Effects of *Escherichia coli* Shiga-like Toxins (Verotoxins) in Pigs

Victor P.J. Gannon, Carlton L. Gyles and Brian P. Wilcock

ABSTRACT

Escherichia coli K12 strains TB1(pCG5), with the genes for Shiga-like toxin IIv from an edema disease isolate of *E. coli* and TB1(pCG5-1), with the toxin genes inactivated by transposon mutagenesis, were used to test the hypothesis that Shiga-like toxin IIv was the same as edema disease principle. Ammonium sulfate precipitated culture supernatants from the pair of *E. coli* K12 strains and from a wild edema disease isolate of *E. coli* (E145) were tested for their ability to induce signs and lesions of edema disease in intravenously inoculated weaned pigs. Similar preparations from *E. coli* which produce Shiga-like toxins I and II were also tested. Preparations from *E. coli* TB1 (pCG5) and E145 contained high levels of Shiga-like toxin IIv and induced signs and lesions similar to those seen in edema disease, whereas preparations from *E. coli* TB1 (pCG5-1) failed to induce signs or lesions of edema disease. All Shiga-like toxin preparations produced delayed neurological signs, fibrinoid necrosis of arterioles and hemorrhages in the cerebellum of pigs. High doses of Shiga-like toxin IIv were associated with superficial necrosis of the colonic epithelium and vasculitis. Shiga-like toxins I and II resulted in kidney lesions but no enteric pathology. Shiga-like toxin II preparations had the lowest median lethal dose for pigs, Shiga-like toxin IIv was intermediate and Shiga-like toxin I was the least toxic.

RÉSUMÉ

Cette expérience portait sur la souche K12 TB1 (pCG5) d'*Escherichia coli*, détentrice des gènes lui permettant de produire la verotoxine IIv et isolée d'un cas de la maladie de l'oedème, ainsi que sur la souche TBl pCG5-1) détentrice des gènes précités, qu'inactive la mutagénése d'un transposon; elle visait à vérifier l'hypothèse selon laquelle la verotoxine IIv correspond à celle de la maladie de l'oedème. On précipita dans du sulfate d'ammonium le surnageant de cultures des deux souches précitées d'*E. coli* et de la souche E145, isolée d'un cas de la maladie de l'oedème; on vérifia ensuite l'habileté de ce surnageant à produire les signes cliniques et les lésions de la maladie de l'oedème, à la suite de son injection intraveineuse à des porcelets récemment sevrés. On vérifia aussi des préparations similaires du surnageant de cultures de colibacilles qui produisent les verotoxines I et II. Le surnageant des souches TBl (pCG5) et E145 contenait beaucoup de verotoxine IIv et provoqua des signes cliniques et des lésions semblables à ceux qu'on observe dans la maladie de l'oedème, contrairement à celui de la souche TB1 (pCG5-1). Toutes les préparations qui contenaient de la verotoxine produisirent des signes nerveux tardifs, de la nécrose fibrinoïde des artérioles et des hémorragies cérébelleuses, chez les porcelets. De fortes doses de la verotoxine IIv, s'accompagnèrent d'une nécrose épithéliale superficielle et d'une vasculite du côlon. Les verotoxines I et II produisirent des lésions rénales, mais pas d'intestinales.

Les préparations de verotoxine II affichèrent la DL50 la plus faible; celles de verotoxine IIv démontrèrent une DL50 intermédiaire, alors que celles de verotoxine I se révélèrent les moins toxiques.

INTRODUCTION

Edema disease (ED) of pigs is a toxemia which results from proliferation of *Escherichia coli* of certain serogroups in the intestine (1,2). The toxic activity is heat-labile and may be recovered from intestinal contents of pigs which have died of ED or extracts of *E. coli* implicated in the disease (1-4). Pigs or mice inoculated parenterally with these preparations develop signs and lesions characteristic of the natural disease (1-4). The toxin in these preparations has been called edema disease principle (EDP) and suggested to be a protein of molecular weight between 50,000 and 100,000 daltons (3), but has never been purified.

Verotoxins or Shiga-like toxins (SLTs) are a class of *E. coli* toxins characterized by cytotoxicity for Vero cells, enterotoxicity and lethality for mice (5-7). Production of SLT is a common feature of *E. coli* of O serogroups 138, 139, and 141 (8-12), the serogroups most frequently associated with ED, and recent studies have suggested that EDP and SLT of

Department of Veterinary Microbiology and Immunology (Gannon, Gyles) and Department of Pathology (Wilcock), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Reprint requests to Dr. C.L. Gyles.

Portions of this paper constituted part of a thesis submitted by the senior author to the Faculty of Graduate Studies, University of Guelph.

Supported by the Natural Sciences and Engineering Research Council, Agriculture Canada and the Ontario Ministry of Agriculture and Food.

Submitted July 22, 1988.

E. coli implicated in ED may be identical (8-12). Cytotoxicity of EDP-containing extracts for Vero cells correlated well with their capacity to induce neurological signs and death in mice (12). Furthermore, Smith *et al* (8) transferred SLT activity from an ED strain to *E. coli* K12 and reproduced ED in pigs inoculated parenterally with extracts of the SLT$^+$ K12 organism. However, since genes other than the SLT genes may also have been transferred to the *E. coli*, it was not certain that ED was the result of the newly acquired SLT activity.

We have used the term SLT-IIv to refer to the SLT associated with ED strains of *E. coli*, to distinguish it from Shiga-like toxins I and II (SLT-I and SLT-II), and to recognize its relationship to SLT-II and its association with edema disease (11,13,14). Strains of *E. coli* of porcine origin occasionally produce either SLT-I or SLT-I and SLT-II (9), but the effects of these types of SLT in pigs are not known. The three SLTs can be differentiated on the basis of neutralization by antisera (6,13) and by rates of heat inactivation (13). There is cross-neutralization between SLT-II and SLT-IIv but not between SLT-I and other SLTs. Furthermore, SLT-II and SLT-IIv have approximately 90% homology in their amino acid and nucleotide sequences (14).

The objectives of this study were to determine whether experimental ED in pigs inoculated with SLT-IIv preparations could be attributed to SLT-IIv and to assess the effects of parenterally administered preparations of SLT-I and SLT-II in pigs. We used an *E. coli* into which the gene for SLT-IIv was cloned and a SLT-IIv$^-$ transposon mutant of this strain to assess the effects of SLT-IIv administered intravenously to weaned pigs. We also compared the effects of extracts from the *E. coli* with the cloned SLT-IIv genes with those from a wild SLT-IIv$^+$ *E. coli*, and determined the effects in pigs of preparations of SLT-I and SLT-II derived from porcine and human isolates of *E. coli*.

MATERIALS AND METHODS

CLONING OF SLT-IIv GENES

Shiga-like toxin-IIv positive *E. coli* strain 412 (O139:K82), received from D.A. Barnum, University of Guelph, was the source of DNA for cloning the SLT-IIv genes. Phage vector lambda EMBL3 and *E. coli* NM539, host for recombinant lambda EMBL3, were purchased from Biocan Scientific (Mississauga, Ontario). Plasmid vector pUC18 was obtained from J. Brunton, Mount Sinai Research Institute, Toronto, Ontario. *Escherichia coli* TB1 *lac pro rpsL ara thi* 80 d *lacZ* M15 *hsdR*, (Bethesda Research laboratories (BRL), Burlington, Ontario), was the host for the pUC18 vector and recombinant plasmids. Phage lambda 467 *b*221 *rex*::Tn*5* *c*I857 *O*am29 *P*am80 was obtained from N.M. Harnett, Agriculture Canada Animal Pathology Laboratory, Guelph, Ontario. *Escherichia coli* C600 was the host strain for propagation of lambda::Tn*5*.

Luria Bertani (LB) broth or agar (15) was used for routine growth of *E. coli* and propagation of phages, and YM broth (15) was used for growth of *E. coli* TB1(pCG5) used in transposon mutagenesis (16).

Chromosomal DNA was prepared from *E. coli* 412 by the method of Roussel and Chabbert (17) and plasmid DNA was prepared by alkaline lysis (18). Strains were transformed with plasmid DNA as described by Curtiss (19). Restriction endonucleases were purchased from Pharmacia (Montreal, Quebec) and BRL and were used as recommended by the suppliers. Restriction mapping was performed by standard techniques (19). Phage lambda DNA digested with *Eco*RI-*Hind*III and a 123 bp DNA ladder (BRL) were used as molecular weight markers.

A library of *E. coli* 412 DNA was constructed by cloning 9-23 Kb fragments produced by partial digestion with *Sau*3A DNA into lambda EMBL3 (20). Following packaging of the lambda DNA, recombinants were selected by plaquing on *E. coli* NM539. Pools of phage were propagated on *E. coli* NM539 and the supernatants of lysates were tested for cytotoxic activity in the Vero cell assay. Insert DNA was subcloned into pUC18. Transposon mutagenesis was performed as described by de Bruijn and Lupski (16). Individual colonies which had acquired plasmids with Tn*5* insertions were tested for production of SLT IIv by the Vero cell assay (9).

SLT PREPARATIONS

Bacterial strains used for SLT preparations are described in Table I. Strains of *E. coli* were grown in brain heart infusion broth (BHI) (Difco Laboratories, Detroit, Michigan) for 24 h at 37°C on a rotary platform shaker (G10 Gyrotory Shaker, New Brunswick Scientific, Edison, New Jersey) at 200 rpm and passed through a cellulose acetate membrane filter of mean pore diameter 0.45 μm. Solid ammonium sulfate was added to the culture filtrates to 60% saturation and the mixture was kept overnight at 4°C, then centrifuged at 8,000 x *g* for 20 min at 4°C. The supernatant was removed, and the sediment resuspended in one-fifth volume (vol) of 0.85% NaCl, and dialyzed against 50 vol of distilled water for 24 h, followed by 50 vol 0.07 M phosphate buffer, pH 8.2, for 6 h. The dialysate was centrifuged at 30,000 x *g* for 60 min and the supernatant was removed and stored at -20°C. Protein concentrations in the preparations were determined by the method of Bradford (21), and cytotoxicity was assayed on Vero cells in microtiter plates (9). Cytotoxic titers were expressed as log median cytotoxic dose (CD_{50}) per mL.

Control SLT$^-$ preparations consisted of preparations from the SLT-IIv$^-$ *E. coli* strain TB1(pCG5-1) or from preparations of SLT which had been inactivated by heating at 80°C for 30 min (13).

ANIMALS

Weaned Yorkshire-Landrace pigs (four to eight weeks of age, 7-20 kg in

TABLE I. Strains of *E. coli* Used as Sources of Shiga-like Toxins

Strain	Serogroup	SLTs Produced
Porcine strains		
E145	O141:K85	SLT-IIv
5481	O130:H38	SLT-II (SLT-I)[a]
Human strains		
CL13	O145:H-	SLT-II (SLT-I)[a]
B2F1/3	O91:H21	SLT-II
K12 strains		
711	O rough	none
7ll(H19)[b]	O rough	SLT-I
TB1(pCG5)[c]	O rough	SLT-IIv
TB1(pCG5-1)[d]	O rough	none

[a]SLT-I constitutes a minor component (< 1%) of the SLT activity in SLT preparations from the strain

[b]*E. coli* donor of the SLT-I phage genome is shown in parentheses

[c]Plasmid containing the cloned SLT-IIv genes is shown in parentheses

[d]The SIT-IIv gene was inactivated by insertion of transposon Tn*5*

weight) were obtained from a specific-pathogen-free herd. The pigs were housed in groups of six to eight animals per pen in animal isolation facilities, fed a commercial ration of hog grower (containing 16% protein) twice daily, and supplied with water *ad libitum*. After a minimum of three to four days in the facility, pigs were inoculated intravenously with SLT preparations, containing sodium deoxycholate at a final concentration of 0.2% (wt/vol) (3).

EXPERIMENTS

SLT-IIv — To minimize the number of pigs required for the study, groups of two pigs were inoculated with SLT-IIv preparations from the wild *E. coli* strain E145 in order to establish a range for median lethal dose (LD_{50}) determinations. Once doses for 0 and 100% mortality were determined, five pigs were tested for each of three dose levels within this range. Pigs were observed every 2 h during the first 12 h postinoculation (PI) and every 4 h thereafter. Pigs that became very ill were euthanized by rapid intravenous administration of concentrated barbiturate and were treated as pigs that died without further intervention. Median lethal dose estimates were calculated by probit analysis (22) and expressed on a log CD_{50}/kg and mg protein/kg basis. Confidence limits and z values were calculated in the comparison of probit values for individual SLTs. Heat-inactivated SLT-IIv preparations at a dose equivalent to twice the LD_{50} of the unheated preparation were administered intravenously to two pigs. One pig which developed neurological signs 14 days after vaccination with an SLT-IIv preparation from *E. coli* TB1(pCG5) (13) was also examined for gross and histopathological lesions.

The effects of SLT-IIv preparations from the K12 SLT-IIv$^+$ *E. coli* strain TB1(pCG5) were compared with those of preparations from the SLT-IIv$^-$ mutant of this strain as follows. Each of four pigs was inoculated with two LD_{50}s of SLT-IIv preparation from *E. coli* TB1(pCG5) and each of four pigs was inoculated with a control preparation from the SLT-IIv$^-$ mutant *E. coli* equivalent to five times the amount of the SLT-IIv preparation. Eighteen hours PI, pigs in both groups were euthanized and necropsied.

Other SLTs — Varying doses of SLT-I preparations from the *E. coli* K12 derivative 711(H19) were administered intravenously to groups of two pigs in an attempt to identify a dose range for 0 and 100% mortality attributable to SLT-I activity. Preparations from the *E. coli* K12 strain 711 served as negative controls.

Shiga-like toxin-II preparations from the porcine *E. coli* strain 5481 and from the human isolate CL13 were administered to pigs as described for *E. coli* E145 above, except that only two doses were tested within the range of 0 and 100% mortality. The SLT-II preparations from *E. coli* strain B2F1/3 were administered as were the other SLT-II preparations, but only two pigs were used for each dose of SLT preparation. Heated preparations of SLT-II (strain B2F1/3) at a dose equivalent to two LD_{50}s of the unheated preparation were used as a negative control.

Gross pathology and histopathology — A necropsy was performed at 12-36 h PI on a minimum of four pigs for each type of SLT and the preparation from the SLT-IIv$^-$ transposon mutant of strain TB1(pCG5-1), and two pigs for each heat-inactivated preparation. Samples of brain, eye, subcutis, large intestine, jejunum, stomach, kidney and lung were taken for histological examination.

Intestinal and ocular tissues were fixed in Bouin's solution while others were fixed in 10% formalin buffered at pH 7.0. Following fixation, tissues were embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin.

RESULTS

CLONING OF THE GENES FOR SLT-IIv

A recombinant phage EMBL3 (L42) containing an 11.5 kilobase pairs (kb) fragment of DNA from *E. coli* strain 412 produced SLT-IIv activity. A 7.5 kb *Sal*I-*Eco*RI fragment of L42 DNA, cloned into plasmid pUC18 (pCG5), contained the genes for SLT-IIv. Transposon mutagenesis of pCG5 generated several insertion mutants, including pCG5-1, which had lost SLT-IIv activity.

COMPARISON OF LD_{50} ESTIMATES FOR SLT-I, SLT-II AND SLT-IIv IN PIGS

Shiga-like toxin-II preparations had the lowest LD_{50} estimates based on cytotoxic dose (Table II). The values for SLT-II from the porcine isolate were not significantly different from those for the isolates of human origin. The LD_{50} estimates for SLT-I in pigs could not be determined because the highest dose tested (log 6.2 CD_{50}/kg) elicited a predominantly endotoxic response and did not result in mortality. Thus the minimum lethal dose and the LD_{50} for SLT-I were $>$ log 6.2 CD_{50} per kg.

EFFECTS OF SLT-IIv

Features associated with endotoxin contamination of preparations (vomition, inappetence, dyspnea and depression) occurred in the majority of pigs within 1 h of inoculation of SLT-IIv preparations. However, with the exception of two pigs given high doses of SLT-IIv preparation from strain E145, which died at 8 and 12 h PI, these clinical signs remitted within 2-6 h PI.

Edema of the temporal region, eyelids, and conjunctiva were noted 10-24 h PI. In some pigs, dyspnea and alteration of the squeal were noted. Neurological signs occurred shortly after subcutaneous edema was apparent (from 12 to 36 h PI) and consisted of mental confusion, ataxia and a staggering gait. In the final stages of illness, pigs were unable to rise and displayed a range of signs including

TABLE II. LD_{50} Estimates for SLT-IIv and SLT-II in Weaned Pigs

SLT	Strain	LD_{50} n	LD_{50} Log CD_{50}/kg[a]	LD_{50} mg Protein/kg[a]
SLT-IIv	E145	15	6.0 (5.8-6.2)	0.21 (0.14-0.34)
SLT-II	5481[b]	10	4.6 (4.0-5.3)	0.005 (0.002-0.024)
SLT-II	CL13[b]	10	4.7 (4.2-5.3)	0.011 (0.004-0.045)
SLT-II	B2F1/3	4	4.4 (3.8-5.0)	0.014 (0.004-0.060)

n = number of pigs inoculated

[a]95% confidence limits are shown in parentheses

[b]SLT preparations from strains 5481 and CL13 each contained a minor component ($<$ 1%) of SLT-I

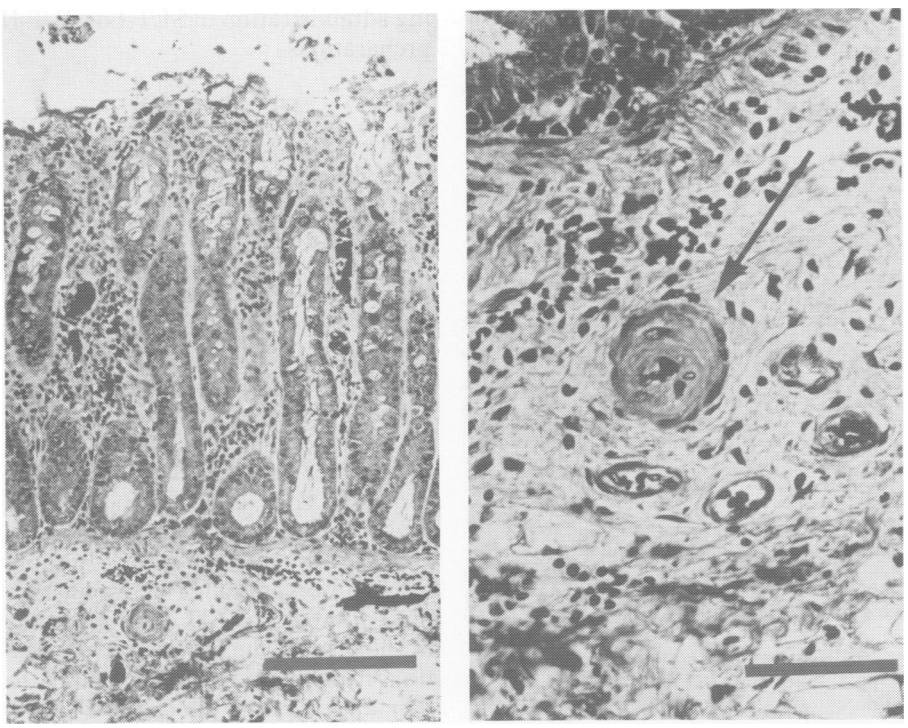

Fig. 1. Colonic lesions (24 hours PI) in a pig given SLT-IIv preparation from *E. coli* strain E145. A. Diffuse necrosis of the colonic epithelium, with hemorrhage into the lumen are evident (H & E; bar = 140 μm). B. Fibrinoid necrosis of a submucosal arteriole, mural edema, and occlusion of the lumen are demonstrated (H & E; bar = 410 μm)

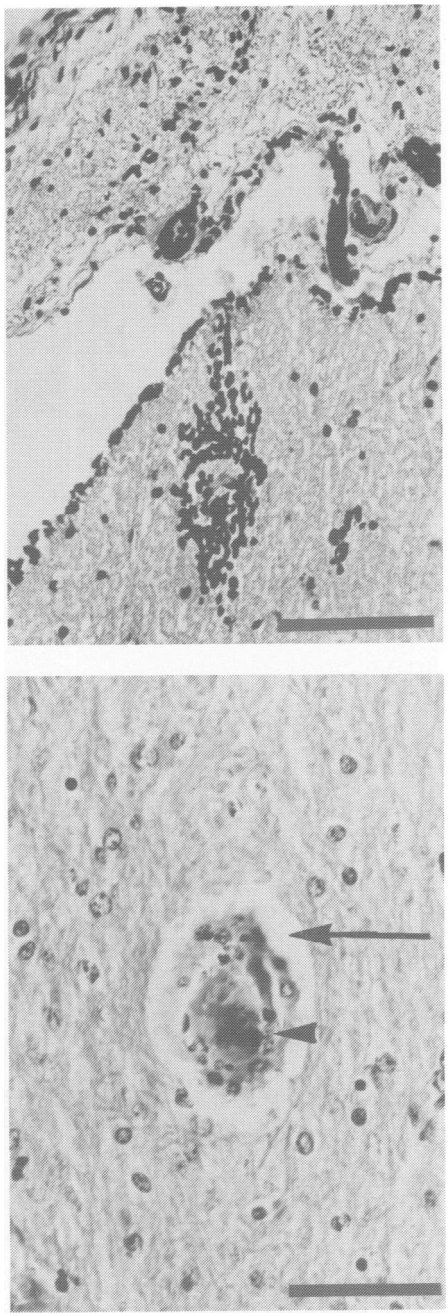

Fig. 2. Hemorrhage and vascular lesions in the cerebellum of a pig given SLT-IIv preparation from *E. coli* strain E145. A. Note meningeal edema and hemorrhage (H & E; bar = 320 μm) B. Perivascular edema (➔) and medial necrosis (▶) of a small cerebellar artery (H & E; bar = 425 μm)

tremors, paddling of the limbs, extensor rigidity, convulsions and coma. Pigs that developed severe neurological signs were euthanized.

In addition to these clinical features, bloody diarrhea was observed at 24 and 18 h in two pigs which received log 6.5 CD_{50}/kg of the SLT-IIv preparations from strains E145 and TB1(pCG5), respectively.

Pathological changes were evident in the spiral colon of all pigs given SLT-IIv preparations but were most severe in pigs given high doses of the toxin. Lesions ranged from colonic edema to ischemic necrosis of the epithelium (Fig. 1A), with hemorrhage into the lumen and fibrin and necrotic debris adherent to the mucosal surface. Necrosis was readily apparent in the small arteries and arterioles of the submucosa (Fig. 1B). These lesions are consistent with those described in naturally occurring and experimentally produced ED.

In the brain, edema of the meninges and petecchial hemorrhage from meningeal vessels was limited to regions overlying the cerebellum. Histologically, hemorrhagic foci were scattered througout the cerebellum (Fig. 2A). In some sections, vascular changes similar to those in the colon were evident (Fig. 2B); in others, only vessel remnants were present in the centers of hemorrhagic foci. Edema, hemorrhage and angiopathy were also noted in the retina of some pigs given high doses of SLT-IIv preparations (Fig. 3). Neither edema of the stomach wall nor degenerative lesions in associated vasculature was apparent.

In the pig which developed neurological signs 14 days after inoculation with an SLT-IIv preparation from TB1(pCG5), fibrinoid necrosis of arterioles in the brain was more widespread than in the acute cases (Fig. 4). A peculiar feature of angiopathy in the brain was the perivascular accumulation of eosinophilic globules of unknown origin (Fig. 4). Colonic lesions and subcutaneous edema were not evident in this pig.

Pigs inoculated with heated SLT-IIv preparations or with preparations from the SLT-IIv$^-$ mutant strain TB1(pCG5-I) remained healthy throughout the period of observation and no significant pathological changes were observed on necropsy.

EFFECTS OF SLT-I AND SLT-II PREPARATIONS

At high doses of SLT-II (10 LD_{50}), death occurred within 9.5 to 12 h PI

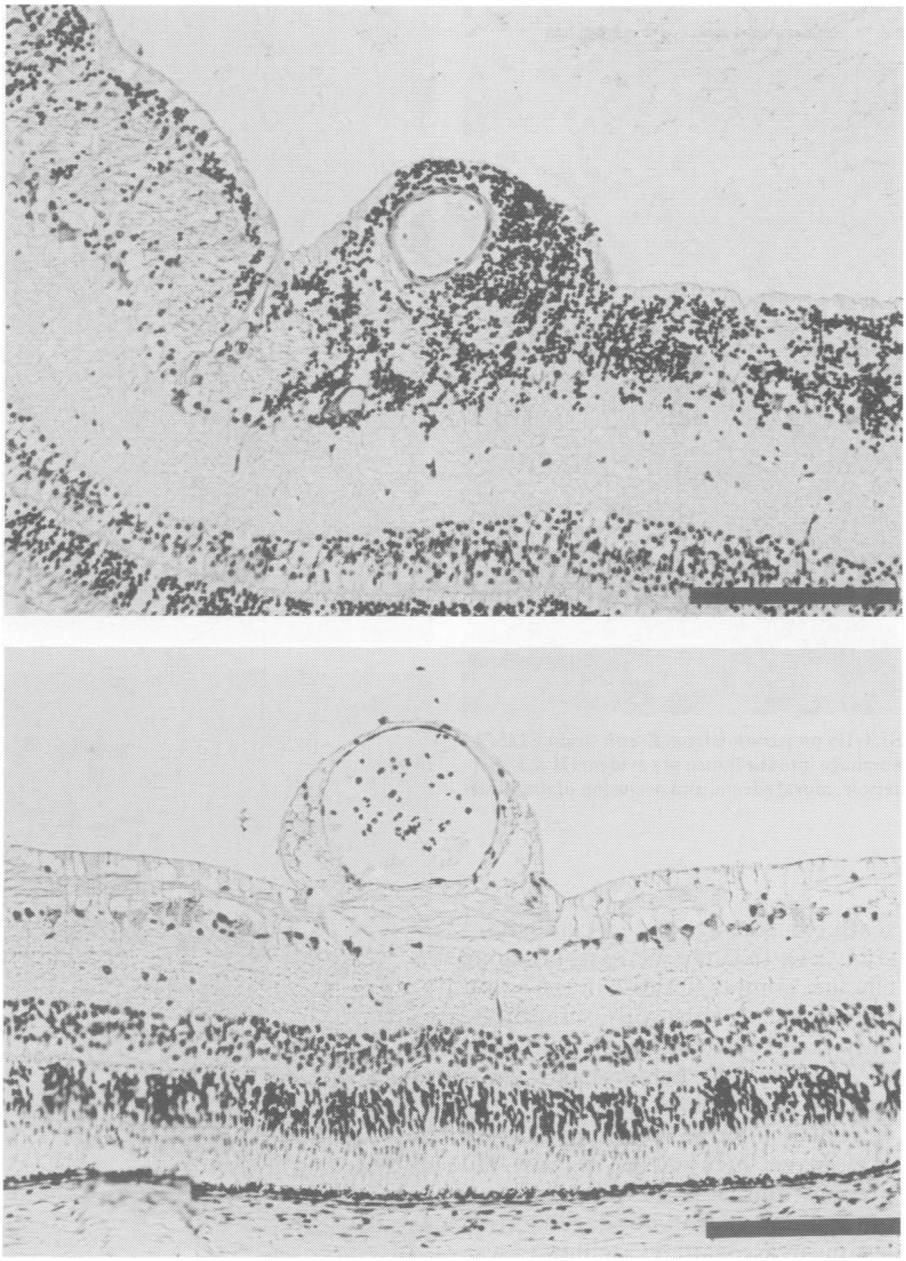

Fig. 3. Comparison of the retinas of two pigs (18 hours PI), one (A) given SLT-IIv+ preparation from *E. coli* TB1(pCG5) and the other (B) given SLT-IIv⁻ preparation from *E. coli* TB1(pCG5-1). A. Note hemorrage and edema surrounding venule traversing the nerve fiber layer (H & E; bar 160 = μm) B. A similar region in a pig given the SLT-IIv- preparation has normal architecture (H & E; bar = 160 μm).

and the time to death increased with decreasing dose. In one pig which had received a low dose of SLT-II, neurological signs were first observed at 100 h PI.

Lesions in pigs which developed delayed neurological signs from SLT-I preparations were similar to those in pigs which died following administration of SLT-II preparations. Gross and histological lesions in the brain and eye were identical to those described for SLT-IIv preparations. However, lesions were not present in the gastrointestinal tract and, in contrast to the response to SLT-IIv preparations, renal pathology was observed in response to SLT-I and SLT-II. Cortical edema and hemorrhage and necrosis of renal tubules were observed in all pigs which developed neurological signs following administration of SLT-I or SLT-II preparations.

DISCUSSION

To determine if SLT-IIv was necessary for clinical and pathological features associated with ED, preparations from the pair of *E. coli* K12 which differed only with respect to the SLT-IIv genes, were compared for their effects in pigs. The cloned DNA containing the SLT-IIv genes has been mapped and sequenced and the data have confirmed the identity of the SLT-IIv genes in the clone and the location of the inactivating transposon in pCG5-1 in the gene encoding the A subunit of SLT-IIv (Gyles, DeGrandis, and Brunton, unpublished observations). The responses in pigs showed that ED developed only in those pigs which received preparations from the SLT-IIv⁺ member of the pair and that the effects of SLT-IIv produced by this laboratory strain of *E. coli* were indistinguishable from those produced by SLT-IIv from a wild ED isolate.

Among the SLTs that were investigated, SLT-IIv produced clinical and pathological features most typical of natural or experimental ED, with edema of the subcutis of the facial region, spiral colon and mesocolon evident (2,23,24). However, certain features in this study differed from those of previous experimental work (2,8,24) and the naturally occurring disease (1,23), namely: 1) edema and vascular changes were not evident in the stomach wall, 2) lesions in the brain were largely confined to the meninges and the cerebellum rather than the brain stem, and 3) hemorrhage was present in the meninges, cerebellum, retina and spiral colon. While it is possible that the dose of toxin, time of death, and composition of the toxin preparations may account for some of these discrepancies, further study is required to define more precisely features of experimental intoxication with SLT-IIv.

Escherichia coli associated with ED have been implicated in hemorrhagic gastroenteritis (25), and mucosal destruction and intestinal hemorrhage have been reported in pigs inoculated orally with ED strains (26). However, in both hemorrhagic gastroenteritis and the study described by Berschin-

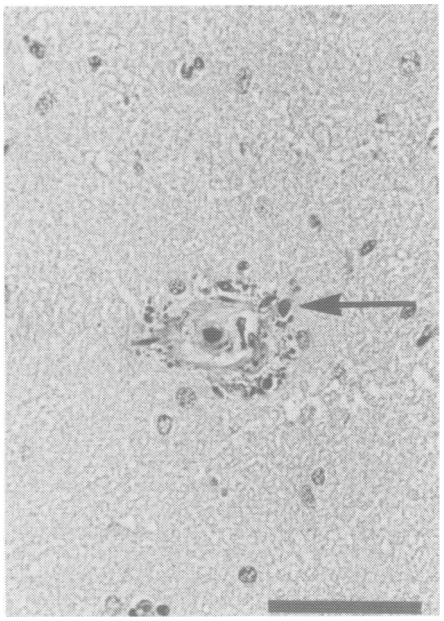

Fig. 4. Arteriole in the midbrain of a pig euthanized 14 days after intravenous administration of SLT-IIv preparation from strain TB1(pCG5). Fibrinoid necrosis of the vessel wall, vessel occlusion and perivascular accumulation of eosinophilic globules (➔) are evident (H & E; bar = 400 μm).

ger and Pohlenz (26), mucosal damage occurred in the small intestine, and edema with no mucosal damage was observed in the large bowel. One explanation for this difference may be the route of toxin exposure: in the natural disease and that produced by oral challenge, necrotic lesions occur in the areas of bacterial colonization, namely, the small intestine. High toxin concentrations in the intestine may account for local damage.

Edema, neurological signs, and damage to the colon are not unique to pigs exposed to SLT-IIv preparations. Shiga-like toxin-producing *E. coli* are associated with hemorrhagic colitis in humans (27) and hemorrhagic colitis and typhlitis occurred following administration of Shiga toxin to rabbits (28,29). Edema and neurological signs are both manifestations of the prodromal stage of the hemolytic uremic syndrome (HUS) in humans (30), a condition associated with both SLT-positive *E. coli* (31) and *Shigella* infection (32). Patients with shigellosis may experience neurological disorders including convulsions and seizures (33).

The effects of preparations from porcine *E. coli* containing the two other types of SLT (I and II) were consistent with the existence of differences and similarities among SLTs (7,11,13). Relative toxicities of the three types of SLT for pigs parallel those reported for mice (6). The SLT-I and SLT-II preparations resulted in neurological signs and pathology identical to those produced by SLT-IIv preparations, but SLT-I and SLT-II induced renal pathology and failed to cause subcutaneous edema or damage to the large intestine. The renal lesions associated with SLT-I or SLT-II may be a result of vascular damage, but the lesions differed from those observed in HUS, in that thrombosis and renal necrosis were not evident. This may be due to a difference in the response of renal tissue for the two species, age of the lesions, or the involvement of factors other than SLT in the pathogenesis of the condition.

Fibrinoid necrosis of small arteries and arterioles was evident with all SLTs and is considered basic to the pathology of ED (24). A similar lesion occurs in the kidneys of patients with HUS (34,35) and in the brain of rabbits given Shiga toxin (36).

The presence of endotoxin in SLT preparations gave rise to characteristic signs and early deaths, as have been described for other studies in which extracts of ED strains of *E. coli* were administered parenterally to pigs (2,8,24,37). The effects of endotoxin were evident despite treatment of the SLT samples with sodium deoxycholate, which is reported to moderate the effects of endotoxin (24). Although it is possible, in this and other studies, to distinguish responses attributable to endotoxin, it is not possible to rule out a role for endotoxin in edema disease until a pure EDP or SLT-IIv preparation is available.

ACKNOWLEDGMENTS

Excellent technical assistance was provided by Marion Wilke and Doug Elliot. Paul Huber's assistance with the photographs is gratefully acknowledged.

REFERENCES

1. **TIMONEY JF.** Oedema disease of swine. Vet Rec 1950; 62: 748-755.
2. **ERSKINE RG, SOJKA WJ, LLOYD MK.** The experimental reproduction of a syndrome indistinguishable from oedema disease. Vet Rec 1957; 69: 301-303.
3. **CLUGSTON RE, NIELSEN NO.** Experimental edema disease of swine (*E. coli* enterotoxemia) I. Detection, and preparation of an active principle. Can J Comp Med 1974; 38: 22-28.
4. **GREGORY DW.** Experimental edema disease (hemolytic *Escherichia coli* toxemia) in pigs and in mice. Am J Vet Res 1960; 21: 88-94.
5. **KONOWALCHUK J, SPEIRS J, STAVRIC S.** Vero response to a cytotoxin of *Escherichia coli.* Infect Immun 1977; 18: 775-779.
6. **STROCKBINE NA, MARQUES LRM, NEWLAND JW, SMITH HW, HOLMES RK, O'BRIEN AD.** Two toxin-converting phages from *Escherichia coli* 0157:H7 strain 933 encode antigenically distinct toxins with similar biologic activities. Infect Immun 1986; 53: 135-140.
7. **PETRIC M, KARMALI MA, RICHARDSON S, CHEUNG R.** Purification and biological properties of *Escherichia coli* verocytotoxin. FEMS Microbiol Lett 1987; 41: 63-68.
8. **SMITH HW, GREEN P, PARSELL Z.** Vero cell toxins in *Escherichia coli,* and related bacteria: transfer by phage and conjugation, and toxic action in laboratory animals, chickens, and pigs. J Gen Microbiol 1983; 129: 3121-3137.
9. **GANNON VPJ, GYLES CL, FRIENDSHIP RW.** Characteristics of verotoxigenic *Escherichia coli* from pigs. Can J Vet Res 1988; 52: 331-337.
10. **GONZALEZ EA, BLANCO J.** Production of cytotoxin VT in enteropathogenic, and non-enteropathogenic *Escherichia coli* strains of porcine origin. FEMS Microbiol Lett 1985; 26: 127-130.
11. **MARQUES LRM, PEIRIS JSM, CRYZ SJ, O'BRIEN AD.** *Escherichia coli* strains isolated from pigs with edema disease produce a variant of Shiga-like toxin II. FEMS Microbiol Lett 1987; 44: 33-38.
12. **DOBRESCU L.** New biological effect of edema disease principle (*Escherichia coli* - neurotoxin), and its use as an *in vitro* assay for this toxin. Am J Vet Res 1983; 44: 31-34.
13. **GANNON VPJ.** Verotoxins of *Escherichia coli* from pigs. PhD Thesis, University of Guelph, 1987.
14. **GYLES CL, DE GRANDIS SA, MacKENZIE C, BRUNTON JL.** Cloning and nucleotide sequence analysis of the genes determining cytotoxin production in a porcine edema disease isolate of *Escherichia coli.* Microbial Pathogenisis 1988; 5: 419-426.
15. **MILLER JH.** Experiments in Molecular Genetics. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1972.
16. **DE BRUIJN FJ, LUPSKI JR.** The use of transposon mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids — a review. Gene 1984; 27: 131-149.

17. **ROUSSEL AF, CHABBERT YA.** Taxonomy and epidemiology of gram-negative bacterial plasmids studied by DNA-DNA filter hybridization in formamide. J Gen Microbiol 1978; 104: 269-276.
18. **MANIATIS T, FRITSCH EF, SAMBROOK J.** Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory, 1982.
19. **FRISCHAUF AM, LEHRACH H, POUSTKA A, MURRAY N.** Lambda replacement vectors carrying polylinker sequences. J Mol Biol 1983; 170: 827-842.
20. **CURTISS III R.** Gene transfer. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips B, eds. Manual of Methods for General Bacteriology. Washington, DC: American Society for Microbiology, 1981: 243-265.
21. **BRADFORD MM.** A rapid, and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 77: 248-253.
22. **HUBERT JJ.** Bioassay. Ed 2. Dubuque, Iowa: Kendall/ Hunt Publishing Co., 1984.
23. **KURTZ HJ, BERGELAND ME, BARNES DM.** Pathologic changes in edema disease of swine. Am J Vet Res 1969; 30: 791-806.
24. **CLUGSTON RE, NIELSEN NO, SMITH DLT.** Experimental edema disease of swine (*E. coli* enterotoxemia) III. Pathology and pathogenesis. Can J Comp Med 1974; 38: 34-43.
25. **THOMLINSON JR, BUXTON A.** Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastroenteritis associated with *Escherichia coli*. Immunology 1963; 6: 126-139.
26. **BERTSCHINGER HU, POHLENZ J.** Bacterial colonization, and morphology of the intestine in porcine *Escherichia coli* enterotoxemia (edema disease). Vet Pathol 1983; 20: 99-110.
27. **JOHNSON WM, LIOR H, BEZANSON GS.** Cytotoxic *Escherichia coli* 0157:H7 associated with haemorrhagic colitis in Canada. Lancet 1983; 1: 76.
28. **CAVANAGH JB, HOWARD JG, WHITBY JL.** The neurotoxin of *Shigella shigae*. A comparative study of the effects produced in various laboratory animals. Br J Exp Pathol 1956; 37: 272-278.
29. **KEUSCH GT, JACEWICZ M, DONOHUE-ROLFE A.** Pathogenesis of *Shigella* diarrhea. J Infect Dis 1986; 153: 238-248.
30. **SHETH K, SWICK HM, HAWORTH N.** Neurological involvement in hemolytic-uremic syndrome. Ann Neurol 1986; 19: 90-93.
31. **KARMALI MA, STEELE BT, PETRIC M, LIM C.** Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin, and cytotoxin-producing *Escherichia coli* in stools. Lancet 1983; 1: 619-620.
32. **KOSTER F, LEVIN J, WALKER L, TUNG KSK, GILMAN RH, RAHAMAN MM, MAJID MA, ISLAM S, WILLIAMS RC.** Hemolytic-uremic syndrome after shigellosis. N Engl J Med 1978; 298: 927-933.
33. **ASHKENAZI S, DINARI G, ZEVULUNOV A.** Convulsions in childhood shigellosis. Am J Dis Child 1987; 141: 208-210.
34. **BRAIN MC, DACIE JV, HOURIHANE DO'B.** Microangiopathic haemolytic anaemia: the possible role of vascular lesions in pathogenesis. Br J Haemat 1962; 8: 358-374.
35. **KAPLAN B, DRUMMOND KN.** The hemolytic-uremic syndrome is a syndrome. N Engl J Med 1978; 298: 964-966.
36. **BRIDGEWATER FAJ, MORGAN RS, ROWSON KEK, WRIGHT GP.** The neurotoxin of *Shigella shigae*. Morphological, and functional lesions produced in the central nervous system of rabbits. Br J Exp Pathol 1955; 36: 447-453.
37. **NIELSEN NO, CLUGSTON RE.** Comparison of *E. coli* endotoxin shock, and acute experimental edema disease in young pigs. Ann NY Acad Sci 1971; 176: 176-189.