T- and B-Cell Immune Responses of Patients Who Had Undergone Colectomies to Oral Administration of Salmonella enterica Serovar Typhi Ty21a Vaccine

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The capacity of an oral live attenuated Salmonella enterica serovar Typhi Ty21a vaccine to induce immune responses in patients who had undergone colectomies because of ulcerative colitis was evaluated, and these responses were compared with those of healthy volunteers. Purified CD4+ and CD8+ T cells from peripheral blood were stimulated in vitro by using the heat-killed Ty21a vaccine strain, and the proliferation and gamma interferon (IFN-γ) production were measured before and 7 or 8 days after vaccination. Salmonella-specific immunoglobulin A (IgA) and IgG antibody responses in serum along with IgA antibody responses in ileostomy fluids from the patients who had undergone colectomies were also evaluated. Three doses of vaccine given 2 days apart failed to induce proliferative T-cell responses in all the six patients who had undergone colectomies, and increases in IFN-γ production were found only among the CD8+ cells from three of the patients. In contrast, both proliferative responses and increased IFN-γ production were observed among CD4+ and CD8+ T cells from 3 and 6 of 10 healthy volunteers, respectively. Salmonella-specific IgA and/or IgG antibody responses in serum were observed for five (56%) of nine patients who had undergone colectomies and in 15 (88%) of 17 healthy volunteers. In ileostomy fluids, significant anti-Salmonella IgA antibody titer increases were detected in six (67%) of nine patients who had undergone colectomies. The impaired T- and B-cell immune responses found after vaccination in the circulation of patients who have undergone colectomies may be explained by a diminished colonization of the Ty21a vaccine strain due to the lack of a terminal ileum and colon.

The induction and dissemination of immune responses after oral administration of enteric vaccines in healthy humans have been extensively studied (2, 12–14, 21, 30). However, there is a paucity of studies done with individuals suffering from diseases that may influence the induction of mucosal immune responses. Patients who have undergone colectomies, e.g., due to inflammatory bowel diseases, malignant tumors, or polyposis coli, may potentially have an impaired ability to develop B- and T-cell responses to antigens presented in the distal ileum and colon.

In typhoid fever, the pathogenesis includes a preferential colonization and penetration of bacteria at the level of the terminal ileum and colon as shown in animal models (7) and studies of typhoid fever in humans (9, 19). A live typhoid vaccine could therefore be a suitable model antigen to address studies of typhoid fever in humans (9, 19). A live typhoid vaccine. Vaccine-specific CD4+ and CD8+ T-cell responses in peripheral blood were analyzed along with immunoglobulin A (IgA) and IgG antibody responses in serum. For the patients who had undergone colectomies, the vaccine-specific IgA antibody responses in ileostomy fluids were also studied.

MATERIALS AND METHODS

Study design. Nine adult patients (two of whom were women), aged 36 to 56 years (mean age, 45 years), who had undergone colectomies due to ulcerative colitis, were recruited from the regular follow-up program for patients with inflammatory bowel disease at the Department of Surgery of the Sahlgrenska University Hospital in Göteborg. Continence surgery had been performed 1 to 20 years earlier (mean, 11.3 years) by the construction of a pelvic pouch with an ileoanal anastomosis. All patients had had very similar amounts of small bowel
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resected. The maximal extent of the small bowel resection was limited to 10 cm of the distal ileum. All patients were generally in good health and had no episodes of acute poutchitis or signs of extraintestinal manifestations of ulcerative colitis for the last 3 years preceding the study. None of the patients had been taking any drugs or specific foods prior to the study period, nor did they take any during the study period. The only drugs regularly administered to the patients who had undergone colectomies were loperamide (four patients) and vitamin B12 (three patients). Seventeen healthy volunteers (10 of whom were women), aged 20 to 66 years (mean age, 35 years), were recruited and served as controls to the patients who had undergone colectomies. None of the individual had a history of typhoid fever or had previously been vaccinated against typhoid fever. All individuals agreed to participate in the study, which was undertaken with due approval from the Human Research Ethical Committee at the Medical Faculty, Göteborg University.

Each individual received, 2 days apart, a total of three doses of a live attenuated S. enterica serovar Typhi Ty21a vaccine (Vivotif; Swiss Serum and Vaccine Institute Bern, Bern, Switzerland). The vaccine was ingested orally in the form of enteric-coated capsules containing at least 2 × 10^{10} bacteria per capsule.

Collection of specimens. To obtain peripheral blood mononuclear cells (MNCs), 50 ml of heparinized venous blood was collected from six of the patients who had undergone colectomies and from 10 of the healthy volunteers prior to the first immunization (day 0), and then 7 or 8 days after the onset of vaccination. Serum specimens were obtained from nine patients who had undergone colectomies and from 10 of the healthy volunteers prior to and after initiation of the cultures, 100 l of fresh culture medium. The supernatants were immediately chilled on ice, centrifuged, and treated with enzyme inhibitors as previously described (18). The ileostomy fluids were frozen at −70°C until used. Biopsies were collected from the ileal pouch by using a gastrointestinal fibroscope and a pair of biopsy forceps (FB 24 K; Olympus, Stockholm, Sweden). On each occasion, 15 pinch biopsies, 1 to 2 mm in diameter, were collected.

Cell preparation. Peripheral blood MNCs were isolated by density gradient centrifugation on Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden), and intestinal MNCs were isolated from ileal pouch biopsies by using an enzymatic dispersion technique as previously described (23). A pool of 15 ileal pouch biopsies yielded 0.6 × 10^{6} to 4.8 × 10^{6} MNCs. After isolation, the blood and intestinal MNCs were washed three times with phosphate-buffered saline (PBS) and resuspended in culture medium (Iscove’s medium supplemented with 5% heat-inactivated AB+ serum, 1% t-glutamine, and 1% gentamicin). CD4+ and CD8+ cells were isolated from the blood MNCs by positive selection by using immunomagnetic techniques as described previously (22). The purity of the blood T-cell populations was monitored by incubation with anti-CD4 fluorescein isothiocyanate (FITC)- and anti-CD8 phycoerythrin (PE)-conjugated antibodies (from Becton-Dickinson, San Jose, Calif.) followed by analysis with a flow cytometer (FACSCalibur, Becton-Dickinson). The purified populations were found to contain at least 97% CD4+ cells and 94% CD8+ cells.

Cell culture. To obtain antigen-presenting cells (APCs), the CD4+ and CD8+ -depleted blood MNCs remaining after the magnetic separation were plated in round-bottomed 96-well plates (NUNC, Roskilde, Denmark) at 10^5 cells per well and incubated for 2 h at 37°C. The nonadherent cells were then removed, the wells were washed twice with PBS, and 10^5 cells per well of the two different T-cell subpopulations were added followed by the addition of 2 × 10^{6} heat-killed serovar Typhi Ty21a bacteria. The heat-killed Ty21a bacteria were obtained by incubating viable vaccine strain bacteria which had been subcultured on horse blood agar plates in an 80°C water bath for 1 h (22). Phthomagglutinin (PHA) (5 µg ml−1; Murex diagnostics Ltd, Temple Hill, United Kingdom) was used as a positive control, and culture medium alone was used as a negative control. The plates were incubated at 37°C and 5% CO2 for 5 days. Two days after initiation of the cultures, 100 µl of the culture medium supernatant was removed and replaced by 100 µl of fresh culture medium. The supernatants (three or six wells) were pooled and stored at −70°C until analyzed for cytokine content. After another 3 days, the wells were pulsed with 0.5 µCi of [3H]thymidine (Amersham, Arlington Heights, Ill.) per well for 8 h, and then the plates were frozen at −20°C. Later on the plates were thawed, and the cells were harvested onto nylon filters and analyzed for proliferative responses by using a scintillation counter. For each specimen, the median counts per minute for a set of unstimulated cultures was subtracted from the median counts per minute for a corresponding set of cultures stimulated with heat-killed Ty21a vaccine strain bacteria. A greater-than-twofold increase in net counts per minute value between pre- and postimmunization specimens was considered a significant proliferative response.

Cytokine detection. The concentration of IFN-γ in ileostomy fluids and cell supernatants from peripheral blood and intestinal MNCs was determined by a recently described enzyme-linked immunosorbent assay (ELISA) method (22). Ileostomy fluids were also analyzed for cytokine concentrations of interleukin-4 (IL-4) and transforming growth factor β with reagents (DuoSet) from R&D Systems (Abingdon, United Kingdom) and for IL-10 with reagents (Opt-EIA) from BD PharMingen (San Diego, Calif.); these assays were performed according to the recommendations of the manufacturers. In each instance the ratio of the amount of released IFN-γ in cell culture supernatants stimulated with heat-killed Ty21a bacteria to the amount stimualted with PHA was calculated. A greater-than-twofold increase in IFN-γ production between pre- and postimmunization specimens was defined as a significant response.

Antibody determinations. Levels of total IgA in ileostomy fluids were determined by a modified microplate ELISA as previously described (3, 25). The antibody responses to Salmonella in serum (IgA and IgG) and in ileostomy fluid (IgA) were analyzed by a modified whole-cell bacterium ELISA (14, 22). Briefly, flat-bottomed polystyrene microtiter plates (NUNC) were coated with 0.01 mg of poly-l-lysine (Sigma) ml−1, and then live S. enterica serovar Typhimurium (strain SL2404; kindly provided by Bruce Stocker, Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, Calif.) (10^9 bacteria per well) were added. The SL2404 strain expresses the same O antigens (i.e., O9 and O12) as the vaccine strain Ty21a and the wild-type S. enterica serovar Typhi (14). The bacteria were fixed in the plates by centrifugation followed by the addition of 0.5% glutaraldehyde (Sigma). Nonspecific binding sites of the plates were blocked with 10% fetal calf serum in PBS. After incubating serial dilutions of pre- and postvaccination specimens for 2 h, horseradish peroxidase-labeled rabbit anti-human IgA or IgG antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) were added, and the plates were further incubated for 90 min followed by the addition of o-phenylenediamine substrate for 30 min. The antibody titers were determined as the interpolated dilutions of the samples giving an absorbance at 492 nm of 0.4 above background.

A greater-than-twofold increase in titer between pre- and postimmunization samples was chosen to signify seroconversion (14). The specific anti-Salmonella O9 and O12 IgA activity in ileostomy fluid was determined by dividing the IgA anti-Salmonella titer by the total IgA concentration (in micrograms per milliliter) of the sample. A greater-than-twofold increase in the ratio of mean IgA anti-Salmonella titer to total IgA concentration between pre- and postimmunization specimens was regarded as a significant response (11, 14).

RESULTS

T-cell immune responses. The T-cell immune responses in peripheral blood after oral immunization with a live attenuated S. enterica serovar Typhi Ty21a vaccine were studied for six patients and 10 healthy volunteers by measuring the proliferative responses and IFN-γ production after stimulation of purified CD4+ and CD8+ cells with heat-killed Ty21a bacteria.

Three doses of vaccine failed to induce any proliferative responses among the various T-cell subsets from the patients, and increases in IFN-γ production were found only among the stimulated CD8+ cells from three (50%) of the patients (Table 1 and Fig. 1). In contrast, 6 (60%) of the 10 healthy volunteers displayed a proliferative response in CD8+ cells (P = 0.034 versus patients; Fisher’s exact test, two-sided), and for 3 of them a proliferative response was also observed for CD4+ cells; the magnitude of the responses among responders was 20-fold for CD8+ cells and 3-fold for CD4+ cells. Increases in IFN-γ production were observed for CD4+ and CD8+ T cells from three and six of the healthy volunteers, respectively (Table 1 and Fig. 1).

The mucosal T-cell response in the distal ileum of the patients who had undergone colectomies was also assessed by obtaining ileal pouch biopsy specimens from three of them. The low cell yield did not allow the use of immunomagnetic
TABLE 1. IFN-γ production and proliferative responses of peripheral blood CD4+ and CD8+ T cells from patients who have undergone colectomies and healthy volunteers after three oral administrations of S. enterica serovar Typhi Ty21a vaccine

<table>
<thead>
<tr>
<th>T-cell population and type of response</th>
<th>T-cell response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
</tr>
<tr>
<td></td>
<td>Frequency†</td>
</tr>
<tr>
<td>CD4+</td>
<td></td>
</tr>
<tr>
<td>IFN-γ production†</td>
<td>0/6</td>
</tr>
<tr>
<td>Proliferation†</td>
<td>0/6</td>
</tr>
<tr>
<td>CD8+</td>
<td></td>
</tr>
<tr>
<td>IFN-γ production†</td>
<td>3/6</td>
</tr>
<tr>
<td>Proliferation</td>
<td>0/6</td>
</tr>
</tbody>
</table>

† Responders were defined as having a greater-than-twofold increase in IFN-γ production or proliferative response between pre- and postimmunization specimens.
‡ For responding subjects 7 or 8 days after the first vaccine dose compared to preimmunization values.
§ IFN-γ production was expressed as the ratio of the amount of released IFN-γ in cell culture supernatants stimulated with Ty21a bacteria to the amount stimulated with PHA.

d Proliferation was expressed as net counts per minute by subtracting the median counts per minute for a set of unstimulated cell cultures from the median counts per minute for a corresponding set of cell cultures stimulated with Ty21a bacteria.

The frequency of both IFN-γ production and proliferation was 5/10 for CD4+ cells and 8/10 for CD8+ cells of healthy volunteers.

**DISCUSSION**

The present study shows that the oral live attenuated S. enterica serovar Typhi Ty21a vaccine has the capacity to induce local B-cell immune responses in the intestines of patients who have undergone colectomies whereas their T- and B-cell immune responses in the circulation were considerably weaker than those of healthy volunteers. Although this study included only a limited number of subjects, we found consistent trends indicating that the antigen-specific immune responses are diminished in patients who have undergone colectomies. We consider it unlikely that other differences between these patients and healthy volunteers, e.g., age, gender, and ongoing medication, could explain the observations. Our findings suggest that the impaired responsiveness, in particular among the T cells, may be of relevance for the future use of live vaccine strains which predominantly colonize the colon, e.g., different genetically attenuated Salmonella and Salmonella vector vaccines including CVD 906, CVD 908, and BRD 509 (17, 20, 26). The observations are also of importance for patients who have cell sorting techniques. No proliferative responses could be detected after vaccination, and stimulation of the intestinal MNCs with heat-killed Ty21a bacteria did not result in any significant increases in IFN-γ production (data not shown). Low levels of IFN-γ, IL-4, IL-10, and transforming growth factor β were detected in some of the ileostomy fluids obtained from the patients before immunization, but there were no appreciable changes in cytokine content seen after administration of Ty21a vaccine.

**B-cell immune responses.** In accordance with the T-cell immune responses seen in peripheral blood after vaccination, the antibody responses in serum were also weaker in the patients than in the healthy volunteers (Table 2). Thus, increases in Salmonella-specific IgA and/or IgG antibody titers were observed for five (56%) of nine patients and for 15 (88%) of 17 healthy volunteers. The magnitudes of the Salmonella IgA as well as the IgG antibody responses were significantly lower for the patients than for the healthy volunteers (Table 2).

To evaluate the ability of the Ty21a vaccine to induce a mucosal B-cell response in the intestines of patients who had undergone colectomies, vaccine-specific IgA antibodies in ileostomy fluids were also measured. Three doses of vaccine induced significant increases in the ratio of Salmonella-specific IgA antibody titers to total IgA concentrations in six (67%) of nine patients, and the maximal geometric mean increase for responders was 12-fold. Peak intestinal IgA antibacterial responses were seen on days 21 to 35 after the onset of vaccination for five of the patients and on day 8 for one patient (Table 3).
undergone colectomies and who are receiving the oral Ty21a vaccine as immunoprophylaxis against typhoid fever before traveling to areas of endemicity.

The pathogenesis of *S. enterica* serovar Typhi is characterized by mucosal invasion and systemic spreading. For protection against serovar Typhi infection, both mucosal and serum antibodies as well as T-cell responses are considered to be important (20). In the present study, strong local IgA responses were observed in ileostomy fluids from 67% of the patients given the oral Ty21a vaccine. Similar frequencies of *Salmonella* IgA antibody responses in the jejunal fluids of healthy volunteers after oral immunization with the same and other derivatives of Ty21a vaccine have been reported (6, 27). In contrast to the comparable immune responses induced by Ty21a vaccine in the intestines of healthy subjects and those who have undergone colectomies, the vaccine did not seem to be as effective in inducing serum antibody responses in patients as in healthy volunteers. Increases in *Salmonella*-specific IgA and/or IgG antibody titers were seen less frequently in patients, and the magnitudes of the responses were significantly lower than those observed for healthy volunteers after vaccination. Interestingly, a similar response pattern with strong vaccine-specific IgA antibody titer rises in ileostomy fluids and more-modest antibody responses in serum were found when an oral inactivated recombinant B subunit whole-cell cholera vaccine was given to patients who had undergone colectomies (18). A deficiency in the uptake of vaccine components by cells presenting them to the systemic immune system may explain the weak serum responses to enteric vaccines in patients who have undergone colectomies.

Although the relative importance of T-cell responses in typhoid fever has been emphasized for several years, only a few studies have characterized the responses in humans after oral typhoid vaccination, and the studies performed have evaluated the responses in healthy volunteers only (24, 26, 28, 29). In the present study, no proliferative responses were observed among circulating T cells from patients who had undergone colectomies, and vaccine-induced IFN-γ production was found only for CD8+ cells from three of the patients on day 7 or 8 after immunization with the Ty21a vaccine. In contrast, a proliferative response and/or increased IFN-γ production was observed for CD4+ cells in 50% of the healthy volunteers and for CD8+ cells in 80% of the healthy volunteers. The Ty21a vaccine-induced T-cell responses observed in our study were comparable to results obtained in other studies using the Ty21a vaccine (30) or other oral live attenuated serovar Typhi vaccine strains (26). Separation of the T cells into CD4+ and CD8+ cell subsets, obtained by using immunomagnetic cell sorting techniques before assessing immune responses, was recently described for healthy volunteers given the Ty21a vaccine (22, 24). In the study by Lundin et al. (22), responses were seen among both circulating CD4+ and CD8+ cells, although the CD8+ cells produced the highest amounts of IFN-γ, and peak T-cell responses were reported on days 7 to 14 after the onset of vaccination. Salerno-Goncalves et al. (24) showed that CD8+ T cells isolated from Ty21a vaccinees are not only able to lyse serovar Typhi-infected blasts but are also potent producers of IFN-γ. A significant association between the frequency of IFN-γ-secreting cells and cytotoxic T-lymphocyte activity was also demonstrated.

According to data from humans challenged with live *S. enterica* serovar Typhi bacteria, the bacterial ileum and colon are involved in the early phases of typhoid fever (9). Moreover, recently published studies of the intestinal histopathology in humans with complications of typhoid fever requiring surgery have shown that bowel perforations were identified exclusively in the last 60 cm of the ileum (5), and in cases with bleeding and intussusception only the terminal ileum and colon were involved (19). All our patients had undergone colectomies and continence surgery with resection of approximately 10 cm of

![TABLE 2](http://cvi.asm.org/) Specific IgA and IgG responses to *Salmonella* O9 and O12 antigens after three oral doses of serovar Typhi Ty21a vaccine

<table>
<thead>
<tr>
<th>Immunization group</th>
<th>Preimmune titera</th>
<th>Maximal postimmune titerb</th>
<th>Fold increasec (responding subjects)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colectomized patients</td>
<td>5/9 (56)</td>
<td>91 (51–160)</td>
<td>1.6 (2.2)</td>
</tr>
<tr>
<td>Healthy volunteers</td>
<td>14/17 (82)</td>
<td>230 (100–500)</td>
<td>5.0 (6.6)</td>
</tr>
</tbody>
</table>

a Frequency of responders in the two groups was compared with Fisher’s exact test (two-sided). The differences were not significant.
b Geometric mean titer (95% confidence interval) before immunization for the whole group of subjects.
c Geometric mean maximal titer (95% confidence interval) obtained on day 7 or 8 or 14 after the first immunization for the whole group of subjects.
d Differences in the magnitudes of immune responses between patients and healthy volunteers were evaluated by Student’s t test (unpaired, two-tailed). P = 0.012 for the IgA response and 0.029 for the IgG response.

![TABLE 3](http://cvi.asm.org/) Specific IgA antibody responses to *Salmonella* O9 and O12 antigens in ileostomy fluid samples from patients before immunization and after three doses of an oral attenuated *S. enterica* serovar Typhi Ty21a vaccine

<table>
<thead>
<tr>
<th>Days post-immunization</th>
<th>Anti-Salmonella IgA titer/total IgA concn (U µg-1)b</th>
<th>Frequency (no. of patients)c</th>
<th>Fold increase (responding subjects)d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.39 (0.15–1.0)</td>
<td>3/9</td>
<td>2.3 (15)</td>
</tr>
<tr>
<td>7 or 8</td>
<td>0.91 (0.49–1.7)</td>
<td>6/8</td>
<td>3.5 (6.9)</td>
</tr>
<tr>
<td>21</td>
<td>1.2 (0.47–3.2)</td>
<td>6/8</td>
<td>3.8 (7.1)</td>
</tr>
<tr>
<td>35</td>
<td>1.5 (0.76–2.9)</td>
<td>6/9</td>
<td>3.8 (7.1)</td>
</tr>
</tbody>
</table>

a The vaccine was administered orally on days 0, 2, and 4.
b Geometric mean anti-Salmonella O-9, 12 IgA antibody titer/total IgA concentration (95% CI) for all subjects. The values have been multiplied by 10.
c Responders were defined as having a greater-than-twofold increase in the ratio of specific IgA antibody titer to total IgA concentration between pre- and postimmunization specimens. One volunteer did not deliver specimens on day 21.
d Geometric mean increase in the ratio of anti-Salmonella O9 and O12 IgA antibody titer to total IgA concentration.
the distal ileum. The impaired T- and B-cell responses observed in the circulation of patients who have undergone colectomies suggest that the terminal ileum and colon may be of importance in the induction of systemic immune responses to the Ty21a vaccine. For these patients, the total gastrointestinal transit time is considerably shorter than for healthy individuals (1, 29) and the mucosal area, where colonization with Ty21a bacteria preferentially seems to occur, is reduced. Thus, it is likely that a possible diminished colonization of the Ty21a vaccine strain in patients who have undergone colectomies would result in a limited “spill-over” of antigen to the circulation and also in an impaired presentation of antigen to non-mucosal immune cells. The impaired T-cell responses found after vaccination may also be due to the fact that patients with ulcerative colitis have some form of deficiency in the antigen presentation. However, recently it was shown that peripheral blood dendritic cells from patients with ulcerative colitis rather had increased stimulatory capacities compared to such cells from healthy controls (10). It has also been shown that CD8+ peripheral blood cells from patients with inactive ulcerative colitis and an intact colon have an increased capacity for IFN-γ production when cocultured with intestinal epithelial cells (4). Furthermore, the prevaccination levels of IFN-γ production in the patients participating in the present study did not differ from those observed for a group of healthy volunteers.

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