8	26
O1 Inaba	2.0 (1.6–2.6)	6.8 (5.0–8.9)	3.8 (2.6–5.6)	1.3 (1.1–1.6)	2.8 (2.1–3.7)	1.4 (1.2–1.6)	1.0 (1)	1.3 (1.2–1.4)	1.1 (1.0–1.2)
%PR	8	48	17	4	13	0	0	21	4
O139	2.0 (1.6–2.5)	128.0 (102–161)	6.5 (4.6–9.1)	1.5 (1.3–1.8)	103.0 (83.4–128)	2.6 (1.9–3.4)	1.0 (1)	7.0 (4.9–10)	1.8 (1.5–2.2)
%PR	17	100	43	0	100	4	0	70	22
n	23	23	18	23	23	18	23	23	18
LPS									
O1 Ogawa	2.3 (1.8–2.9)	2.4 (1.7–2.8)	2.9 (2.2–3.8)	1.2 (1.0–1.4)	1.6 (1.5–2.2)	1.3 (1.0–1.4)	1.1 (1.0–1.2)	1.15 (1.0–1.2)	1.3 (1.2–1.4)
%PR	39	30	44	4	4	0	4	13	13
O1 Inaba	2.4 (2.05–2.8)	3.6 (3.3–3.9)	3.4 (2.5–4.6)	1.5 (1.3–1.7)	2.0 (1.7–2.3)	1.5 (1.3–1.6)	1.0 (1)	1.3 (1.2–1.4)	1.1 (1.1–1.2)
%PR	13	26	9	0	0	0	4	0	0
O139	2.1 (1.6–2.7)	198.0 (163–241)	8.0 (5.8–11.5)	2.0 (1.6–2.4)	133.0 (105–169)	2.7 (1.9–3.7)	1.0 (1)	9.0 (6.3–12.9)	1.8 (1.5–2.2)
%PR	4	100	33	0	83	0	0	61	13
n	23	23	18	23	23	18	23	23	18
CT	2.0 (1.5–2.6)	215.0 (168–273)	6.6 (4.8–9.0)	1.3 (1.1–1.6)	2.5 (1.8–3.5)	1.5 (1.2–1.9)	1.6 (1.2–2.1)	655.0 (492–871)	7.6 (5.0–11.6)
%PR	9	91	21	4	13	0	9	100	50
n	23	23	18	23	23	18	23	23	18

[a] *n*, number of patients studied; %PR, percentage of positive responses of patients indicated by isotype-specific ASC/10^6 MNC > GM + 3 SD of control values.
[b] Results are geometric means (±1 standard error of the mean) at days 2, 7, and 11 after onset of cholera.

TABLE 2. ASC responses in *V. cholerae* O1-infected patients[a]

V. cholerae antigen	GM and range								
	IgA			IgM			IgG		
	Day 2	Day 7	Day 11	Day 2	Day 7	Day 11	Day 2	Day 7	Day 11
WC									
O1 Ogawa	2.0 (1.6–2.9)	104.0 (71–142)	5.5 (3.3–7.6)	4.1 (2.7–6.3)	98.0 (1.9–129)	5.0 (3–8)	1.6 (1.0–1.6)	18.9 (9.6–33)	1.2 (1.0–1.5)
%PR	7	83	20	22	72	20	5	67	7
O1 Inaba	2.5 (1.8–3.5)	131.0 (79–163)	4.3 (2.6–6.7)	2.9 (1.9–4.4)	63.53 (48–83)	3.8 (2.4–6.0)	1.2 (1.0–1.5)	16.2 (9–29)	2.3 (1.6–3.3)
%PR	11	78	33	17	83	17	5	67	28
O139	2.7 (2.1–3.5)	5.8 (4–8.5)	2.8 (2.1–4.0)	3.1 (2–4.6)	2.9 (2.6–3.5)	3.1 (2.0–4.8)	1.0 (1)	1.6 (1.2–2.0)	1.0 (1)
%PR	11	44	22	17	0	11	0	22	6
n	18	18	15	18	18	15	18	18	15
LPS									
O1 Ogawa	1.9 (1.5–2.2)	103.0 (69.7–152)	6.4 (4.9–8.5)	3.1 (2.2–4.5)	122.0 (97.5–153)	4.9 (3.6–6.8)	1.2 (1.2–1.3)	20.0 (12.8–32)	1.8 (1.5–2.2)
%PR	8	93	52	18	93	24	3	64	18
O1 Inaba	2.4 (1.8–3.0)	112.5 (81–156)	6.1 (4.5–8.2)	3.6 (2.3–5.5)	77.0 (44.8–128.5)	3.4 (2.2–5.1)	1.0 (1)	14.7 (8.0–26.5)	1.8 (1.4–2.2)
%PR	16	79	21	21	74	13	0	58	20
O139	1.8 (1.5–2.5)	2.9 (2.2–3.6)	2.5 (2.3–3.8)	2.1 (1.5–2.4)	2.3 (1.8–3.0)	2.5 (1.9–3.2)	1.0 (1)	1.15 (1–3)	1.3 (1.1–1.5)
%PR	4	7	9.5	7	7	0	0	4	5
n	28	28	21	28	28	21	28	28	21
CT	3.9 (2.8–5.3)	218.0 (161–295)	13.1 (9.5–18)	1.4 (1.2–1.6)	3.6 (2.6–5.0)	1.7 (1.5–2.0)	1.9 (1.5–2.5)	550.0 (411–704)	16.0 (11–23)
%PR	18	93	38	0	31	0	3	96	57
n	28	28	21	28	28	21	28	28	21

[a] See the footnotes to Table 1.

DISCUSSION

Cholera patients responded with antigen-specific ASC responses which increased sharply at around day 7 of the onset of the disease and decreased by day 11. This transient increase in specific ASCs is typical of a mucosal response and similar to observations made previously in patients with diarrhea (19, 29, 33) or vaccinees orally immunized against enteric diseases (18, 20, 21). Thus, both the O139- and O1-infected patients exhibited strong antibacterial B-cell responses in the IgA and IgM isotypes after natural disease, which are indicative of a mucosal immune response (9, 10). As expected, the response to the bacterial antigen was mainly against the serogroup of the infecting strain. Weak ASC responses to the heterologous antigen were seen mainly in the IgA isotype in some O1- and O139-infected patients. In the O139-infected patients, this may be due to an anamnestic response, as these patients may have been exposed previously to the O1 antigens. However, some O1-infected patients as well as controls showed a response to the new O139 antigen. It is possible that these individuals had been recently exposed to this pathogen or to bacteria which cross-react with *V. cholerae* O139. For example, *V. cholerae* serogroups O22 and O155 and *Aeromonas trota* share antigens with *V. cholerae* O139 (3). Otherwise, this could be explained by nonspecific polyclonal B-cell activation, which has previously been documented in patients with diarrhea and in healthy controls in other studies (19, 33).

We have compared the pathogen-specific ASC response, which can be considered a proxy measure of the mucosal immune response in the gut (9, 10), with the vibriocidal antibody response, which in previous studies has been used as an indirect indicator of the intestinal immune response (14). This comparison showed the LPS-specific IgA ASC assay to have a sensitivity of 93 to 100% compared to the vibriocidal response. This finding suggests that as with the vibriocidal antibody re-

FIG. 1. IgG and IgA antitoxin responses in plasma of patients with cholera due to *V. cholerae* O1 and *V. cholerae* O139. GM titers and SE are shown for day 2 (d2), day 7 (d7), day 11 (d11), and day 22 (d22) after onset of cholera. *n*, number of patients studied; PR, number of patients showing a positive antibody response to CT.

TABLE 3. Vibriocidal antibody titers in patient sera collected after onset of disease[a]

Patient group	GM (±1 SE) vibriocidal antibody titer (range) to:					
	V. cholerae O139			*V. cholerae* O1		
	Day 2	Day 7	Day 11	Day 2	Day 7	Day 11
V. cholerae O139 infected	11.5 (9.4–14.0)	209.0 (139–315)	437.0 (312–611)	83.0 (63–110)	76.0 (57–102)	76.0 (54–107)
n	23	23	18	23	23	18
V. cholerae O1 infected	6.0 (5.0–6.4)	6.0 (5–7)	6.0 (5.0–6.4)	66.0 (47–99)	1,748.0 (1,171–2,610)	2,630.0 (1,832–3,775)
n	28	28	22	28	28	22

[a] See the footnotes to Table 1.

sponse, the LPS-specific IgA ASC response can also be used as an indirect marker of the mucosal immune response in cholera. The antigen-specific ASC response, although sensitive and useful as a proxy indicator of the mucosal immune response of the gut, must be interpreted with caution since weak responses to the heterologous antigen in cholera-endemic regions are also seen.

In this study, both the O1- and O139-infected patients mounted a stronger CT-specific IgG ASC response than IgA ASC response, unlike the immunologically naive Swedish volunteers, who had a stronger IgA ASC response than IgG ASC response when orally immunized with vaccines containing CTB (31, 39). In patients with diarrhea, IgG ASC may simply reflect the induction of a memory response and/or leakage of antigens to the systemic compartment due to an inflamed gut, which can generate specific ASC of systemic origin (18, 20, 33). The antitoxic IgG antibody response in serum has previously been shown to be a good correlate of the gut mucosal immune response to CT in cholera (14). Our data indicate that measuring the CT-specific antibody response in plasma corresponds well with the CT-specific ASC response in the peripheral blood. Thus, for cholera diagnosis and for vaccine evaluation based on antitoxin immunity, determinations of toxin-specific ASC responses will be useful.

As expected, the kinetics of the antibody responses in plasma were different from those of the specific ASC responses. This is to be expected since the plasma antibody responses induced by natural cholera infection are believed to be in part a systemic immune response and in part a spillover from the intestine into the circulation (10). Systemic antibodies in cholera (14, 29) or other enteric infections (25, 33) persist longer, whereas specific ASC make only a transient appearance. The circulating ASC mainly represent gut-derived B-cell blasts en route to the gut mucosa.

Since *V. cholerae* O139 possesses a polysaccharide capsule (16, 37, 38), it had initially been speculated that it may shield critical surface antigens, resulting in a decrease of certain immunological responses in the host (11, 16, 24). However, it is apparent that *V. cholerae* O139-infected patients show WC- and LPS-specific ASC responses comparable to those seen in *V. cholerae* O1 patients. This may be due to the fact that the CPS and LPS are antigenically related; CPS is a polymer of the side chain of the LPS (7). In a study of the immune response of North American volunteers after challenge with *V. cholerae* O139, responses to LPS, CPS, and CT were demonstrated (24). However, a vibriocidal antibody response was not seen in this study. In other studies carried out in North America and Sweden with live (6) or killed O139 candidate vaccines (15), volunteers responded with vibriocidal antibodies. In an earlier study (30) carried out by us, we optimized conditions under which these antibodies can be detected in O139 patients by using a modification of the vibriocidal test for *V. cholerae* O1 (14). All of these studies show that by a suitable modification of the vibriocidal assay that is used for *V. cholerae* O1, including the selection of an appropriate *V. cholerae* O139 strain, a vibriocidal antibody response to *V. cholerae* O139 infection can be detected.

This is the first study in a region where cholera is endemic in which immune responses of patients suffering from O1 or O139 cholera to both the homologous and heterologous causative agents of cholera were examined. The immunological assays carried out were also more comprehensive than in previous studies. We measured ASC responses to WC, LPS, and CT in all isotypes of immunoglobulins, plasma antibody to CT, and the vibriocidal antibody. Even though we have not measured the immune response to the CPS, since it is a polymerized form of the side chain of the LPS (7), it is likely that CPS-specific ASC and antibodies will be detected in O139 patients as was observed in North American volunteers challenged with *V. cholerae* O139 (24). The data presented here can be used to assess immunity after natural disease or after vaccination.

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