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RESPONSE OF INTESTINAL EPITHELIAL CELLS TO *TRICHURIS SUIS* EXCRETORY–SECRETORY PRODUCTS AND THE INFLUENCE ON *CAMPYLOBACTER JEJUNI* INVASION UNDER IN VITRO CONDITIONS

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ABSTRACT: We previously developed a swine animal model in which natural host resistance to *Campylobacter jejuni* is altered by experimental infection with low numbers of the nematode *Trichuris suis*. Pigs naturally colonized with *C. jejuni* experience colitis because of the invasion of the bacterium approximately 21 days after exposure to *T. suis*. To better understand the mechanism of *T. suis*–dependent *C. jejuni* colitis, we evaluated the effects of *T. suis* excretory–secretory products (ESPs) on intestinal epithelial cells (IECs) and the influence of ESP on *C. jejuni* invasion in IECs under in vitro conditions. Viability assays revealed a dose-dependent cytotoxic response in ESP-treated IECs, particularly IPEC-1 and INT407 cells. Transepithelial electrical resistance dropped significantly in IPEC-1 cells treated on apical and basolateral surfaces, but not in those treated only on apical surfaces. Using the gentamicin-killing assay, reduced numbers of intracellular *C. jejuni* were recovered from IECs treated with ESP at 1 mg protein/ml concentration. This observation can be at least partially explained by a novel antibacterial activity in ESP. Contrary to our hypothesis, ESP at subtoxic concentrations did not enhance invasion. In addition to mechanical damage from worms, these results suggest that soluble products released by *T. suis* contribute to IEC damage at the site of worm attachment.

Campylobacter jejuni is the most commonly isolated human enterobacterial pathogen in the United States, causing an estimated 4 million cases of enteritis per year (Altekruse et al., 1998). Although diarrhea is the most common presenting symptom and is usually self-limiting within 5–7 days, more severe consequences occur in immunocompromised hosts (Altekruse et al., 1998). *Campylobacter jejuni* infection is also the number one antecedent infection associated with the development of the Guillain-Barré syndrome, an autoimmune neuropathy (Buzby et al., 1997; Nachamkin et al., 1998). The primary source of human infection is improperly prepared poultry, but there are multiple additional reservoirs including swine (Borch et al., 1996).

Pigs are one of the best available experimental models to study *C. jejuni* pathogenesis (Boosinger and Powe, 1988; Vitovec et al., 1989; Babakhani et al., 1993). The observation that opportunistic *C. jejuni* become invasive in conventionally reared pigs infected with *Trichuris suis*, the swine whipworm (Mansfield and Urban, 1996), led us to pursue studies to determine the mechanisms of pathogenesis in this dual infection model. Our model is unique in that whipworm infection is necessary to condition the host for *C. jejuni* susceptibility. Whipworm-induced conditions, such as a weakened intestinal epithelial barrier or a compromised immunologic background, may simulate conditions in humans that are incapable of resisting infection by *C. jejuni*.

The interaction between *T. suis* and the normal colonic flora in pigs was first noted by Rutter and Beer (1975). They hypothesized that migrating *T. suis* larvae damaged the epithelium, thus creating an environment favorable for penetration by microorganisms. Mansfield and Urban (1996) extended these findings when they found intracellular bacteria in epithelial cells near the site of worm attachment, in addition to recovering *C. jejuni* from inflamed lymphoglandular complexes (LGCs) in the distal colon of *T. suis*–infected pigs. One of the hypotheses derived from the observation that *C. jejuni* invasion occurred at a site far-removed from worms in the proximal colon was that resistance to *C. jejuni*, mediated by the intestinal epithelial barrier, is diminished by soluble substances released by the worms.

The colonic intestinal epithelium is a primary target for tissue damage by whipworms and their excretory–secretory products (ESPs). Ingested *Trichuris* eggs containing infective L1 larvae hatch in the distal small intestine and the proximal large intestine (Rutter and Beer, 1975; Panesar, 1981). L1 larvae enter crypts in the cecum and colon, migrate to the base of the lumen, and penetrate the epithelial layer from within the crypt (Wakelin, 1969; Panesar, 1981). *Trichuris suis* larvae, although embedded in a tunnel of fused host epithelial cells, undergo 4 molts in the process of migration to the mucosal surface (Beer, 1973). Invasion and maintenance of this syncytial niche as the larvae develop and migrate to the mucosal surface may be aided by proteases and pore-forming proteins in ESPs, based on observations in a *Trichuris muris* infection model (Drake, Bianco et al., 1994, Drake, Korchev et al., 1994; Drake et al., 1998). As the worm matures, the posterior region emerges from the tunnel to lie free in the lumen, whereas the filamentous anterior portion remains buried within a shallow epithelial tunnel. During the adult *T. suis* stage, secretory products are released from the anterior end embedded superficially in the host epithelium, whereas excretory products are released from the posterior end protruding into the host gut lumen. Among the known proteins in ESPs from adult whipworms maintained in tissue culture media are a zinc metalloprotease (Hill et al., 1993), a thiol protease (Hill and Sakanari, 1997), a phenol oxidase (Fetterer and Hill, 1994), and a glycoprotein (Hill et al., 1997). The remaining constituents of ESPs, as well as their activity and fate in vivo are unknown.

Using in vitro model systems of the intestinal epithelial barrier, the goal of the present study was to identify a mechanism

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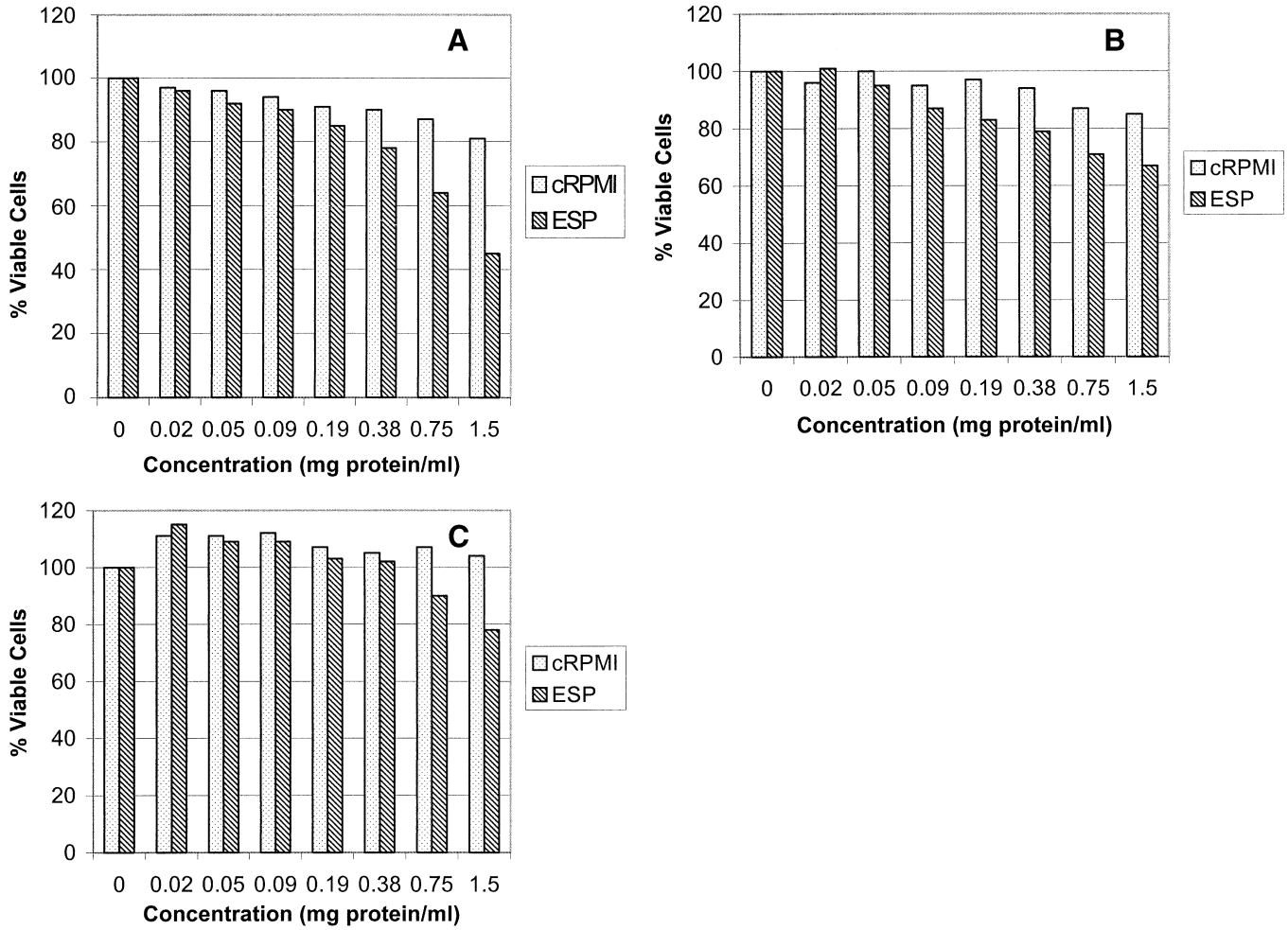

FIGURE 1. Effect of *Trichuris suis* ESP on the viability of IECs in culture. Serial 2-fold dilutions of ESP and control cRPMI were applied to INT407 (**A**), IPEC-1 (**B**), and Caco-2 (**C**) cells in 96-well plates (n = 3). Viability was measured by the spectrophotometic MTT assay after 72 hr exposure. INT407 and IPEC-1 cells were more susceptible to ESP than Caco-2 cells ($P < 0.05$). The ESP effect was different from the background cRPMI effect in INT407 and IPEC-1 cells, but not in the Caco-2 cells ($P < 0.01$).

by which *T. suis* ESP affects intestinal epithelial cells (IECs) in a manner consistent with the enhanced *C. jejuni* invasion observed in the in vivo model. Toward this aim, cell lines of pig and human origin were used to investigate the direct effect of *T. suis* ESP on IECs. In addition, the invasion of IECs by *C. jejuni* was measured in the presence and absence of ESPs to determine if ESPs rendered IECs more permissive for *C. jejuni* internalization.

MATERIALS AND METHODS

Preparation of *Trichuris suis* ESP

The *T. suis* ESP used in these experiments was prepared from adult whipworms pulled free from the colonic mucosa of experimentally infected pigs as described previously (Hill et al., 1993). After washing in sterile saline prewarmed to 37 C, worms were washed in sterile Hanks balanced salt solution (HBSS) to remove fine debris not visible under the microscope. This was followed by incubation in a 5×-concentrated antibiotic cocktail in Roswell Park Memorial Institute (medium) (RPMI)-1640 for a 16- to 24-hr period. The original 5× cocktail contained 500 U/ml penicillin, 500 μg/ml streptomycin, 1.25 μg/ml amphotericin B, and 350 μg/ml chloramphenicol (CAP). A second incubation in a 1× antibiotic cocktail without chloramphenicol was performed for an additional 16- to 24-hr period. Worms were then washed repeatedly in sterile HBSS, at least 3 changes for a minimum of 2 hr each, to remove residual antibiotics. Finally, worms were incubated for 10 days in RPMI-1640 containing 1% glucose (4 worms/ml) at 37 C, with humidified 5% CO_2 for the collection of whipworm-conditioned media-containing ESP. To confirm sterility, aliquots of ESPs were plated on blood agar plates and incubated aerobically and anaerobically for at least 48 hr. The batches with contamination were discarded. ESPs were collected daily, pooled, and concentrated at 4 C by ultrafiltration using an Amicon stirred cell (Millipore, Bedford, Massachusetts) with a 10,000 MW cutoff to 1/20 of the original volume. Using the Bradford assay (Bio-Rad, Hercules, California) the total protein content of 20×-concentrated ESP, which ranged from 3–4 mg protein/ml, was determined. The concentrated ESP was sterile filtered (0.22 μm; Millipore) and stored at −80 C. As a control for the volume reduction step, RPMI-1640 media containing 1% glucose without worms was concentrated under the same conditions. Bovine serum albumin (BSA) added to concentrated RPMI (cRPMI) was also used as a control for protein content.

Maintenance of tissue culture cells

Caco-2 cells are a transformed cell line originally derived from a human colonic adenocarcinoma (Pinto et al., 1983). These cells were selected because they are polarized cells that express markers of differentiated small intestine. The Caco-2 cells used were derived from the BBe clone of Caco-2 (Peterson and Mooseker, 1992). INT407 cells, derived from embryonic human small intestine, were purchased from

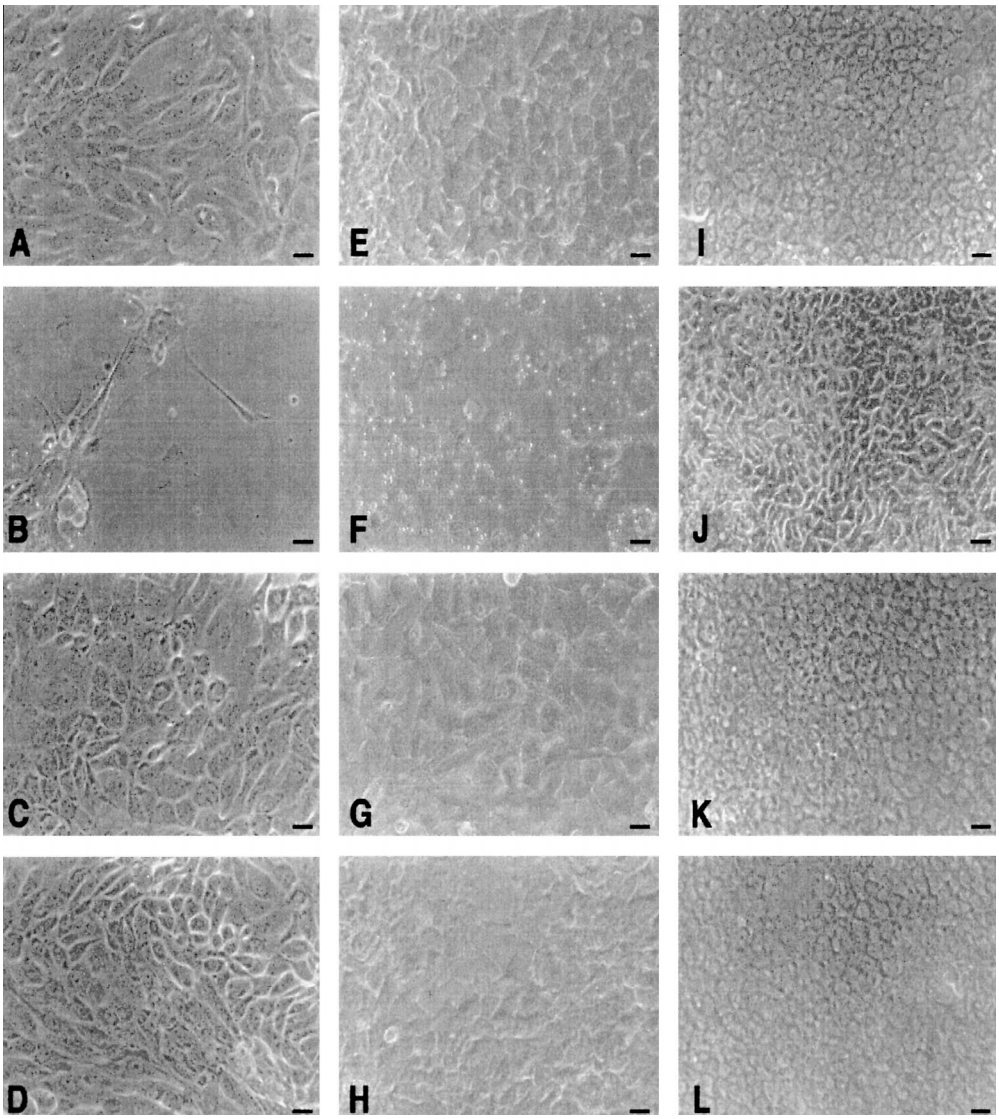

FIGURE 2. *Trichuris suis* ESP damages IECs in culture. IPEC-1 (**A**–**D**), INT407 (**E**–**H**), and Caco-2 (**I**–**L**) cells were untreated (**A, E, I**); treated with ESP at 1.5 mg protein/ml (**B, F, J**); treated with cRPMI at an equivalent dilution (**C, G, K**); or treated with cRPMI containing BSA at 1.5 mg/ml (**D, H, L**) for 72 hr. Cytopathic effects are most apparent in ESP-treated IPEC-1 (**B**) and INT407 (**F**) cells. Bar = 25 μm.

ATCC as a model representing undifferentiated cells of intestinal crypts. INT407 cells form homogeneous monolayers of flattened cells. Also used was an IEC cell line from the small intestine of a neonatal piglet, IPEC-1 cells (Gonzalez-Vallina et al., 1996).

Caco-2 and INT407 cells were grown in modified Eagle's medium (MEM) containing 15% fetal bovine serum. IPEC-1 cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 5% fetal bovine serum, epidermal growth factor (5 μg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), and selenium (5 ng/ml). For the growth of IPEC-1 cells on semipermeable supports, 1×10^5-cells were plated onto a rat tail collagen-coated 0.33-cm^2 surface area Transwell (Corning-Costar, Acton, Massachusetts) with a 0.4-μm pore size. The media were replenished thrice weekly and the cells were studied 14–21 days after plating. The cells were used between passages 32–46 (IPEC-1), 281–298 (INT407), and 59–69 (Caco-2). All tissue culture reagents were purchased from Gibco (Rockville, Maryland) unless otherwise stated.

Viability assays

The response of IECs to the ESP was evaluated by testing for growth stimulatory or cell-damaging effects under in vitro conditions. Preliminary experiments using trypan blue to selectively stain nonviable cells indicated IEC damage in response to ESP treatment. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) method, based on spectrophotometric quantitation of a colored reagent in viable cells, was used for more detailed study to generate data amenable to statistical analysis. Serial 2-fold dilutions of ESPs at a starting concentration of 1.5 mg protein/ml were added to confluent cells grown in 96-well plates ($n = 3$) that had been seeded at a density of ~10–20,000 cells/well. IPEC-1 and INT407 cells were used at 2–3 days postseeding, whereas Caco-2 cells were used 9–10 days after seeding to ensure that they were differentiated. Matched dilutions of cRPMI were used as negative controls to detect any background effect caused by osmotic disturbances associated with the volume reduction step in the ESP preparation protocol. To avoid unknown protein interactions between ESPs and fetal bovine serum, serum-free medium (QBSF 56, Sigma, St. Louis, Missouri) was the diluent for INT407 and Caco-2 cells, although a complete medium had to be used for IPEC-1 cells because serum-free media were insufficient for their growth needs. Fresh ESP and cRPMI were added to the cells daily. The MTT experiments approximated long-term ESP exposure with endpoint measurements taken at 72 hr post-exposure. At 72 hr postexposure, MTT was added to cells at a final

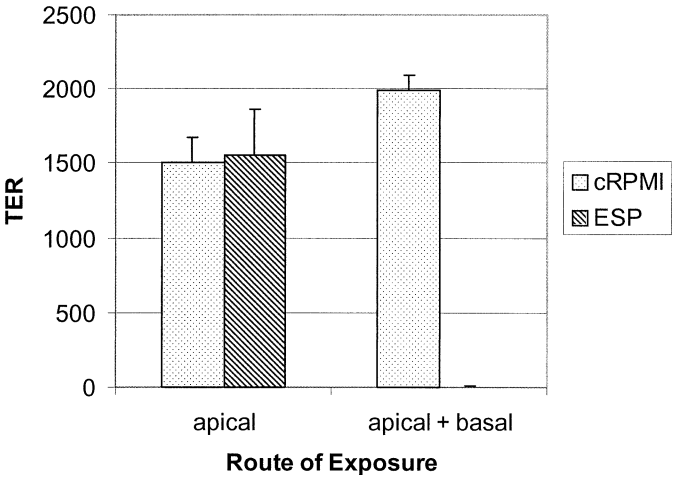

FIGURE 3. The effect of *Trichuris suis* ESP on TER of differentiated IPEC-1 cells. The cells were treated either on the apical surface alone or on both apical and basal surfaces with ESP at 1 mg protein/ml or cRPMI at an equivalent dilution for 72 hr (n = 4). Monolayer integrity was maintained in all treatment groups except ESP-treated apically and basally.

concentration of 0.5 mg/ml, and the assays were conducted after 3 hr. The MTT assay is based on the endocytosis of a water-soluble tetrazolium salt exclusively by viable cells that is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenases (Mosmann, 1983; Liu et al., 1997). Cells laden with these deposits were homogenized in acidic isopropanol to solubilize the colored product and then measured for absorbance at 562 nm in an automated platereader (Bio-Tek Instruments, Inc., Winooski, Vermont). In separate experiments, photographs were taken of cells treated with concentrated ESP (1.5 mg protein/ml), cRPMI at an equivalent dilution, and cRPMI containing BSA (1.5 mg/ml) for 72 hr to examine morphological changes in response to ESP exposure. The photos were taken using the 40× objective on an inverted Nikon TMS microscope with a Nikon FDX camera.

Electrophysiology

For treatment with ESP, monolayers were incubated with 50 μl of ESP or cRPMI in the apical chamber and 175 μl of ESP or cRPMI in the basal chamber. Before electrophysiological analysis, the monolayers were rinsed in HBSS with 15 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid. Measurements were made with 200 μl of HBSS in the apical chamber and 1 ml of HBSS in the basal chamber. Electrophysiological measurements of ESP- and cRPMI-treated IPEC-1 cells were made with agar bridges and Ag–AgCl calomel electrodes, as described previously (Turner et al., 1997). Potential differences were measured before and during application of a 25-μA current. Transepithelial electrical resistance (TER) was calculated using Ohm's law, as described previously (Turner et al., 1997).

Confocal immunofluorescence of IPEC-1 monolayers

After culture with ESP or cRPMI, monolayers were fixed with 2% paraformaldehyde in phosphate-buffered saline (PBS) with 1 mM $CaCl_2$ and 1 mM $MgCl_2$. After fixation, the cells were permeabilized in PBS with 0.5% Triton X-100 and 5% BSA. F-actin was stained with 5 U/ml Alexa 488-conjugated phalloidin (Molecular Probes, Eugene, Oregon) in PBS with 5% BSA for 30 min. After staining, the filters were cut from the Transwells and mounted in a Slowfade antifade reagent (Molecular Probes). The cells were analyzed on a Zeiss LSM 310 laser scanning confocal microscope.

Growth of *Campylobacter jejuni*

We tested various media and conditions to optimize the growth of ATCC strain 33292 of *C. jejuni*, originally isolated from a human with enteritis. To test for virulence and to generate low passage isolates, a 3-day-old colostrum-deprived piglet was orally inoculated with ~5×10^9 colony forming units (cfu) of *C. jejuni* 33292. The piglet developed clinical signs of diarrheal disease within 2 days postinoculation, and *C. jejuni* were reisolated from feces and amplified to generate low passage bacterial stocks. Freezer stocks were maintained in sheep blood at −80 C. For in vitro experiments, *C. jejuni* (passage 4–5) was grown overnight (18–24 hr) on *Brucella* agar supplemented with 5% sheep blood. Bacteria were harvested from plates with a sterile swab into MEM or DMEM/F12 tissue culture medium (for invasion and translocation experiments) or *Brucella* broth (for antibacterial assays). Absorbance was adjusted to 0.1 OD_{560}, which corresponds to ~5×10^8 cfu/ml based on growth curves performed in our laboratory.

Invasion assays

The IECs were tested for susceptibility to *C. jejuni* invasion using a standard gentamicin-killing assay (Konkel et al., 1992). *Campylobacter jejuni* (passage 5–6) was added to IECs in 96-well plates, which had been treated with ESP in a dose–response design (ESP concentrations of 0–1 mg protein/ml for 24 hr). For these experiments, IPEC-1 and INT407 cells were used at 3–4 days postseeding, and Caco-2 cells were used at 9–10 days to ensure they had reached the differentiated phenotype. Approximately 5×10^7 cfu of *C. jejuni* was added to each well of cells at roughly 50,000 cells/well to achieve a multiplicity of infection (MOI) of ~1000. Before addition of *C. jejuni*, the cells were washed in serum-free medium to remove the ESP. The cells were incubated with *C. jejuni* for 6 hr to allow internalization followed by aspiration of *C. jejuni*–laden media and treatment with media containing gentamicin (0.1 mg/ml) for 1 hr to kill extracellular *C. jejuni*. The media were aspirated and the cells were washed with the media to remove gentamicin. Finally, 50 μl of 0.5% sodium deoxycholate (Sigma) was added to lyse cells and release the intracellular *C. jejuni*. The cell lysate was homogenized in 0.2 ml PBS, serially diluted, and plated to quantitate internalized *C. jejuni*.

Statistical analyses

For the viability assays, multiple analysis of covariance was used to predict the dependent variable, DELTA, defined as the difference between treatment with ESP and cRPMI, on the basis of the categorical variable CELL (IPEC-1, INT407, Caco-2) and the continuous variable DOSAGE. The reported *P* values are for the type III sum of squares. Specific contrasts were requested between IPEC-1 versus INT407, IPEC-1 versus Caco-2, and between INT407 and Caco-2.

IEC model systems

We modeled the vertical (crypt to surface) axis of the intestine to examine in vitro effects of *T. suis* ESP on IECs in different stages of differentiation. INT407 (and IPEC-1 when grown on solid substrates) represented cells in the base of intestinal crypts having a flattened to cuboidal morphology. The Caco-2 cells (and IPEC-1 when grown on semipermeable supports) resembled polarized enterocytes on the mucosal surface with microvilli and tight junctions, although the Caco-2 cell clone used did retain a secretory phenotype typical of undifferentiated cells. The horizontal axis (proximal to distal) of the intestine was also taken into consideration. Although no data is available on the stability and turnover of ESP in vivo, we considered it possible that biologically active factors in ESP could be passively carried distally in the pig colon and retain activity. Concentrations of ESP for in vitro experiments were selected to span a range predicted to be physiologically relevant along the horizontal axis of the colon of a pig with a moderate whipworm infection, in which it is not unusual to find greater than a thousand whipworms. Conservatively extrapolating from in vitro measurements, we estimated ESP amounts in the proximal colon to exceed 1 mg protein. Each adult worm is capable of producing 10–100 μg protein/ml/day under in vitro conditions (data not shown). Therefore, if each of 100 worms produced 10 μg protein in a single day, a minimum of 1 mg ESP protein would be generated in the proximal colon, regardless of the relative contribution and localization of excreted versus secreted products.

The concentration of ESP intuitively diminishes away from the site of worm attachment because the number of worms declines. Hypothetically, ESP at maximal concentrations (proximal colon) would cause overt cytotoxicity, particularly orally secreted products that accumulate

in the syncytial tunnel. Lower concentrations (distal colon) would be predicted to have more subtle effects on downstream IECs, such as decreased tight junction permeability or modification of IEC surface receptors. Each of these 3 scenarios would enable *C. jejuni* to penetrate the IEC barrier more efficiently by paracellular or transcellular pathways. The following experiments were conducted to test for these types of ESP-induced changes in an effort to explain the in vivo observation that *C. jejuni* invasion occurred distal to the site of worm attachment.

RESULTS

Effect of ESP on IEC viability

ESP-treatment consistently reduced cell viability in a dose-dependent fashion, although the dose–response was not the same for the 3 cell types tested (Fig. 1). IPEC-1 and INT407 cells had a similar response pattern to ESP and were significantly more susceptible to damage by ESP than were Caco-2 cells ($P < 0.05$). Viability in both INT407 and IPEC-1 cells began to taper off at the 50-μg ESP protein/ml dose. Although ESP was toxic to Caco-2 cells, cytotoxicity was limited to the 2 highest concentrations tested. Overall, the effect of ESP was significantly different from that of the cRPMI effect in INT407 and IPEC-1, but not in Caco-2 cells ($P < 0.01$).

Morphological changes consistent with cytotoxicity occurred in ESP-treated IPEC-1 and INT407 cells (Fig. 2). IPEC-1 cell monolayers were destroyed in focal areas; the remaining cells were enlarged and distorted, often having fibrous extensions. INT407 cells showed diffuse damage and membrane blebbing resulting in a solubilized appearance. Caco-2 cells showed no overt damage in response to ESP treatment; instead they appeared to be more irregularly shaped compared with the polygonally shaped control cells. Cells treated with cRPMI and BSA as controls for volume reduction and protein content, respectively, were no different from untreated controls in all 3 cell types examined (Fig. 2).

Effects of ESP on TER and the perijunctional actomyosin ring of IPEC-1 cells

Experiments to determine the TER of ESP-treated IPEC-1 cells revealed differential susceptibility of cells based on which cell surfaces were treated. IPEC-1 cells treated on both apical and basolateral surfaces at 1 mg ESP protein/ml for 72 hr lost all TER compared with cells treated with ESP only apically and to cells treated with cRPMI (Fig. 3). A subsequent experiment revealed dose- and time-dependent effects of concomitant apical and basolateral ESP treatment on IPEC-1 cells (Fig. 4).

Because changes in TER have been related to reorganization of the perijunctional actomyosin ring (Turner, 2000), cells were stained for f-actin and examined by confocal laser microscopy (Fig. 5). The monolayers of IPEC-1 cells treated apically and basolaterally with ESPs had a dramatic reorganization of the actin cytoskeleton. This was most apparent as an increased separation between the perijunctional actomyosin ring of adjacent cells, resulting in a fluorescent image in which the perijunctional actomysin rings of adjacent cells had a double parallel line appearance (see arrow, Fig. 5). Also, the typical fusiform shape of the perijunctional actomyosin ring in the IPEC-1 cells was modified to a rigid polygon. These changes were evident within 24 hr of treatment with 0.1 mg/ml ESP. Interestingly, the morphologically detectable reorganization of the perijunctional actomyosin ring was closely correlated with effects on TER.

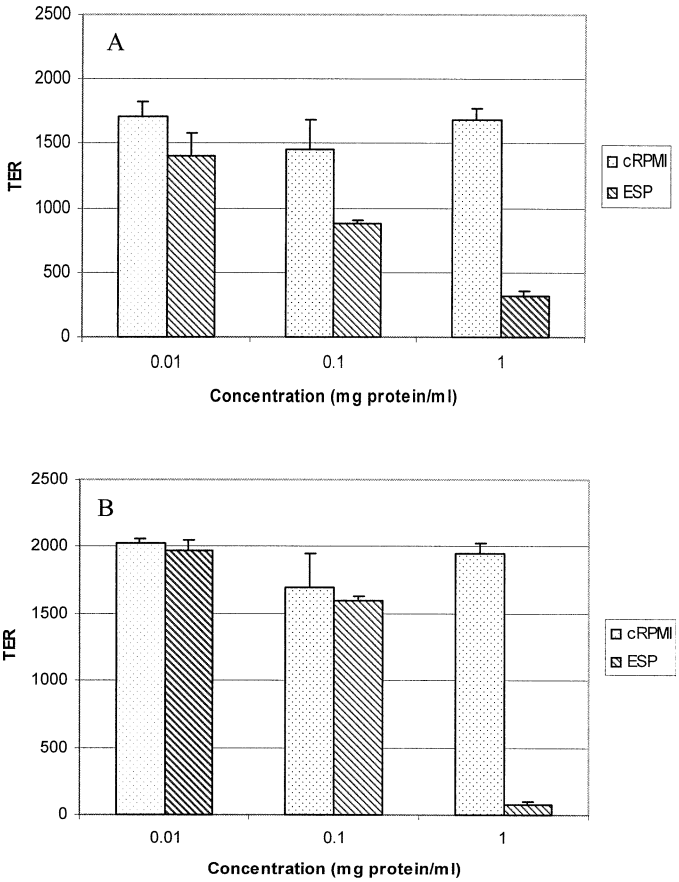

FIGURE 4. Effect of *Trichuris suis* ESP on TER of differentiated IPEC-1 cells treated apically and basolaterally. The cells (n = 3) were treated apically and basolaterally with ESP at 1, 0.1, and 0.01 mg protein/ml or cRPMI at an equivalent dilution for 24 (**A**) and 72 hr (**B**). There were dose- and time-dependent effects on monolayer integrity.

Monolayers treated with 0.1 mg/ml ESP for 72 hr regained both baseline TER (Fig. 4) and an actin distribution that was indistinguishable from control monolayers incubated with cRPMI (morphological data not shown).

Effect of ESP on IEC invasion by *Campylobacter jejuni*

Exposure of IECs to subtoxic concentrations (0.01 and 0.1 mg/ml) of ESP was not associated with an increase in intracellular *C. jejuni*, as hypothesized (Fig. 6). However, there was a dramatic decrease in the number of internalized *C. jejuni* recovered from IECs treated with ESP at 1 mg/ml, particularly in IPEC-1 cells. This outcome was consistently observed and was associated with an antibacterial activity in the residual ESP (Abner et al., 2001).

DISCUSSION

Intestinal *C. jejuni* is nonpathogenic in the absence of swine whipworm infection, but transitions to pathogen status when pigs are infected with *T. suis* (Mansfield and Urban, 1996). To investigate the mechanisms by which opportunistically invasive *C. jejuni* overcome host resistance in *T. suis*–infected pigs, we tested the hypothetical contributions of the *T. suis* ESP to this disease process using in vitro IEC model systems.

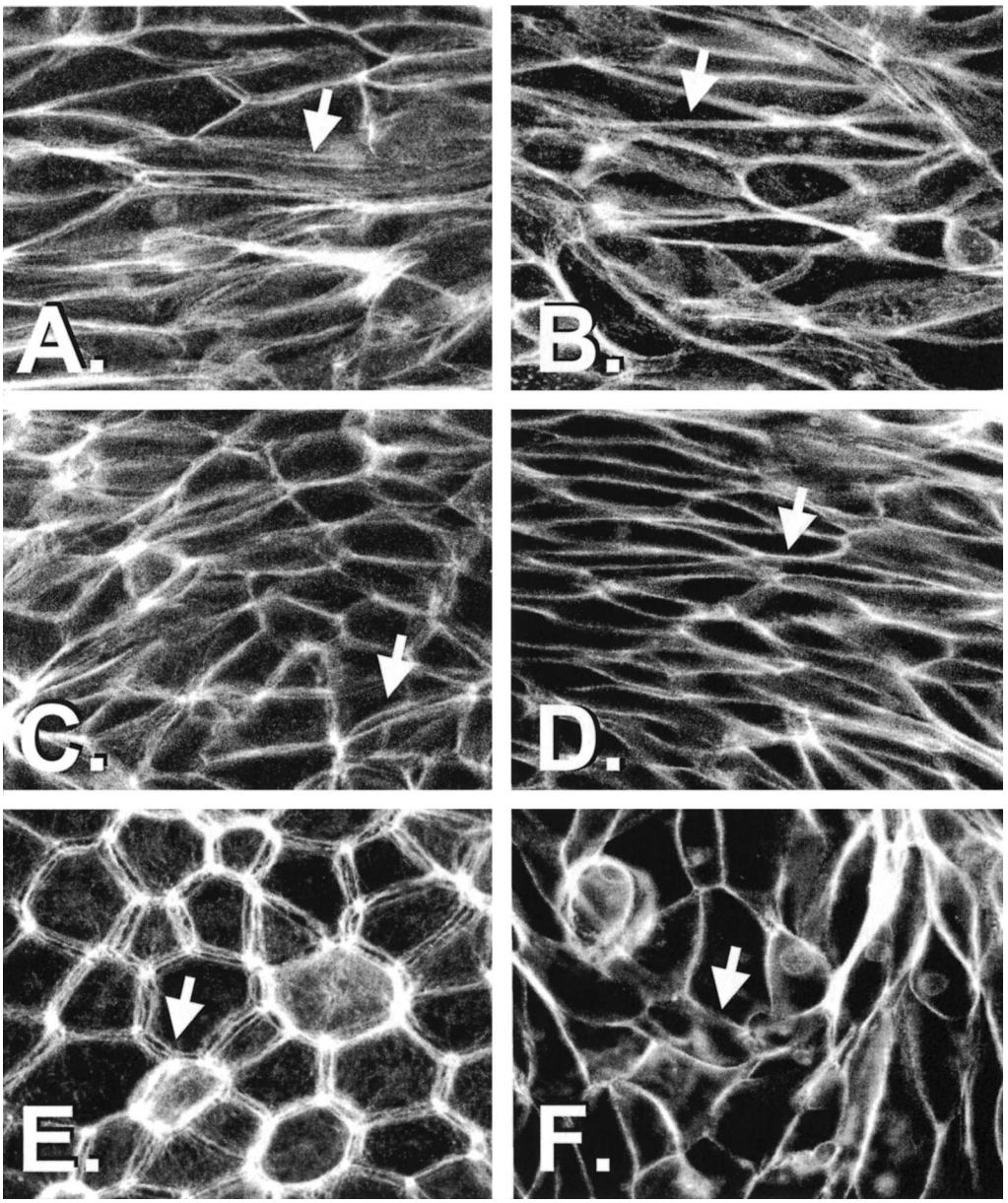

FIGURE 5. Actin distribution in IPEC-1 cells treated with ESP. The IPEC-1 monolayers were incubated with ESP (**A, C, E**) or cRPMI (**B, D, F**) at 0.1 mg/ml (**A, B**) or 1 mg/ml (**C**–**F**). The monolayers were fixed after 24 hr (**A**–**D**) or 72 hr (**E, F**) of culture. The perijunctional actomyosin ring was examined by confocal laser microscopy. The ESP induced both shape change and a double parallel line pattern where adjacent cells touched (arrows).

The integrity of IEC monolayers was assessed because whipworms and their ESP are in intimate contact with the intestinal epithelium throughout their lifecycle. When viability was measured in cells grown on solid substrates, ESP from adult *T. suis* caused dose-dependent cytotoxic effects. Those cells modeling undifferentiated cells in the base of intestinal crypts (INT407 and IPEC-1) were more susceptible to ESP cytotoxicity, whereas the model of differentiated cells (Caco-2) was relatively resistant. These data suggest that immature IECs in the proximal colon would be particularly sensitive to ESP, which would contribute to mechanical damage by whipworms (Fig. 7).

Because of their pig origin and sensitivity in the viability assays, IPEC-1 cells were selected for experiments to measure electrical resistance as an indicator of tight junction integrity. Apical treatment of these polarized cells with high concentrations of ESP did not affect TER. Conversely, the cells were overtly damaged and TER was completely lost when they were treated both apically and basolaterally. Furthermore, at lower ESP concentrations modeling sites distal to worm attachment, separated perijunctional actomyosin rings were demonstrated in cells treated apically and basolaterally. These data, along with the relative insensitivity of Caco-2 cells in viability assays, suggest that exposure of apical surfaces of differentiated cells is not sufficient for the cytotoxic phenotype. In vivo, basolateral surfaces of differentiated IECs in the promixal colon would be exposed to ESP where the epithelium is denuded or in the mi-

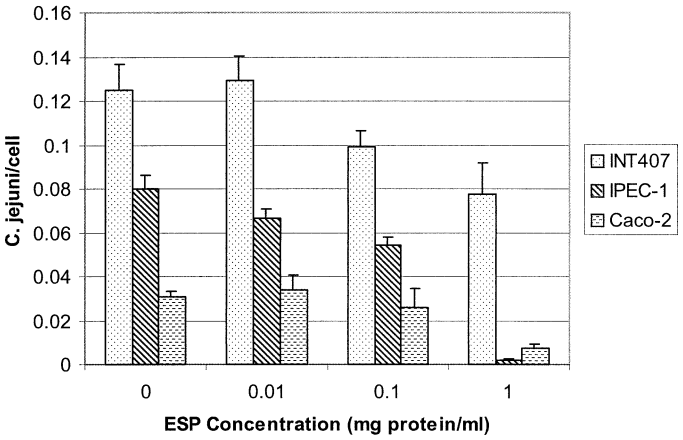

FIGURE 6. Effect of ESP on *Campylobacter jejuni* invasion in IECs. The cells were treated with ESP over a range of concentrations for 24 hr to simulate subtoxic levels of exposure before addition of *C. jejuni* at an MOI of 1,000 for 6 hr to measure internalization. Values represent the mean of 6 samples ± standard error of the mean.

croniche occupied by the adult worm. Histologic data are consistent with cytotoxicity of surface-differentiated cells at the site of adult worm attachment (Mansfield and Urban, 1996). Pore-forming proteins from *T. muris* and *T. trichiura* are thought to facilitate invasion, as well as syncytial tunnel formation and maintenance in the host cecal epithelium through a postulated lysis mechanism (Drake, Korchev et al., 1994). Our data using unfractionated *T. suis* ESP support this hypothesis.

Increased intracellular *C. jejuni* could not be demonstrated in IECs treated with subtoxic concentrations of ESP. The counter-intuitive observation of decreased *C. jejuni* recovery from ESP-treated cells led to the discovery that ESP has a direct growth inhibitory effect on *C. jejuni* (Abner et al., 2001). Although the monolayers of all 3 cell types were visually intact after ESP treatment at 1 mg/ml, there is no way to exclude the possibility that gentamicin was better able to penetrate the ESP-treated cells and kill any internalized *C. jejuni*. However, the observation that there was relatively little drop in internalized bacteria in INT407 cells, which were just as susceptible to ESP-induced cytotoxicity as the IPEC-1 cells would argue against this possibility.

Interpretation of these data and extrapolation to the in vivo scenario are challenging and confounded by the dual cytotoxic and antibacterial activities present in *T. suis* ESP, both of which would be maximal in the proximal colon where worms are concentrated. Collectively, these data suggest that ESP is more likely to enhance *C. jejuni* translocation by a paracellular rather than a transcellular mechanism. ESP-induced cell damage could expose the underlying fibronectin in the extracellular matrix that would promote *C. jejuni* attachment via the CadF adhesin (Konkel et al., 1997). Fibronectin is differentially expressed in higher amounts in the base of crypts (Beaulieu, 1992), where *C. jejuni* thrives in the mucus (Beery et al., 1988). Although the ESP used in these experiments was derived from adult worms, it is likely that similar biologically active factors would be released by larval worms.

Histologic data show bacteria in close proximity to the worms and in the lamina propria underlying the damaged epithelium in pigs experimentally infected with *T. suis* (Mansfield

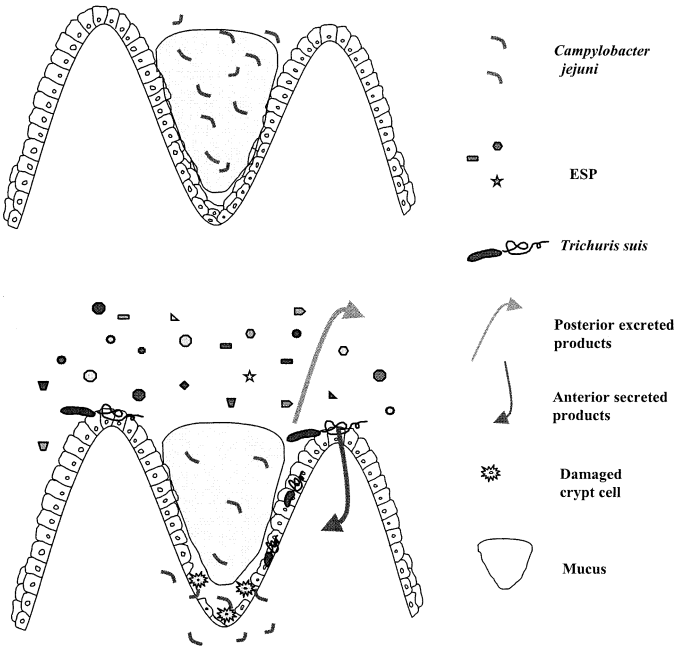

FIGURE 7. Predictive model of in vivo scenario in the proximal colon of pigs infected with *Trichuris suis*. ESP damages IECs, particularly undifferentiated cells in the base of crypts. Opportunistic bacterial pathogens that can overcome the deleterious antibacterial action of ESP, e.g., those embedded in mucus within crypts, would have a selective advantage for penetration into the underlying lamina propria.

and Urban, 1996). Although it is not possible to assess the viability of those bacteria in fixed tissues, bacteria are differentially susceptible to ESP (Abner et al., 2001). It is also conceivable that *C. jejuni* is protected against deleterious ESP effects by being embedded in mucus, which is produced excessively in *T. suis*–infected pigs (Rutter and Beer, 1975). In support of this hypothesis, there is evidence that microcolonies of intestinal bacteria embedded in a mucus matrix are protected against hostile environmental factors (Costerton et al., 1983; Costerton, 1984). In addition, intestinal mucus is a diffusion barrier against drug absorption (Larhed et al., 1998). Mucus is chemotactic for *C. jejuni* (Hugdahl et al., 1988) and we have found *C. jejuni* associated with mucus-laden goblet cells in the pig colon (Mansfield, unpubl. data).

Rutter and Beer (1975) hypothesized that migrating whipworms damage the epithelium, such that opportunist pathogens can reach underlying tissues. This damage could be caused by any combination of physical movement of the parasite, soluble ESP, and immunopathology from the host response. We tested the direct effect of ESP from adult *T. suis* on IECs under in vitro conditions, in the absence of whipworms and an intact immune system. The data presented in this article provide in vitro support for a mechanism by which *T. suis* ESP could contribute to the disruption of the IEC barrier. The in vivo consequence would be lowered natural resistance against opportunistic pathogens in the proximal colon that are capable of overcoming the antibacterial ESP effects, either naturally or with the aid of a host factor such as mucus (Fig. 7).

Tissue culture models, particularly the IPEC-1 cells, have been beneficial as a reductionist means to assay for ESP effects that could contribute to the overall pathological process in the

animal model of *T. suis*–dependent *Campylobacter* susceptibility. Although confounded by the obvious complexity of in situ interactions, this approach has provided an explanation for a nonmechanical, parasite-dependent host cell destruction mechanism and a novel nematode-dependent antibacterial activity. Additional experiments are needed to explain the differential response of IPEC-1 cells treated apically versus those treated apically and basolaterally. Future efforts will focus on the isolation and the characterization of the factor(s) in ESP responsible for decreased viability in eukaryotic and prokaryotic cells.

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