8. Henle W, Henle GE, Horwitz CA. Epstein-Barr virus specific diagnostic tests in infectious mononucleosis. Hum Pathol 1974;5:551–65.
9. Reedman BM, Klein G. Cellular localization of an Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. Int J Cancer 1973;11:499–520.
10. de Miranda P, Blum MR. Pharmacokinetics of acyclovir after intravenous and oral administration. J Antimicrob Chemother 1983; 12(suppl B):29–37.
11. Sixbey JW, Nedrud JG, Raab-Traub N, Hanes RA, Pagano JS. Epstein-Barr virus replication in oropharyngeal epithelial cells. N Engl J Med 1984;310:1225–30.
12. Marklund G, Ernberg I, Lundberg C, Henle W, Henle C. Differences in EBV-specific antibody patterns at onset of infectious mononucleosis. Scand J Infect Dis 1986;18:25–32.
13. Henle G, Henle W, Horwitz CA. Antibodies to Epstein-Barr virus–associated nuclear antigen in infectious mononucleosis. J Infect Dis 1974;130:231–9.
14. Halprin J, Scott AL, Jacobson L, et al. Enzyme-linked immunosorbent assay of antibodies to Epstein-Barr virus nuclear and early antigens in patients with infectious mononucleosis and nasopharyngeal carcinoma. Ann Intern Med 1986;104:331–37.
15. Rhodes G, Carson DA, Valbracht J, Houghten R, Vaughan JH. Human immune responses to synthetic peptides from the Epstein-Barr nuclear antigen. J Immunol 1985;134:211–6.

Safety and Immunogenicity of Human Rotavirus Vaccine Strain M37 in Adults, Children, and Infants

Karen Midthun, Neal A. Halsey, Mary Jett-Goheen, Mary Lou Clements, Mark Steinhoff, James C. King,* Ruth Karron, Modena Wilson, Barbara Burns, Victoria Perkis, Roberta Samorodin, and Albert Z. Kapikian

Center for Immunization Research, Department of International Health, Johns Hopkins University, Baltimore, and Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

Rotavirus vaccine strain M37 (serotype 1), recovered from the stool of an asymptomatic newborn infant and serially passaged in cell culture, was given orally to adults, children, and infants. Serologic responses were detected by neutralization assay or EIA in 59% of 17 adults (10^5-pfu dose), 55%–60% of 21 infants and children (10^4-pfu dose), and 70% of 10 infants (10^5-pfu dose); vaccine virus was shed by 24%, 20%–36%, and 70%, respectively. In adults, neutralizing antibody rises to strain M37 and the related serotype 1 strain Wa occurred with equal frequency (41% vs. 47%). In pediatric subjects, the former were more frequent (36%–40%) than the latter (10%–18%). This was also true of 8 infants who received two doses of vaccine. Mild gastrointestinal illnesses occurred with equal frequency in pediatric subjects who received vaccine or placebo. Thus, strain M37 was well tolerated and immunogenic in young infants, but elicited primarily vaccine-strain–specific rather than serotype-specific neutralizing antibody responses.

Because rotavirus is the single most important etiologic agent of severe diarrheal disease in infants and young children worldwide, the development of a safe and effective rotavirus vaccine is a high priority. Vaccine strains of animal rotavirus origin have induced variable levels of protection (0–>80%) [1–4], and serotype-specific immunity to each of the epidemiologically important human rotavirus serotypes (serotypes 1–4) may be required to protect against rotavirus diarrhea in young infants not primed by previous rotavirus infection. For this reason, a quadrivalent vaccine preparation containing rhesus rotavirus (RRV, serotype 3) and three human-RRV reassortants (serotypes 1, 2, and 4) is currently under evaluation [5].

Another approach to vaccination is the use of human rotavirus strains that usually infect neonates without causing illness and therefore appear to be naturally attenuated [5, 6]. Natural infection with one such strain was associated subsequently with less frequent and less severe episodes of rotavirus illness [7]. M37 is an asymptomatic neonatal strain that belongs to serotype 1 by virtue of its major neutralization protein, VP7 [8]. It is also related to asymptomatic human rotavirus strains belonging to serotypes 2, 3, and 4 by virtue of its other major neutralization protein, VP4 [8, 9]. The M37 strain is an attractive vaccine candidate because serotype 1 is prevalent among cases of rotavirus diarrhea worldwide. Furthermore, although the VP4 protein of M37 differs

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Reprints or correspondence: Dr. Karen Midthun, Johns Hopkins University, Center for Immunization Research, 624 N. Broadway, Room 125, Baltimore, MD 21205.
* Present address: Department of Pediatrics, University of Maryland, Baltimore.

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from that of symptomatic strains [9], it may be more likely than the VP4 of animal strains to induce cross-reactive neutralizing antibodies directed at other human strains. Therefore, we evaluated the safety and immunogenicity of human rotavirus vaccine candidate strain M37 in adults, children, and infants.

Materials and Methods

Vaccine. Human rotavirus strain M37 was isolated from the stool specimen of an asymptomatic neonate in Caracas, Venezuela, on 16 February 1982 [10]. It was propagated in primary African green monkey kidney (AGMK) cells for a total of 21 passages, including triple plaque purification by the Laboratory of Infectious Diseases, National Institutes of Health. It was passaged 9 additional times in pretested primary, secondary, or tertiary AGMK cells to produce the vaccine pool (titer, 3×10^5 pfu/ml) designated lot M37-1 by Flow Laboratories (Mclean, VA). The vaccine was prepared in accordance with guidelines established by the US Food and Drug Administration.

Study sequence and subjects. Subjects were studied in a stepwise fashion with respect to age and dose. The criteria for selection of adult and pediatric subjects and the clinical study details have been described [11]. Briefly, healthy 18- to 40-year-old adults in an isolation facility were vaccinated orally with 1 ml of undiluted vaccine (10^5 pfu) after drinking distilled water with 2 g of $NaHCO_3$ and subsequently observed for 7 days. They fasted for 1 h before and after vaccination. The vaccine was first given to two adults with higher titers of serum neutralizing antibody (<1:320) and then to additional adults with lower titers. Diarrhea was defined as a single liquid stool ≥300 ml, two liquid stools totaling ≥200 ml, or more than three unformed stools within 48 h.

Healthy pediatric subjects were given vaccine or placebo (2:1 ratio) by a blinded investigator; 1 ml of vaccine diluted 10-fold in buffered formula (10^4 pfu) was given orally to children 6–60 months old and then to infants 1.5–5.0 months old. Subsequently, 1 ml of undiluted vaccine (10^5 pfu) was given to the younger age group. A subset of infants 1.5–5.0 months old at the time of their first dose was given a second dose (10^4 or 10^5 pfu) within 1.0–2.5 months. All pediatric subjects drank 30 ml of formula (Similac; Ross Laboratories, Columbus, OH) that lacked rotavirus-neutralizing activity and contained 0.4 g of $NaHCO_3$ immediately before vaccination and fasted for 1 h before and after vaccination. The placebo for the 10^4-pfu-dose group was buffered formula and for the 10^5-pfu-dose group was sterile Eagle's minimal essential medium.

Subjects were observed for up to 1 h/day for 2 days before and 7 days after vaccination. Parents monitored their children for symptoms of illness and collected stool specimens from their children for examination by study personnel. Rectal temperatures were measured each day by study personnel and each evening at home by parents. Fever was defined as a rectal temperature >38.1°C confirmed by repeat examination in 10–15 min. Diarrhea was defined as three or more unformed stools in a 24-h period.

Laboratory studies. Serum specimens were collected from all subjects before and 4–5 weeks after vaccine or placebo administration. Sera were tested by rotavirus-specific IgA and IgG ELISA, using RRV as antigen, and by plaque reduction neutralization assay (PRNA) to the following human rotavirus strains (serotype): M37 (1), Wa (1), DS-1 (2), P (3), and ST3 (4). The ELISA and PRNA were done as described previously [6, 12].

A significant antibody response was defined as a fourfold or greater rise in titer. Stool or rectal swab specimens were collected from all subjects daily starting 1–2 days before through 7 days after vaccination and at follow-up 4–5 weeks later; samples were made into 10% stool suspensions in veal infusion broth. The suspensions were tested for rotavirus by confirmatory ELISA and then by inoculation into MA104 cell culture roller tubes with one subsequent blind passage [11]. After incubation at 37°C for 7 days, the cultures were frozen, thawed, and tested by confirmatory ELISA. Diagnostic studies to detect enteric pathogens in stool specimens from subjects with diarrhea included an examination for parasites, bacterial cultures for *Salmonella, Shigella, Campylobacter, Aeromonas, Yersinia,* and *Vibrio,* an ELISA for adenovirus, and in some cases electron microscopic studies.

Results

Gastrointestinal illness after vaccination. Of 17 adults, 1 developed diarrheal illness 2 days after vaccination that resolved within 1.5 days and was not associated with fever, vomiting, or dehydration. This subject did not respond serologically to or shed rotavirus, and examination of diarrheal stool specimens revealed *Blastocystis hominis* and *Entamoeba coli.* In the 6- to 60-month-old age group, none of 10- to vaccine or 6 placebo recipients developed gastrointestinal illness.

In the <6-month-old age group, mild illnesses occurred with equal frequency in vaccine and placebo recipients. Diarrhea occurred in 1 of 11 10^4-pfu recipients, none of 10 10^5-pfu recipients, and 2 of 14 placebo recipients, lasted only 1 day, and was characterized by three to six unformed stools; no adventitious agents were identified. The vaccine and placebo groups did not differ with respect to the mean number of unformed stools or total stools per child per day. Only 2 infants had fever after vaccination; a 10^4-pfu recipient had fever (39.2°C) in association with otitis media on days 6 and 7, and a 10^5-pfu recipient had an isolated low-grade fever (38.2°C) on day 1. Vomiting was reported in 9%–30% of 10^4-pfu and 10^5-pfu recipients and 36% of placebo recipients and was characterized by only 1–2 episodes of emesis. Eight infants who were vaccinated with either 10^4 pfu ($n = 3$) or 10^5 pfu ($n = 5$) at <6 months of age were revaccinated with the same dose 1.0–2.5 months later. None developed illness after their booster dose except for one who had a low-grade fever (38.4°C) after a 10^5-pfu dose.

Vaccine virus shedding and serologic responses. Of 17 adults vaccinated with 10^5 pfu, 24% shed rotavirus and 59% responded serologically (table 1). Of 10 6- to 60-month-old-

Table 1. Shedding of virus and serologic responses after one dose of human rotavirus vaccine strain M37.

Age group (*n*)	Dose (pfu)	% of subjects: Shedding virus	With a fourfold or greater antibody rise by: PRN to strain (serotype) M37* (1)	Wa (1)	ST3 (4)	IgG or IgA ELISA	Any assay
18–40 years (17)	10^5	24	41	47	18	47	59
6–60 months (10)	10^4	20	40	10	20	40	60
1.5–5.0 months (11)	10^4	36	36	18	9	27	55
1.5–5.0 months (10)	10^5	70	40	10	10	40	70

NOTE. PRN, plaque-reduction neutralization; pfu, plaque-forming units.
* M37, the vaccine strain, has a Wa-like VP7 and an ST3-like VP4. Serotype specificity as defined by hyperimmune animal sera is largely determined by VP7.

children vaccinated with 10^4 pfu, 20% shed rotavirus and 60% responded serologically. Among <6-month-old infants, 36% shed rotavirus and 55% responded serologically after a 10^4-pfu dose (n = 11), whereas 70% shed rotavirus and 70% responded serologically after a 10^5-pfu dose (n = 10). Serologic responses occurred more frequently in vaccinees without preexisting serum neutralizing antibody to the M37 strain (titer <1:40) than in vaccinees with detectable antibody (titer ≥1:40) (14/17 vs. 15/31; P = .02, χ^2 test). The percentages of subjects in each age and dose group with detectable rotavirus antibody in their prevaccination sera and their corresponding geometric mean titers are shown in table 2. In contrast to the adults and 6- to 60-month-old children, most <6-month-old infants lacked rotavirus-specific serum IgA (19/21) and neutralizing antibody to the vaccine strain (12/21).

After vaccination, the frequency of neutralizing antibody responses to M37 and to Wa, a serotype 1 strain related to M37 by VP7, was comparable in adults (41% vs. 47%, respectively; table 1). In contrast, responses to M37 (36%–40%) were more frequent than responses to Wa (10%–18%) among pediatric vaccine recipients. Neutralizing antibody responses to ST3, an asymptomatic serotype 4 strain related to M37 by VP4, occurred with lesser frequency (9%–20%) in all age groups. Neutralizing antibody responses to serotype 2 strain DS-1 and serotype 3 strain P were detected in 12% and 6%, respectively, of adults and were not tested for in pediatric subjects. Seven of eight infants who received two doses of 10^4 pfu (n = 3) or of 10^5 pfu (n = 5) had serologic responses after the first dose (n = 3), the second dose (n = 3), or both doses (n = 1). Neutralizing antibody responses to M37, Wa, and ST3 were noted in 63%, 13%, and 38% of these two-dose vaccine recipients, respectively. Although none of 20 placebo recipients shed vaccine virus, 1 had a neutralizing antibody response to M37. This subject was the 12-month-old sibling of a 45-month-old vaccine recipient who had a neutralizing antibody response to both M37 and ST3 but did not shed virus during the observation period.

Of 48 vaccinated subjects, 17 shed virus as follows: day 1, 0; day 2, 6%; day 3, 24%; day 4, 47%; days 5 and 6, 53%; day 7, 35%; and day 30, 0. Of 13 pediatric subjects who shed virus, 8 shed amounts sufficient to be detected by ELISA of their 10% stool suspensions. These 8 subjects all had a serologic response compared with 6 of 9 subjects who shed rotavirus but in an amount detectable only after amplification in cell culture.

Table 2. Subjects (%) with detectable rotavirus antibodies in prevaccination serum specimens and corresponding reciprocal geometric mean titers (GMT).

Age group (*n*)	Mean age (months)	Dose (pfu)	% with detectable antibody* (GMT, range): By PRN to strain (serotype) M37 (1)	Wa (1)	ST3 (4)	By IgA
18–40 years† (17)	—	10^5	82 (158, 40–1280)	88 (316, 80–5120)	65 (100, 40–320)	100 (230, 50–1600)
6–60 months (10)	25.7	10^4	80 (1000, 320–5120)	100 (720, 80–5120)	70 (125, 80–320)	70 (398, 100–3200)
1.5–5.0 months (11)	3.2	10^4	45 (63, 40–80)	64 (126, 80–320)	36 (80, 80)	18 (200, 200)
1.5–5.0 months (10)	2.4	10^5	40 (80, 40–320)	40 (112, 40–320)	10 (40, 40)	0

NOTE. GMT, geometric mean titer; pfu, plaque-forming units.
* Defined as titer ≥1:40 for plaque-reduction neutralization (PRN), ≥1:50 for IgA.
† Adults were selected for study participation on the basis of serotype 1 neutralizing titers; 2 with screening titers >1:320 and 15 with titers ≤1:320 were chosen.

Discussion

Neonatal human rotavirus vaccine strain M37 was well-tolerated in adults, children, and infants. The diarrheal episode that occurred in 1 of 17 adults did not appear to be vaccine-induced and may have been related to *B. hominis*. Mild gastrointestinal illnesses occurred with similar frequency in the <6-month-old vaccine and placebo recipients, suggesting that they were not vaccine-related. Thus, the vaccine seems to be attenuated, although this observation is based on a small number of subjects and must be considered a preliminary finding. The basis for attenuation of the M37 strain is unclear, since it is possible that attenuation was conferred entirely by 30 passages in cell culture; alternatively, the strain may have been naturally attenuated not only for neonates [10] but also for older subjects.

Both the 10^4- and 10^5-pfu doses were moderately immunogenic in infants <6 months old, the target population for vaccination. Serologic responses and vaccine virus shedding were found in more 10^5-pfu recipients than in 10^4-pfu recipients, but the difference was not significant. The serum-neutralizing antibody rises in the pediatric vaccine subjects were primarily vaccine-strain specific, with neutralizing antibody rises to Wa, another serotype 1 strain, detected less frequently. These results suggest that M37 and Wa differ with respect to immunodominant epitopes on VP7, which is consistent with their different reactivity patterns to certain serotype 1–specific monoclonal antibodies directed at VP7 [13].

The percentages of adults with neutralizing antibody responses to M37 and Wa were similar, consistent with the fact that adults develop more broadly cross-reactive antibody responses [14]. However, the frequency of heterotypic responses to serotype 2, 3, and 4 human rotaviruses in adults vaccinated with strain M37 was low. The neutralizing antibody responses to ST3, a serotype 4 strain related to M37 via VP4, were low in all age groups, suggesting that the VP4 protein of M37 is not particularly effective in stimulating the production of cross-reactive antibodies to the VP4 proteins of other human rotavirus strains. The effect of giving two doses of vaccine (10^4 or 10^5 pfu) within a 1.0–2.5-month period increased the overall serologic response rate in infants <6 months of age to 88%. Although serotype-specific responses to M37 and ST3 were noted in more infants after two than after one dose, responses to Wa remained low.

Most pediatric subjects who shed virus had quantities sufficient to be detected by ELISA of their 10% stool suspensions. This finding is in contrast to that with RRV and human-RRV reassortant vaccines, which usually are detected in stool specimens only after inoculation into cell culture [11, 15]. The ELISA-positive stool suspensions suggest that larger quantities of vaccine virus were being shed, which might theoretically predispose to transmission. Transmission was suspected but not confirmed in the case of the placebo recipient who developed a neutralizing antibody response to M37 and was the sibling of a vaccinee.

Our safety and immunogenicity findings are similar to those of a phase 1 study in Venezuela [5], in which M37 (10^4 pfu) was given to <6-month-old infants. More Venezuelan infants developed a neutralizing antibody response to M37 than did our infants, but the difference was not significant (14/22 vs. 4/11; $P = .13$, χ^2 test).

In conclusion, strain M37 (10^4 or 10^5 pfu) was well-tolerated in adults, children, and infants. In <6-month-old infants, M37 was moderately immunogenic, with two doses increasing the overall serologic response rate, but neutralizing antibody responses were primarily vaccine-strain specific. This raises concern about the potential efficacy of this vaccine candidate because an association between vaccine failures and lack of a serologic response to the infecting rotavirus serotype has been noted previously [14]. An efficacy trial is currently under way and should provide important information as to the effectiveness of the M37 strain as a vaccine candidate.

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References

1. Vesikari T, Isolauri E, Delem A, et al. Clinical efficacy of the RIT 4237 live attenuated bovine rotavirus vaccine in infants vaccinated before a rotavirus epidemic. J Pediatr **1985**;107:189–94.
2. Clark HF, Borian FE, Bell LM, Modesto K, Gouvea V, Plotkin SA. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J Infect Dis **1988**;158:570–87.
3. Flores J, Perez-Schael I, Gonzalez M, et al. Protection against severe rotavirus diarrhea by rhesus rotavirus vaccine in Venezuelan infants. Lancet **1987**;1:882–4.
4. Santosham M, Letson GW, Wolff M, et al. A field study of the safety and efficacy of two candidate rotavirus vaccines in a Native American population. J Infect Dis **1991**;163:483–7.
5. Flores J, Perez-Schael I, Blanco M, et al. Comparison of reactogenicity and antigenicity of the M37 rotavirus vaccine and rhesus rotavirus based quadrivalent vaccine. Lancet **1990**;2:330–4.
6. Hoshino Y, Wyatt RG, Flores J, Midthun K, Kapikian AZ. Serotypic characterization of rotavirus derived from asymptomatic human neonatal infection. J Clin Microbiol **1985**;21:425–30.
7. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. N Engl J Med **1983**;309:72–6.
8. Hoshino Y, Sereno MM, Midthun K, Flores J, Kapikian AZ, Chanock RM. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc Natl Acad Sci USA **1985**;82:8701–4.
9. Gorziglia M, Green K, Nishikawa K, et al. Sequence of the fourth gene of human rotaviruses recovered from asymptomatic or symptomatic infections. J Virol **1988**;62:2978–84.
10. Perez-Schael I, Daoud G, White L, et al. Rotavirus shedding by newborn children. J Med Virol **1984**;14:127–36.

11. Halsey NA, Anderson EL, Sears SD, et al. Human-rhesus reassortant rotavirus vaccines: safety and immunogenicity in adults, infants, and children. J Infect Dis **1988**;158:1261–7.
12. Midthun K, Pang L, Flores J, Kapikian AZ. Comparison of immunoglobulin A (IgA), IgG, and IgM enzyme-linked immunosorbent assays, plaque reduction neutralization assay, and complement fixation in detecting seroresponses to rotavirus vaccine candidates. J Clin Microbiol **1989**;27:2799–804.
13. Green KY, James HD Jr, Kapikian AZ. Evaluation of three panels of monoclonal antibodies for the identification of human rotavirus serotype by ELISA. Bull World Health Organ **1990**;68:601–10.
14. Green KY, Taniguchi K, Mackow ER, Kapikian AZ. Homotypic and heterotypic epitope-specific antibody responses in adult and infant rotavirus vaccinees: implications for vaccine development. J Infect Dis **1990**;161:667–79.
15. Wright PF, Tajima T, Thompson J, Kokubun K, Kapikian A, Karzon DT. Candidate rotavirus vaccine (rhesus rotavirus strain) in children: an evaluation. Pediatrics **1987**;80:473–80.

Heterogeneity of Immunotypes of Heat-Labile Enterotoxins of Enterotoxigenic *Escherichia coli* of Human Origin

Zhen-Hai Qu,* Mary Boesman-Finkelstein, Mohammad Kazemi,† and Richard A. Finkelstein

Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia

A new technique, checkerboard immunoblotting (CBIB), has been applied to detect and to differentiate heat-labile enterotoxins, (LTs), from enterotoxigenic strains of *Escherichia coli* of human origin using polyclonal and monoclonal antibodies. Optimal conditions of production and release of LTs were defined using CBIB. LT release was enhanced when *E. coli* cells were treated with 8 *M* urea. LT production was highest when *E. coli* strains were incubated with shaking (200 rpm) at 37°C for 12 h in CAYE-2 medium. Two hundred and five strains of *E. coli*, isolated from patients with diarrhea in Japan, Thailand, the United States, Mexico, and Brazil, were examined for LT. Of 133 LT-positive strains, 4 (3%) produced an LT that reacted like H-LT-1 (originally isolated from *E. coli* strain H-74-114) while 126 strains (94.7%) produced LT that reacted like H-LT-2 (originally isolated from strain H-10407) or H-LT-3 (from strain H-240-3). Three strains of human origin (2.3%) produced an LT that reacted like P-LT (produced by *E. coli* strains of porcine origin). The study shows that CBIB, a simple, efficient, and practical assay, might be useful for epidemiologic surveys and for evaluation of serologic responses to LTs and antitoxic vaccines.

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhea in children and adults in developing countries and of traveler's diarrhea, particularly in the tropics. ETEC produces either or both heat-labile enterotoxin (LT) and heat-stabile enterotoxin (ST). Two major types of LT, LT-I and LT-II, have been identified so far [1–4]. LT-I includes at least three distinct H-LTs, that is LT from *E. coli* strains of human (H) origin: H-LT-1 from strain H-74-114 [5], H-LT-2 from strain H-10407 [6], and H-LT-3 from strain H-240-3 [7] as well as P-LT from *E. coli* strains of porcine (P) origin. LT-II resembles LT-I in some biologic properties but is not cross-reactive with LT-I [3, 4]. Many assay methods for detecting LT-producing ETEC have been reported, but few assays have been used to differentiate distinct LTs produced by different strains of ETEC. These immunologic differences could be important from the standpoints of vaccine development and epidemiology.

We used checkerboard immunoblotting (CBIB), a newly developed immunoassay [8], to investigate the conditions for optimal production and release of LT-I and to detect and to differentiate LT-Is from 205 isolates of *E. coli* from patients with diarrhea in different parts of the world. The distribution of LT-II was recently reported [4].

Materials and Methods

E. coli strains. We studied 205 clinical isolates of *E. coli*. One hundred, isolated at Osaka International Airport Quarantine Station (Japan) from patients with diarrhea who had just returned from cholera-infected areas of Southeast Asia, were provided by Y. Takeda and T. Honda [9] (Osaka University, Japan). Sixty *E. coli* strains, isolated from patients with diarrhea from the United States and Mexico, were provided by J. Matthewson (University of Texas, Houston). Forty LT^+ strains iso-

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Reprints or correspondence: Dr. Richard A. Finkelstein, Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO 65212.
* Permanent address: Department of Microbiology, Xuzhou Medical College, Jiangsu, China.
† Present address: Hyland Division, Baxter Healthcare, Duarte, California.

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