DNA Probes To Identify Shiga-Like Toxin I- and II-Producing Enteric Bacterial Pathogens Isolated from Patients with Diarrhea in Thailand

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Received 22 February 1988/accepted 10 May 1988

When Shigella species, Escherichia coli, and five other bacterial enteric pathogens isolated from children with diarrhea in Thailand were tested for hybridization under stringent conditions with probes for Shiga-like toxins I and II, only 30 Shigella dysenteriae I hybridized with the Shiga-like toxin I probe.

High-level Shiga-like toxin (SLT)- or verocytotoxin (VT)-producing Escherichia coli has been associated with cases of hemolytic uremic syndrome (HUS) and hemorrhagic colitis. Low levels of cytotoxic activity for HeLa cells have been reported to be produced by a number of enteric pathogens, including strains of Shigella, Vibrio cholerae, Vibrio parahaemolyticus, Salmonella typhi, E. coli, and derivatives of E. coli K-12 (1, 3, 8, 12-14). Cytotoxic activity was detected in sonicated lysates of bacteria when these organisms were grown in an iron-depleted medium. The cytotoxicity from these organisms was reported as Shiga-like because the toxin activity was neutralized with polyclonal or monoclonal antibodies to purified Shiga toxin (1, 8, 12-14).

In an earlier study by Scotland et al. (16), culture filtrates of 253 enteropathogenic E. coli (EPEC) strains, isolated from infants with diarrhea, which belonged to 11 E. coli serogroups were tested for cytotoxicity to Vero cell monolayers. Of these strains, 25 were cytotoxic to Vero cells, of which 23 belonged to serogroup O26 and 2 belonged to serogroup O128. VT+ E. coli O26 and O128 also hybridized with the VT probes (17). In another study, E. coli of classical enteropathogenic serogroups that were isolated from infants in Thailand who were hospitalized for diarrhea were examined for cytotoxin production (3). Sonicated extracts of 53 (55%) of 96 EPEC strains tested at a dilution of 1:100 were cytotoxic to HeLa cell monolayers. Of 53 cytotoxic sonic extracts, 50 (94%) were inhibited to various degrees (29 to 89%) by a 1:500 dilution of rabbit antiserum to purified Shiga toxin. Vero cell cytotoxicity was not detected in culture supernatants of these isolates grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.6% yeast extract (3). Thus, it was unclear whether these isolates specifically produced Shiga toxin or cross-reacting substances.

Specific DNA probes for genes coding for SLT I and SLT II have been constructed (11). These probes hybridized with 90 VT+ E. coli and none of 12 VT- E. coli isolates.

To determine the prevalence of bacterial enteric pathogens containing genes coding for SLT I and SLT II, Shigella strains, as well as E. coli strains that were not enteroinvasive, enterotoxigenic, or enterohaemorrhagic, isolated from children less than 5 years of age with bloody diarrhea and from children without diarrhea were tested for hybridization with the SLT probes under stringent conditions (9) and for Vero cell cytotoxin production (6). To determine whether bacterial pathogens previously reported to produce Shiga toxin contained genes coding for SLT I and SLT II, bacterial enteric pathogens of different species isolated from persons with diarrhea were examined with SLT-specific DNA probes under stringent hybridization conditions (9).

The DNA probe for SLT I was a BamHI 1.142-base-pair fragment isolated from recombinant plasmid pN3I-19, and the probe for SLT II was a Smal-PstI 842-base-pair fragment isolated from recombinant plasmid pNN110-18 (11). These fragments were labeled with α-32P by nick translation (7). The following strains were spotted on Whatman 541 filters (Whatman, Inc., Clifton, N.J.), processed as previously described (18), and tested for colony hybridization with SLT I and SLT II DNA probes under stringent conditions (9): (i) Shigella dysenteriae I (30 isolates) isolated in Thailand in 1987, (ii) E. coli of classical enteropathogenic serogroups isolated from infants hospitalized with diarrhea at a hospital in Thailand (96 isolates) (3), (iii) E. coli strains (5 per patient) isolated from 40 children less than 5 years of age with bloody diarrhea (200 isolates), and (iv) E. coli strains isolated from 28 children less than 5 years old without diarrhea (140 isolates). Groups iii and iv were seen at the outpatient department of Children’s Hospital, Bangkok, from February to June 1985, and the patients affected had not taken antibiotics before specimens were obtained. Isolates of E. coli O157:H7 (10 isolates) from Canadian patients with hemorrhagic colitis were used as control strains in the hybridization assays. E. coli isolates from children with bloody diarrhea and from controls were also inoculated into 20 ml of Penassay broth (Difco Laboratories, Detroit, Mich.) in 125-ml Erlenmeyer flasks and incubated with shaking at 200 rpm at 37°C for 24 h. Fivefold dilutions of sterile culture supernatants were tested for cytotoxicity to Vero cells as described by Konovalchuk et al. (6).

Bacterial enteric pathogens isolated from patients with diarrhea in Thailand from 1980 to 1987 and lyophilized within 1 month of isolation were examined by colony hybridization with the SLT I and II probes under stringent hybridization conditions. Enterotoxigenic E. coli was identified by the Y-1 adrenal and suckling mouse assays (2, 15), enteroinvasive E. coli was identified with a specific probe and confirmed by the

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Serena test (18), and EPEC adhesin factor-positive E. coli was identified with a probe coding for plasmid-mediated genes coding for mannose-resistant localized adherence to HEP-2 cells (10).

All 30 S. dysenteriae 1 isolates tested hybridized with the SLT I DNA probe and produced high levels of VT. None of 200 E. coli isolates from children less than 5 years of age with bloody diarrhea and none of 140 E. coli isolates from children of the same age without diarrhea hybridized with the SLT I or SLT II probes; none were cytotoxic to Vero cells. All 10 E. coli O157:H7 control strains were cytotoxic to Vero cells; 5 hybridized with the SLT I and SLT II, and 5 hybridized with the SLT I probe. By hybridization under high stringency, genes coding for SLT I or SLT II were not found in 3 other Shigella species (93 isolates), Aeromonas species (60 isolates), O1 V. cholerae (90 isolates), non-O1 V. cholerae (60 isolates), V. parahaemolyticus (30 isolates), Campylobacter jejuni (30 isolates), Salmonella typhimurium (10 isolates), other nontyphoid Salmonella species (38 isolates), enterotoxigenic E. coli (82 isolates), enteroinvasive E. coli (56 isolates), EPEC adhesin factor-positive E. coli (120 isolates), and EPEC adhesin factor-negative E. coli of classical EPEC serogroups (40 isolates). None of 96 E. coli of classical enteropathogenic serogroups, 50 of which produced low levels of cytotoxins to HeLa cells, hybridized with SLT I or SLT II probes. Genes coding for SLT I and SLT II were not found in isolates of other species of bacterial enteric pathogens examined.

Other forms of cytotoxins may be important in the pathogenesis of Shigella, EPEC, and other species of bacteria, but genes coding for SLT I or SLT II were not detected in isolates from Thailand. If other cytotoxins are involved, genes coding for these cytotoxins have less than 80% nucleotide sequence homology with genes coding for either SLT I or SLT II. Whether these isolates produce cytotoxins that are encoded by genes that are related but not homologous enough with the SLT I and II probes to be detected under a high degree of stringency remains to be determined. The use of specific DNA probes to identify DNA sequences that code for SLT I and SLT II is considerably faster than, and probably as specific as, testing culture supernatants or sonicate lysates in tissue cultures for cytotoxicity and inhibiting their effect with antibodies to SLT I and II. A study in which O-and H-serotyped E. coli will be examined will be the SM I and SLT II-specific DNA probes is planned to determine the role of SLT I and SLT II E. coli as a cause of diarrhea in children less than 6 months of age in Thailand.

We thank Chittima Pitarangsri, Prani Ratrasarn, Orapan Chivaratanond, Thamma Sakuldaipere, Pradit Nabnumruang, Sajee Pinnoi, Suchitra Changchawati, Songmuang Piaphong, Vitaya Khunvaiert, and Nattakarn Sirirapraivan for their excellent technical assistance. We are grateful to Samuel Ratnam, Newfoundland Public Health Laboratories, for providing E. coli O157:H7 control strains.

LITERATURE CITED