

Protection in Rats Immunized with *Escherichia coli* Heat-Stable Enterotoxin

FREDERICK A. KLIPSTEIN,^{1*} RICHARD F. ENGERT,¹ AND JOHN D. CLEMENTS^{1,2}

Departments of Medicine¹ and Microbiology,² University of Rochester Medical Center, Rochester, New York 14642

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Rats immunized with a semipurified preparation of the *Escherichia coli* heat-stable (ST) enterotoxin conjugated with a protein carrier were protected against challenge with semipurified or purified ST and viable organisms of multiple heterologous serotypes that produce only ST (LT⁻/ST⁺), but they were not protected against heat-labile (LT) toxin or viable strains which produce LT either alone (LT⁺/ST⁻) or together with ST (LT⁺/ST⁺).

Intestinal contamination by enterotoxigenic strains of *Escherichia coli* which elaborate the heat-labile (LT) or heat-stable (ST) enterotoxins, either singly or together, is a common cause of acute diarrhea in humans, particularly among visitors to, or children resident in, tropical areas. These organisms together with rotavirus are considered to be the principal cause of severe diarrheal disease, which results in between five and ten million deaths per year among young children in underdeveloped countries (3). The development of a program of immunological protection against these pathogens seems to be the only practical approach for prevention.

Immunization of experimental animals with LT has been shown to provide protection against challenge with either the toxin itself or viable homologous or heterologous strains which produce LT, either alone (LT⁺/ST⁻) or together with ST (LT⁺/ST⁺) (6-9). Immunization with LT does not, however, provide protection against either ST toxin or viable ST-only-producing strains (6), which are also a significant cause of acute diarrhea in humans (13-15).

Until recently, the low-molecular-weight ST toxin had been thought to be nonantigenic. Within the past several years, however, several laboratories have achieved purification of this toxin and shown that antibody raised against the purified toxin is capable of neutralizing the secretory effect of ST in the suckling mouse model (1, 10, 11). Since no information is available regarding whether active immunization with ST yields protection against direct challenge with viable ST-producing strains, we have evaluated this, using rats as the experimental animal model.

Weanling 120-g Sprague-Dawley rats were immunized with either LT or ST. Purified LT holotoxin was prepared by the methods de-

scribed by Clements and Finkelstein (4) from strain 711 (F1LT), a transformed K-12 derivative bearing LT gene(s) of the Ent plasmid from porcine strain P 307. Homogeneity of the LT toxin was documented by polyacrylamide gel electrophoresis. The ST toxin was prepared by the methods reported by Staples and his associates (16) from strain Texas 452 (O78:H12), an LT⁻/ST⁺ strain isolated from a child with acute diarrhea (14). The activity of samples obtained during the various purification procedures was monitored by the suckling mouse assay (5). The purified ST toxin was highly potent with a minimal effective dosage of 2 ng in the suckling mouse assay, although some heterogeneity was apparent on thin-layer chromatography. Because the yield of purified ST toxin achieved by this procedure was insufficient to provide the quantities of toxin necessary to immunize large groups of rats, immunization was performed using a semipurified preparation of ST obtained after the initial purification steps of adsorption chromatography on Amberlite XAD-2 and acetone extraction. The minimal effective dose of this material in the suckling mouse assay was 5 ng.

Rats were immunized at 4-day intervals on three occasions by concomitant parenteral and peroral routes. Parenteral immunization was given intraperitoneally with Freund complete adjuvant for the primary immunization and Freund incomplete adjuvant for the booster immunizations. Peroral immunizations were given 2 h after the peroral administration of cimetidine (Tagamet; Smith Kline and French Laboratories, Carolina, Puerto Rico) at a dosage sufficient to ablate gastric secretions (6). Toxin dosages are expressed in terms of protein as determined by the method of Lowry et al. (12). The LT holotoxin was given at a dosage of 100 µg par-

enterally and 250 μg perorally. The semipurified ST toxin was conjugated at a ratio of 2:1 with porcine immunoglobulin G by the carbodiimide reaction (2) using 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide at a carbodiimide-protein ratio of 45/1. This ST conjugate was given at a dosage of 500 μg parenterally and 1,000 μg perorally.

Rats were challenged 5 days after the final booster immunization by the instillation of toxin or viable organisms into ligated loops according to published techniques (6, 7). Challenge dosages of LT and purified ST were those concentrations which yielded 50% of the maximum secretory response in unimmunized rats (the "50% effective dosage"); these values were 0.19 ng for LT and 6.5 ng for purified ST (Fig. 1). Cultures of LT^+/ST^- strain PB-258 (serotype O15:H), LT^+/ST^+ strain H-10407 (serotype O78:H11), and LT^-/ST^+ strain Texas 452 (serotype O78:H12) containing 10^9 viable organisms per ml were used for challenge; this concentration of organisms has been found to be the minimum amount necessary to yield maximum secretion in ligated loops of unimmunized rats (6). The values reported are the mean \pm standard error of the mean percent reduced secretion in groups of two or more immunized rats as compared with groups of unimmunized rats given the same challenge material. Reduced secretion of more than 50% is regarded as evidence of strong protection (7).

Rats immunized with LT had strong protection against LT toxin and the viable LT^+/ST^- and LT^+/ST^+ strains but no protection against purified ST toxin or the LT^-/ST^+ strain (Table

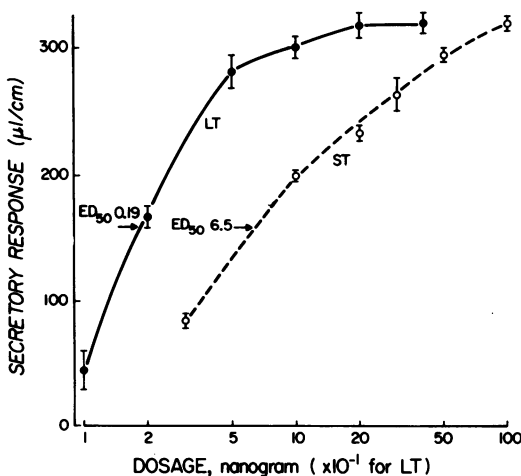


FIG. 1. Secretion induced by graded dosages of purified LT and ST in ligated loops of unimmunized rats. ED_{50} , 50% effective dosage.

1). Rats immunized with semipurified ST were strongly protected against purified ST toxin and the LT^-/ST^+ strain but were not protected against either LT or the LT^+/ST^- or LT^+/ST^+ strains. Rats immunized with ST were also protected against challenge with the semipurified ST material that was used to immunize them and with viable cultures of heterologous serotypes of LT^-/ST^+ strains: secretion was reduced by $57 \pm 4\%$ in rats challenged with 8.0 ng (the 50% effective dosage) of semipurified ST, by $61 \pm 1\%$ in rats challenged with LT^-/ST^+ strain 214-4 (not typable), and by $62 \pm 3\%$ in rats challenged with LT^-/ST^+ strain E7740 (O27:H7). Rats immunized with just the ST protein carrier, porcine immunoglobulin G, at the same dosage used for ST toxin immunization had no protection whatsoever against challenge with purified ST or an LT^-/ST^+ strain.

These results indicate that ST is immunogenically active when conjugated to a carrier and that immunization with this material provides protection against strains which produce just ST. Although the ST toxin material used for immunization was impure, it would seem most likely that the observed protection was directed against ST since (i) protection was evident both against the semipurified ST used for immunization and purified ST, and (ii) protection extended to heterologous serotypes of LT^-/ST^+ strains. Rats immunized with LT are strongly protected against LT^+/ST^+ strains when challenged either by the ligated loop technique (7, 8) or by colonization of the intact intestine (9); this observation, coupled with our finding that immunization with ST provided only slight protection against an LT^+/ST^+ challenge in the present study, suggests that LT is the predominant toxin elaborated by LT^+/ST^+ strains.

The demonstration that immunological protection can be achieved in an experimental animal model against enterotoxigenic *E. coli* strains which produce either LT or ST suggests that development of an effective immunization program against these organisms in humans is feasible.

TABLE 1. Results of challenge in immunized animals

Immuno- gen used	% Reduced secretion after challenge with ^a :				
	LT toxin	LT^+/ST^-	LT^+/ST^+	ST toxin	LT^-/ST^+
LT	94 \pm 1	66 \pm 2	48 \pm 1	0	3 \pm 1
ST	0	0	7 \pm 4	83 \pm 9	69 \pm 2

^a Mean \pm standard error of the mean percent reduced secretion in immunized rats as compared to similarly challenged unimmunized animals.

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