Successful Colonization and Immunization of Adult Rabbits by Oral Inoculation with \textit{Vibrio cholerae} O1

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Adult rabbits were inoculated orally (or duodenally) with virulent \textit{Vibrio cholerae} O1. Jejunal colonization occurred only when hypoperistalsis was induced at the time of inoculation by tincture of opium given intraperitoneally (or by temporary ileal obstruction). For oral inoculation, prior neutralization of gastric acid was also required. Inoculation with $10^{9}$ \textit{V. cholerae} caused jejunal colonization for 1 to 2 days and ileal colonization for 5 to 6 days. The extent of small bowel colonization 18 h after oral inoculation was related to inoculum size but also reflected limited multiplication of small inoculum sizes and net death, clearance of large inoculum sizes, or both. Serious diarrhea occurred only in rabbits fed large inoculum sizes, i.e., $10^{10}$ \textit{V. cholerae}, and then rarely. Rabbits colonized once with $10^{10}$ \textit{V. cholerae} became highly resistant to recolonization with either the same or opposite serotype. After 18 weeks, these rabbits were still partially protected, whereas twice-colonized rabbits were highly protected. Protection against recolonization appeared to be due, at least partly, to interference with the adherence of \textit{V. cholerae} to the bowel mucosa, thus allowing rapid removal of \textit{V. cholerae} when peristalsis resumed. Prior colonization also protected against cholera-like diarrhea in rabbits challenged by the removable intestinal tie-adult rabbit diarrhea technique, the 50\% effective dose for severe or lethal diarrhea being increased more than 100-fold, and probably more than 10,000-fold, for challenge with either the homologous or heterologous serotype of \textit{V. cholerae}. The described rabbit model appears well suited for the study of immunity evoked by enteric colonization with \textit{V. cholerae} O1.

Intestinal colonization of humans with virulent \textit{V. cholerae} O1 stimulates substantial, lasting immunity against reinfection (2, 9, 12). Such protection is probably due to secretory immunoglobulin A (IgA) antibodies produced in the intestinal lamina propria and directed against various antigens of the organism or its secreted antigenic products. These observations are the basis for current attempts to develop an effective oral vaccine for cholera by using antigenic products (16, 22) or live avirulent mutants (1, 10) of \textit{V. cholerae}.

This effort would be aided by an animal model in which enteric colonization with \textit{V. cholerae} was possible, a vigorous mucosal immune response occurred, and resistance to subsequent reinfection could be studied; such a model would be especially useful for the study of candidate live oral vaccines. Until now, however, no such model has been described, the major difficulty being that most adult animals resist intestinal colonization with \textit{V. cholerae}. Although infants of several species are susceptible to intestinal colonization, and may develop cholera-like diarrhea, this is only true for the first few days or weeks of life (3, 23). Under these conditions, longitudinal studies of actively induced intestinal immunity, which may require several weeks or months between immunization and assessment of protection, have not been possible.

This report describes studies on colonization and immunization of adult rabbits with virulent \textit{V. cholerae} O1. Colonization was aided by producing transient intestinal hypoperistalsis at the time of oral or intestinal inoculation. This approach was suggested by previous reports that the susceptibility of guinea pigs to experimental shigellosis, and that of rabbits to experimental cholera, is enhanced by procedures that diminish intestinal motility (6, 21). For oral inoculation it was also necessary to carefully neutralize gastric acid before inoculation. With this approach, enteric colonization with \textit{V. cholerae} was achieved, and it was shown that a single transient colonization evoked marked protection against attempted recolonization or experimental cholera.
MATERIALS AND METHODS

Rabbits. Locally supplied male New Zealand white rabbits were used. These weighed 2 to 2.5 kg (age, 9 to 11 weeks) when studies were begun, unless otherwise indicated.

V. cholerae inocula. Two strains of V. cholerae O1 were used, Ogawa 395 and Inaba B36237. Both are of the classic biotype and are toxigenic and virulent (16). Stock cultures were stored at -70°C in 10% skim milk or brain heart infusion broth with 15% glycerol. To prepare each inoculum, a sample was thawed, inoculated into 1% peptone water (pH 7.4), and cultured overnight at 37°C. A 50-ml Erlenmeyer flask containing 10 ml of Casamino yeast extract medium (4) was inoculated with 0.05 ml of the overnight culture and shaken at 180 cycles per min for 4 h in a 37°C water bath. Bacteria were then centrifuged and suspended in 0.01 M phosphate-buffered saline, pH 7.4. Viable bacterial counts were determined on 3% gelatin agar (19) by the drop-plate method (11). Inocula were prepared by diluting the bacterial suspension in fresh Casamino yeast extract medium.

Rabbit inoculation for enteric colonization. Rabbits were fasted overnight before inoculation. In the first study, the inoculum was given intraduodenally through a small laparotomy. Rabbits were sedated with 0.05 ml of Inoveran given intramuscularly (Pitman-Moore, Inc., Washington Crossing, N.J.) and restrained; the incision site was anesthetized with 2% lidocaine. The 10-ml inoculum was injected directly into the duodenum through a no. 25 needle, the ileum having first been obstructed with a no. 11 umbilical tape tie in a slip knot about 5 cm from the mesoappendix. Ends of the slip knot were brought out through the incision, which was then closed. After 2 h, the ileal tie was gently removed, and the remaining skin opening was sutured.

In all subsequent studies, the inoculum was given intragastrically, usually after neutralization of gastric acid. The latter procedure involved 50 mg of cimetidine per kg, given intravenously at time 0, and 15 ml of NaHCO3 (5 g/100 ml of H2O), administered by gastric tube at 15 and 30 min. Preliminary studies showed that this fully neutralizes gastric acidity, i.e., pH >6.5 for at least 30 min after the second dose of NaHCO3. The V. cholerae inoculum in 15 ml of Casamino yeast extract medium was given by gastric tube immediately after the second dose of NaHCO3. At 30 min, 2 ml of tincture of opium, containing 10 mg of morphine, was given intraperitonially (i.p.). In some studies (see below), the tincture of opium, the gastric neutralization procedure, or both were omitted.

Quantitation of intestinal colonization. At the indicated interval after inoculation, rabbits were killed with intravenous pentobarbital. The abdomen was opened, and the distal ileum was located. Beginning 10 cm proximal to the mesoappendix and moving cephalad, a 10-cm segment of ileum was isolated by ties. In similar fashion, a 10-cm segment of jejunum was isolated, beginning 10 cm distal to the ligament of Treitz and moving caudal. Each segment was removed, measured, and any fluid content collected in a sterile glass cylinder. The segment was then opened and washed by dipping and rotating it 10 times in 20 ml of sterile phosphate-buffered saline. After excess fluid was drained away, the segment was weighed and homogenized in a Potter-Elvehjem homogenizer containing 5 ml of sterile phosphate-buffered saline using a teflon-coated pestle. The homogenate and the wash fluid, including any fluid in the segment, were then each cultured quantitatively for V. cholerae. Bacteria recovered from washed intestine were considered adherent, and those in the intestinal washing were considered nonadherent. Rectal swab cultures were also obtained, plated directly onto thiosulfate-citrate-bile salts-sucrose agar, and incubated overnight at 37°C. V. cholerae were identified by typical colonial appearance.

In one study, the number of V. cholerae in the entire small bowel was determined. Ties were placed immediately below the pylorus and just proximal to the ileocecal valve. The entire small bowel was removed, divided into several segments, and homogenized (with its contents) as above. The homogenates were then pooled, and quantitative cultures for V. cholerae were performed.

Rabbits challenged by the RITARD technique. In some studies, rabbits were challenged intraduodenally with varying inocula of V. cholerae Ogawa 395 by the removable intestinal tie-adult rabbit diarrhea (RITARD) technique as described by Spira et al. (21). Briefly, this involved permanent obstruction of the cecal orifice with an umbilical tape tie, intraduodenal inoculation with viable V. cholerae, and simultaneous ileal obstruction for 2 h with a removable tie as described above. After inoculation, rabbits were caged separately over a germicidal liquid bedding, given food and water freely, and observed for diarrhea twice daily for 5 days. When previously colonized rabbits were challenged, age-matched controls not previously colonized were given the same inoculum. Results were recorded as: no diarrhea, mild diarrhea (minimal perirectal fecal staining), severe diarrhea (marked perirectal fecal staining), and lethal diarrhea. Rectal swabs for culture of V. cholerae were taken on days 1 and 3 after inoculation.

RESULTS

Intestinal colonization with V. cholerae. (i) Duration of colonization after intraduodenal inoculation. Intraduodenal inoculation with 109 V. cholerae Ogawa 395, combined with temporary ileal obstruction, caused jejunal colonization for 1 to 2 days and ileal colonization for 5 to 6 days (Fig. 1). Bacterial counts on washed, homogenized intestines were greatest on day 1 (nearly 109 and 107 per g of jejunum and ileum, respectively) and then declined steadily. No rabbits developed diarrhea.

(ii) Development of an effective oral inoculation technique. The preceding study showed that V. cholerae can colonize rabbit intestines when transient hypoperistalsis is induced by ileal obstruction at the time of duodenal inoculation. Further studies determined whether similar colonization could be achieved without surgical manipulation, the inoculum being given intragastrically. Specifically evaluated were the importance of careful neutralization of gastric acid prior to inoculation, and the induction of tempo-
rinary hypoperistalsis by i.p. tincture of opium, rather than a temporary ileal obstruction. At 18 h after gastric inoculation of $10^{10}$ V. cholerae Ogawa 395, comparable colonization of the jejunum and the ileum was achieved, provided that gastric acid was fully neutralized before inoculation and temporary hypoperistalsis was produced either by a 2 h ileal obstruction or i.p. opium (Table 1). Omission of the ileal tie or opium caused colonization levels that were 2.4 to 3.4 logs lower; when gastric neutralization was also omitted, virtually no colonization occurred. Because of its simplicity, all subsequent colonization studies used the technique of intragastric inoculation preceded by gastric neutralization and followed by i.p. opium.

(iii) Colonization achieved by various gastric inocula of V. cholerae. Figure 2 shows that gastric inocula of $10^{8}$ or greater caused jejunal and ileal colonization after 18 h equal to that seen in the previously studied, surgically manipulated rabbits (Fig. 1). Lower inocula caused proportionately lower levels of colonization. The dose-response curve, however, had a slope of less than 1, i.e., the colonization achieved per log of inoculum was greater with small inocula than with large ones. Similar results were obtained using either V. cholerae Ogawa 395 or Inaba B36237. As in previous studies, bacterial counts were consistently greater in the ileum than the jejunum. After inoculation, diarrhea occurred only in rabbits given inocula of $10^{10}$ bacteria. Of these animals, 2.4% died with evidence of severe intestinal fluid secretion; among the remainder, however, diarrhea was rare.

In additional studies, groups of three rabbits

![FIG. 1. Time course of jejunal and ileal colonization after intraduodenal inoculation of V. cholerae Ogawa 395. Fasting rabbits were inoculated intraduodenally with $10^8$ viable V. cholerae Ogawa 395, and the terminal ileum was obstructed for 2 h with a tie. At the indicated intervals rabbits were killed and washed segments of jejunum and ileum cultured quantitatively for V. cholerae. Each point represents data from a single rabbit.](http://iai.asm.org/download/.../737)

![FIG. 2. Dose-response curve for intestinal colonization with V. cholerae. Rabbits were inoculated orally with the indicated inoculum and strain of V. cholerae. Results shown are mean V. cholerae per gram of washed intestine taken 18 h after inoculation. Each point contains data from 3 to 9 rabbits.](http://iai.asm.org/download/.../737)

### Table 1. Effect of various inoculation techniques on intestinal colonization with V. cholerae Ogawa 395

<table>
<thead>
<tr>
<th>Inoculation route</th>
<th>Inoculum</th>
<th>Gastric acid neutralized$^a$</th>
<th>Ileal tie$^b$ or i.p. opium$^c$</th>
<th>V. cholerae colonization$^d$ in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Jejunal homogenate</td>
<td>Ileal homogenate</td>
</tr>
<tr>
<td>Duodenum</td>
<td>$10^9$</td>
<td>-</td>
<td>Tie</td>
<td>4.8 (0.5)</td>
</tr>
<tr>
<td>Gastric</td>
<td>$10^{10}$</td>
<td>No</td>
<td>Tie</td>
<td>3.8 (0.5)</td>
</tr>
<tr>
<td>Gastric</td>
<td>$10^{10}$</td>
<td>Yes</td>
<td>Tie</td>
<td>4.5 (1.1)</td>
</tr>
<tr>
<td>Gastric</td>
<td>$10^{10}$</td>
<td>Yes</td>
<td>Ileum</td>
<td>None</td>
</tr>
</tbody>
</table>

$^a$ See text for gastric neutralization technique; —, not applicable.

$^b$ Ileum obstructed for 2 h after inoculation.

$^c$ Tincture of opium (2 ml) i.p. 30 min after intragastric inoculation.

$^d$ Mean $\log_{10} V. cholerae (\pm SE)$ recovered per gram of washed, homogenized intestine 18 h after reinoculation. None indicates mean $\log_{10} \leq 1.2$. $n = 3$ to 6 rabbits for each mean.
were fed either a low dose ($4 \times 10^3$) or high dose ($2 \times 10^{10}$) of \textit{V. cholerae} Ogawa 395. The entire small bowel was removed after 18 h and its total content of \textit{V. cholerae} determined. From rabbits given the smaller inoculum, 48-fold more \textit{V. cholerae} were recovered than originally fed, whereas only 1.7% of the bacteria given were recovered from rabbits fed the larger inoculum. Rectal swabs taken 18 h after inoculation were positive in all 3 rabbits given the large inoculum, but in none given the smaller inoculum.

Protection against recolonization or diarrhea in previously colonized rabbits. (i) Resistance to homologous and heterologous recolonization. Rabbits were colonized once with $10^{10}$ \textit{V. cholerae} Ogawa 395 or Inaba B36237. After 3 to 18 weeks, these rabbits and age-matched controls not previously colonized were inoculated with $10^{10}$ \textit{V. cholerae} Ogawa 395. Rabbits colonized once were almost completely resistant to homologous recolonization for 8 weeks, and were still partially resistant after 18 weeks; when colonized twice with a 3-week interval, nearly complete resistance to homologous recolonization lasted more than 18 weeks (Table 2). Complete resistance to recolonization with the heterologous serotype of \textit{V. cholerae} was also demonstrated 3 weeks after primary colonization. The number of viable \textit{V. cholerae} recovered from the intestinal wash and from washed homogenized intestine were nearly identical under all conditions studied (Table 2).

(ii) Time course of intestinal colonization and recolonization. Figure 3 shows the time course of jejunal and ileal colonization, measured for washed homogenized intestine, after the first or second gastric inoculation with $10^{10}$ \textit{V. cholerae} Ogawa 395. The first inoculation caused ileal colonization that was relatively constant, between $10^6$ and $10^8$ \textit{V. cholerae} per g of washed intestine, after 2.5, 5, 7.5, and 18 h. Jejunal colonization was $10^6$ to $10^7$ \textit{V. cholerae} per g between 2.5 and 7.5 h, and declined to $10^5$ per g at 18 h. In contrast, the time course of enteric colonization, when rabbits were reinoculated after a 3-week interval, differed markedly. Bacterial counts for homogenates of washed jejunal and ileal wash fluids showed resistance to homologous and heterologous recolonization. Rabbits were orally inoculated with $10^{10}$ \textit{V. cholerae} Ogawa 395 (first colonization). In some rabbits this procedure was repeated after 19 days (second colonization). At the indicated intervals after either the first or second colonization, rabbits were killed, and the number of vibrios on washed segments of jejunal and ileum was determined. Each point represents mean values from 3 to 6 rabbits. Results of quantitative cultures of intestinal wash specimens were virtually identical with those from washed homogenized bowel (data not shown).
TABLE 3. Effect of repeated doses of i.p. opium on intestinal bacterial counts 7.5 h after second inoculation with V. cholerae Ogawa 395

<table>
<thead>
<tr>
<th>Colonization*</th>
<th>Time of i.p. opium (min)</th>
<th>V. cholerae on washed intestinec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Jejunum</td>
</tr>
<tr>
<td>First</td>
<td>30</td>
<td>6.0 (0.7)</td>
</tr>
<tr>
<td>Second</td>
<td>30</td>
<td>≈ 1.1</td>
</tr>
<tr>
<td>Second</td>
<td>30, 210</td>
<td>4.0 (0.7)</td>
</tr>
</tbody>
</table>

a Each colonization was with 1010 V. cholerae Ogawa 395. The second was 19 days after the first.
b Time of i.p. opium dose(s) after intragastric inoculation.
c Mean log10 V. cholerae (±SE) recovered per gram of washed, homogenized intestine. n = 4 to 7 rabbits for each mean.

d Significantly greater than in recolonized rabbits given only a single i.p. dose of opium (P < 0.02 by Student’s t test).

Colonization in jejunum and ileum were constant for the first 5 h, but were 1 to 2 logs lower than after primary colonization; thereafter, however, V. cholerae numbers declined sharply, becoming nearly undetectable in the jejunum by 7.5 h, and being 3 and 5 logs lower in the ileum, after 7.5 and 18 h, respectively. The sharp decline in V. cholerae counts in the jejunum at 7.5 h, was significantly diminished, however, when rabbits were given a second i.p. dose of tincture of opium 210 min after inoculation (Table 3). In all of the preceding studies, bacterial counts in jejunal and ileal washings were essentially identical to those in homogenates of washed intestine (data not shown).

(iii) Protection against experimental cholera in previously colonized rabbits. The effect of previous colonization with 1010 V. cholerae Ogawa 395 on the outcome of homologous challenge 3 weeks later by the RITARD technique is summarized in Table 4. In nonimmune control rabbits, the ED50 for severe or lethal diarrhea was 107 V. cholerae, and 1011 bacteria caused severe disease in 93% of animals. In contrast, previously colonized rabbits were highly protected against challenge with V. cholerae of either the homologous (108) or heterologous (1011) serotypes. Additionally, postchallenge rectal swabs were positive for V. cholerae in only 29 to 38% of previously colonized rabbits, but were positive in 83 to 92% of similarly challenged controls.

DISCUSSION

This study shows that adult rabbits can be enterically colonized with virulent V. cholerae O1 provided that intestinal peristalsis is temporarily slowed so that the bacteria can adhere to small bowel mucosa rather than be swept downstream to the colon. This effect was achieved equally by tincture of opium given i.p. or by temporary ileal obstruction. Both procedures diminish intestinal peristalsis for several hours and have been previously used to experimentally promote gut colonization with enteric pathogens (6, 20, 21); the former has the practical advantage of being nonsurgical. When V. cholerae cells were given orally, it was also essential to neutralize gastric acid before inoculation; otherwise, the bacteria, which die quickly at pH values of less than 5.0 (14), do not survive transit of the stomach. This observation agrees with studies of volunteers, in whom the ED50 for severe diarrhea was reduced 4 logs by neutralizing stomach acid before oral inoculation with V. cholerae (13).

Although enteric colonization occurred, and lasted 5 to 6 days in the ileum, the process was

TABLE 4. Protection against experimental cholera by prior gut colonization with virulent V. cholerae

<table>
<thead>
<tr>
<th>Primary colonization</th>
<th>No. with severe or lethal diarrhea/no. challenged (%) after challenge with indicated inoculum of V. cholerae Ogawa 395a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^6</td>
</tr>
<tr>
<td>None (controls)</td>
<td>4/12 (33)</td>
</tr>
<tr>
<td>10^10 V. cholerae</td>
<td>NTb</td>
</tr>
<tr>
<td>Ogawa 395</td>
<td></td>
</tr>
<tr>
<td>10^10 V. cholerae</td>
<td>NT</td>
</tr>
<tr>
<td>Inaba B36237</td>
<td></td>
</tr>
</tbody>
</table>

a Challenge by RITARD technique 19 days after primary colonization.
b NT, not tested.
c One rabbit developed nonlethal diarrhea 5 days after challenge; culture of its diarrheal stool was negative for V. cholerae. P = 0.006, compared with controls. Rectal swab cultures were positive for V. cholerae, on either day 1 or 3 postchallenge, in 83% of controls and 38% of previously colonized rabbits, 0.10 > P > 0.05 by Fischer’s exact test.
d No diarrhea occurred during the 5 days after challenge. P < 0.001, compared with controls. Rectal swab cultures were positive for V. cholerae, on either day 1 or 3 postchallenge, in 92% of controls and 29% of previously colonized rabbits, P = 0.014.
relatively inefficient; the bacteria did not multiply extensively, and diarrhea did not usually develop. The failure of a small inoculum (e.g., $10^3$ bacteria) to multiply extensively in vivo, and the actual decline in total bacterial counts 18 h after a large inoculation ($10^{10}$ bacteria), show that high-level colonization could not be sustained and probably explain the failure of diarrhea to develop, except rarely. These results differ from those in adult rabbits challenged by the RITARD technique; with that method adherent bacteria rapidly multiply to levels of $10^8$ per g of intestine, and severe diarrhea often follows (21). The greater multiplication of V. cholerae in the RITARD model probably reflects greater alterations in intestinal physiology due to the surgical modifications employed (i.e., temporary ileal obstruction and cecal ligation). These results also contrast to those in susceptible humans, in whom a small inoculum (e.g., $10^4$ bacteria) usually multiply until diarrhea occurs (13). The reasons for species differences in the efficiency of mucosal colonization by V. cholerae are not known, but are likely to become clearer when its mucosal adherence mechanisms are better understood.

Rabbits inoculated once with $10^{10}$ virulent V. cholerae were markedly resistant to recolonization with the same strain for 8 weeks, and partially resistant for at least 18 weeks. Although specific antibody responses in serum or gut secretions were not measured, it is likely that protection against this noninvasive organism was mediated by intestinal secretory immunoglobulin A antibodies stimulated by the initial colonization. The higher level of protection after 18 weeks in twice-colonized rabbits suggests that the second colonization evoked antibody responses that were greater, longer lasting, or of higher specific affinity (or some combination of these) than occurred after the first colonization. Evidence that rabbits colonized once were also highly resistant to recolonization with the heterologous serotype of V. cholerae indicates a significant protective role for antibodies directed against one or more antigens shared by both serotypes of V. cholerae O1. These antigens are bacterial surface antigens (e.g., the group-specific portion of capsular lipopolysaccharides, flagellar antigens, and outer membrane proteins) and secreted antigens that possibly aid the colonization process (e.g., protease, certain hemagglutinins (5), and cholera toxin). Several of these antigens have already been shown to stimulate protective immune responses in experimental animals (16, 17, 22, 24).

One evidence of resistance to recolonization was the abrupt decline in small bowel bacterial counts between 5 and 7.5 h after reinoculation. This could have at least two explanations. First, preexisting secretory antibodies might prevent mucosal colonization by binding to superficial colonization factor antigens (5), or by immobilizing or agglutinating bacteria so that they could not migrate efficiently to the epithelial surface (18). In such a case, the viable inoculum would adhere poorly and might be easily swept away when normal peristalsis resumed. This possibility is supported by evidence that a second i.p. dose of opium, 3 h after the first, caused prolonged high-level colonization of the jejunum, and to a lesser extent the ileum. Apparently, the second dose of opium delayed the return of normal peristalsis and, thus, postponed bacterial clearance. However, attempts to show that V. cholerae were actually less adherent in immunized rabbits, by comparing the relative proportions of adherent (washed intestine) and nonadherent (intestinal washing) bacteria with those in nonimmune rabbits, were unsuccessful. A second possibility, not examined in this study, is that secretory antibodies had a bactericidal effect, directly inhibited bacterial multiplication, or both. That both mechanisms may have contributed to the observed protection is not excluded.

In most aspects of this study, colonization-induced immunity was measured by resistance to recolonization. It was also shown, however, that prior colonization with $10^{10}$ V. cholerae protected rabbits against experimental cholera when challenged by the RITARD technique. Although such protection may have been due partly to antitoxic immunity, its demonstration accords with the view that immunization which protects against mucosal colonization will also protect against clinical cholera. The extent of this protection was not determined precisely, but was at least 100-fold, and probably in excess of 10,000-fold, as measured by the increased ED$_{50}$s for homologous and heterologous serotype challenges, respectively.

The ease with which immunity to recolonization and disease was induced by oral colonization with viable V. cholerae contrasts with prior studies on enteric immunization of rabbits with a nonliving antigen, cholera toxin. In those studies, 15 to 20 oral doses of antigen were required before antitoxic protection was demonstrable (7). Although several explanations of this apparent difference in immunizing efficiency are possible, two are of particular interest. First, it is possible that motile V. cholerae reach the epithelial surface more efficiently than does a soluble antigen and thus are more available for uptake by antigen sampling mechanisms of the mucosa. Second, immunity to recolonization may reflect highly efficient, synergistic protection by antibodies against several bacterial antigens; evidence for such synergistic protection
has been described in animals immunized with combined antigens of \textit{V. cholerae} (15, 16, 22).

The adult rabbit model described here appears well suited for studies of mucosal colonization with \textit{V. cholerae} and of the protective immune mechanisms which colonization evokes; these include comparative studies on the ability of candidate live avirulent \textit{V. cholerae} vaccine strains to colonize the small bowel and immunize against subsequent colonization by fully virulent strains. Among the advantages of this model are the several similarities between protection observed in previously colonized rabbits and that which follows infection in humans. Thus, volunteers convalescent from experimentally induced cholera also develop prolonged resistance to symptomatic reinfection (9), which includes resistance to recolonization of small bowel mucosa (8); volunteers challenged once resist reinfection with the homologous, as well as the homologous, serotype (8); and naturally acquired immunity to cholera among persons living in cholera-endemic areas increases with age, due to repeated accidental ingestions of viable \textit{V. cholerae} (12). Such similarities suggest that results obtained with this model will directly aid understanding of the pathogenesis and immunology of cholera in humans. Additional advantages are that surgical techniques are not required, colonization is easily achieved with very little risk of morbidity due to diarrhea, and the extent of colonization can be controlled by varying the inoculum size.

\section*{ACKNOWLEDGMENTS}

This work was supported by Public Health Service research grants AI-14480 and AI-18818 from the National Institute of Allergy and Infectious Diseases and by a grant from the Global Program for Control of Diarrheal Diseases of the World Health Organization. E.T. was supported by a grant from the Government of Japan. Research facilities were provided by the Gerontology Research Center, National Institute of Aging, under its Guest Scientist program.

\section*{LITERATURE CITED}


