Immunological Control Mechanism Against Cholera Toxin: 
Interference with Toxin Binding to Intestinal Receptors

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The immunological control mechanism against cholera toxin (CT) in the small intestine of rats was studied in vivo. CT binding to intestinal receptors was determined by injecting radiolabeled CT into the loops of rat small intestine and subsequently separating purified microvillus membranes from mucosal scrapings of those loops. Substantial radioactivity (10³ cpm/mg of microvillus protein) was present in microvillus fractions of small intestine exposed to [¹²⁵I]-labeled CT compared to radioactivity (10² cpm/mg) in fractions from intestine exposed to radiolabeled bovine serum albumin (BSA) used as a control. CT binding to intestinal receptors was significantly inhibited (P < 0.02) in rats immunized with crude toxin by a combined intraperitoneal and oral method compared to CT binding in animals immunized with BSA or controls, suggesting a specific relationship between intestinal antitoxin and inhibition of binding. Furthermore, ligated ileal loops from CT-immunized animals showed a significant decrease in fluid accumulation when exposed to CT compared to loops from control or BSA-immunized animals, suggesting that antitoxins also interfered with the biological action of CT under conditions of immunization. These studies provide direct evidence that intestinal antitoxins protect against CT-induced diarrhea by interfering with the attachment of the toxin to the intestinal microvillus surface.

Although experimental animal studies have shown that the diarrheal effect of cholera toxin (CT) can be inhibited by prior immunization with either toxin (2, 8) or toxoid (19-21), no known mechanism for antitoxin control of CT activity within the small intestine has been reported. Purified CT can now be separated into two subunits, the activating component (A) and the binding component (B), each of which is necessary for the biological effect on intact intestinal cells (5, 6, 15). Presumably, the B component binds with specific receptors on the intestinal microvillus surface (5, 11, 26) which allows the A component to activate membrane-associated adenylate cyclase (13, 23), thereby increasing cellular cyclic adenosine 3',5'-monophosphate (13, 22) and causing hypersecretion of fluid and electrolytes into the intestinal lumen (1, 4, 22, 23). Since the biological effect of CT depends on initial binding to the intestinal surface, one possible mechanism of immunological protection against cholera is intestinal antibody interference with CT attachment to intestinal receptor sites. Previous in vitro studies from this laboratory suggest that antitoxin may interfere with toxin binding to intestinal receptor sites (27). The present investigation was designed to further study the possible role of local antibodies on the attachment of CT to the microvillus membrane under more physiological in vivo conditions. This study provides direct evidence that antitoxin interferes with CT binding to the intestinal mucosal surface and thereby prevents excessive fluid secretion into the intestinal lumen.

MATERIALS AND METHODS

Preparation of labeled CT and BSA. Purified CT prepared by the method of Finkelstein and LoSpalluto (6) was either obtained from the SEATO Cholera Research Program (lot no. 0972, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) or purchased from Schwarz/Mann, Div. of Becton, Dickinson & Co., Orangeburg, N.Y. Purified bovine serum albumin (BSA; Nutritional Biochemicals Corp., Cleveland, Ohio) was used as a control protein for binding studies and as a control immunogen for immunization studies. CT and BSA were labeled with Na¹²⁵I (New England Nuclear Corp., Boston, Mass.) using a modification of the method of Greenwood et al. (10) as previously reported (27). Random Na¹²⁵I-CT preparations were compared with equivalent quantities of unlabeled CT for biological activity using ligated ileal loops in rats. No appreciable loss (less than 5%) in biological ac-
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munogenicity was reported previously. A partially purified preparation of CT (provided by SEATO cholera Research Program, National Institute of Allergy and Infectious Diseases) was emulsified with incomplete Freund adjuvant (Difco Laboratories, Detroit, Mich.) and given intraperitoneally (i.p.; 0.5 ml of a 4-mg portion of CT per ml) at 10-day intervals. Animals were injected six times and subsequently orally boosted by administration of 100 mg of crude toxin in 1 ml of saline (0.13 M NaCl) and NaHCO₃ (1.3%, wt/vol) by gastric gavage according to the method of Pierce et al. (19). Animals were studied between 8 and 12 days after the oral boost. An additional group of rats injected i.p. with BSA (0.5 ml of a 2-mg/ml portion emulsified with incomplete Freund adjuvant) and boosted orally (100 mg of BSA in saline and NaHCO₃) according to the same immunization schedule that was used as immunized controls. At the time of the study, the peritoneal cavity of all animals was examined. There was no evidence of peritonitis or adhesions and the intestine was not visibly inflamed.

Quantification of antibody activity and immunofluorescence studies. Serum samples, intestinal washings, and extracts of intestinal mucosal scrapings from CT-immunized rats were analyzed for antibody activity. Small intestinal washings and mucosal extracts were prepared for analysis of antibody activity according to a modification of the method of Walker et al. (28), and the antitoxin antibody levels were determined by radioimmunodiffusion; rabbit antiserum to rat immunoglobulin A (IgA), IgG, and IgGa used in this procedure was prepared as described (25) and kindly provided by Kurt Bloch (Clinical Immunology Unit, Massachusetts General Hospital). Precipitin titer were determined by radioimmunodiffusion techniques on serial dilutions of serum, portions of mucosal extracts, and luminal washings from CT-immunized rats. Radioimmunodiffusion was done as a two-step procedure. The first step included the interaction of rat serum with rabbit anti-rat gamma globulin to precipitate rat antibodies in agar; this step was followed by washing with phosphate-buffered saline and a second diffusion with 125I-labeled CT. The maximum titer for a test material was considered to be the greatest dilution to show a positive radioactive precipitin line.

In previous studies we have reported that radiolabeled antigen introduced into the intestinal cavity of immunized animals interferes with the measurement of antibody activity in secretions and mucosal extracts from these animals (29). Therefore, the intestinal antibody activity of immunized animals used in CT-binding studies could not be measured directly. Instead, serum, intestinal secretions, and mucosal extracts from animals immunized with CT but not actually used in binding experiments were analyzed and compared. A correlation between serum titers of antitoxin and antitoxin levels in mucosal extracts from these animals was noted. Based on this correlation, serum samples collected from CT-immunized animals used in toxin-binding studies were assumed to generally reflect the local intestinal antitoxin activity in these same animals.

Previous reports have suggested that a combined i.p. and oral CT immunization was more effective in producing secretory antibodies than i.p. immunization alone; accordingly, immunofluorescent techniques (9) to confirm this observation were done to identify the local plasma cell response to CT immunization under conditions of these experiments. Loops of small intestine from CT-immunized and control rats were distended with embedding medium (Ames Co., Elkhart, Ind.) to maintain villus position, frozen rapidly in liquid nitrogen, and then prepared as thin sections of intestinal rings. Rings were sequentially exposed to purified CT, rabbit antitoxin, and then fluorescein-conjugated goat anti-rabbit gamma globulin; all sections were washed with phosphate-buffered saline between exposures.

CT binding to intestinal microvillus membranes. CT binding to intestinal microvillus membrane was studied in vivo. Age- and weight-matched control, CT-immunized, and BSA-immunized animals were starved overnight and a laparotomy was performed under ether anesthesia. The small intestine was washed with 20 ml of warm (37°C) saline to clear residual contents and then ligated from proximal jejunum to the terminal ileum as an isolated loop. No apparent disruption of intestinal blood supply was noted in loops prepared by this surgical technique. Freshly dialyzed radiolabeled CT (20 μg of 131I-labeled CT in 10 ml of saline, 3 x 10⁶ cpm/μg) was injected into the loop, and the laparotomy incision was closed with surgical clips. After a 10-min exposure to the toxin, the animal was sacrificed, and the loop was removed from the animal, washed thoroughly with 10 ml of cold saline to free any loosely or nonspecifically adherent toxin, and scraped for microvillus membrane preparation using the modified method of Hopfer et al. (12). Three animals were used in each immunized and control group per experiment to obtain measurable amounts of microvillus membranes for further analysis.

The purity of membrane preparations was evaluated morphologically by phase-contrast microscopy and biochemically by the specific activity of sucrase (3). In general, the sucrase activity in the partially purified microvillus fraction increased 15- to 20-fold over that in the initial homogenate, and a 40- to 50-fold increase was achieved in the final membrane preparations. Membrane-protein concentration was determined by the method of Lowry et al. (16). An average of 1.5 mg of purified microvillus protein per group of animals in each experiment was prepared. The radioactivity in the purified membrane fraction was counted in a crystal scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) with a count-
ing efficiency of 58%, and the amount of 125I-labeled CT bound was calculated using the original specific activity of 125I-labeled CT injected.

The specificity of CT binding was investigated by exposing additional loops of small intestine in control animals to equivalent counts of 125I-labeled BSA and then measuring the amount of radioactivity recovered in the microvillus membrane fraction after separation. In other control experiments, microvillus membrane preparations were preexposed to unlabeled CT or BSA before adding experimental quantities of 125I-labeled CT to the preparation. Comparison of specific binding in all groups was always made in those animals tested on the same day as matched pairs to minimize the possible variation among different preparations of radiolabeled toxin and/or any variation in the complicated process involved in microvillus membrane preparation.

Characterization of CT in membrane preparations and intestinal secretions of control and CT-immunized animals. Microvillus membrane preparations from the small intestine of control and CT-immunized animals were analyzed by sucrose density gradient ultracentrifugation as previously described (27). After centrifugation, 2-drop fractions were collected from a puncture site at the bottom of the gradient in tubes containing 0.5 ml of saline; the radioactivity in each tube was determined. It was assumed that radioactivity in the membrane fraction of the gradient after ultracentrifugation would reflect CT bound to receptor sites on the membrane and not free 125I contamination or 125I-labeled CT loosely associated with the membrane preparation. The location of radioactivity on sucrose gradient fractions from 125I-labeled CT-exposed intestinal microvillus preparations was compared with radioactivity of gradient fractions from 14C-labeled membranes alone (prepared as previously described [27]) and 125I-labeled CT alone.

In addition experiments, the fluid rinsed from the small intestinal lumen of normal and immunized animals after exposure to 125I-labeled CT was concentrated by negative pressure dialysis and analyzed by density gradient ultracentrifugation. The gradient location of radioactivity in intestinal rinses from control, BSA-immunized, and CT-immunized animals was compared.

Ileal loops studies. To further study the protective role of local antibodies against the toxigenic effect of CT on the small intestine, the accumulation of fluid in ligated ileal loops from control and immunized animals was compared. Animals were restricted to a 5% glucose and water ad libitum intake for 24 h prior to study. Under ether anesthesia, the small intestine was exteriorized through a midline incision and cleaned thoroughly by infusion of warm saline (25 ml, 37°C) into the lumen to flush the contents out through a small antimesenteric incision made at the distal end of the ileum. Saline was introduced slowly to avoid necessary distention of intestinal wall and was finally emptied using a wet swab gently rolled the intestinal surface. Negligible amounts (less than 0.5 ml) of fluid remained in the intestinal lumen using three preparations. Two ileal loops, approximately 10 cm in length and 2 cm apart, beginning approximately 5 cm above the ileocecal valve and extending proximally were prepared. The intestinal incision site used for rinsing was not included in test loops, nor was there evidence of altered mesenteric blood supply to the isolated loops using this surgical technique. Varying doses of CT (0.5 to 2 μg) in 0.5 ml of saline or in 0.5 ml of saline alone were injected into either loop using an alternating sequence. The small intestine was then returned to the abdominal cavity, and the abdominal incision was closed with two layers of running suture using 4-0 silk. After an 8-h incubation period with ad libitum H2O intake the animals were sacrificed, and the fluid volume, length, and weight of loops were measured. The volume of fluid accumulated per unit of wet weight was expressed as an estimate of surface area to allow for comparison among experimental groups.

Statistical analysis on all experiments was done by paired t test or normal Student's t test.

RESULTS

Characterization of antitoxin antibodies. Antitoxin activity in serum samples from all animals (24 rats) and in the intestinal washings and mucosal extracts of representative animals (6 rats) that were immunized with CT but not used in binding experiments were analyzed for the specific class of antibody response and for precipitin titers. Both IgG1 and IgG2 but not IgA antibodies to CT were identified in serum samples tested from all immunized rats, including those used in binding studies. Intestinal secretions and mucosal extracts also showed levels of IgG1 and IgG2 antibodies. Approximately 80% of CT antibody activity in intestinal washings and extracts was IgG1 antibodies. Antibody titers in serum samples from CT-immunized rats ranged from 1:100 to 1:1,250, and antibody activity in mucosal extracts ranged from 1:8 to 1:32. Only undiluted samples of intestinal washings showed antibody activity. In previous studies (28) we have reported a direct correlation between serum antibody activity and intestinal antibody activity using the i.p. immunization technique; although for these studies in which use of a combined i.p. and oral immunization schedule a direct correlation between serum and intestinal antibody activity was not maintained, there was nonetheless an association between intestinal and serum antibody activity. Therefore, we assumed that the measurement of anti-CT titers in serum reflected the antibody activity in secretions and mucosal extracts of CT-immunized animals used in the binding studies.

To assess the relative contribution of plasma cells present in the lamina propria of the small intestine to the production of intestinal antitoxin antibodies, immunofluorescent studies

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were done on representative sections of small intestine from CT-immunized animals and control animals. Figure 1 shows a typical pattern in control CT-immunized (A) versus (B) animals. Large numbers of fluorescent-positive plasma cells were noted in the lamina propria of small intestinal sections from CT-immunized animals compared to negligible numbers of cells in control animals, suggesting that local plasma cells were producing antitoxin antibodies.

Binding and characterization of CT attachment to intestinal microvillus membranes. In experiments designed to quantitate specific binding of CT to intestinal microvillus membranes, equivalent amounts of 125I-labeled CT or 125I-labeled BSA were infused into small intestinal loops from nonimmunized rats. After a 10-min exposure, the loops were rinsed, the mucosal surface was scraped, and microvillus membranes were prepared. Radioactivity in purified microvillus membranes from animals (six experiments) exposed to 125I-labeled CT was extremely high, approximately 10^6 cpm was recovered in each milligram of microvillus protein (Fig. 2). In contrast, negligible amounts of radioactivity (less than 10^3 cpm/mg) remained with the microvillus fraction of animals (three experiments) exposed to 125I-labeled BSA (Fig. 2). Microvillus membranes from these animals were further analyzed by sucrose gradient ultracentrifugation to characterize the attachment of CT to specific receptors on the intestinal surface. When microvillus membranes from animals exposed to 125I-labeled CT were placed on the gradient, radioactivity appeared at the bottom of the gradient (Fig. 3B). This location was identical to the position of purified 14C-labeled microvillus membranes alone (Fig. 3A) on a gradient ultracentrifuged under the same conditions and was clearly different from the position of free 125I-labeled CT on the gradient (Fig. 3C). Furthermore, no radioactivity was apparent in the membrane fraction of gradients exposed to 125I-labeled BSA.

To examine the specificity of CT binding, the small intestine from additional animals (three experiments) was first incubated with unlabeled CT (50 μg) to saturate the membrane-binding sites before exposure to 125I-labeled CT. The amount of 125I-labeled CT bound to the membrane was negligible (less than 10^3 cpm/mg), suggesting that radiolabeled CT and not simply 125I counts had been bound previously to the microvillus membrane. Furthermore, when the intestine was preincubated with BSA before 125I-labeled CT exposure (three experiments), no appreciable difference in CT binding to microvillus membrane was noted compared to binding in experiments with exposure to 125I-labeled CT alone, suggesting that nonspecific adherence contributed minimally to CT binding in these experiments.

CT binding in immunized animals. The binding of 125I-labeled CT to microvillus membranes of rats immunized with either CT (six experiments) or with BSA (four experiments) was also studied. An average of 55% inhibition of binding was observed in CT-immunized rats compared to controls (P < 0.02) (Fig. 4A), whereas no significant difference was shown between control and BSA-immunized animals (Fig. 4B).

Furthermore, an association between antitoxin activity in serum from immunized rats and the degree of inhibition of CT binding to microvillus membranes isolated from immunized rats was noted. In immunized animals with high serum titers (greater than 1:1,000 titer, six animals) inhibition of binding was 50% or greater, whereas the immunized animals with low serum titer (less than 1:250 titer, four animals) had 25% or less inhibition of binding.

Characterization of CT in intestinal washings. In selected experiments, intestinal washings obtained from immunized and control animals exposed to 125I-labeled CT were concentrated and applied to a sucrose gradient. After ultracentrifugation, radioactivity was detected in fractions collected near the middle of the gradient (Fig. 5A) in control and BSA-immunized animals. In contrast, washings obtained from CT-immunized animals showed radioactivity near the bottom of the gradient (Fig. 5B).

Ileal loop studies. To further examine the biological significance of these observations, the effect of CT on intestinal secretions measured in ligated ileal loops was compared in control and immunized animals. Whereas the volume of fluid accumulated in all saline-injected control loops prepared from both groups was negligible (less than 0.1 ml/loop), the fluid secretion rate in CT-treated control loops was shown to be dose related (Table 1). The results also showed that prior immunization with CT was effective in protecting the intestinal against subsequent challenge with CT. Intestinal secretion rate was almost entirely suppressed in loops from immunized animals injected with 1 μg of CT (only three out of seven loops showed fluid accumulation) compared to loops of control animals (P < 0.001) and was maintained as a significant decrease in loops from immunized animals injected with 2 μg of CT compared to controls (P < 0.01).
FIG. 1. Small intestine from control CT-immunized (A) and (B) rats exposed to CT, rabbit anti-CT, and fluorescein-conjugated goat anti-rabbit gamma globulin. Large numbers of fluorescent-positive plasma cells appear in the lamina propria of (A) compared to negligible numbers of cells in (B).
FIG. 2. Radioactivity, defined as (counts per minute ± standard error of the mean, SEM) × 10^3)/milligram of protein, in the microvillus fraction from small intestine of rats exposed to ^125^-labeled CT and ^125^-labeled BSA. (107 ± 25) × 10^3 cpm/mg was present in CT-exposed animals (N = 6) compared to (0.7 ± 0.1) × 10^3 cpm/mg in BSA-exposed (N = 3) animals.

DISCUSSION

Several studies in experimental animals have reported that immunization with CT or toxoid can protect against subsequent orogastric challenge with purified, active toxin (2, 8, 19-21). Since the diarrhea associated with Vibrio cholerae does not involve the bacterial penetration of the intestinal mucosal barrier, the antitoxin protection against CT presumably occurs within the intestinal lumen or on the intestinal surface. Although the actual site and mechanism of antitoxin protection have not been elucidated, one likely possibility is an interference with CT binding to intestinal epithelial cells. Recent studies have shown that CT consists of two subunits which have specific functions with respect to the biological activity of the toxin (5, 6). Subunit B, an aggregate of polypeptides (10,000 daltons), binds rapidly and tightly to specific receptor sites on the intestinal epithelial cell surface, whereas subunit A, a fragment of 26,000 daltons, activates adenylate cyclase, resulting in elevated cellular cyclic adenosine 3',5'-monophosphate and hypersecretion of fluid and electrolytes into the intestinal lumen (1, 4, 22, 23). Both fragments of the toxin as a complete molecule are necessary for the biological activation of intact epithelial cells (31). Since the attachment of CT to the intestinal surface is required before hypersecretion occurs, antitoxins in intestinal secretions or on the intestinal surface may interact with the binding component (subunit B) and thereby

FIG. 3. Sucrose density gradient separation of CT and isolated microvillus membranes from rat small intestine. [1^4^C]Glucosamine activity, representing intestinal microvillus membranes, appeared in a distinct zone at the bottom of the gradient (A). ^125^-labeled CT, when exposed to the small intestine, appeared in the same zone of the gradient as the microvillus membrane fraction (B), whereas ^125^-labeled CT alone appeared only at the top of the gradient in a zone clearly separated from the membrane (C).
disrupt the toxin binding to intestinal receptors. In a previous publication, we have reported indirect evidence that antitoxin may inhibit CT binding to isolated microvillus membranes (27).

The present study provides direct evidence to support this hypothesis. Under physiological, in vivo conditions, radiolabeled CT was exposed to loops of small intestine, and the binding of toxin was characterized and quantitated by preparing purified microvillus membranes from mucosal scrapings of the intestinal surface. The specificity of CT binding to receptor sites was established in comparative experiments using radiolabeled BSA as a control protein. Negligible radioactive BSA was present in microvillus fractions of small intestine compared to that of radioactive CT. Since BSA is a glycoprotein of comparable size to CT and is known to adhere to but not bind to specific receptors on the intes-

![Graph](https://via.iast.org/)

**Fig. 4.** CT bound (picograms per microgram of microvillus protein) to intestinal microvillus membrane from normal, CT-immunized, and BSA-immunized rats. Significant inhibition of CT binding was found in CT-immunized (19.9 ± 2.8 pg of CT per μg of protein, N = 6) compared to normal (44.4 ± 8.5 pg of CT per μg of protein, N = 6) rats (A). However, no difference was observed in CT binding between BSA-immunized (34.8 ± 6.3 pg/μg of protein, n = 4) and normal (31.4 ± 6.3 pg/μg of protein, N = 4) rats (B).

![Graph](https://via.iast.org/)

**Fig. 5.** Sucrose density gradient localization of radioactivity in concentrated rinse fluid of 3H-labeled CT-exposed intestine from control and CT-immunized rats. In control rats (A) radioactivity is located near the top of the gradient; this position is the same as that of 3H-labeled CT alone. In contrast, radioactivity in rinse fluid of intestine from CT-immunized rats appears in the bottom (B) of the gradient, suggesting the formation of CT-anti CT complexes.

**Table 1.** Fluid secretion rate in CT-injected ileal loops of control and immunized rats

<table>
<thead>
<tr>
<th>Toxin dose (µg)</th>
<th>Group</th>
<th>Rats (no.)</th>
<th>Wt of ileal loops (g)</th>
<th>Fluid secretion rate (ml/g of tissue h)</th>
<th>P*</th>
</tr>
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<tbody>
<tr>
<td>0.5</td>
<td>A</td>
<td>4</td>
<td>1.09 ± 0.02</td>
<td>0.31 ± 0.10*</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>6</td>
<td>0.92 ± 0.07</td>
<td>0.78 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>7</td>
<td>1.07 ± 0.06</td>
<td>0.09 ± 0.04</td>
<td>&lt;0.001</td>
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* Group A, control animals; group B, animals immunized with CT (serum antitoxin titer > 1:250).
* Mean ± standard error of the mean.
* Significant difference between groups A and B.
CT binding in CT-immunized rats was noted to be associated with the level of serum antitoxin activity in these animals. Since antitoxin activity in mucosal extracts and washings studied in separate experiments was shown to be associated with the activity present in serum (28), the results suggested that interference with the attachment of CT to mucosal receptor sites most likely was related to the level of antitoxin antibody present in the small intestine. The association between serum and intestinal antitoxin, however, can only reflect relative levels of antibodies within the gut, since intestinal antitoxin activity is derived from local and systemic lymphoid tissue (18). When intestinal washings from CT-immunized animals exposed to 125I-labeled CT were analyzed by sucrose density ultracentrifugation and compared to washings from control animals, radioactivity appeared in fractions near the bottom of the gradient. In previous reports (29), intestinal washings from immunized animals exposed to 125I-labeled antigen were analyzed by immunoprecipitation and sucrose gradient techniques; these studies clearly showed that radioactivity in fractions from the bottom of the gradient were antigen-antibody complexes. We have, therefore, assumed that the larger molecular size of CT in washings from the intestinal lumen of CT-immunized animals represented CT complexed with anti-CT antibody on the intestinal surface. The biological significance of the binding inhibition was assessed in ileal loop experiments in which the effect of CT-immunization on fluid accumulation after CT exposure was compared to fluid accumulation in control animals. A significant inhibition of fluid accumulation was noted in CT-immunized rats compared to controls when varying doses of CT was used. Thus, the present study suggests that antitoxins present in intestinal secretions or on the mucosal surface combine with toxin to form complexes. Complex formation interferes with the attachment of toxin to specific receptor sites on the intestinal surface, thereby preventing hypersecretion of fluid into the small intestinal lumen.

The immunological control mechanism against CT reported in this study is consistent with other known secretory antibody functions against bacterial proliferation (7, 32) and antigen uptake within the gut (29, 30). Williams and Gibbons (32) have reported that salivary IgA antibodies against bacterial organisms can prevent these organisms from adhering to and proliferating on epithelial surfaces. Fubara and Freter (7) have also noted that intestinal antibodies can interfere with the adherence of V. cholerae to the intestinal epithelial surface. In previous studies from this laboratory, we have shown that local antibodies present on the intestinal surface can interfere with the pinocytosis of soluble antigens (29) and can facilitate proteolysis of these antigens by pancreatic enzymes (30). Therefore, it would appear that a primary function for intestinal antibodies may be the interference of antigen attachment to the intestinal mucosal surface.

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