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Two Toxin-Converting Phages from *Escherichia coli* O157:H7 Strain 933 Encode Antigenically Distinct Toxins with Similar Biologic Activities

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***Escherichia coli* O157:H7 strain 933 contains two distinct toxin-converting phages (933J and 933W). The biologic activities and antigenic relationship between the toxins produced by 933J and 933W lysogens of *E. coli* K-12, as well as the homology of the genes that encode the two toxins, were examined in this study. The 933J and 933W toxins, like Shiga toxin produced by *Shigella dysenteriae* type 1, were cytotoxic for the same cell lines, caused paralysis and death in mice, and caused fluid accumulation in rabbit ileal segments. The cytotoxic activity of 933J toxin for HeLa cells was neutralized by anti-Shiga toxin, whereas the activity of 933W toxin was not neutralized by this antiserum. In contrast, an antiserum prepared against *E. coli* K-12(933W) neutralized 933W toxin but not 933J toxin or Shiga toxin. For *E. coli* 933, most of the cell-associated cytotoxin was neutralized by anti-Shiga toxin, whereas most of the extracellular cytotoxin was neutralized by anti-933W toxin. However, a mixture of these antisera indicated the presence of both toxins in cell lysates and culture supernatants. Among 50 elevated cytotoxin-producing strains of *E. coli*, we identified 11 strains isolated from cases of diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome that produced cell-associated cytotoxins which were neutralized by the 933W antitoxin. Southern hybridization studies showed that the cloned toxin structural genes from phage 933J hybridized with DNA from phage 933W under conditions estimated to allow no more than 26% base-pair mismatch. These findings indicate that *E. coli* produces two genetically related but antigenically distinct cytotoxins with similar biologic activities which we propose to name Shiga-like toxins I and II. Strains of *E. coli* that produce elevated levels of Shiga-like toxin I or Shiga-like toxin II, or both, have been associated with the clinical syndromes of diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome.**

Escherichia coli serotype O157:H7 was incriminated as the agent responsible for two outbreaks of hemorrhagic colitis that occurred in the United States during 1982 (23, 26). The *E. coli* O157:H7 strains isolated during these outbreaks did not produce either heat-labile or heat-stable enterotoxin, nor were they enteroinvasive (26). However, two isolates from patients and an isolate (strain 933) from ground beef, which was implicated as the vehicle in these outbreaks, produced high levels of cell-associated and cell-free cytotoxin active on both HeLa and Vero cells (A. D. O'Brien, T. A. Lively, M. E. Chen, S. W. Rothman, and S. B. Formal, Letter, Lancet i:702, 1983). In Canada, strains of *E. coli* O157:H7 have been isolated from patients with hemorrhagic colitis (20; W. M. Johnson, H. Lior, and G. S. Bezanson, Letter, Lancet i:76, 1983) and hemolytic uremic syndrome (6; M. A. Karmali, B. T. Steele, M. Petric, and C. Lim, Letter, Lancet i:619–620, 1983), and these strains have been shown to produce a cytotoxin active on Vero cells. Toxin from *E. coli* 933 was purified to apparent homogeneity and shown to have biologic activities similar to those of *Shigella dysenteriae* type 1 (Shiga) toxin: both toxins were cytotoxic for selected cell lines in vitro, paralytic and lethal for mice, and enterotoxic in ligated rabbit ileal segments (8; A. D. O'Brien, T. A. Lively, T. W. Chang, and S. W. Gorbach, Letter, Lancet ii:573, 1983).

Production of elevated levels of a HeLa or Vero cell cytotoxin by *E. coli* is associated with temperate bacteriophages (17, 24; S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe, Letter, Lancet ii:216, 1983; H. R. Smith, N. P. Day, S. M. Scotland, R. J. Gross, and B. Rowe, Letter, Lancet i:1242–1243, 1984). *E. coli* 933 harbors two distinct toxin-converting phages designated 933J and 933W (17). The cytotoxin produced in large amounts by *E. coli* K-12 lysogenized with phage 933J can be neutralized by antiserum against Shiga toxin (17). The structural genes for this toxin were cloned from phage 933J DNA and shown by Southern hybridization to have homology with DNA from *S. dysenteriae* type 1 and *Shigella flexneri* (13). Recent studies in our laboratory demonstrated that the cytotoxin produced by *E. coli* K-12(933W) was not neutralized by anti-Shiga toxin, in contrast with our original report on 933W toxin (17). The purposes of this investigation were to compare the biologic activities and antigenic specificities of the 933J and 933W toxins and to determine whether their structural genes are homologous.

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MATERIALS AND METHODS

Bacterial strains. *E. coli* O157:H7 strain 933 was obtained from G. K. Morris, Centers for Disease Control, Atlanta, Ga. *E. coli* C600(933J) and *E. coli* C600(933W) were constructed by lysogenizing *E. coli* K-12 strain C600 with phage 933J or phage 933W (17). *E. coli* H30 (O26:H11) was obtained from J. Konowalchuk, Bureau of Microbial Haz-

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ards, Ottawa, Canada. *S. dysenteriae* type 1 strain 60R was obtained from S. B. Formal, Walter Reed Army Institute of Research, Washington, D.C. Clinical isolates of *E. coli* were obtained from M. A. Karmali, Hospital for Sick Children, Toronto, Canada; from D. Sherwood, Moredun Research Institute, Edinburgh, Scotland; from J. G. Wells, Centers for Disease Control, Atlanta, Ga.; and from B. Rowe, Central Public Health Laboratory, London, United Kingdom. Strains were routinely stored at −70°C in Penassay broth (Difco Laboratories, Detroit, Mich.) supplemented with 15% glycerol.

Toxins. Bacteria were grown for 48 h in Chelex-treated syncase medium as previously described (16). Culture supernatants were collected and bacterial lysates were prepared either by the French press method (16) or by sonication (14).

Polyclonal rabbit antisera. Antibody to purified Shiga toxin was raised in rabbits according to published procedures (15). Antibody to crude 933W toxin was raised in rabbits by a series of 13 intramuscular inoculations of extracellular culture fluid (0.2 ml) or cell extracts (0.05 to 0.2 ml) prepared from *E. coli* C600(933W) as previously described (24), mixed 1:1 with incomplete Freund adjuvant, and administered at irregular intervals over a period of 53 days.

Cytotoxicity assays. Filter-sterilized culture supernatants and bacterial lysates were tested for cytotoxicity (4) on HeLa cells, Vero cells, Chinese hamster ovary cells (CHO), and mouse Y-1 adrenal cells. HeLa cells were grown in Eagle minimal essential medium (Flow Laboratories, Inc., McLean, Va.); Vero and Y-1 adrenal cells were maintained in RPMI 1640 medium (Flow Laboratories); and CHO cells were maintained in alpha Eagle minimum essential medium (Flow Laboratories). Media were supplemented with 10% (vol/vol) fetal calf serum, 0.8 mM glutamine, 500 U of penicillin G per ml, and 500 μg of streptomycin per ml. Microtiter plates (96-well; Costar, Cambridge, Mass.) were inoculated with approximately 10,000 cells per well (100 μl) and incubated overnight at 37°C in the presence of 5% CO_2. Serial dilutions of culture supernatants or bacterial lysates were made in cell culture medium. Samples containing 100 μl of each dilution were added to wells of the microtiter plates, and incubation was continued at 37°C in 5% CO_2. Vero cells were examined daily for 4 days by light microscopy for cytotoxicity, whereas the HeLa, CHO, and Y-1 adrenal cells were examined after 24 h. In each case, the 50% cytotoxic dose (CD_{50}) corresponded to the amount of toxin required to kill 50% of the cells in a well.

Neutralization of cytotoxicity. Toxin from culture supernatants or sonic lysates was serially diluted (twofold for low toxin producers or 10-fold for moderate or high toxin producers) in cell culture medium. A sample of each toxin dilution (100 μl) was mixed with an equal volume of one of the antibodies described below and incubated at 37°C for 1 h followed by overnight incubation at 4°C. Samples (100 μl) of each toxin-serum mixture were added to individual wells of microtiter plates containing HeLa cells, and the cells were examined for cytotoxic effects after incubation overnight at 37°C in 5% CO_2. The antibodies used for neutralization tests included undiluted hybridoma culture supernatant containing monoclonal antibody (MAb) 13C4 specific for the B subunit of Shiga-like toxin (25), MAb 32D3 specific for the B subunit of cholera toxin (5) as a negative control, and 1:50 dilutions in cell culture medium of rabbit antiserum against purified Shiga toxin, rabbit antiserum against crude 933W toxin, or normal rabbit serum as a negative control. Lysates of *E. coli* H30 and *S. dysenteriae* type 1 strain 60R were included in the neutralization assays as toxin controls to demonstrate the neutralizing activities of MAb 13C4 and the rabbit antiserum against Shiga toxin. A sample was considered to contain neutralizable cytotoxin if the dose required to kill 50% of the HeLa cells in the presence of antitoxin antibody was at least fourfold greater for samples diluted twofold or tenfold greater for samples diluted tenfold than the amount of toxin required for equivalent cytotoxicity in the presence of control antibody. These assays for Shiga-like toxins are similar in principle to the assays used to determine L_+ and L_r doses of diphtheria toxin (28).

Mouse lethality. The lethality of crude French press lysates from *E. coli* 933, *E. coli* C600, *E. coli* C600(933J), and *E. coli* C600(933W) was tested according to published procedures (18). The 50% lethal dose (LD_{50}) of each toxin was determined in the following way. Groups of five female (6 to 8 weeks old) CD-1 mice (Charles River Breeding Laboratories, Inc., Kingston, N.Y.) were inoculated intraperitoneally with various doses of bacterial lysates diluted in phosphate-buffered saline that contained 0.1% (wt/vol) gelatin. The mice were observed daily over a 10-day period for hind-leg paralysis and death. The LD_{50}s were calculated by the method of Reed and Muench (21).

Enterotoxicity assay. French press lysates of *E. coli* 933, *E. coli* C600, *E. coli* C600(933J), and *E. coli* C600(933W) were tested for enterotoxic activity in ligated rabbit ileal segments as previously described (3). One milliliter of bacterial lysate (undiluted) was inoculated per segment. Each sample was tested in eight ligated segments of different rabbits. A sample was considered to contain enterotoxin if the average of the volume-to-length ratios of the eight inoculated segments was ≥1.0 ml/cm.

Restriction endonuclease digestion and hybridization analysis of 933W phage DNA. DNA from phages 933W and 933J was prepared as previously described (17) and digested with *Bam*HI, *Sal*I, or *Eco*RI (International Biotechnologies, Inc., New Haven, Conn.) according to the recommendations of the manufacturer. DNA fragments were separated electrophoretically on 0.7% (wt/vol) agarose gels, blotted onto nitrocellulose paper, and probed as previously described (2, 12). Probe pJN25 contains a 3.0-kilobase insert from phage 933J that encodes the 933J cytotoxin (13). Approximately half of the 3.0-kilobase insert from phage 933J is estimated to code for toxin, while the other half represents phage sequences. The pJN25 probe was radiolabeled by nick translation using ^{32}P-labeled dCTP and a commercially available kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). The hybridization with the ^{32}P-labeled pJN25 probe was performed in 5× SSPE (0.9 M NaCl, 50 mM $NaH_2PO_4 \cdot 2H_2O$, 5 mM disodium EDTA; pH 7.4) at 50°C or 65°C overnight. Washing was done at room temperature if the hybridization was performed at 50°C or at 50°C if the hybridization was performed at 65°C. As a control, the digested 933W phage DNA was also probed with ^{32}P-labeled pBR328 (the vector plasmid contained in pJN25) under conditions allowing approximately 36% mismatch. The blots were exposed to Kodak X-AR film (Eastman Kodak Co., Rochester, N.Y.) in the presence of intensifying screens.

RESULTS

Biologic activities of the 933J and 933W toxins. As shown in Table 1, lysates of *E. coli* 933, *E. coli* C600, *E. coli* C600(933J), and *E. coli* C600(933W) had the same specificity for killing cultured cells. Lysates from all four strains killed HeLa and Vero cells but did not affect Chinese hamster ovary cells or Y-1 adrenal cells. Culture supernatants of *E.*

TABLE 1. Comparison of the biologic activities of Shiga-like toxins in cell extracts of *E. coli*[a]

E. coli strain	Cytotoxicity for:				Paralysis and death in mice	Enterotoxicity in ligated rabbit ileal segments
	HeLa cells	Vero cells	CHO cells	Y-1 cells		
933	+ (high)	+ (high)	−	−	+	+
C600	+ (low)	+ (low)	−	−	−	−
C600(933J)	+ (high)	+ (high)	−	−	+	+
C600(933W)	+ (moderate)	+ (moderate)	−	−	+	+

[a] Symbols: +, presence of biologic activity; −, absence of biologic activity. The level of cytotoxicity (Marques et al., in press) is given in parentheses: high, 10^5 to 10^8 CD_{50}/ml in sonic lysates; moderate, 10^3 to 10^4 CD_{50}/ml in sonic lysates; low, 2×10^1 to 6×10^2 CD_{50}/ml in sonic lysates.

coli 933, *E. coli* C600(933J), and *E. coli* C600(933W) were also selectively cytotoxic for HeLa cells (Table 2) and Vero cells (not shown), but the culture supernatant of *E. coli* C600 contained no demonstrable cytotoxic activity. This is the same pattern of cytotoxicity that has been reported for Shiga toxin from *S. dysenteriae* type 1 (9, 19).

The amounts of cytotoxin present in extracts of *E. coli* 933, *E. coli* C600, *E. coli* C600(933J), and *E. coli* C600(933W) differed greatly (Tables 1 and 2). Typically, sonic lysates of *E. coli* 933 were 10,000- to 100,000-fold more toxic than lysates from *E. coli* C600. Lysates from *E. coli* C600(933J) were approximately 1,000-fold more toxic than lysates from *E. coli* C600(933W). In contrast, the toxicity of sonic lysates of *E. coli* C600 did not exceed 100 CD_{50}/ml and was much lower than the toxicity of lysates from C600(933J) or C600(933W). The cytotoxicity of extracts prepared by the French press method was typically greater than the cytotoxicity of sonic lysates by about 10-fold.

French press lysates of *E. coli* 933, *E. coli* C600(933J), and *E. coli* C600(933W) were capable of paralyzing and killing mice. The lysate of *E. coli* C600 did not cause these effects (Table 1), presumably because the amount of cytotoxin in *E. coli* C600 lysates was below the level necessary for mouse lethality (O'Brien, et al., Lancet ii:573, 1983). In two experiments, the number of LD_{50}s per milligrams of protein for *E. coli* C600(933J) was 160 to 170, whereas for *E. coli* C600(933W) the number of LD_{50}s per milligrams of protein was 8 to 14. The number of CD_{50}s corresponding to one LD_{50}s was 40- to 70-fold greater for 933J toxin than for the 933W toxin.

Lysates of *E. coli* 933, *E. coli* C600(933J), and *E. coli* C600(933W) also had enterotoxic activity in the rabbit ligated ileal segment assay. The *E. coli* C600 lysate did not cause fluid accumulation (Table 1), but because the number of CD_{50}s contained in 1 ml of C600 lysate was also expected to be below the threshold for enterotoxicity (O'Brien et al., Lancet ii:573, 1983). Dose-response curves were not performed in ileal segments because the number of rabbits available was limited. The data in Tables 1 and 2 demonstrate that the 933J and 933W toxins have the same kinds of biologic activities. The greater lethality relative to cytotoxicity for 933W toxin was reproducible and was the most striking difference between the biologic activities of these two toxins.

Antigenic relationships between the 933J and 933W toxins. Cross-neutralization studies were done to assess the antigenic relationship between the 933J and 933W toxins (Table 2). In these assays, a constant amount of antibody was mixed with various amounts of lysate or supernatant. Under these circumstances, cytotoxicity is detected if the amount of homologous cytotoxin exceeds the neutralizing capacity of the antitoxin, or heterologous (non-neutralizable) cytotoxin is present, or both. To distinguish among these alternatives, samples of lysates or supernatants were also treated with mixtures of antisera capable of neutralizing the 933J and 933W toxins. The cytotoxic activities in the lysate and culture supernatant of *E. coli* C600(933J) were neutralized by monoclonal antibody against the B subunit of Shiga-like toxin (MAb 13C4) or by anti-Shiga toxin, but they were not neutralized by anti-933W toxin. In contrast, the cell-associated and cell-free cytotoxins from *E. coli* C600(933W) were neutralized by anti-933W toxin, but they were not

TABLE 2. HeLa cytotoxicity of culture supernatants and lysates from *E. coli* strains in the presence or absence of antisera against Shiga and Shiga-like toxins[a]

E. coli strain	CD_{50}/ml	No. of CD_{50} required for cytotoxicity in the presence of:					
		α-CT MAb 32D3	NRS	α-SLT MAb 13C4	Rabbit α-Shiga	Rabbit α-933W	Rabbit α-Shiga + α-933W
C600(933J)							
Lysate	10,000,000	1	1	1,000	10,000	1	10,000
Supernatant	100,000	1	1	1,000	1,000	1	1,000
C600(933W)							
Lysate	10,000	1	1	1	1	100	100
Supernatant	10,000	1	1	1	1	100	100
933							
Lysate	10,000,000	1	1	100	100	1	10,000
Supernatant	1,000,000	1	1	1	1	100	10,000

[a] Rabbit sera were used at dilutions of 1:50, and the monoclonal antibodies were used as undiluted hybridoma culture supernatant. Neutralization controls included lysates from *S. dysenteriae* 1 strain 60R and *E. coli* H30. The activity in these lysates was neutralized by anti-Shiga toxin and α-Shiga-like (α-SLT) MAb but not by α-cholera toxin (α-CT) MAb, normal rabbit serum (NRS) or rabbit anti-933W. The lysates were prepared from suspensions of bacterial cells that had been concentrated approximately 50-fold with respect to the original cultures from which the supernatants were obtained, and the data shown are representative of the results of many experiments. The titer of a sonic lysate of *E. coli* C600 was 100 CD_{50}/ml. Culture supernatants of *E. coli* C600 were not cytotoxic.

neutralized either by MAb 13C4 or by anti-Shiga toxin. This lack of cross-neutralization demonstrates that the phage-encoded 933J and 933W toxins are antigenically distinct.

The cytotoxicity of extracts of *E. coli* C600 was usually neutralized by MAb 13C4 and by rabbit anti-Shiga toxin (data not shown), confirming previous reports (16, 25), but the rabbit anti-933W toxin did not neutralize the *E. coli* C600 cytotoxin. Occasionally either no activity could be detected with *E. coli* C600 lysates or they exhibited low titers of nonspecific cytotoxicity that could not be neutralized either with anti-Shiga toxin or with anti-933W toxin.

The lysate of *E. coli* 933 contained a high level of cytotoxic activity (Table 2) which was neutralized by MAb 13C4 or anti-Shiga toxin, but which was not neutralized by rabbit anti-933W toxin. However, a mixture of rabbit anti-Shiga toxin and rabbit anti-933W toxin neutralized the toxicity of the *E. coli* 933 lysate to a greater extent than anti-Shiga toxin alone, suggesting that a second toxin was present at a lower level. In contrast, the mixture of antitoxins was no more effective against the toxin from C600(933J), or C600(933W) than was the single, active antitoxin in the mixture. These control experiments demonstrated that anti-Shiga toxin and anti-933W antitoxins did not act additively or synergistically to enhance the neutralization of 933J or 933W cytotoxin. Therefore, the results obtained with lysates of strain 933 demonstrated the presence of two distinct cytotoxins which were antigenically related to the toxins encoded by phages 933J and 933W.

Culture supernatants of *E. coli* 933 also contained 933J and 933W toxin, but their relative titers varied from one supernatant to another. This variability has led to inconsistent neutralization results for the extracellular cytotoxicity with anti-Shiga toxin. Our findings with this antiserum and culture supernatants from strain 933 have ranged from complete neutralization (O'Brien et al., Lancet **i**:702, 1983) to partial or no demonstrable neutralization. A second problem that has also led to inconsistent neutralization results is the loss of 933W activity in some cultures of 933 left at 4°C on agar plates for more than 3 months.

In the experiment presented in Table 2, the relative titers of 933J and 933W toxin in the culture supernatant of strain 933 were the reverse of those seen in cell lysates. The anti-933W toxin neutralized cytotoxic activity in culture supernatants, but neither anti-Shiga toxin nor monoclonal antibody to Shiga-like toxin had this effect. That the 933J toxin was in fact present in culture supernatants was evident from the more dramatic reduction in cytotoxic activity when a mixture of anti-Shiga toxin and anti-933W toxin was used rather than anti-933W toxin alone.

Neutralization of the cytotoxic activity from clinical isolates of *E. coli*. Marques et al. (L. R. M. Marques, M. A. Moore, J. G. Wells, I. K. Wachsmuth, and A. D. O'Brien, J. Infect. Dis., in press) recently surveyed 418 *E. coli* strains for production of Shiga-like toxin and identified 49 elevated cytotoxin-producing isolates (moderate and high producers). The cell-associated cytotoxicity of 39 of these strains was neutralized by anti-Shiga toxin, but for 10 strains the cell-associated cytotoxicity could not be neutralized by anti-Shiga toxin. In the present study, the antiserum prepared for analysis of the 933W-encoded toxin was used (on the basis of the neutralization data in Table 2) to determine whether the cell-associated cytotoxicity of the 10 strains not neutralized by anti-Shiga toxin could be neutralized by anti-933W crude toxin. The isolates that produced toxin which was not neutralized by anti-Shiga toxin included four strains (O157:H7) from patients with hemorrhagic colitis, two strains (O113:H21) from patients with hemolytic uremic syndrome, one strain (O157:H7) from a patient with diarrhea, and three strains (O4:NM, O8:H9, O149:H8) from calves with diarrhea. The cytotoxic activity in lysates from all 10 isolates was neutralized by the rabbit antiserum against crude 933W toxin. In addition, the cell-associated cytotoxic activity of one *E. coli* O157:H^- strain (E32511) described by Scotland et al. (S. M. Scotland, H. R. Smith, and B. Rowe, Letter, Lancet **i**:885–886, 1985) was only neutralized by rabbit anti-933W toxin. The cytotoxic activity in culture supernatants of E32511 and of 27 strains reported by Marques et al. (Marques et al., in press) as not neutralized by anti-Shiga toxin, was found in this investigation to be neutralized by either anti-Shiga toxin plus anti-933W toxin or anti-933W toxin alone (the 10 strains described above). Therefore, some clinical isolates produce cytotoxin antigenically related to the 933W toxin, and this antigenic variant of Shiga-like toxin may play a role in the pathogenesis of diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. The relative contributions of the 933J and 933W types of Shiga-like toxin to the virulence of *E. coli* strains that produce both toxins remains to be determined.

Homology of the 933J and 933W toxin genes. To compare the relatedness of the toxin genes at the DNA level, Southern hybridization experiments were performed between restriction fragments of phage 933W DNA and the pJN25 DNA probe for the intact structural genes for Shiga-like toxin from phage 933J. Under the more stringent hybridization conditions tested, estimated to permit no more than 26% base-pair mismatch, pJN25 hybridized to at least one band from phage 933W DNA digested with *Eco*RI, *Sal*I, or *Bam*HI, either singly or in pairwise combinations (Fig. 1). In control experiments, labeled DNA from the pBR328 vector did not hybridize to the restricted 933W phage DNA under less stringent conditions, estimated to permit up to 36% mismatch (data not shown). These results demonstrate that the cloned DNA insert is responsible for the hybridization of the pJN25 probe with the 933W DNA and provide evidence that the 933J and 933W toxins have homologous nucleotide sequences. Definitive proof for their homology, however, will require the use of probes that are known to be completely internal to the toxin structural genes.

DISCUSSION

The major conclusion from this study is that the Shiga-like toxin encoded by phage 933W is antigenically distinct from, but homologous with, the Shiga-like toxin encoded by phage 933J. Data from the cross-neutralization experiments with the polyclonal antisera and with MAb 13C4, which is specific for an epitope on the B subunit of Shiga-like toxin (25), did not demonstrate any shared epitopes between the 933W and 933J toxins. Nevertheless, the 933J- and 933W-encoded toxins appear to be homologous both functionally and genetically. Their similar biologic activities, as well as their identical target cell specificities (Table 1), suggest that both their receptor-binding function and their mode of action, which in the case of Shiga toxin is inhibition of protein synthesis (22), may be similar. One difference in the biologic activities of the 933W and 933J toxins is the ratio of LD_{50} to CD_{50} per milligram of cell lysate protein. The genetic relatedness of the 933W and 933J toxins is suggested by the homology between the cloned 933J toxin genes and 933W phage DNA in the Southern hybridization studies.

Evidence supporting the existence of antigenically distinct cytotoxins from *E. coli* has been reported by several investigators (7, 10, 11, 24, 27; Scotland et al., Lancet **i**:885–886,

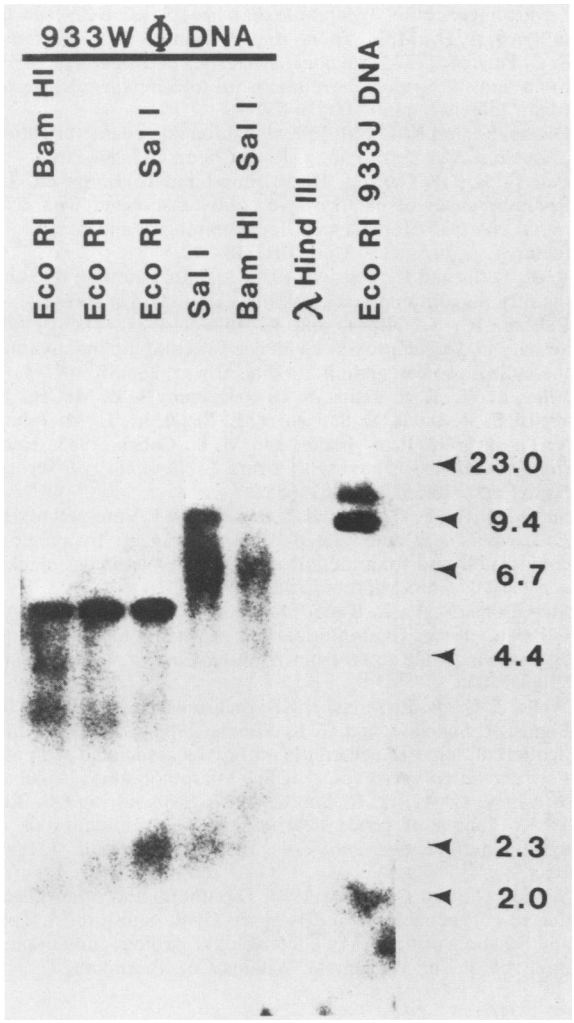

FIG. 1. Southern hybridization of 933W phage DNA probed with ^{32}P-labeled pJN25, a plasmid containing the structural genes for Shiga-like toxin from phage 933J. Hybridization of pJN25 was performed in an aqueous solution using 5× SSPE overnight at 65°C. The autoradiograph represents a 4-day exposure in the presence of an intensifying screen. Fragments of lambda DNA cut with *Hin*dIII (Bethesda Research Laboratories) were used as molecular-size markers. The multiple bands observed in some of the lanes indicate that the restriction enzyme(s) cut one or more times within the DNA sequences which show homology with the probe.

1985). To avoid confusion, the nomenclature for the antigenic variants should be addressed. We propose that the name Shiga-like toxin I be applied to the 933J toxin and related toxins which are neutralized by antiserum against Shiga toxin from *S. dysenteriae* type 1 strain 60R. The name Shiga-like toxin II is suggested for the 933W toxin and toxins neutralized by antibodies to 933W toxin. Specific antisera raised against each purified prototype toxin should be used to identify which antigenic variant is produced. We have made antibodies against purified Shiga toxin and monoclonal antibodies against Shiga-like toxin I from *E. coli*, and we are now in the process of purifying the 933W toxin and raising polyclonal and monoclonal antibodies against it. Once purified 933W toxin is available, we will also be able to compare directly the specific activities of the 933J and 933W toxins in each of the available bioassays.

Scotland et al. (Scotland et al., Lancet **i**:885–886, 1985) have suggested the names verotoxin 1 (VT1) for the toxin encoded by genes which hybridize with *S. dysenteriae* type 1 and *E. coli* H19 (O26:H11) and verotoxin 2 (VT2) for an antigenically distinct toxin produced by *E. coli* E32511 (O157:H$^-$). It is now clear that Shiga-like toxin I and VT1 are different names applied to the same serological group of toxins (13; O'Brien et al., Lancet **i**:702, 1983; Scotland et al., Lancet **i**:885–886, 1985). The name verotoxin has been used in reference to the Vero cell cytotoxin from *E. coli* that was originally described in 1977 by Konowalchuk et al. (11). Thus, the term verotoxin was coined before the recognition that the Vero cell- and HeLa cell-active toxins produced by *E. coli* and *S. dysenteriae* 1 are antigenically related (25; O'Brien et al., Lancet **i**:702, 1983). We prefer the name Shiga-like toxin I to VT1 for the following reasons. (i) It acknowledges the precedence of the name Shiga toxin (1; M. Neisser, and K. Shiga, Letter, Dtsch. Med Wochenschr. **29**:61, 1903) for toxins with the biological activities summarized in Table 1. (ii) It emphasizes the antigenic and genetic relationships of the toxin with Shiga toxin. (iii) Cytotoxicity for Vero cells is a property of many cytotoxins that is not unique to Shiga-like toxin. Shiga-like toxin II and VT2 appear to be the same toxin as determined by neutralization of *E. coli* E32511 cytotoxic activity with rabbit anti-933W toxin. It is not yet established whether Shiga-like toxin II is produced by bacteria other than *E. coli*. The definitive relationships between the members of the family of Shiga-like toxins will ultimately be defined at the genetic level by the nucleotide sequences of their structural genes. Such information will also provide a definitive basis for establishing a nomenclature that accurately reflects the relationships between Shiga toxin and the Shiga-like toxins.

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