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# Cholera Toxoid Boosts Serum *Escherichia coli* Antitoxin in Humans

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Sera from individuals vaccinated with a cholera toxoid were tested to determine if sera with significant titers of cholera antitoxin could neutralize *Escherichia coli* thermolabile enterotoxin. Individuals who responded to immunization with significant increases in serum cholera antitoxin titers also showed increases in serum *E. coli* enterotoxin-neutralizing capacity. These findings suggested the possibility that the cholera toxoid vaccine may be useful in preventing disease caused by *E. coli*.

Recent reports of pathophysiological similarities and cross-neutralization between cholera enterotoxin and thermolabile *Escherichia coli* strain LT delayed-onset enterotoxin suggest that the two toxins may be identical, or at least very similar, and may share antigenic determinants (2, 6-8). We therefore tested paired sera from volunteers immunized with cholera toxoid to determine if toxoid immunization caused an increase in serum antitoxic activity against the *Escherichia coli* LT delayed-onset enterotoxin. This enterotoxin is designated delayed onset because, as in the case of cholera toxin, there is a delay of several hours between exposure of intestinal mucosa to the toxin and the onset of toxin-induced intestinal fluid accumulation (6, 7).

## MATERIALS AND METHODS

**Volunteers.** A group of 276 citizens of Bangladesh who live in Matlab, an area where cholera and other diarrheal diseases are endemic, gave informed consent for the procedures described below.

**Immunization.** A lyophile of cholera toxoid in phosphate buffer (IND 405 prepared by Wyeth Laboratories under contract 70-2102 from the National Institute of Allergy and Infectious Diseases), which was prepared by glutaraldehyde treatment of purified cholera enterotoxin, was mixed with diluent containing protamine and aluminium chloride. This toxoid was administered to volunteers intramuscularly by Ped-O-Jet injectors. Volunteers received either 25, 50, or 100 μg of toxoid. Sera were obtained prior to injection and 14 days after the injection. Serum vibriocidal antibody titers were measured by the technique of McIntyre and Feeley (5). Serum cholera antitoxin titers were determined by the hemagglutination technique of Hochstein et al. (4) and are expressed as antitoxin units per milliliter, based on a simultaneous titration of cholera antitoxin reference serum. When hemagglutination titers became available, sera from a subsample of 27 patients were tested for *E. coli* antitoxin titers. There were no significant differences in age and sex distribution between the whole group and the subsample. Of the 27 patients under consideration, age distribution was: 1 to 4 years (4%); 5 to 14 (40%), and 15 and over (56%). The sex distribution was 50% male, 50% female.

**Serology.** Serum *E. coli* antitoxin was measured by the rabbitskin bluing method as described by Evans et al. (1, 3). Measurements were made in duplicate on each of two rabbits (four determinations per sample on 57 sera) or one rabbit (two determinations per sample on 3 sera). Rabbits weighed between 1.7 and 2.5 kg.

Concentrates (50-fold) of *Escherichia coli* culture filtrates were prepared by the Evans method (3) using the enterotoxin-producing strain CRL 10400, isolated from a patient with profuse watery diarrhea from whom no other pathogen could be isolated. The toxic filtrate was tested in dog jejunal loops and caused fluid accumulation as previously described (6).

**Assay.** A slight modification of the Evans (1) method for rabbitskin toxin assay was made, in that concentrated filtrates were initially diluted 1:25 with borate-gelatin buffer (pH 7.5) and then mixed with equal volumes of the same buffer. A 0.1-ml volume of the resulting mixture was used as the intradermal toxin dose. In our rabbits this amount gave the clearest and most reproducible positive results, and induced an arbitrarily defined positive bluing response with a mean of at least 7 mm of induration, with at least 5 mm of bluing. This response was used as the standard dose of *E. coli* LT enterotoxin (delayed onset) in the assay. When the standard dose was used as positive controls in duplicate with each set of sera tested, the mean diameter of bluing obtained was 5.9 ± 1.5, standard deviation (SD). Negative controls of borate-gelatin buffer (diluent for sera) were used with each set of tests, and neither induration nor bluing was ever observed.

The neutralizing dilution of serum was defined as the dilution of serum which completely eliminated induration or bluing when mixed with an equal volume of twofold-concentrated *E. coli* filtrate and injected intradermally (0.1 ml). Bluing was measured

22 h later and 1 h after intravenous (i.v.) injection of 1.2 ml of 5% pontamine sky-blue (diluted 1:1 in normal saline solution) per kg of body weight. Sera were coded and randomly numbered by an independent party before rabbitskin assays were carried out, and the code was not broken until tests were completed.

## RESULTS

**Effects of immunization sera on cholera and E. coli enterotoxins.** Preimmunization sera had little or no antitoxin against cholera or *E. coli* enterotoxins. Postimmunization sera fell into two groups. In the nonresponsive group (12 individuals), there was little or no increase in antitoxin; in the responsive group (15 individuals), antitoxin levels increased significantly. Of the volunteers who received 25, 50, or 100 μg of toxoid, 33, 50, and 75%, respectively, fell into the responsive group. Individuals who responded to immunization with a rise in serum cholera antitoxin titers had a parallel rise in serum *E. coli* antitoxin titers, and individuals who did not respond to immunization had no rise in serum cholera or *E. coli* antitoxins. (Table 1). There was no significant rise in vibriocidal titers in the paired sera. This result confirmed the relative freedom of the toxoid vaccine from somatic antigens.

## DISCUSSION

Our results, which are consistent with the data derived from recent studies in animals (6-8), indicated that in vitro human cholera antitoxin cross-neutralized *E. coli* strain LT delayed-onset enterotoxin. In the present study the striking parallelism of cholera and *E. coli* antitoxin titers suggested that both titrations were measuring a single cross-neutralizing antibody which could provide a possible basis for cross-immunity.

Both cholera and diarrhea associated with enterotoxigenic *E. coli* are endemic in the study population, and repeated infection, often subclinical, can boost individual immune responses. A field demonstration of cross-immunity to clinical disease would have obvious epidemiological significance.

If serum antitoxic activity is related to immunity against clinical cholera or *E. coli* diarrhea, then the cholera toxoid vaccine should protect against both diseases in recipients who respond with a significant increase in serum antitoxin levels. The possibility of cross-neutralization by cholera antitoxin of additional bacterial enterotoxins other than that of *E. coli* deserves further study.

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TABLE 1. *Antitoxin levels after cholera toxoid immunization*[a]

| Group A antitoxin | | | | Group B antitoxin | | | |
|---|---|---|---|---|---|---|---|
| Cholera | | *E. coli* | | Cholera | | *E. coli* | |
| Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| 5.6 | 11 | 1:16 | 0 | 2.8 | 355 | 1:16 | 1:1,024 |
| 2.8 | 5.6 | 0 | 1:2 | 2.8 | 355 | 0 | 1:1,024 |
| 5.6 | 11 | 0 | 1:16 | 11 | 710 | 1:16 | 1:256 |
| 5.6 | 44 | 1:16 | 1:2 | 2.8 | 1,420 | 0 | 1:1,024 |
| 11 | 44 | 1:16 | 1:2 | 5.6 | 2,840 | 0 | 1:1,024 |
| 5.6 | 5.6 | 1:2 | 1:2 | 5.6 | 1,420 | 1:16 | 1:1,024 |
| 11 | 44 | 1:2 | 1:16 | 5.6 | 1,420 | 1:2 | 1:1,024 |
| 11 | 44 | 1:2 | 1:2 | 2.8 | 1,420 | 1:2 | 1:1,024 |
| 11 | 11 | 1:16 | 1:16 | 22 | 1,420 | 1:2 | 1:1,024 |
| 5.6 | 11 | 0 | 1:2 | 44 | 2,840 | 1:16 | 1:1,024 |
| 22 | 178 | 1:16 | 1:16 | 5.6 | 1,420 | 1:16 | 1:1,024 |
| 2.8 | 178 | 1:2 | 1:16 | 11 | 1,420 | 0 | 1:1,024 |
| | | | | 22 | 2,840 | 1:16 | 1:1,024 |
| | | | | 11 | 1,420 | 1:16 | 1:1,024 |
| | | | | 22 | 1,420 | 1:16 | 1:1,024 |

[a] Antitoxin levels in pre- and postimmunization sera expressed as hemagglutination units (cholera) or neutralizing dilution of serum (*E. coli*). Dilutions over 1:1,024 were not tested. Zero = no neutralization even with undiluted serum. Group A, Nonresponders; group B, responders.

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