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Interaction of Verotoxin 2e with Pig Intestine

THOMAS E. WADDELL,[1]* CLIFFORD A. LINGWOOD,[2] AND CARLTON L. GYLES[1]

Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1,[1] and Department of Microbiology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8[2]

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In pigs with edema disease, verotoxin 2e (VT2e) is produced in the intestine and transported to tissues, but neither the mechanism by which toxin passes through the intestine nor its failure to induce an enterotoxic reaction is understood. Binding of VT2e to pig intestine was examined by enzyme-linked immunosorbent assay involving microvillus membranes (MVM) and crude mucus; thin-layer chromatographic overlay immunoassay with total lipids extracted from MVM; and indirect immunofluorescence of toxin bound to thin sections of jejunum, ileum, and colon. VT2e bound significantly to MVM from pig jejunum and ileum but not to crude mucus. Verotoxin 2e-binding glycolipids, globotetraosylceramide and globotriaosylceramide, were detected by thin-layer chromatographic overlay immunoassay in extracts of MVM from jejunum and ileum. Indirect immunofluorescence showed that VT2e bound to vessels within the submucosa and muscularis mucosa of the jejunum, ileum, and colon and to enterocytes at the lower portion but not at the tips of villi in the jejunum and ileum. Receptors for VT2e are therefore present in the intestine of the pig, but their role in absorption of VT2e is unclear since intraintestinal inoculation of pigs with large quantities of VT2e does not result in edema disease. Previously reported lack of enterotoxicity of verotoxins in pig intestine may be explained by the absence of toxin receptors in the villus absorptive enterocytes.

Verotoxins (VTs) are a family of protein cytotoxins produced by certain strains of *Escherichia coli* that are implicated in disease in humans and animals. Humans are affected by both intestinal and extraintestinal manifestations of infection seen as hemorrhagic colitis and the hemolytic uremic syndrome, respectively (12). Pigs, on the other hand, show only the extraintestinal effects of infection, resulting in edema disease (ED), which is a verotoxemia (2). In the extraintestinal diseases, VTs are absorbed across the intestinal epithelium and taken up by the vasculature, where they are believed to damage susceptible endothelial cells in target organs. The mechanism of absorption of VTs from the intestine is not known.

There are two antigenically related groups of VTs (9); the verotoxin 1 (VT1) group includes VT1 and Shiga toxin, whereas the VT2 group includes all of the other known VTs. All VTs bind to globotriaosylceramide (Gb3) (20, 21, 31, 37), which is considered to be a functional receptor (26, 36). VT2e also binds to globotetraosylceramide (Gb4), a functional receptor for the toxin (4, 16, 31), and to Forssman antigen and galactosylglobotetraosylceramide (4, 31). The distribution of VT receptor glycolipids in enterocytes (reviewed in reference 17), tissues (42), and endothelial cells (34) is thought to account for the local intestinal effects of VTs in rabbits and the systemic effects of VTs in natural and experimental verotoxemia.

ED is a frequently fatal disease of weaned pigs which follows intestinal colonization by verotoxigenic *E. coli* (VTEC) strains which produce VT2e (1, 33). The disease is characterized by vascular damage in target organs (18). ED is the only natural extraintestinal VTEC-associated disease for which a clearly defined role of a VT has been established; intravenous inoculation of pigs with purified VT2e reproduces the common clinical signs and pathologic lesions found in the natural disease (24).

In experimental ED, absorption of VT2e occurred without visible damage to the intestinal epithelium in pigs whose intestines had been colonized with an ED strain of *E. coli* (1, 25). Since the intestine of the postweaning pig generally prevents the transmucosal passage of macromolecular proteins (41) and intraintestinal inoculation of pigs with VT2e alone does not reproduce ED (38), it is likely that specialized conditions are required for the transport of toxin from the gut to the systemic vasculature. The possible mechanisms of absorption of VT2e from the intestine of the pig include specific receptor-mediated transcytosis, nonspecific transcytosis through enterocytes, paracellular transcytosis, and antigenic sampling through M cells.

To investigate the mechanism of intestinal absorption of VT2e from the intestine, this study examined the interaction of VT2e with the pig intestine. Comparisons were made with the rabbit small intestine because the rabbit has been used extensively as a model to study the pathogenesis of VTEC-associated intestinal and extraintestinal disease (10) and the effects of VTs in the small intestines of rabbits of different ages have been well characterized (7, 11, 13, 15, 26, 27). For these reasons, the interactions of VT2e with the intestines of postweaning pigs, 1-week-old rabbits, and 6-week-old rabbits were compared.

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MATERIALS AND METHODS

VT2e purification. VT2e was purified from *E. coli* JM101(pGT110) (35) (obtained from J. Brunton, Department of Microbiology, University of Toronto, Toronto, Ontario, Canada). Briefly, a polymyxin B sulfate (Sigma, St. Louis, Mo.) extract of an isopropylthio-β-D-galactopyranoside (Xymotech, Montreal, Quebec, Canada)-induced overnight culture was subjected to differential ammonium sulfate precipitation, and protein precipitated between 40 and 60% am-

* Corresponding author. Present address: Health of Animals Laboratory, Agriculture and Agri-Food Canada, 110 Stone Road West, Guelph, Ontario, Canada N1G 3W4. Phone: (519) 822-3300. Fax: (519) 822-2280. Electronic mail address: waddellt@em.agr.ca.

monium sulfate saturation was dissolved in water and desalted with a Sephadex G-50 column (Pharmacia, Baie d'Urfe, Quebec, Canada) equilibrated with 10 mM Na_2HPO_4 buffer containing 100 mM NaCl (pH 6.0). The resulting material was applied with a syringe to a 1-ml Econo-Pac S cation-exchange column (Bio-Rad, Mississauga, Ontario, Canada) equilibrated with 10 mM Na_2HPO_4 buffer containing 100 mM NaCl (pH 6.0). Bound toxin was eluted isocratically with 10 mM Na_2HPO_4 buffer containing 250 mM NaCl (pH 6.0).

Toxin activity was assessed by the Vero cell cytotoxicity assay (22), with modifications. After 3 days of growth, the cell monolayers were fixed, stained with crystal violet, washed with water, and air dried. The residual crystal violet was solubilized with 200 μl of 70% ethanol, and 50 μl was transferred to 200 μl of phosphate-buffered saline (PBS) in 96-well plates. For each sample, the absorbance (optical density [OD]; λ = 550 nm) was measured in an enzyme-linked immunosorbent assay (ELISA) plate reader, and the 50% tissue culture cytotoxic dose was determined by comparing the absorbance with that produced by control monolayers not treated with toxin. The purity of toxin was assessed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and the lipopolysaccharide content was determined by a colorimetric *Limulus* amebocyte assay (BioWhittaker, Walkersville, Md.). Protein concentrations were determined by the bicinchoninic acid assay (Pierce, Chromatographic Specialties, Brockville, Ontario, Canada).

Purified VT2e had a specific activity of 3.2×10^9 tissue culture cytotoxic doses per mg of protein and contained 0.4 ng of lipopolysaccharide per mg of protein. Very minor contaminating bands were occasionally observed in toxin preparations following Coomassie blue staining of SDS-polyacrylamide gels.

Purified VT2 was kindly provided by C. Clark (Health of Animals Laboratory, Agriculture and Agri-Food Canada, Guelph, Ontario, Canada).

Production of rabbit immune serum to VT2e. Immune serum to VT2e was produced by subcutaneous injection of New Zealand White rabbits (two sites five times at 3-week intervals) with 25 μg of glutaraldehyde-inactivated VT2e adsorbed to aluminum hydroxide [Alhydrogel; Langford (Cyanamid), Inc., Guelph, Ontario, Canada] containing 0.01% saponin (kindly provided by S. Rosendal, University of Guelph). VT2e toxoid was prepared from toxin purified from *E. coli* TB1(pCG6) by the method of MacLeod and Gyles (22), with the exception that cation-exchange fast protein liquid chromatography (FPLC; Pharmacia) employing a linear gradient of 100 to 500 mM NaCl containing 10 mM NaH_2PO_4 (pH 6.0) was performed before anion-exchange FPLC. The immune serum neutralized 4 tissue culture cytotoxic doses of VT2e with a titer of 10^6 in a toxin neutralization assay performed on Vero cells (22) and was found to be specific for VT2e by Western blotting (immunoblotting), radial immunodiffusion, and immunoelectrophoresis.

MVM and crude mucus isolation. Intestinal microvillus membranes (MVM) were prepared from the jejuna and ilea of three 5- to 6-week-old weaned female Yorkshire-Landrace pigs and from the small intestines of three 1-week-old and three 6-week-old New Zealand White rabbits by the method of Kessler et al. (14). The enrichment of MVM was assessed by measuring alkaline phosphatase activity by the method of Weiser (40). Purified MVM were enriched between 10 and 30 times for alkaline phosphatase activity. Crude mucus from the jejunum, ileum, and colon was prepared from three 5- to 6-week-old female Yorkshire-Landrace pigs by gently scraping the mucosa into PBS and subjecting the samples to centrifugation at 27,000 × *g* for 30 min.

ELISA detection of VT2e binding to intestinal samples. MVM or crude mucus preparations were diluted to a concentration of 10 μg/ml in Tris-buffered saline (TBS; 50 mM Tris-HCl–100 mM NaCl [pH 7.6]), added to wells of Falcon Pro-Bind ELISA plates (Fisher), and incubated at 4°C overnight. Unbound material was removed by washing, and residual binding sites were blocked with TBS containing 1% bovine serum albumin (BSA; Boehringer Mannheim, Laval, Quebec, Canada) (BSA-TBS). VT2e or VT2 (0 to 1,000 ng/ml) was added to the wells, and the plates were incubated for 2 h. After washing, rabbit anti-VT2e, diluted 1/2,000 (vol/vol), and enzyme immunoassay-grade affinity-purified anti-rabbit immunoglobulin G (IgG; heavy plus light chain)-horseradish peroxidase conjugate (Bio-Rad), diluted 1/3,000 (vol/vol), were added sequentially for 2 h each. Dilutions were prepared in BSA-TBS, and all incubations and washings were performed at 4°C. The plates were developed with 2,2′-azino-di-[3-ethylbenzthiazoline-6-sulfonate] (Boehringer Mannheim). Mean absorbance values were compared by using Student's *t* test.

The amount of MVM protein bound to the ELISA plate was determined by measuring the alkaline phosphatase activity remaining on the plate after washing and then comparing this activity with that of a standard curve generated with MVM in suspension. This was accomplished by modifying the method of Weiser (40) to a 96-well plate format. MVM bound to the ELISA plate or in suspension were incubated at 37°C with 0.5 M Tris-HCl (pH 9.4), 0.3 mM $ZnCl_2$, 10 mM $MgCl_2$, and 0.23 mM *p*-nitrophenyl phosphate, and released *p*-nitrophenol was determined spectrophotometrically (λ = 405 nm) in an ELISA plate reader.

VT2e binding to lipid extracts. Total lipids were extracted from pig and rabbit MVM by the method of Folch et al. (6). Lipid extracts and glycolipid standards (glycosylceramide, lactosylceramide, Gb3, Gb4, and Forssman antigen) were separated by ascending chromatography in chloroform-methanol-water (60/40/9, vol/vol/vol) and examined for VT2e binding by thin-layer chromatographic (TLC) overlay immunoassay (3) using rabbit anti-VT2e, alkaline phosphatase-conjugated protein A (Pierce, Chromatographic Specialties), and nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate substrate (Bio-Rad). Glycolipids in MVM samples were identified by comparison with authentic glycolipid standards.

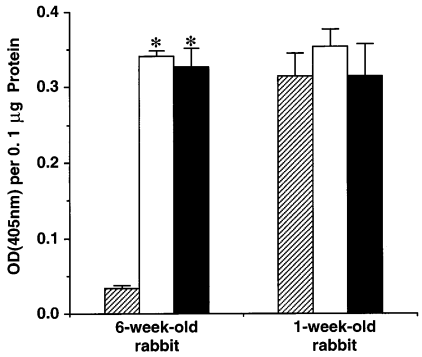

FIG. 1. Binding of VT2e and VT2 to rabbit small intestinal MVM. MVM were immobilized in ELISA plate wells, blocked with BSA, and incubated with no toxin (hatched bars), 1,000 ng of VT2e per ml (open bars), or 1,000 ng of VT2 per ml (closed bars). Bound toxin was detected with polyclonal rabbit anti-VT2e and horseradish peroxidase-conjugated goat anti-rabbit IgG. The data were corrected for the relative amount of MVM immobilized in the ELISA plates (see Results). The values are means ± SD from one experiment performed in triplicate. Statistically significant binding ($P < 0.005$) is indicated by asterisks. The results are representative of two separate experiments.

Immunofluorescence detection of VT2e binding to pig intestine. Air-dried frozen sections of intestines from five 5- to 6-week-old weaned female Yorkshire-Landrace pigs were overlaid sequentially with VT2e (2 μg/ml), rabbit anti-VT2e (1/1,000, vol/vol), and fluorescein-conjugated goat anti-rabbit $F(ab')_2$ (1/100, vol/vol; Boehringer Mannheim) for 1 h each. The toxin and antibodies were diluted in BSA-TBS. BSA-TBS containing no VT2e was applied to control sections, which were incubated with rabbit anti-VT2e and then with fluorescein-conjugated goat anti-rabbit $F(ab')_2$.

RESULTS

VT2e binding to MVM and lipid extracts. An ELISA was developed to study the interaction of VT2e with intestinal MVM and mucus. Since MVM are particulate and it was not known if they could be immobilized in ELISA plates in a reproducible manner, the amount of MVM protein that bound to ELISA plates was assessed by comparing the residual intestinal alkaline phosphatase activity that was not removed from the plate by washing with that of MVM in suspension. Using this approach, we found that MVM from pigs and rabbits bound to polystyrene ELISA plates in a reproducible manner; binding levels, expressed as micrograms of MVM protein immobilized, were 0.104 ± 0.011, 0.326 ± 0.050, 0.196 ± 0.035, and 0.187 ± 0.036 (means ± standard deviations [SD] for three experiments each performed in quadruplicate) for 1-week-old rabbit small intestine, 6-week-old rabbit small intestine, weaned-pig jejunum, and weaned-pig ileum, respectively.

To characterize the utility of ELISA to detect binding of VTs to MVM, 6- and 1-week-old rabbit small intestinal MVM were immobilized in ELISA plates; VT2e, VT2, or no toxin was added to the wells; and bound toxin was detected with polyclonal rabbit anti-VT2e (Fig. 1). MVM from a 6-week-old rabbit, which have been reported to contain Gb3 (27), bound both VT2 and VT2e significantly ($P < 0.005$). MVM from a 1-week-old rabbit, which have been reported not to contain Gb3 or Gb4 (27), the receptors for VT2 and VT2e (4, 30, 35), did not bind VT2 or VT2e significantly. The pattern of VT2 and VT2e binding by the small intestinal MVM from rabbits of different ages correlated with the ability of the VTs to cause fluid accumulation in ligated loops of rabbit small intestine (8, 17, 22). MVM from pigs and rabbits were next immobilized in ELISA plates and incubated with various amounts of VT2e. The binding of toxin was found to be saturable, with maximal

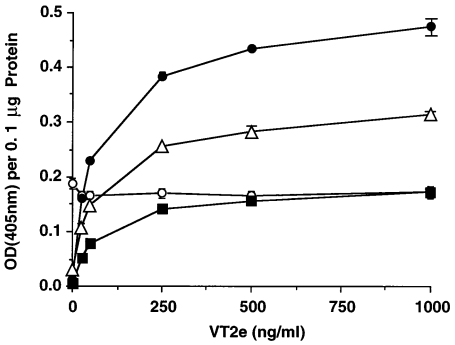

FIG. 2. Binding of VT2e 1-week-old rabbit small intestinal MVM (○), 6-week-old rabbit small intestinal MVM (■), and weaned-pig jejunal MVM (△) and ileal MVM (●). MVM were immobilized in ELISA plate wells, blocked with BSA, and incubated with 0 to 1,000 ng of VT2e per ml. Bound toxin was detected with polyclonal rabbit anti-VT2e and horseradish peroxidase-conjugated goat anti-rabbit IgG. The data were corrected for the relative amount of MVM immobilized in the ELISA plates (see Results). The values are means ± SD from one experiment performed in quadruplicate. The SD in most cases are indicated within the symbols. The results are representative of two separate experiments.

binding occurring at approximately 250 ng of VT2e per ml (Fig. 2). Similar saturation binding curves were observed for all of the membrane preparations that bound VT2e significantly. We therefore considered that the ELISA method would be useful to characterize the interaction of VT2e with intestinal samples.

MVM from three weaned pigs, three 6-week-old rabbits, and three 1-week-old rabbits were examined for VT2e binding by ELISA (Fig. 3). VT2e bound to jejunal MVM from two of the three pigs ($P < 0.005$), to ileal MVM from the three pigs ($P < 0.005$), and to small intestinal MVM from all of the 6-week-old rabbits ($P < 0.005$) but not to small intestinal MVM from the 1-week-old rabbits. Total lipid extracts from rabbit and pig MVM samples were separated by ascending TLC and examined qualitatively for VT2e-binding glycolipids by TLC overlay immunoassay (Fig. 3, top). VT2e bound to lipid species from pig intestinal MVM displaying the mobility of Gb3 and Gb4. The pig intestinal MVM which failed to bind toxin in ELISA did not contain detectable Gb3 or Gb4. Neither Gb3 nor Gb4 was detected by TLC overlay immunoassay in the ileal MVM from one pig which repeatedly bound VT2e in very low but significant amounts in ELISA. Six-week-old rabbit small intestinal MVM, but not 1-week-old rabbit small intestinal MVM, contained the toxin-binding glycolipid Gb3.

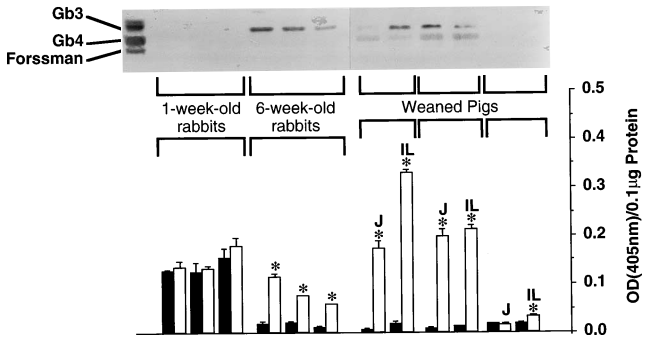

FIG. 3. Binding of VT2e to three 1-week-old rabbit small intestinal MVM, three 6-week-old rabbit small intestinal MVM, and three weaned pig jejunal (J) and ileal (IL) MVM and to corresponding lipid extracts which are shown directly above. MVM were immobilized in ELISA plate wells, blocked with BSA, and incubated with no VT2e (solid bars) or 250 ng of VT2e per ml (open bars). Bound toxin was detected with rabbit anti-VT2e and horseradish peroxidase-conjugated anti-rabbit IgG. For the rabbits, each pair of bars represents one animal. For the pigs, two pairs of bars (J, IL) represent one animal. The data were corrected for the relative amount of MVM immobilized in the ELISA plates (see Results). The values are means ± SD from one experiment performed in quadruplicate. Statistically significant binding ($P < 0.005$) is indicated by asterisks. The results are representative of two separate experiments. (Top) Lipid extracts (equivalent to 25 µg of protein) were separated by TLC and examined for VT2e-binding MVM glycolipids by overlay immunoassay with VT2e, rabbit anti-VT2e, and alkaline phosphatase-conjugated protein A. The top panel is reprinted from reference 38a with permission of the publisher.

VT2e binding to mucus. VT2e did not bind to immobilized crude jejunal, ileal, and colonic mucus from three pigs in ELISA (not shown).

VT2e binding to intestinal tissue. VT2e was overlaid on thin sections of pig jejunum, ileum, and colon, and bound toxin was detected by indirect immunofluorescence. VT2e bound to arterioles and venules at all levels of the mucosa and to interstitial cells of the laminae propriae of villi in tissue samples from all of the pigs examined. The binding of toxin to arterioles is shown in Fig. 4. The tunicae mediae of arterioles appeared to be strongly labeled with VT2e. VT2e bound faintly to the apical membrane of enterocytes at the lower portion of the villi (Fig. 5) in the jejuna and ilea of three of the five pigs examined. The binding of toxin to enterocytes was sporadic, and not all villi were labeled. VT2e did not bind to enterocytes in the colon.

DISCUSSION

All VTs and Shiga toxin are thought to cause fluid accumulation in ligated loops of the rabbit small intestine by the same mechanism, which has been studied in detail for Shiga toxin. Mobassaleh et al. (26) found that rabbits of different ages differed in susceptibility to the enterotoxic effects of Shiga toxin. Rabbits younger than 16 days of age did not respond to Shiga toxin in the intestine, rabbits between 16 and 18 days of age showed variable responses, and rabbits older than 18 days of age showed a uniformly high response (26). The onset of development of the enterotoxic response was correlated with the onset of binding of ^{125}I-labeled Shiga toxin to MVM derived from the small intestine (26) and the Gb3 content of the MVM (26, 27). Keusch et al. (15) and Keenan et al. (13) found that VTs induced apoptosis and expulsion of enterocytes from the tips of villi in rabbit small intestine. Together, these observations suggest that Shiga toxin and other VTs act directly on apical enterocytes to induce fluid accumulation and that this effect is mediated by the glycolipid Gb3. Currently, Shiga toxin is thought to cause fluid accumulation in ligated loops of rabbit small intestine by inhibiting Na^+ absorption by apical enterocytes (11).

Although specific receptors for VTs are present in the intestines of rabbits (17) and rabbits are susceptible to the lethal effects of VT administered by the intravenous route (30, 42), there is no evidence that these receptors can mediate absorption of VT to the vasculature. Indeed, evidence suggests that they cannot. Intraintestinal administration of VT to rabbits (28) or VTEC infection of rabbits (5, 19, 28, 29, 32) does not result in clinical signs of verotoxemia.

Intraintestinal inoculation of pigs with large quantities of purified VT2e has failed to reproduce ED (38). Indeed, VT2e alone does not cause fluid accumulation (23) or local damage to the intestinal mucosa (38) in ligated loops of the pig small intestine. The reason for the lack of sensitivity of the pig to intraintestinal VT2e is not clear since the toxin is stable in pig intestinal washings (23), and Gb3 and Gb4, receptors for VT2e (4, 31), have been reported to be present in pig intestinal mucosal scrapings (31). The fact that other VTs also fail to

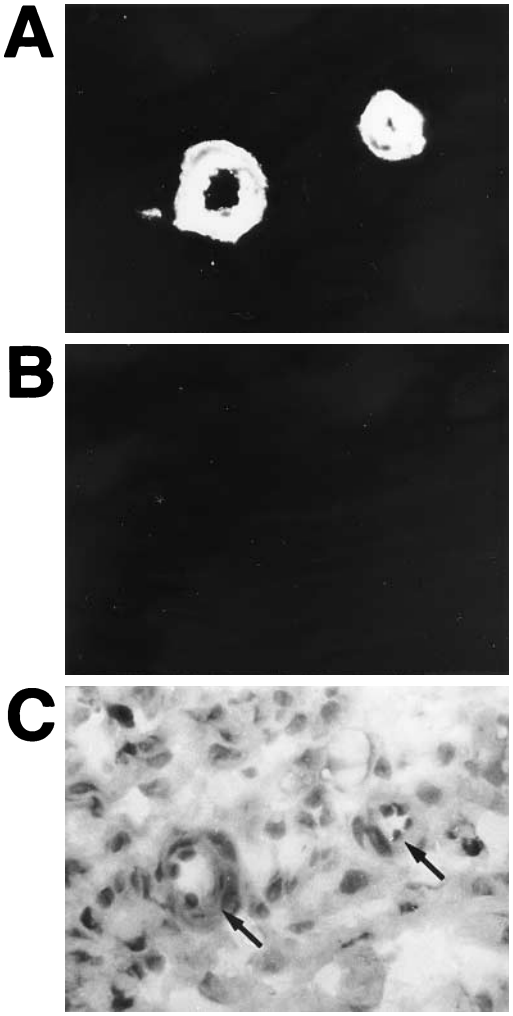

FIG. 4. Binding of VT2e to small arterioles in the submucosa of the colon of a weaned pig. Three consecutive frozen sections are shown. (A) A frozen section was overlaid with VT2e, and bound toxin was detected with polyclonal rabbit anti-VT2e and fluorescein-conjugated goat anti-rabbit $F(ab')_2$. Two arterioles appear brightly fluorescent. (B) A frozen section incubated with polyclonal rabbit anti-VT2e and fluorescein-conjugated goat anti-rabbit $F(ab')_2$ shows background fluorescence. (C) A frozen section fixed with formalin and then stained with hematoxylin and eosin shows the arterioles (arrows). Magnification, ×300.

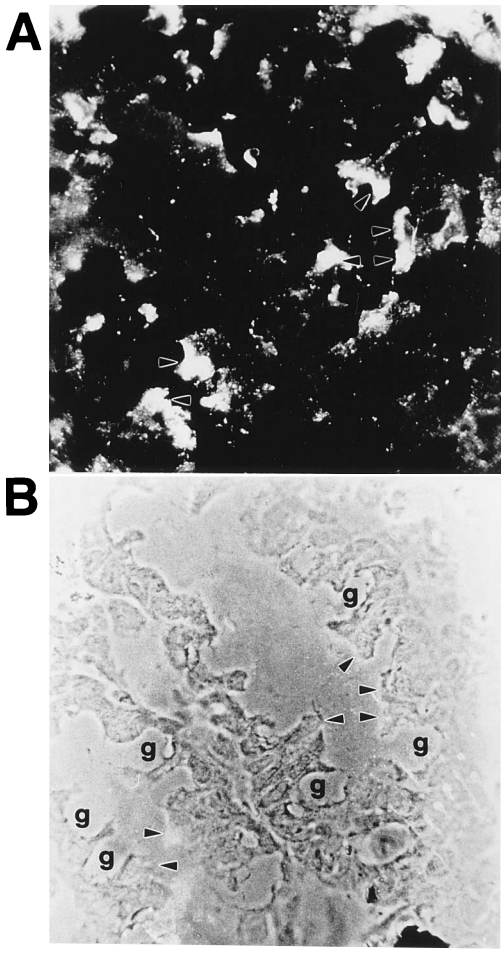

FIG. 5. Binding of VT2e to brush border of enterocytes at the lower portion of villi in the ileum of a weaned pig. (A) A frozen section was overlaid with VT2e, and bound toxin was detected with polyclonal rabbit anti-VT2e and fluorescein-conjugated goat anti-rabbit $F(ab')_2$. Weakly fluorescent brush borders are indicated by arrowheads. An adjacent frozen section incubated with polyclonal rabbit anti-VT2e and fluorescein-conjugated goat anti-rabbit $F(ab')_2$ displayed background fluorescence (not shown). (B) Phase-contrast image of panel A. Regions corresponding to those fluorescent in panel A are indicated with arrowheads. g, goblet cells.

cause fluid accumulation in ligated loops of the pig small intestine (8) suggests that there is something different in the way in which the pig intestine (compared with the rabbit intestine) interacts with VTs. One would predict that intestinal receptor in the pig would confer sensitivity to VTs by the intestinal route. Since this is not the case, differences in the nature of interaction of VT2e with the intestines of the pig and the rabbit may account for the propensity of VT2e-producing *E. coli* to cause systemic but not enteric disease in pigs.

In this study, VT2e was shown to bind to MVM from the jejuna and ilea of weaned pigs. The small intestinal MVM from 6-week-old rabbits, which are at an age which is responsive to the enterotoxic effects of Shiga toxin and VT1 (13, 15) and VT2e (22), were also shown to bind VT2e. The MVM from 1-week-old rabbits, which are at an age which is not responsive to the enterotoxic effects of VT (17), did not bind VT2e. The binding of VT2e to MVM from pigs and rabbits was correlated with the presence of VT receptor glycolipids. However, VT-binding glycolipids were not detected in one preparation of pig ileal MVM which bound VT2e reproducibly in ELISA. Since only Gb3 was detected in 6-week-old rabbits, it is likely that this glycolipid mediates the enterotoxic effect of VT2e in the rabbit. The low enterotoxic potential of VT2e in the rabbit may be explained by the poorer ability of Gb3 to mediate the cytotoxicity of VT2e as demonstrated in cultured cell lines (16, 31).

The lack of enterotoxicity of VTs in the weaned pig (8, 23) seems to conflict with the presence of receptor in intestinal MVM and suggests that toxin may not bind to receptor on enterocytes because of interaction with intestinal mucus. In a solid-phase binding assay, VT2e did not bind to mucus from any region of the pig intestine, suggesting that mucus does not act as a barrier to access of toxin to intestinal enterocytes. However, it has been suggested that mucus may act as a barrier to the physical diffusion of toxins (39).

Another possible explanation for the lack of enterotoxicity of VT2e in the pig is that the toxin binds to a population of enterocytes which would not result in generation of net fluid

secretion in the intestine or that the concentration of receptor in the apical enterocytes is too low to result in a biological response. To investigate these possibilities, the location of VT2e binding to frozen sections of small intestine and colon was examined by indirect immunofluorescence. VT2e was observed to bind to the brush border of enterocytes in the lower portion of the villi of the jejunum and ileum but not the colon. In two of five pigs examined, binding to enterocytes was not detected. Variability has been noted in rabbit MVM Gb3 content (27) and the number of binding sites for Shiga toxin (26). It is not known whether the Gb3 and Gb4 content of pig intestine is developmentally regulated as is the Gb3 content of the rabbit small intestine. It is possible that the expression of Gb3 and Gb4 in the pig intestine is influenced by weaning stress.

In all of the pigs examined, VT2e bound to blood vessels in the submucosa and muscularis mucosa and to some interstitial cells of the villi of the jejunum, ileum, and colon when overlaid on thin sections of tissue. It should be noted that a limitation of the overlay technique is that sites labeled with toxin may not be targeted in vivo. However, the results suggest that VT2e receptor is expressed widely in the pig intestinal vasculature. In natural ED, necrotizing arteritis of the small and large intestine is commonly observed (18), and vascular damage in the intestine has been observed following experimental infection of pigs with an ED-causing strain of *E. coli* (25) or injection of pigs with purified VT2e (24). Methyiapun et al. (25) noted that intestinal vascular lesions observed following infection of pigs with an ED-causing strain of *E. coli* did not necessarily coincide with areas of mucosal colonization. Ischemia resulting from the action of small amounts of absorbed VT2e on areas of the intestinal vasculature may lead to an increased absorption of toxin.

The interaction of VT2e with the pig intestine is different from that of Shiga toxin with the rabbit intestine. In the pig, VT2e binds to the enterocytes at the base of the villi and not to the apical absorptive enterocytes, as does Shiga toxin in the rabbit (11). The lack of enterotoxicity of VT2e and other VTs in pigs may therefore be explained by the apparent lack or cryptic nature of VT receptor within apical absorptive enterocytes. Although VT receptor glycolipids are present in the intestine of the pig, they apparently cannot mediate uptake of VT2e to the systemic vasculature since intraintestinal administration of VT2e to pigs does not result in toxemia (38). Intestinal colonization of pigs with an ED strain of *E. coli* has been shown to decrease from the apex to the base of the villi (1). Therefore, a local environment at the villus tip lacking specific VT2e receptors may allow toxin to traverse the gut barrier by a nonspecific mechanism.

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