Immune Responses in Ileostomy Fluid and Serum after Oral Cholera Vaccination of Patients Colectomized because of Ulcerative Colitis

JAN KILHAMN,1,2 HANS BREVINGE,3 ANN-MARI SVENNERHOLM,1 AND MARIANNE JERTBORN1,2*

Departments of Medical Microbiology and Immunology,1 Infectious Diseases,2 and Surgery,3 Göteborg University, Göteborg, Sweden

Received 23 December 1997/Returned for modification 18 February 1998/Accepted 22 May 1998

The capacity of an oral inactivated B-subunit–whole-cell cholera vaccine to induce immune responses in patients colectomized due to ulcerative colitis was studied. Two doses of vaccine induced significant mucosal immunoglobulin A (IgA) antibody responses in ileostomy fluid against cholera toxin in 14 of 15 (93%) patients and against whole vibrios in 9 of 15 (60%) cases. The serological responses were lower (but not significantly) than those observed in healthy Swedish volunteers. Increased IgA antitoxin levels were found in ileostomy fluid as late as 2 years after vaccination.

Ulcerative colitis is a severe inflammatory bowel disease leading to colectomies in more than 30% of patients, with approximately 10% of the patients having colectomies in the year after diagnosis (10). In most patients, a continent ileostomy ad modum Kock or today a pelvic pouch with ileoanal anastomosis is constructed after the colectomy (16, 19). Patients with continent ileostomies are likely not to feel they must restrict their travel, which is of special concern, since diarrheal diseases pose a risk to them (17). Under normal conditions, there is an increased loss of sodium and water from the intestines of colectomized patients that is compensated by an increased renal reabsorption (5, 6). Enterotoxin-mediated diarrheal diseases such as cholera and enterotoxigenic *Escherichia coli* (ETEC) diarrhea are likely to further increase the intestinal losses, which might lead to severe dehydration and sodium deficiency.

The aim of the present study was to examine whether a licensed oral inactivated B-subunit–whole-cell (B-WC) cholera vaccine could induce intestinal immune responses in patients who had had colectomies due to ulcerative colitis and to compare the antitoxic and antibacterial antibody responses in sera from colectomized patients with those found in a group of healthy volunteers (without any history of gastrointestinal illness) given the same cholera vaccine. We also evaluated whether determination of specific immunoglobulin A (IgA) immune responses in ileostomy fluid could be used to assess the kinetics of intestinal immune responses after oral immunization.

**Study design.** Fifteen adult patients (eight women), aged 30 to 73 years (mean age, 42 years), who had had colectomies due to ulcerative colitis 3 to 27 years (mean, 14 years) earlier were recruited from the regular follow-up program for patients with inflammatory bowel disease at the Department of Surgery, Sahlgrenska University Hospital in Göteborg, Sweden. Continenence surgery had been performed 3 to 25 years (mean, 11 years) earlier by construction of either a continent ileostomy ad modum Kock (11 patients) or a pelvic pouch with an ileoanal anastomosis (4 patients). The maximal extent of the small bowel resection was limited to 10 cm of the distal ileum. All patients were in general good health and had had no signs of acute pouchitis for the year preceding the study. Twenty healthy adult Swedish volunteers (15 women), aged 22 to 48 years (mean age, 31 years), with no history of gastrointestinal illness or inflammatory bowel disease served as controls. None of the study subjects had been previously vaccinated against cholera or had travelled to areas where cholera or ETEC is endemic in the 2 years preceding the study. All subjects agreed to participate in the study, which was approved by the Research Ethical Committee at the Medical Faculty, Göteborg University. Each subject received two oral doses of the B-WC cholera vaccine with an interval of 2 weeks between the doses. The vaccine, containing 1.0 mg of recombinantly produced cholera B subunit (CTB) and 10^11 heat- and formalin-killed O1 vibrios per dose, was produced by SBL Vaccin, Stockholm, Sweden, as previously described (11) and was administered in 150 ml of a 2.8% sodium bicarbonate buffer solution (Samarin; Cederroths Nordic AB, Upplands Väsby, Sweden) (13).

From the 15 colectomized patients, ileostomy fluid and blood specimens were collected immediately before the first immunization (day 0) and then 9 days after the second dose. From 9 of these patients, additional specimens were also obtained after 5 and 21 days (only fluid) and 4, 8, 12, and 24 months. From the 20 healthy volunteers, serum samples were collected before the first immunization (day 0) and 9 days after the second vaccine dose. Ileostomy fluids were collected within 3 h after the last emptying of the reservoir. A 50-ml portion of fluid was immediately chilled on ice and then centrifuged at 20,000 × g for 30 min. Twenty milliliters of the supernatant was saved and treated with enzyme inhibitors essentially by the method of Gaspari et al. (9). Soybean trypsin inhibitor (STI; Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 100 μg ml⁻¹ followed by addition of EDTA (pH 7.2 to 7.4; Merck, Darmstadt, Germany) to a final concentration of 0.05 M. Then 100 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.) diluted in 99% methanol was added to the STI-EDTA-treated ileostomy fluid to a final concentration of 2% (vol/vol) and kept at room temperature for 15 min. Finally, bovine serum albumin (Sigma Chemical Co.) and NaN₃ were added to final concentrations of 1 mg ml⁻¹ and 0.02% (vol/vol), respectively. The ileostomy fluid was divided into two portions; one aliquot was frozen at −70°C, and the other portion was lyophilized and...
TABLE 1. Antitoxic and antibacterial IgA antibody responses in ileostomy fluid samples from colectomized patients 9 days after the second oral immunization with B-WC cholera vaccine

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Frequency of responders</th>
<th>Specific IgA/total IgA (U μg⁻¹)</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preimmunization</td>
<td>Postimmunization</td>
<td>All subjects</td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>14/15 (93)</td>
<td>0.26</td>
<td>9.9</td>
</tr>
<tr>
<td>V. cholerae LPS</td>
<td>6/15 (40)</td>
<td>0.24</td>
<td>0.53</td>
</tr>
<tr>
<td>Whole vibrios</td>
<td>7/15 (47)</td>
<td>0.58</td>
<td>1.3</td>
</tr>
</tbody>
</table>

a Responders were defined as having a greater than twofold increase in specific IgA antibody titer/total IgA concentration between pre- and postimmunization specimens. The number of responders/total number of patients is shown. The frequency as a percentage is shown in parentheses.
b Geometric mean specific IgA antibody titer/total IgA concentration for all subjects. The values have been multiplied by 10.
c Geometric mean increase in specific IgA antibody titer/total IgA concentration.

Antibody and immunoglobulin determinations. Levels of total IgA in ileostomy fluid were determined by a modified microplate enzyme-linked immunosorbent assay (ELISA) (4, 24). IgA antibody responses to cholera toxin in ileostomy fluid were studied by the GM1 ELISA (23) and antibacterial antibodies by an ELISA in which the WC component of the B-WC vaccine or Vibrio cholerae O1 lipopolysaccharide (LPS) was used as a solid-phase antigen (14, 15). Threefold (antitoxin) or twofold (antibacterial) serial dilutions of pre- and postvaccination specimens were tested side by side in duplicate. The specific antitoxic and antibacterial activities of IgA in ileostomy fluid were determined by dividing the GM1 ELISA antibody titers by the total IgA concentration (in micrograms per milliliter) of the sample. Based on previous calculations, a greater than twofold increase in the mean IgA antibody titer/total IgA concentration between pre- and postimmunization specimens was chosen to signify seroconversion (1, 14). Serum antitoxic responses of IgA and IgG classes were determined by the GM1 ELISA mentioned above (23). Antibacterial antibodies in serum were determined by a microtiter vibriocidal assay (18). Threefold (GM1 ELISA) or twofold (vibriocidal test) serial dilutions of pre- and postvaccination specimens were tested in duplicate. The antibody titer ascribed to each sample was the mean of the duplicate determinations. An increase of twofold (antitoxin) or fourfold (vibriocidal), or more, in the endpoint titer between pre- and postvaccination specimens was used to signify seroconversion at a P value of <0.05 (12, 13). Confidence intervals (CI) were calculated by using the t distribution in a two-tailed fashion.

Adverse reactions. Surveillance for possible side effects to the B-WC cholera vaccine in colectomized patients was performed during 5 consecutive days after each immunization. Gastrointestinal symptoms, i.e., abdominal discomfort, abdominal cramps, and/or an increase in the reservoir-emptying frequency up to twice the normal rate were reported by three (20%) of the patients after the first immunization and by two (14%) of them after the second dose. The symptoms did not affect the patients’ daily activities. The frequency and severity of symptoms were consistent with previous findings in healthy individuals who had been immunized with the same cholera vaccine and were probably due to the intake of bicarbonate buffer (13, 14).

Antibody responses in ileostomy fluid. To date, the most reliable approach to assess gut mucosal immune responses is to determine specific IgA antibodies in intestinal secretions (2, 12, 24). The lavage procedure has been used extensively in our laboratory for evaluations of intestinal immune responses in humans after oral immunization with cholera as well as inactivated ETEC vaccines (1, 14, 24). However, since the method is laborious and repeated intestinal lavages are difficult to perform, it is not suitable for studies of the kinetics of gut mucosal immune responses after vaccination. More recently, it has been described that IgA antibody responses in feces may reflect intestinal immune responses in volunteers receiving an oral ETEC vaccine, although the sensitivity of such determinations has been lower than that of lavage analyses (3, 25). In an attempt to identify an easily accessible specimen that allows collection on multiple occasions and possibly has a high sensitivity in reflecting intestinal immune responses, we evaluated whether ileostomy fluid could be used to study locally produced secretory IgA antibodies in the intestine. Ileostomy fluid specimens collected from 15 colectomized patients given B-WC vaccine were found to contain a mean of 97% water, with negligible variations in water content between samples collected from different patients and on various days. The geometric mean total IgA concentrations were similar in fluids collected before and after immunization, being 2,630 and 2,512 μg ml⁻¹, respectively. The concentration of total IgA was approximately 20 times higher in ileostomy fluids than in intestinal lavages or fecal extracts (2, 3, 9). At least part of this difference might be explained by the dilution effect obtained by the lavage fluid treatment or fecal extraction procedure. In agreement with findings in earlier vaccine trials using lavage fluid for assessment of intestinal immunity (2, 3), the total IgA content in consecutive ileostomy fluid specimens from one subject was very consistent. The interindividual variation in total IgA concentrations in ileostomy fluids was comparable to that in lavage specimens (approximately 7-fold) but lower than in fecal extracts (approximately 23-fold) (2, 3). The small variations in total IgA concentration found might be due to the standardized conditions under which the specimens were collected. Thus, all ileostomy fluids were obtained within 3 h after the last emptying of the reservoirs and immediately chilled on ice in order to minimize the time for degradation of immunoglobulins by proteolytic enzymes. In comparison with determination of specific IgA antibodies in intestinal lavages, analyses of ileostomy fluids seem to have some advantages, such as the ease of obtaining specimens and the possibility of being able to monitor the kinetics of the gut immune responses after immunization. However, the number of colectomized patients available for such studies is limited.

It has been suggested that patients with ulcerative colitis might have defective immunoregulation. In active ulcerative colitis, an upregulation of peripheral blood mononuclear cells as well as mucosal lymphocytes with regard to surface activation markers has been noted (22). In the present study, two doses of B-WC vaccine induced significant increases in CTB-specific IgA antibody titers/total IgA concentrations in ileostomy fluid in 14 of the 15 (93%) colectomized patients, and the geometric mean fold increase for responders was 51-fold (95% CI, 15- to 178-fold) (Table 1). The frequency of CTB-specific IgA antibody responses in ileostomy fluid samples from the patients was comparable to that previously observed in lavage.
fluid and stool specimens of healthy Swedish volunteers given CTB orally together with either killed *V. cholerae* (14) or killed ETEC bacteria (1, 3). The magnitude of the gut mucosal IgA antitoxin response was at least as high in the colectomized patients as in healthy volunteers (1, 14). Although there was a tendency towards a less frequent antibacterial immune response in colectomized patients than in lavage fluid samples from healthy volunteers after cholera vaccination (14), antibacterial IgA antibody responses were still found in ileostomy fluid in 9 of 15 (60%) of the patients; of these patients, 6 responded to LPS and 7 responded to the WC component of the vaccine. The magnitudes of the increases in antibacterial titers against the two different types of antigens were also very similar (Table 1).

**Antibody responses in serum.** In contrast to the strong immune response seen in the intestine after immunization, the B-WC cholera vaccine did not seem to be as efficient in inducing serum antibody responses in colectomized patients as in healthy volunteers. Thus, two doses of B-WC vaccine induced significant increases in IgA antitoxin titer in serum in 10 of 15 (67%) of the patients, and 7 patients (47%) developed IgG antitoxin responses (Table 2). The magnitude of the increases in antitoxin titer among responders was approximately 7.5-fold. Vibriocidal antibody responses in serum were found in 4 of 15 (27%) of the patients, and the magnitude of the titer increases for these responders was 10-fold (Table 2). The serological responses were lower than those observed in the group of healthy volunteers given the same vaccination (Table 2). Thus, both the frequencies and magnitudes of IgA and IgG antitoxin responses as well as vibriocidal antibody responses were higher in the healthy volunteers than in the colectomized patients, although the differences were not statistically signifi-

<table>
<thead>
<tr>
<th>Immunization group</th>
<th>Immune responsea</th>
<th>IgA antitoxin</th>
<th>IgG antitoxin</th>
<th>Vibriocidal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequencyb</td>
<td>Magnitudec</td>
<td>Frequency</td>
<td>Magnitude</td>
</tr>
<tr>
<td>Colectomized patients (<em>n</em> = 15)</td>
<td>10 (67)</td>
<td>7.5 (3.6–15)</td>
<td>7 (47)</td>
<td>7.7 (3.4–17)</td>
</tr>
<tr>
<td>Healthy volunteers (<em>n</em> = 20)</td>
<td>17 (85)</td>
<td>22 (14–35)</td>
<td>16 (80)</td>
<td>6.6 (4.5–9.8)</td>
</tr>
</tbody>
</table>

a Differences in frequencies and magnitudes of antibody responses between the immunization groups were determined by Fisher's exact test and Student's *t* test, respectively, and were found to be nonsignificant.

b The number of responders is shown. Response was evaluated in relation to prevaccination titers. The frequency (number of responders/number of patients or volunteers tested; shown as a percentage) is shown in parentheses.

c Increase in geometric mean titer for responding subjects in relation to prevaccination titer. 95% CI are shown in parentheses.

**FIG. 1.** Kinetics of the geometric mean IgA antitoxin titer/total IgA concentration in ileostomy fluid samples from nine colectomized patients after immunization with two oral doses of B-WC cholera vaccine. Frequencies of responders are indicated above the bars. The error bars show the standard errors of the means.
The relation between the immune responses in ileostomy fluid and serum samples from the colectomized patients was also studied. Of the 14 patients responding with IgA anti-toxin titer rises in ileostomy fluid, 10 developed significant IgA anti-toxin responses and 7 responded with IgG antitoxin in serum. Only one of nine patients with increases in bacterial IgA antibody titers in ileostomy fluid responded with a significant virobioidal antibody titer increase in serum.

**Kinetics of immune responses.** The ease in obtaining additional specimens of ileostomy fluid made it possible to study the kinetics of the gut mucosal immune responses in colectomized patients up to 24 months after oral immunization with B-WC cholera vaccine. In earlier studies in Bangladesh, IgA antibody responses in lavage fluid have been monitored for up to 28 days after two doses of cholera vaccine, and by that time, significant antitoxic and antibacterial IgA immune responses were found in approximately two-thirds of the volunteers who had initially responded to the vaccine (12, 24). More recently, analyses of fecal IgA antibodies in healthy Swedish volunteers have shown that increases in vaccine-specific gut mucosal IgA antibody titers can be demonstrated in 40 to 50% of initially responding vaccinees 6 months after cholera vaccination (15). In the present study, we could show that eight of nine (89%) initially responding colectomized patients still had significantly elevated IgA anti-toxin levels in ileostomy fluid 8 months after vaccination and in six of these patients (67%), antitoxin levels remained elevated after 24 months (Fig. 1). Peak intestinal IgA anti-toxin responses were found in fluids collected 9 days after vaccination; the geometric mean level being 51-fold higher than that before vaccination. Thereafter, the IgA anti-toxin levels decreased but still showed a mean increase of 6.2-fold after 24 months compared to that before vaccination (Fig. 1). Intestinal antibacterial IgA antibody responses were found in three of six initially responding patients 4 months after immunization, and only one patient had a demonstrable intestinal anti-bacterial IgA antibody response after 12 months. The antibacterial IgA response peaked in fluids collected either 9 or 21 days after vaccination. In contrast to the long-lasting anti-toxin response seen in the intestine, only 33% of the patients had elevated IgA and IgG anti-toxin levels in serum after 2 years. The kinetics of the virobioidal antibody response in serum could not be studied, since only one patient responded to the vaccine.

In conclusion, the present study shows that an oral inactivated B-WC cholera vaccine was safe and gave rise to significant mucosal IgA antibody responses in ileostomy fluid when given to adult patients who had had colectomies due to ulcerative colitis. Large field trials have shown that the B-WC cholera vaccine confers protection against cholera (8, 21) and that through its B-subunit component (which cross-reacts immunologically with B subunits of the heat-labile toxin [LT] of ETEC), the vaccine is also capable of inducing substantial (ca. 70%) short-term protection against LT-producing *E. coli* diarrhea in adult travellers and children in areas where LT-producing *E. coli* is endemic (7, 20). The results of the present study suggest that the B-WC cholera vaccine may also be used for immunoprophylaxis against cholera and LT-producing ETEC in patients with ileostomies who intend to travel to areas in Africa, Asia, and Latin America where cholera and ETEC are endemic.

This work was supported in part by a grant from the Swedish Medical Research Council (16X-09089).

We are grateful to Camilla Johansson and Marie Bengtsson for skilful technical assistance and to Elisabet Lindholm and Harriet Törnqvist for collecting the specimens.

**REFERENCES**

21. Sanchez, J. L., B. Vasquez, R. E. Buege, R. Meza, G. Castellares, C. Cabezas, and...