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Role of a 60-Megadalton Plasmid and Shiga-Like Toxins in the Pathogenesis of Infection Caused by Enterohemorrhagic *Escherichia coli* O157:H7 in Gnotobiotic Piglets

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Enterohemorrhagic *Escherichia coli* (EHEC) of serotype O157:H7 has two putative virulence factors: (i) a fimbrial adhesin, specified by a 60-megadalton (MDa) plasmid, and (ii) bacteriophage-specified cytotoxin(s), known as Shiga-like toxin (SLT) or verotoxin. The contribution of these factors to the pathogenesis of EHEC-induced disease in gnotobiotic piglets was examined. The bacterial strains included the following: two EHEC strains and their corresponding plasmid-cured derivatives; another EHEC isolate and its derivative which had spontaneously lost the ability to produce SLT; one *E. coli* K-12 transconjugatant containing a 60-MDa plasmid from an EHEC strain; two K-12 strains into which an SLT-producing phage had been transduced (one of these strains also carried a 60-MDa EHEC-derived plasmid); and the parent K-12 strain. Each strain was fed to four piglets, which were observed for diarrhea and examined for development of characteristic mucosal lesions 3 or 5 days after inoculation. All 24 piglets inoculated with the three EHEC strains and their respective derivatives (two plasmid cured and one SLT negative) showed the typical mucosal lesions of bacterial attachment: effacement of microvillous border and cell membrane dissolution culminating in destruction of surface and glandular epithelium in the cecum and colon. No such lesions were observed in 12 piglets inoculated with three strains of *E. coli* K-12, including the strain which carried both the 60-MDa plasmid and a phage which specified production of SLT. Moderate to severe diarrhea was observed in 16 piglets inoculated with two EHEC strains and their derivatives (one plasmid cured and one SLT negative). The third EHEC strain and its plasmid-cured derivative produced fewer typical mucosal lesions and no diarrhea. The reason for the reduced virulence of this strain was not clear. These results demonstrate that neither the 60-MDa plasmid nor the capacity to produce SLT is essential for expression of virulence by *E. coli* O157:H7 in gnotobiotic piglets.

Hemorrhagic colitis, caused by *Escherichia coli*, is a newly recognized disease which is clinically and epidemiologically distinguishable from other infections caused by pathogenic *E. coli*. Hemorrhagic colitis has been attributed to *E. coli* strains belonging to serotype O157:H7 (26; W. M. Johnson, H. Lior, and G. S. Bezanson, Lancet i:76, 1983), although recent studies have demonstrated the existence of strains from other serogroups that fulfill the same clinical and pathological criteria (17; S. Tzipori, I. K. Wachsmuth, J. Smithers, and C. Jackson, Gastroenterology, in press). Strains of *E. coli* O157:H7 have also been implicated in sporadic cases (22) and outbreaks (28a) of hemolytic uremic syndrome and possibly other conditions (4; W. R. Grandsen, M. A. S. Damm, J. D. Anderson, J. E. Carter, and H. Lior, Lancet ii:150, 1985). *E. coli* strains that cause these clinical syndromes have been referred to as enterohemorrhagic *E. coli* (EHEC) (15).

The mechanism(s) by which EHEC strains cause diarrhea is not clear. They possess a 60-megadalton (MDa) plasmid which encodes fimbriae that mediate bacterial attachment to cultured Henle 407 intestinal cells (9). They also liberate one or two bacteriophage-determined toxin(s) which are cytotoxic to Vero and HeLa cells and are known as Shiga-like toxins 1 and 2 (SLT-I and SLT-II) or verotoxins 1 and 2 (VT-1 and VT-2) (29). Recent experiments in newborn rabbits (25) and mice (2) have implicated VT as a probable factor contributing to diarrhea.

E. coli O157:H7 induces characteristic, reproducible mucosal lesions in the ceca and colons of gnotobiotic piglets (7, 31). This animal model has been used to investigate the pathogenesis of the infection and to demonstrate the ability of *E. coli* serotypes other than O157:H7 to induce similar colonic lesions (Tzipori et al., in press).

The present study utilized gnotobiotic piglets to investigate the contribution of the 60-MDa plasmid and SLT to the pathogenesis of EHEC. This was achieved by infecting gnotobiotic piglets with strains of *E. coli* O157:H7 which no longer carried the plasmid or bacteriophages specifying the SLT gene and with *E. coli* K-12 strains to which the plasmid and bacteriophage specifying production of either SLT-I or SLT-II had been transferred.

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TABLE 1. Summary of experimental inoculation of piglets[a] with strains of *E. coli* serotype O157:H7, *E. coli* K-12, and their derivatives

Strain	Serotype	60-MDa plasmid present	SLT type produced	Diarrhea	Characteristic mucosal lesions
EDL 933	O157:H7	+	I, II	+	+
EDL 933c	O157:H7	−	I, II	+	+
A7785-C3B	O157:H7	+	I, II	−	+
A7785-C3A	O157:H7	−	I, II	−	+
84-289	O157:H7	+	I, II	+	+
85-170	O157:H7	+	None	+	+
C600 (K-12)		−	None	−	−
C600/2 (K-12)		−	II	−	−
C600/1 (K-12)		+	I	−	−

[a] Four piglets per strain.

MATERIALS AND METHODS

Bacteria. The bacterial strains used in this study are listed in Table 1. *E. coli* EDL 933 and A7785-C3 were isolated during an outbreak of hemorrhagic colitis in Michigan (26), and strain 84-289 was isolated from a food handler in a nursing home in Canada. Strain A7785-C3B is a streptomycin-resistant derivative obtained by cultivating the parent strain A7785-C3, which was isolated from the same patient as EDL 933, on agar containing 20 μg of streptomycin per ml. The 60-MDa plasmid carried by strain A7785-C3B was eliminated by conjugally transferring into it a temperature-sensitive F′ *lac* plasmid containing Tn*5*, a kanamycin-resistance transposon. The resulting transconjugates, selected on media containing kanamycin and streptomycin, had lost the 60-MDa plasmid. Subsequently, the F′ *lac*::Tn*5* plasmid was lost spontaneously to produce strain A7785-C3A. Strain 85-170 is an SLT-negative derivative of *E. coli* 84-289 which, during storage in the laboratory, spontaneously lost the ability to produce SLT-I and SLT-II. Strain EDL 933c was obtained by eliminating a 60-MDa plasmid, pEDL933, from strain EDL 933 by treatment with ethidium bromide. This plasmid was also transferred to *E. coli* C600, a substrain of *E. coli* K-12. To accomplish this, pEDL933 was first marked by insertion of a transposon, Tn*801*, which encodes ampicillin resistance. This was achieved by transferring the temperature-sensitive donor plasmid pMR5 (27), which contained Tn*801*, to EDL 933 by conjugation. Transconjugants selected on ampicillin-containing agar were cultured at the restrictive temperature of 42°C. This facilitated transposition of Tn*801* into pEDL933 and loss of pMR5. The pEDL933::Tn*801* conjugate was then transformed into *E. coli* C600 by using the technique described by Cohen et al. (5). This strain was subsequently transduced with a bacteriophage specifying production of SLT-I to yield strain C600/1.

E. coli K-12 strain C600 was transduced with a bacteriophage containing the SLT-II gene from *E. coli* O157:H7 to become C600/2, which produces SLT-II.

Loss of the 60-MDa plasmid in the cured strains EDL 933c and A7785-C3A was confirmed by hybridization studies using a DNA probe which hybridizes with EHEC plasmid (17).

Transduction with bacteriophage. For phage induction, 100 μl of an overnight culture of the SLT-producing strain was inoculated into 20 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and grown to an optical density of 0.5. Bacteria were harvested by centrifugation and suspended in 5 ml of 10 mM $CaCl_2$. This suspension was induced for 1 min under a UV lamp, and 0.5 ml of the irradiated bacteria was then incubated in 20 ml of fresh Trypticase soy broth in foil-covered tubes with shaking for 5 h at 37°C.

After the bacteria were removed by centrifugation, the supernatant was sterilized by membrane filtration (0.45-μm pore size). Serial dilutions of supernatant were then mixed with 100 μl of the recipient strain of *E. coli* C600 and incubated for 30 min at 37°C. A 100-μl sample of this mixture was plated in 3 ml of top layer (0.6% agar). One plaque, consisting of 10^6 phage particles, was picked with a Pasteur pipette, suspended in 0.5 ml of buffer (10 mM Tris, pH 7.5, 50 mM NaCl, 7 mM $MgCl_2$), and incubated for 4 h at room temperature with shaking. This suspension was sterilized by filtration; 100 μl of the filtrate was mixed with 100 μl of log-phase indicator cells (optical density, 0.5) and plated as described above. This procedure was repeated twice to plaque purify the phage. The growth from within a plaque was picked, suspended in broth, incubated overnight, and plated on Trypticase soy agar. Plates were flooded with an undiluted preparation of the stock phage suspension and incubated for a further 24 h.

Stable derivatives lysogenized with the phage were identified by their resistance to the phage. After single colonies were subcultured, culture supernatants and lysates from *E. coli* C600 lysogenized with the phage were tested on HeLa cells for cytotoxicity and cytotoxin-neutralization by anti-Shiga toxin or anti-SLT-II. One phage that was transduced from the wild-type strain was considered to be the 933J phage on the basis of its appearance on electron microscopy and the capacity of anti-Shiga toxin to completely neutralize the cytotoxic activity of lysates of strain C600/1 bacteria lysogenized with this phage. Cytotoxic activity of strain C600/2 supernatant was not neutralized by anti-Shiga toxin but was neutralized by antitoxin to SLT-II.

Preparation of inocula. Bacteria were stored at −20°C in 34% (vol/vol) glycerol. Before use they were cultured on 5% horse blood agar at 37°C overnight. Approximately 20 colonies of each strain were inoculated into 50 ml of Trypticase soy broth containing 0.6% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) in a 250-ml conical flask. Flasks were incubated at 37°C overnight in an orbital shaker set at 300 rpm. Bacteria were pelleted by centrifugation at 10,000 × *g* for 10 min and resuspended in 1/10 the original volume of spent medium. The number of bacteria inoculated was adjusted to contain between 1×10^{10} and 2×10^{10} CFU in 2 ml of inoculum. Bacteria used to inoculate piglets were characterized according to plasmid profile and biotype (24) and, where appropriate, by serotyping and resistogram typing. The plasmid content of each strain was determined by performing agarose gel electrophoresis on DNA extracted from bacteria by the method of Birnboim and Doly (3). The multiple typing scheme was also used to confirm the identity of bacteria recovered from blood or intestinal contents of infected piglets.

Experimental animals. The derivation and maintenance of gnotobiotic piglets have been described previously (18). Briefly, 36 piglets derived from four litters were inoculated 24 h after delivery, when it was clear that they were healthy and drinking well. The piglets were divided into nine groups, one for each bacterial strain (Table 1). They were inoculated orally, in pairs, each strain being tested on two occasions in piglets from two separate litters. Piglets were observed regularly for signs of diarrhea and illness. At 3 or 5 days after inoculation, or when severe illness developed, repre-

sentative piglets from each group were euthanized by injection of sodium pentobarbitone.

Necropsy procedure. Piglets were examined for gross pathological changes. Samples were taken from five equally spaced sites in the small intestine and from the stomach, cecum, colon, and mesenteric lymph nodes. After fixation in buffered Formalin, samples were sectioned for light and electron microscopy (31).

The proximal, mid, and distal small intestine, spiral colon, and heart blood were cultured quantitatively. Serial 10-fold dilutions of homogenized gut contents and mucosal scrapings or of blood were prepared in sterile physiological saline, plated on paired 5% horse blood and MacConkey agar plates, and incubated at 37°C.

Cytotoxicity assay. Serum samples collected from piglets at necropsy were filtered through 0.2-μm-pore-size disposable membrane filters (Millipore Corp., Bedford, Mass.). Serial two-fold dilutions in phosphate-buffered saline were then tested in HeLa cells for cytotoxicity. The nine bacterial strains used to inoculate the piglets were also tested for cytotoxicity in vitro (Table 2). Samples of 0.5 ml of overnight broth culture of each strain were added to 10 ml of Evans medium (6), modified by omission of iron, and incubated with shaking at 37°C for 5 h (10), after which each culture was centrifuged to pellet the cells and the supernatant was passed through a 0.2-μm-pore-size membrane filter. Samples were tested either undiluted or after serial twofold dilutions starting at 1:10. Confluent monolayers of HeLa cells in 96-well microtiter trays were inoculated with 50 μl of each dilution of serum or culture fluid and examined over a period of 48 h. Each dilution was tested in duplicate and repeated on two occasions. Microtiter trays were incubated at 37°C with 5% CO_2 and examined daily for typical cytotoxicity (13). The cytotoxicity titer of each sample tested was taken as the reciprocal of the highest dilution which killed 50% or more of the cell monolayer after 48 h of incubation.

Gut loop experiments. The effect of SLT on fluid accumulation in rabbit and pig gut loops was investigated to determine whether the intestines of these two species were equally sensitive to the action of SLT. *E. coli* strains which were investigated included the following: EDL 933 (O157:H7); H30 (O26), a well-known producer of SLT-I (13,22); HS (O9:H4), a nonpathogenic human *E. coli* strain (23); and a pig enterotoxigenic *E. coli* strain of serogroup O64, which produces heat-stable enterotoxin detectable in infant mice and in gut loops of piglets. The three human strains were grown in 20 ml of modified Evans medium with shaking for 5 h, pelleted, and suspended on 1 ml of polymyxin B suspension (0.1 mg/ml). After incubation for 30 min at 37°C, the supernatant was filtered through a 0.2-μm-pore-size Millipore filter and stored in 1-ml fractions at −70°C. Nine-day-old conventional piglets were anesthetized, and 10-cm-long jejunal loops were inoculated with 1 ml of each of the three human *E. coli* strains or with 1 ml of culture supernatant of the pig enterotoxigenic *E. coli* strain grown overnight in Trypticase soy broth. Intestinal loops were exteriorized, and the amount of fluid which had accumulated per centimeter of loop was measured 6 h after inoculation (19). The same procedure was repeated in 2-month-old rabbits (12). Each preparation was assayed twice.

TABLE 2. Cytotoxic activity on HeLa cells of sera from gnotobiotic piglets infected with nine strains of *E. coli* and of filtrates obtained from cultures of 10^{10} bacteria

E. coli strain	Cytotoxicity titer per 50 μl of:	
	Serum[a]	Culture filtrate
EDL 933	8	40
EDL 933c	8	40
A7785-C3B	4	20
A7785-C3A	8	20
84-289	4	80
85-170	<1	<1
C600	<1	<1
C600/2	4	20
C600/1	8	20

[a] Values are the means from four animals.

RESULTS

Clinical observations. Diarrhea of variable severity occurred in all 16 piglets inoculated with two strains of *E. coli* O157:H7 (EDL 933 and 84-289) and with their derivatives cured of either the 60-MDa plasmid (EDL 933c) or the ability to produce SLT (85-170). None of the other five strains caused diarrhea or any other illness (Table 1).

Diarrhea began usually on day 3 and continued until necropsy in piglets killed 5 days after inoculation. The diarrhea was voluminous, but not watery. Piglets with diarrhea appeared thinner and had soiled perineal regions. Five piglets also developed anorexia on day 4 or 5 (strains EDL 933, EDL 933c, and 84-289). Two piglets from the same litter developed signs consistent with endotoxic shock within 48 h after inoculation (EDL 933 and C600/1), and one piglet infected with EDL 933c developed ataxia 4 days after inoculation. There was some variation in the severity of diarrhea in piglets from different litters even when given the same bacterial strain.

Necropsy findings. Piglets with diarrhea had excessive amounts of fluid in the distal small intestine and the large intestine. The large intestine was congested and often had a gelatinous appearance due to edema.

Extensive mucosal lesions were only present in piglets which had diarrhea. The eight piglets infected with strain A7785-C3B and its plasmid-cured derivative A7785-C3A experienced no diarrhea but had characteristic focal lesions in the mucosa. Therefore all strains of *E. coli* O157:H7, with or without the 60-MDa plasmid and with or without the phage-determined SLT gene, were capable of inducing the characteristic lesions in the mucosa of the large intestine. The distribution of the lesions was similar in all the animals examined and resembled those described in earlier studies (7, 31). The cecum was the organ most extensively affected, while in the spiral colon, lesions were patchy and varied from extensive at the proximal end to focal at the distal end. The terminal ileum was less frequently affected (in 7 of 28 piglets), usually with only a few foci present. Involvement of the terminal ileum was unrelated to strain of bacterium, time of necropsy, or severity of diarrhea.

The sequence of events and the nature of the mucosal lesions have been described in detail elsewhere (31). Briefly, lesions were characterized by bacteria attaching to surface and glandular epithelium, effacement of microvillous border, and dissolution of the underlying cell membrane, which resulted in destruction of the cells (Fig. 1 through 4). Invasion into deeper tissue was accomplished by several mechanisms which included sequestration and destruction of cells and infiltration of bacteria into the lamina propria and interglandular spaces either by penetration through the intercellular space or by limited endocytosis (Fig. 2 through 5).

Bacterial counts. Colonization of the gastrointestinal tract by bacteria was extensive and quite uniform in the lower

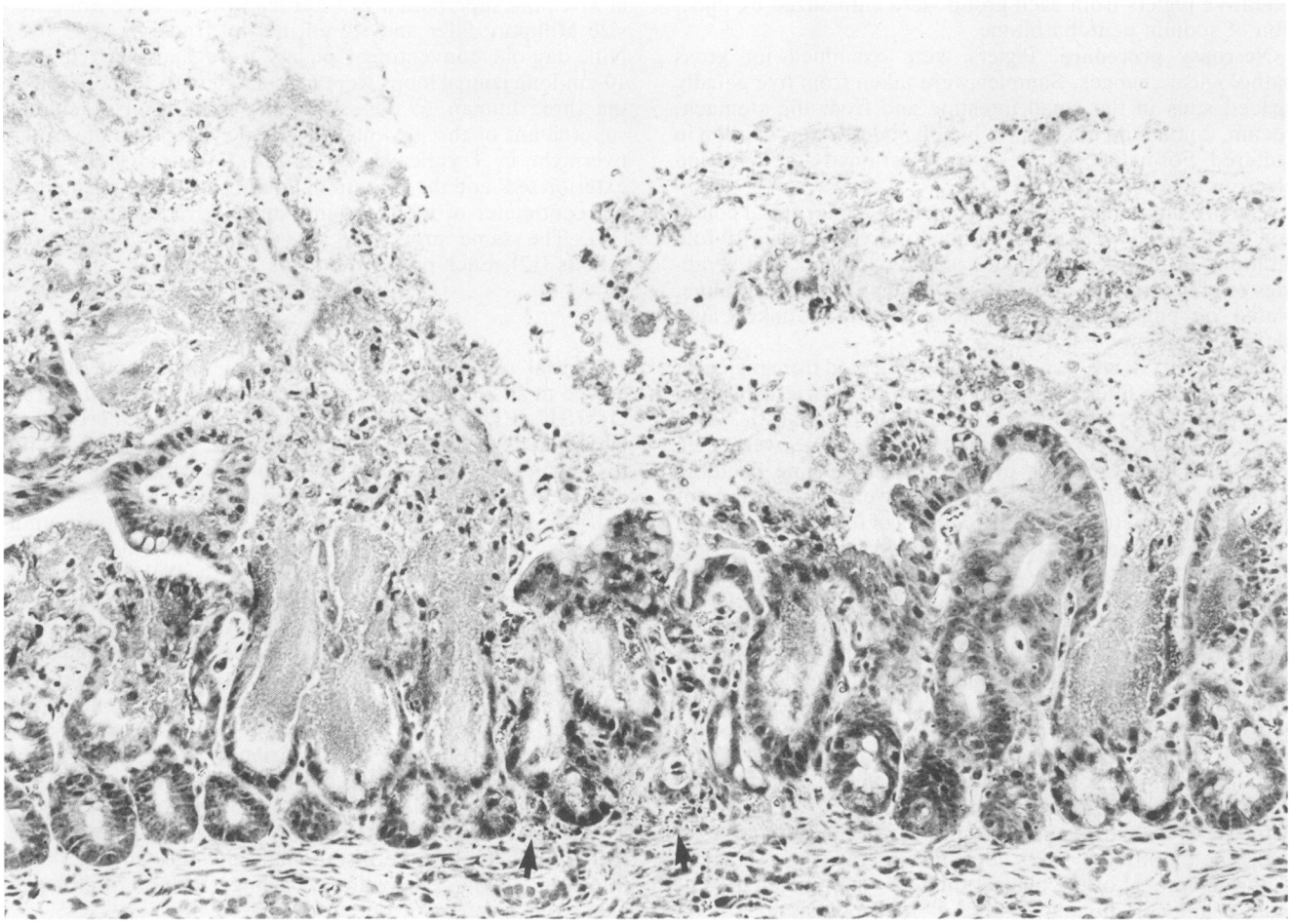

FIG. 1. Section of the cecum of a piglet necropsied 5 days after inoculation with *E. coli* O157:H7 strain EDL 933c (plasmid cured). Note the complete loss of mucosal architecture. In some crypts, which are filled with bacteria, the glandular epithelium is completely destroyed. There are areas of direct communication between the submucosa, through the interglandular space, and the lumen. Cellular necrosis can be seen in some areas below crypts (arrows). Cell debris, erythrocytes, leukocytes, bacteria, mucus, and, presumably, plasma proteins form a pseudomembrane. Note little or no inflammation. (Hematoxylin plus eosin stain; magnification, ×100.)

small intestine and in the large bowel, irrespective of the bacterial strain. Bacterial counts were between 10^8 and 10^{10} CFU/g of gut scrapings in the terminal ileum and from 10^9 to 10^{10} CFU/g in the large intestine. Piglets inoculated with strains of *E. coli* K-12 tended to have counts at the lower end of the range. The counts indicated that the distribution of lesions and diarrhea were not due to differences in the capacity of various bacterial strains to colonize these regions. Colonization of the proximal small intestine was less uniform, and bacterial counts ranged from 10^4 to 10^8 CFU/g. Higher counts in this region were usually, but not always, associated with earlier necropsies.

Bacteremia at necropsy was detected in two of the four piglets (both from the same litter) that were inoculated with strain 84-289. They also had severe anorexia in addition to diarrhea.

Cytotoxicity assay. Serum levels of cytotoxicity and the amount of SLT produced by each bacterial strain in vitro corresponded closely. By contrast, there was no correlation between the amount of SLT produced and the occurrence of diarrhea or the extent of mucosal lesions. Levels of cytotoxicity measured in the sera of infected piglets corresponded well with the capacity of the strains to produce SLT as assayed in vitro (Table 2).

Confirmation of strain identity. In every case, strains isolated from the intestinal contents or blood of piglets were identical, in terms of biotype, serotype, plasmid profile, and capacity to produce SLT, to those used to infect the pigs.

Gut loop experiments. The amount of fluid which accumulated in ligated gut loops of piglets and rabbits after inoculation with SLT or control preparations is shown in Table 3. It is clear that the heat-stable enterotoxin caused fluid accumulation in both species of animal, whereas bacterial extracts from the VT-producing strains EDL 933 and H30 were active only in rabbits.

DISCUSSION

This study in gnotobiotic piglets failed to clarify a role for either the 60-MDa plasmid or the capacity to produce SLT in the pathogenesis of enteric infection with *E. coli* O157:H7. Diarrhea and characteristic mucosal lesions of equal severity were produced in all piglets infected with two wild-type EHEC strains and their plasmid-cured or SLT-negative

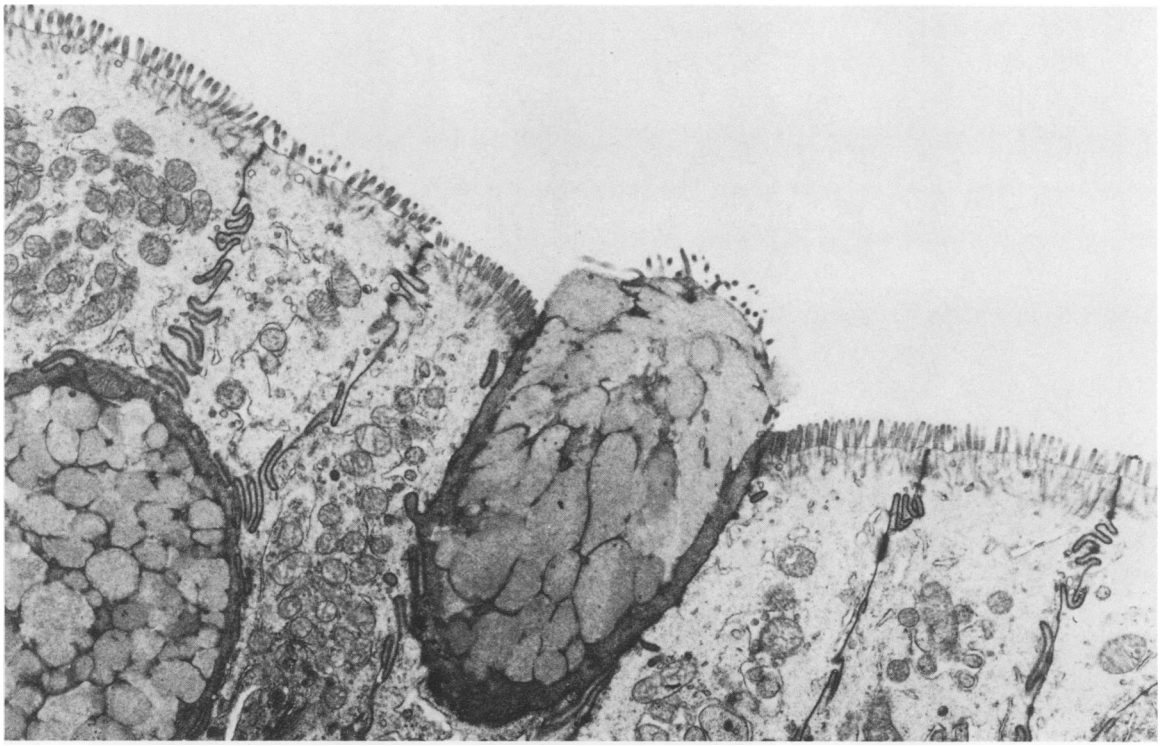

FIG. 2. Electron micrograph of an ultrathin section of the cecum from a piglet necropsied 5 days after inoculation with *E. coli* K-12 strain C600. Note surface epithelium, including one goblet cell in the center, coated with intact microvillous border. (Magnification, ×8,500.)

derivatives. Strain A7785-C3B, which is also *E. coli* O157:H7 (and shares the same origin as EDL 933), caused no diarrhea, nor did its plasmid-cured derivative strain A7785-C3A. Nevertheless, both strains induced characteristic lesions, albeit focal and mild. This indicated a correlation between the extent of mucosal injury and clinical signs caused by a strain. In the course of the selection for streptomycin resistance in the laboratory to obtain strain A7785-C3B from A7785-C3, a cryptic virulence property may have been lost, which reduced the efficiency of bacterial association with the surface epithelium.

Strains of *E. coli* C600 which received either the 60-MDa plasmid or either of the two known SLT-encoding bacteriophages were nonpathogenic to piglets, as was the parent strain. Bacterial counts indicated that all strains became well established in the large intestine and that the presence or absence of lesions, which were clear cut in each animal, could not be attributed to differences in the capacity of the strains to proliferate to higher numbers. Two piglets infected with K-12 developed infection, characteristic lesions, and diarrhea after exposure to an environment contaminated with strain EDL 933c. The absence of the plasmid from strain EDL 933c did not prevent the organism from displacing *E. coli* K-12 from the gastrointestinal tract in these piglets, causing extensive proliferation, mucosal lesions, and diarrhea (data not shown).

TABLE 3. Fluid accumulation in piglet or rabbit ligated gut loops[a]

Serogroup (strain)	Type of toxin[b]	Fluid accumulation (ml/cm) in:		Cytotoxicity for HeLa cells[c]
		Piglet jejunum	Rabbit ileum	
064 (AT/82)	STa	2.0	0.94	<10
026 (H30)	SLT-I	0.1	1.08	6,400
0157 (EDL 933)	SLT-I+II	0.3	1.01	3,200
09 (HS)	None	0.3	0.25	<10

[a] Fluid in jejunum of 9-day-old piglets or ileum of 2-month-old rabbits, 6 h after inoculation with 1 ml of either culture filtrate (O64) or one of three bacterial cell preparations extracted with polymyxin B.

[b] STa, Heat-stable enterotoxin of enterotoxigenic *E. coli* of porcine origin.

[c] Values are the reciprocal of the highest dilution of a 50-μl sample which killed 50% or more of cells grown in microtiter trays in 48 h.

Rigorous testing of strains recovered from infected piglets showed that plasmids and phages were stably maintained in the *E. coli* C600 strains into which they had been transferred. These findings indicate that within the plasmid- and SLT-cured *E. coli* O157:H7 strains, there were no subpopulations of bacteria which retained the plasmid or the phages, as these bacteria, had they been more virulent, would be expected to have proliferated more extensively than their nontoxigenic counterparts.

E. coli O157:H7 and classical enteropathogenic *E. coli* induce similar mucosal lesions in gnotobiotic piglets (20, 30). Whereas a 60-MDa plasmid in EPEC encodes for an adhesion factor, demonstrable in vitro using HEp-2 cells (21), that appears to facilitate colonization of and attachment to the intestines of colostrum-deprived piglets (1), the 60-MDa plasmid in EHEC strains codes for fimbriae that mediate attachment to Henle 407 cells (9). The presence or absence of the plasmid encoding these fimbriae in *E. coli* O157:H7 did not seem to influence the attachment of bacteria to the mucosa in gnotobiotic piglets. However, whether the fimbriae, or indeed the plasmid, are determinants essential for promoting colonization and attachment in competition with other gut microflora, as was shown with enteropathogenic *E. coli* in colostrum-deprived piglets (1), is unknown.

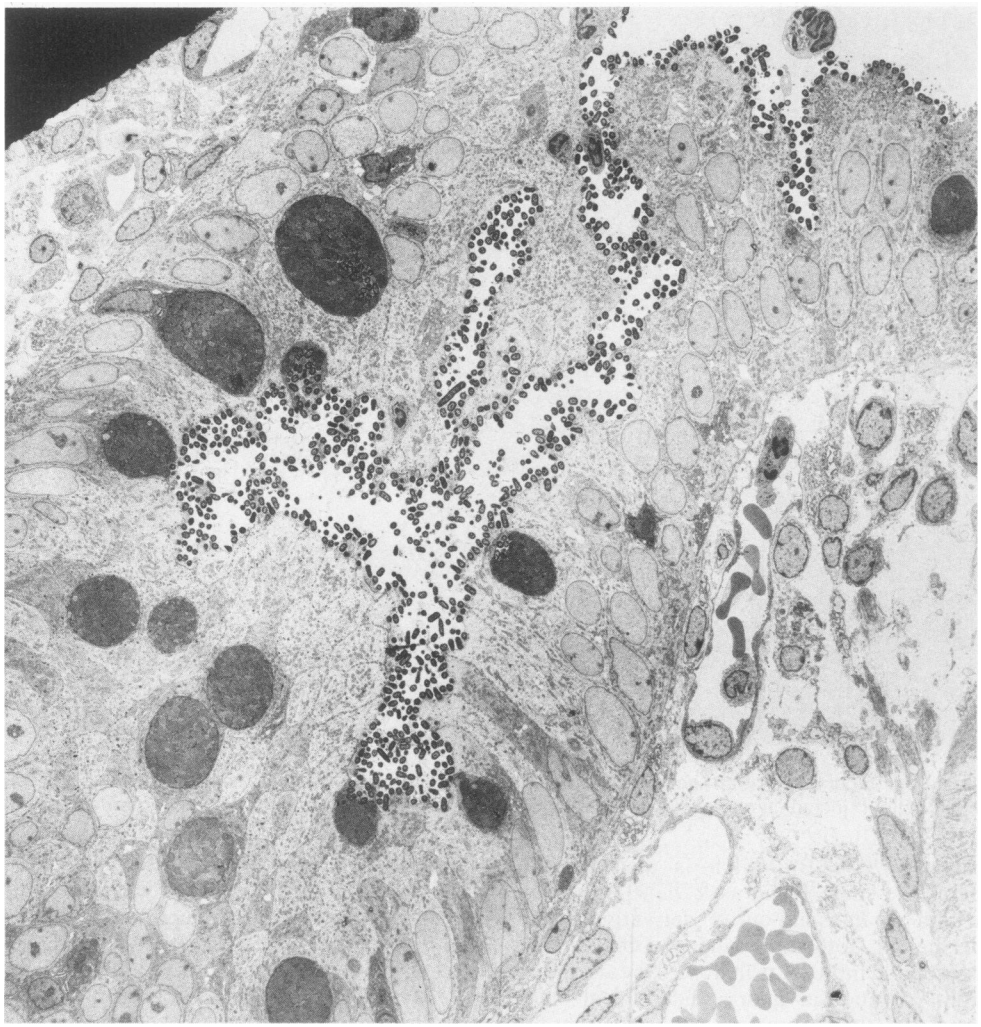

FIG. 3. Oblique section through the cecum of a piglet necropsied 5 days after inoculation with *E. coli* O157:H7 strain 85-170 (SLT negative). Note edema and vascular congestion in the lamina propria associated with bacterial attachment and proliferation in the crypt. (Magnification, ×1,000.)

The role of SLT in the pathogenesis of diarrhea and its contribution to lesions in this model is questionable. This is contrary to current thinking, based on epidemiological observations, as to its role in humans and to the interpretation of experiments conducted in rabbits (25) and mice (2). It seems that piglets are not as susceptible to the diarrheagenic effect of SLT as rabbits, an observation which was partially confirmed in intestinal loops of the two animal models, in which SLT-producing strains induced fluid transudation in rabbits but not in pigs. It is evident that the gnotobiotic piglet may not be a suitable animal to study the diarrheagenic activity of SLT, as there was no question that the strains used produced SLT, and with bacterial counts in excess of 10^{10} organisms per g in the distal ileum and the large intestine, a large quantity of SLT was present in the gastrointestinal tract. We elected to measure the amount of SLT in the serum of the infected piglets, rather than in the feces, because we found it to be less variable (unpublished data). The serum levels correlated well with the quantity of SLT produced in vitro by each of the strains. The absorption of SLT from the gut into the blood circulation, and presumably into other extracellular compartments, of infected piglets may lend support to the possible role of this toxin in nongastrointestinal conditions reported recently in humans (4, 22, 28a). The role of Shiga toxin, to which SLT-I is apparently identical, in shigellosis is also not clear (8, 16), despite its being one of the most potent toxins in nature. Studies in volunteers fed a proliferating noninvasive attenuated *Shigella dysenteriae* I strain that retained its ability to produce the toxin resulted in no symptoms (14); this may indicate that the reaction of the gastrointestinal tract of humans to the toxin resembles that observed in the pig.

While EHEC strains are noninvasive in the true sense, they nevertheless lead to the destruction of surface and glandular epithelium, which results in extensive erosions and often mucosal ulcers from which blood cells escape. These lesions, which are clearly unrelated to the production of SLT, are more likely to lead to loss of erythrocytes from exposed lamina propria, often with damaged vasculature in the mucosa, than from the relatively mild mucosal changes attributed to SLT described in the rabbit model (25). It is more difficult, in this context, to assess the significance of the studies conducted in mice, in which the inoculum used was whole bacterial cell extracts that contained products other than SLT and which were given intraperitoneally (2). It remains to be determined whether hemorrhagic colitis in

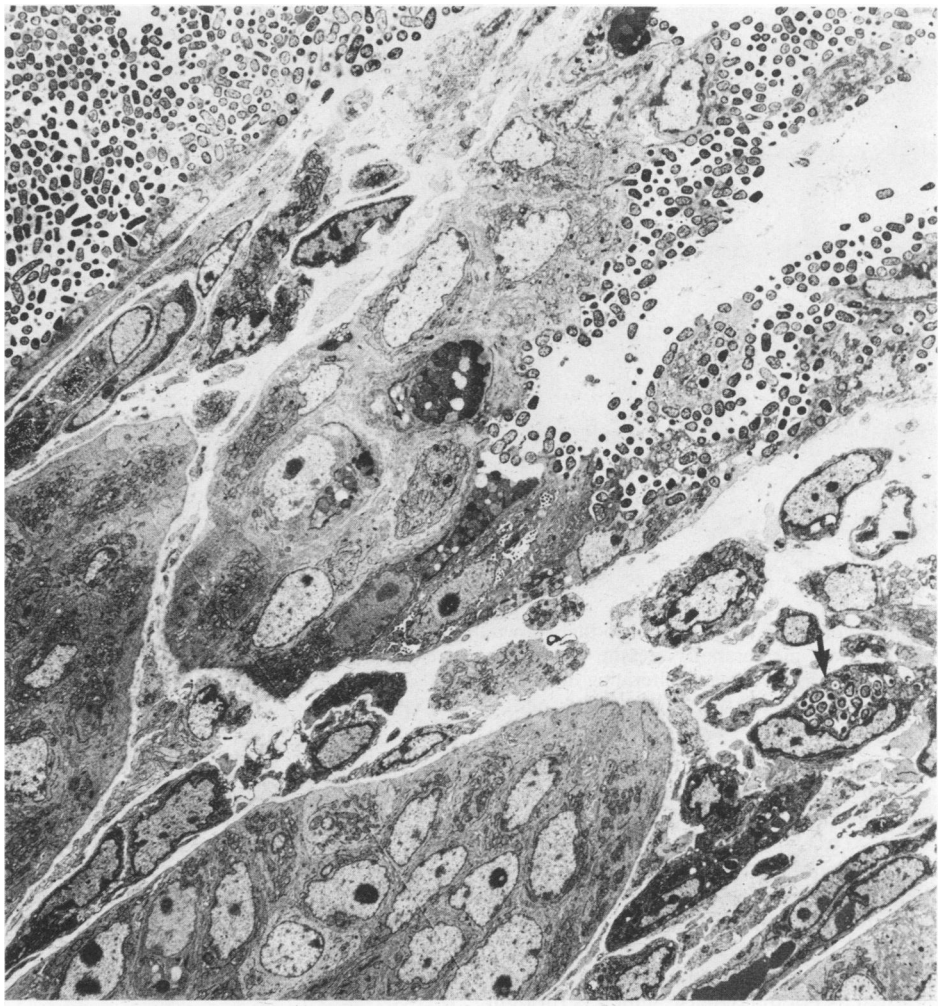

FIG. 4. Electron micrograph of the cecum from a piglet necropsied 5 days after inoculation with strain EDL 933c (plasmid-cured *E. coli* O157:H7). Note the complete destruction of the crypt in the top left, partially destroyed crypt in the middle, and still intact and partly obscured crypt at the bottom. Bacteria can be seen within mononuclear cells (arrow) inside a markedly edematous interglandular space. (Magnification, ×1,300.)

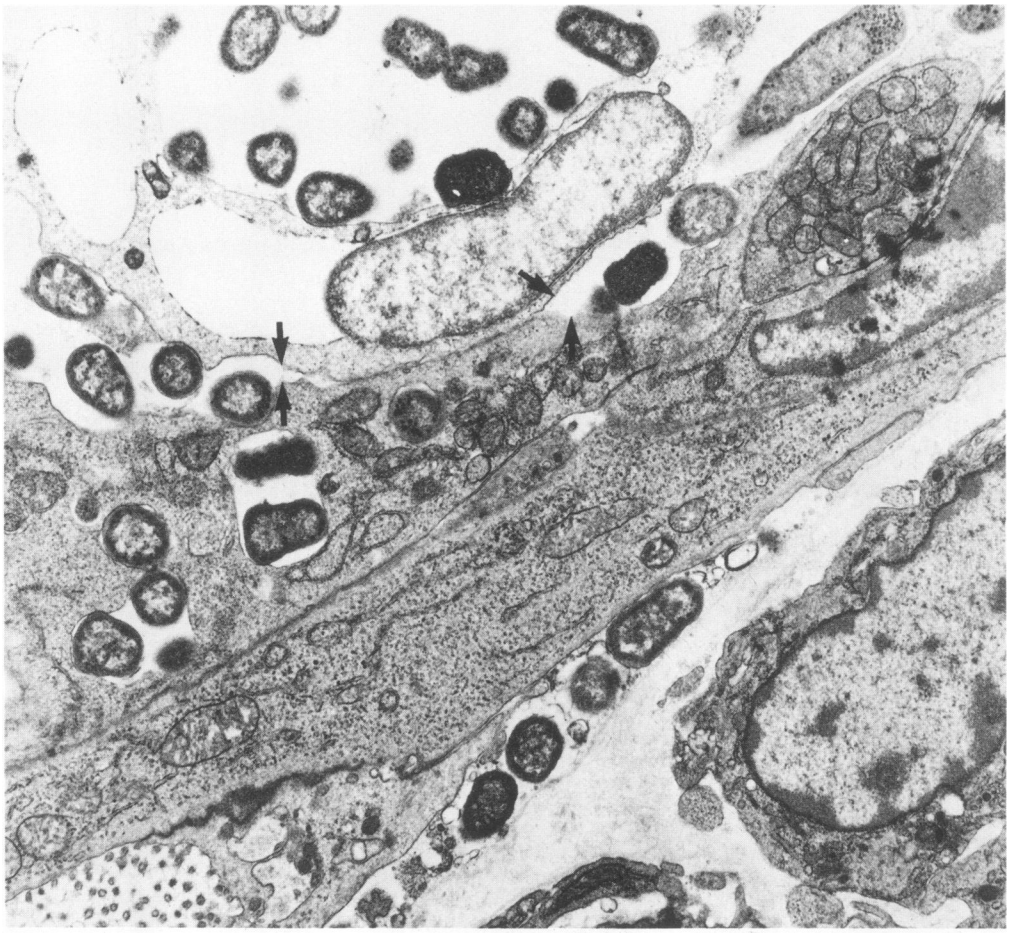

FIG. 5. Higher magnification of an area adjacent to that shown in Fig. 4, showing bacteria proliferating within the interglandular space, having penetrated the basement membrane. Note the extensively vacuolated glandular cell, with intracellular bacteria, which is being separated from underlying cells by bacteria proliferating in the interjunctional space (arrows). (Magnification ×10,000.)

humans is due largely to the effect of SLT, as suggested by the rabbit model, or to the extensive non-plasmid-, non-SLT-mediated mucosal injury seen in the piglet model, or perhaps to both.

SLT is also thought to contribute to nongastrointestinal diseases such as hemolytic uremic syndrome (10, 11, 22, 28a), thrombotic thrombocytopenic purpura (4), and possibly others (Grandsen et al., Lancet **ii**:150, 1985). In conventional pigs raised on farms, SLT-producing *E. coli* serotypes cause a syndrome known as edema disease which is thought to be associated with a related toxin (28). Neurological signs, presumably caused by the toxin, which often occur in affected piglets may explain the occurrence of neurological abnormalities observed in one animal in this study and in several animals in a previous one (Tzipori et al., in press).

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