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The *pef* Fimbrial Operon of *Salmonella typhimurium* Mediates Adhesion to Murine Small Intestine and Is Necessary for Fluid Accumulation in the Infant Mouse

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We investigated the role of the *pef* operon, containing the genes for plasmid-encoded (PE) fimbriae of *Salmonella typhimurium*, in adhesion to the murine small intestine. In an organ culture model, a mutant of *S. typhimurium* carrying a tetracycline resistance cassette inserted in *pefC* was found to be associated in lower numbers with murine small intestine than the wild type. Similarly, heterologous expression of PE fimbriae in *Escherichia coli* increased the bacterial numbers recovered from the intestine in the organ culture model. Adhesion to villous intestine mediated by PE fimbriae was further demonstrated by binding of an *E. coli* strain expressing PE fimbriae to thin sections of mouse small intestine. The contribution of *pef*-mediated adhesion on fluid accumulation was investigated in infant mice. Intragastric injection of *S. typhimurium* 14028 and SR-11 caused fluid accumulation in infant mice. In contrast, *pefC* mutants of *S. typhimurium* 14028 and SR-11 were negative in the infant mouse assay. Introduction of a plasmid containing *pefBACD* and *orf5*, the first five genes of the *pef* operon, into the *pefC* mutant complemented for fluid accumulation in the infant mouse assay. However, heterologous expression of PE fimbriae in *E. coli* did not result in fluid accumulation in the infant mouse, suggesting that factors other than fimbriae are involved in causing fluid accumulation.

Salmonella typhimurium is the most common cause of acute gastroenteritis in humans in the United States. However, the mechanism by which *S. typhimurium* causes diarrhea in humans is not well defined. Although at least three different toxic activities of *S. typhimurium* have been found in several animal and cell culture models, their contribution to the generation of diarrhea in humans has never been conclusively demonstrated (3, 10, 13, 23, 24, 31, 32, 37–39, 51). In fact, salmonellosis appears to be a complex, multifactorial process (43), and the ability of *S. typhimurium* to multiply in the lamina propria and cause inflammation may contribute significantly to diarrheal disease (8, 9, 11).

Bacterial adhesins are known to support colonization of the host's alimentary tract, thereby increasing the bacterial load in proximity to the epithelial lining. As a consequence, fimbriae of enterotoxigenic *Escherichia coli* and *Vibrio cholerae* are necessary for diarrhea (5, 14, 18, 42, 45, 46). Although several fimbrial adhesins have been found in *S. typhimurium* (1), fimbriae have so far not been implicated in fluid accumulation in animal models. In this report, we present evidence that plasmid-encoded (PE) fimbriae of *S. typhimurium* mediate adhesion to mouse small intestine and are necessary for fluid accumulation in the infant mouse assay.

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MATERIALS AND METHODS

Bacterial strains, cell lines, and growth conditions. Bacterial strains used in this study are listed in Table 1. All bacteria were cultured in Luria-Bertani broth (LB; 5 g of yeast extract, 10 g of tryptone, and 10 g of NaCl per liter) or on plates (LB broth containing 15 g of agar per liter) at 37°C. Antibiotics, when required, were included in the culture medium or plates at the following concentrations: carbenicillin, 100 mg/liter; kanamycin, 60 mg/liter; nalidixic acid, 50 mg/liter; chloramphenicol, 30 mg/liter; and tetracycline, 10 mg/liter. HeLa and T84 cells were cultivated in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 1% nonessential amino acids, and 1 mM glutamine (DMEMsup). For adhesion assays, 24-well microtiter plates were seeded with HeLa or T84 cells at a concentration of 5×10^5 cells per well in 0.5 ml of DMEMsup and incubated overnight at 37°C in 5% CO_2. Analytical-grade chemicals were purchased from Sigma. All enzymes were purchased from Boehringer Mannheim.

Recombinant DNA and genetic techniques. Plasmid DNA was isolated by using ion-exchange columns from Qiagen. Standard methods were used for restriction endonuclease analyses, ligation and transformation of plasmid DNA, transfer of plasmid DNA by conjugation, and isolation of chromosomal DNA from bacteria (27, 30). Plasmids were constructed by using the vector pBluescript SK+ (40) or the suicide vector pEP185.2 (21).

Southern transfer of DNA onto a nylon membrane was performed as previously described (27). Labeling of DNA probes, hybridization, and immunological detection were done by using the DNA labeling and detection kit (nonradioactive) from Boehringer Mannheim. The DNA was labeled by random-primed incorporation of digoxygenin-labeled dUTP. Hybridization was performed at 65°C in solutions without formamide. Hybrids were detected by an enzyme-linked immunoassay, using an antidigoxygenin-alkaline phosphatase conjugate and the substrate AMPPD [3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxethane; Boehringer Mannheim]. The light emitted by the dephosphorylated AMPPD was detected by X-ray film.

Production of rabbit anti-PefA serum. The nucleotide sequence of a DNA region encoding PE fimbriae which has been reported recently (7) was used to design primers for PCR amplification of *pefA*. A DNA fragment encoding the C-terminal 167 amino acids of PefA was amplified by using the primers 5′-GGGAATTCTTGCTTCCATTATTGCACTGGG-3′ and 5′-TCTGTCGACG GGGGATTATTTGTAAGCCACT-3′. The 520-bp PCR product was digested with *Eco*RI and *Sal*I and cloned into the expression vector pGEX-4T-1 to create an in-frame translational fusion with the N terminus of gluthathione *S*-transferase and amino acids 6 to 172 of PefA. Purification of the glutathione *S*-transferase–PefA fusion protein from sonic lysates was performed by using a gluthathione-Sepharose affinity matrix (Pharmacia). The purified fusion protein was used to produce antiserum by injecting a rabbit subcutaneously at six dif-

TABLE 1. Bacterial strains used

Strain	Genotype	Reference or source
E. coli		
ORN172	*thr-1 leuB thi-1* Δ(*argF-lac*)*U169 malA1 xyl-7 ara-13 mtl-2 gal-6 rpsL fhuA supE44* Δ(*fimBEACDFGH*) Km^r	49
DH5α	*endA* 1 *hsdR17 supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*)*U169 deoR* [ϕ80 d*lac* Δ(*lacZ*)*M15*]	Laboratory collection
S17-1 λ*pir*	*prp thi recA hsdR*; chromosomal RP4-2 (Tn*1*::ISR1 *tet*::Mu Km::Tn*7*); λ*pir*	41
S. typhimurium		
14028	Wild type	American Type Culture Collection
$14028P^-$	14028 cured from virulence plasmid	Laboratory collection
IR715	14028 Nal^r	44
AJB7	IR715 *pefC*::Tet^r	This study
14028rck	14028 *rck*::Km^r	D. Guiney
SR-11	Wild type	Laboratory collection
χ4252	SR-11 Δ[*ahp-11*::Tn*10*]-251	26
χ4253	SR-11 Δ[*fim-ahp-11*::Tn*10*]-391	26
AJB9	SR-11 Δ[*ahp-11*::Tn*10*]-251, *pefC*::Tet^r Nal^r	This study
S. enteritidis		1
S. dublin		1
S. heidelberg		1

ferent locations with a total of 1 mg of fusion protein suspended in Titermax adjuvant (Cytrx). A booster injection was administered 4 weeks later.

Electron microscopy. Bacteria were grown overnight in a static culture and were allowed to adhere to a Formvar-coated grid for 2 min. The bacteria were fixed with 0.1% glutaraldehyde in sodium cacodylate buffer (100 mM, [pH 7.4]) for 1 min. The grid was rinsed with water, and fimbriae were negatively stained with 0.5% (wt/vol) aqueous uranyl acetate (pH 4.6) for 30 s. The grids were allowed to dry before they were analyzed by electron microscopy.

Virulence studies in mice. Virulence of *S. typhimurium* mutants was tested by infection of 6- to 8-week-old female BALB/c mice. To calculate the 50% lethal dose (LD_{50}), serial 10-fold dilutions of overnight cultures were made in LB and administered intragastrically to groups of four mice in a 0.2-ml volume. Mortality was recorded at 4 weeks postinfection, and the LD_{50} was calculated by the method of Reed and Muench (35).

Ligated ileal loop model. Ligated intestinal loops were prepared as described previously (17), using 6- to 8-week-old female BALB/c mice. In brief, mice were starved for 24 h prior to intraperitoneal injection of 1.5 to 2 mg of Nembutal (Abbott Laboratories, North Chicago, Ill.) per mouse. A small incision was then made through the abdominal wall, and the small bowel was exposed. A loop was formed by ligating the intestine with silk thread at the ileocecal junction and at a site ~4 to 5 cm proximal to the cecum. Bacteria (200 ml of a 5×10^9-CFU/ml culture) were injected through a 25-gauge needle. The bowel was then returned to the abdomen, and the incision was stapled closed. Mice were killed after 8 h by cervical dislocation, and fluid accumulation in intestinal loops was evaluated.

Cell culture techniques and adhesion assay. HeLa and T84 cells were fixed in 2% glutaraldehyde in phosphate-buffered saline (PBS) for 1 h at 4°C. Glutaraldehyde was removed by three 30-min washes with 1 ml of PBS at 4°C, and 1 ml of DMEMsup was added to each well. The bacterial cultures were diluted, and about 5×10^6 bacteria in 0.25 ml of DMEMsup were added to each well. Both 1 and 2 h after incubation at 25°C, nonadherent bacteria were removed by five washes with 1 ml of PBS. Wells were sampled by lysing the fixed cells with 0.5 ml of 1% deoxycholate and rinsing each well with 0.5 ml of PBS. Adherent bacteria were quantified by plating dilutions made in sterile PBS on LB agar. All experiments were performed independently three times.

IOC. On the basis of the conditions described for tissue preservation (50), we established an intestinal organ culture model (IOC) which allowed us to study association of salmonellae to the lumen of the small bowel in vitro. In brief, bacteria were grown as standing overnight cultures in 1 ml of LB at 37°C in 5% CO_2, harvested, and resuspended in DMEMsup. The small intestines were removed from 8-week-old female BALB/c mice, starved for 24 h prior to the experiment, and placed into a petri dish containing DMEMsup. The intestine (~20 cm) was ligated at the distal end, filled with 1 ml of a bacterial suspension containing 10^9 CFU, then ligated at the proximal end, and incubated for 30 min at 37°C in 5% CO_2. The intestine was opened at both ends, rinsed with 1 ml of PBS, and opened longitudinally. Nonadherent bacteria were removed by three washes in 10 ml of PBS in petri dishes, and 3-cm sections of intestinal wall were homogenized in 5 ml of PBS, using a Stomacher (Tekmar, Cincinnati, Ohio). Dilutions were plated on LB containing the appropriate antibiotics to quantify the bacteria associated with the organ. Experiments were repeated with organs from six different animals. A paired difference test was used to evaluate the significance of differences in adhesion observed for different strains.

In vitro adhesion assay to thin sections from mouse small intestine. Six- to eight-week-old female BALB/c mice were anesthetized with Avertin (2.5%, 0.03 ml/g of body weight). For perfusion with picric acid-paraformaldehyde (2% paraformaldehyde, 15% picric acid, 0.1 M monobasic sodium phosphate [pH 7.3]), the thoracic cavity was opened, and a perfusion needle, pressured by a peristaltic pump (Pharmacia model P-1), was inserted into the left ventricle. After flow of saline solution into heart had begun, the atria were cut, allowing blood to exit. Perfusion with saline was followed by perfusion with picric acid-paraformaldehyde. The mouse small intestine was then removed and fixed in picric acid-paraformaldehyde for 2 h. The tissue was washed with PBS and allowed to stand in 10% sucrose–PBS for 4 h. Tissues were immersed in OCT embedding medium (Tissue-Tek, Miles Scientific) in a mold and quick-frozen in a liquid nitrogen-cooled bath of 2-methyl butane, and 10-mm histological sections were placed on microscope slides. Nonspecific binding to sections was blocked by a 30-min incubation in 0.05% Tween 20–0.2% bovine serum albumin in PBS at 37°C. Bacteria were labeled with fluorescein isothiocyanate (Sigma) as described previously (36). Fluorescein isothiocyanate-labeled bacteria were diluted in PBS containing 0.01% (vol/vol) Tween 20. A few drops of this suspension were placed directly over the tissue specimen, which was then incubated at 37°C in a moist chamber. After 30 min, nonadherent bacteria were removed by six 5-min changes in PBS, and the sections were fixed for 10 min on ice in 3% paraformaldehyde. The slides were mounted in slow-fade mounting medium (Molecular Probes Inc.) and examined by fluorescence microscopy. Adhesion was performed for each strain on three slides, carrying at least four specimens, in parallel. Each experiment was repeated three times with tissues from different animals, and adhesion was evaluated by two different persons independently.

Infant suckling mouse model. The infant suckling mouse assay has been described previously (4) and has been modified for *S. typhimurium* by Koupal and Deibel (24). In brief, bacteria were grown in LB to an optical density at 578 nm of between 1.0 and 1.5, harvested by centrifugation, and resuspended in an equal volume of sterile PBS. The inoculum contained between 2×10^7 and 5×10^7 bacteria, as determined by performing colony counts. Groups of four 3- to 5-day-old mice were injected intragastrically with 0.1 ml of bacterial suspension. After 2.5 h, the alimentary tract was removed, and the ratio between intestinal weight and the weight of the remaining body was determined for each mouse. For each bacterial strain, the mean of these ratios from at least four different mice was calculated, and the significance of differences observed was analyzed by Student's *t* test. If the mean of a ratio was significantly greater than that of the PBS control ($P < 0.025$), it was scored positive. The values obtained were always consistent with the apparent fluid accumulation observed during removal of the mouse alimentary tract.

RESULTS

Cloning and analysis of cosmids containing the *S. typhimurium pef* operon. The PCR product containing *pefA* was used as a probe to screen for cosmids containing the *pef* operon by colony hybridization of a *S. typhimurium* gene bank constructed in pLAFR II (25). Cosmids which gave positive hybridization signals were isolated from 10 colonies and designated pFB1, pFB2, pFB4, pFB5, pFB6, pFB7, pFB8, pFB9, pFB10, and pFB11. The cosmids and the *S. typhimurium* viru-

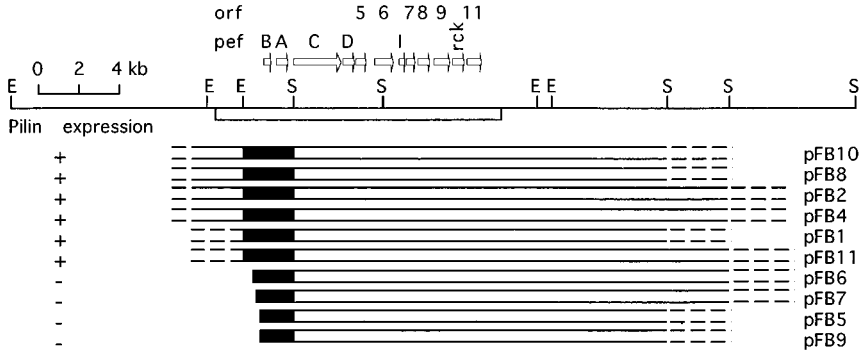

FIG. 1. Restriction map of a region from the *S. typhimurium* virulence plasmid (7, 48). Positions of open reading frames identified previously (7) are shown as outlined arrows above the map. Locations of inserts from cosmids used in this study are shown below. Dashed lines indicate that the exact endpoint of the cosmid insert was not determined. Black bars indicate the *Sal*I-*Eco*RI fragment hybridizing with a *pefA* DNA probe. Results from Western blotting with a rabbit anti-PefA serum of *E. coli* strains harboring these cosmids are shown on the left. +, signal with anti-PefA serum; −, no signal with anti-PefA serum. S, *Sal*I; E, *Eco*RI.

lence plasmid were digested with *Eco*RI, *Sal*I, and *Eco*RI-*Sal*I, and the restriction patterns were compared with those reported for the *pef* operon and the *S. typhimurium* virulence plasmid (Fig. 1) (7, 48). Restriction fragments from an *Eco*RI-*Sal*I digest were transferred onto nylon membranes and hybridized, using the *pefA* PCR product as a probe. The virulence plasmid and cosmids pFB1, pFB2, pFB4, pFB8, pFB10, and pFB11 contained a 2.4-kb *Eco*RI-*Sal*I fragment which hybridized with the *pefA* probe. Hybridization with the *pefA* probe identified *Eco*RI-*Sal*I restriction fragments of 2 kb (pFB6), 1.7 kb (pFB7), and 1.6 kb (pFB5 and pFB9), which indicated that the *Eco*RI-*Sal*I fragments are located on one end of the insert in the corresponding cosmids (Fig. 1). Comparison of cosmid restriction patterns and analysis of data obtained by hybridization with the *pefA* probe enabled the approximate localization of these cosmids on the virulence plasmid to be determined (Fig. 1).

Expression of PE fimbriae in *E. coli*. Expression of PE fimbriae from the *pef* operon has previously been demonstrated in *E. coli* and *S. typhimurium* (7). We investigated whether all cosmids cloned by hybridization with the *pefA* probe mediated expression of PE fimbriae in *E. coli*. For this purpose, cosmids were introduced into a nonfimbriated *E. coli* strain (ORN172) (49), and expression of PefA was investigated by Western blotting (immunoblotting) with rabbit anti-PefA serum. A band of 17 kDa was detected in strains carrying cosmid pFB1, pFB2, pFB4, pFB8, pFB10, or pFB11 but not in strains carrying cosmid pFB6, pFB7, pFB5, pFB9, or pLAFR II (Fig. 1 and 2). These results were confirmed by transmission electron microscopy of negatively stained bacteria. Again, only strains carrying cosmid pFB1, pFB2, pFB4, pFB8, pFB10, or pFB11 were fimbriated (Fig. 3). These data indicated that the 2.4-kb *Eco*RI-*Sal*I fragment contains upstream DNA sequences necessary for *pef* expression (Fig. 1).

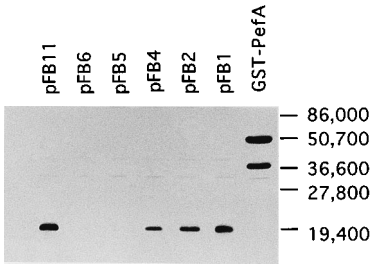

FIG. 2. Western blot of bacterial extracts or purified glutathione *S*-transferase (GST)–PefA fusion protein after separation by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis with a rabbit anti-PefA serum. Positions and molecular masses (in daltons) of standard proteins are given on the right. Bacterial extracts were prepared from *E. coli* strains carrying the cosmids indicated above the first six lanes.

Construction of a *pefC* insertional mutant of *S. typhimurium*. In other fimbrial operons, mutations in genes encoding assembly proteins have always resulted in absence of adhesins from the bacterial surface. The gene product of *pefC* has homology to fimbrial outer membrane ushers (7). To inactivate this gene, a 1,403-bp DNA fragment of the *pefC* open reading frame was amplified by using the primers 5′-AAGAATCAGCAAATG CCCTGTG-3′ and 5′-GCGAATTCTAAAGGAGAGCGAC GTG-3′. The PCR product was digested with *Eco*RI and ligated into the vector pBluescript digested with *Eco*RI-*Sma*I. The resulting plasmid, pPE1, was digested with *Sma*I, thereby deleting 374 bp of the *pefC* gene, and the 2-kb *Sma*I fragment carrying the tetracycline resistance gene of pAK1900 (34a) was ligated into this site. From the resulting plasmid, pPE2, a *Kpn*I-*Xba*I fragment was cloned into the suicide vector pEP185.2 (21). This construct (pPE3) was introduced into *E. coli* S17 λ*pir* and then conjugated into *S. typhimurium* IR715 (44). One exconjugant sensitive to chloramphenicol (vector) and resistant to tetracycline was designated AJB7. Chromosomal DNA of AJB7 was analyzed by Southern hybridization with the *pefA* probe to confirm insertional inactivation of *pefC* (Fig. 4).

Influence of PE fimbriae on adhesion of *E. coli* and *S. typhimurium* to murine small intestine in vitro. We next investigated whether the *pef* operon is involved in adhesion to epithelial cells in vitro. No difference in adhesion between IR715 and AJB7 or between ORN172 and ORN172(pFB11) was observed when we used HeLa and T84 cells, two human epithelial cell lines (data not shown). We next studied the effect of a *pefC* mutation on adhesion in an IOC. The work by Worton et al. has established that the intestinal epithelium remains intact for up to 2 h if sections of murine small bowel are placed into tissue culture medium (50). The IOC allowed us to restrict bacterial contact to the luminal surface of the intestine. The influence of variations between animals on the IOC was minimized by performing mixed infection experiments. When a 1:1 mixture of IR715 and AJB7 was used as the inoculum, IR715 was found to be associated in larger numbers with sections of the small intestine than the *pefC* mutant AJB7 ($P < 0.005$) (Fig. 5). We next investigated whether heterologous expression of PE fimbriae in *E. coli* confers increased adhesion to murine

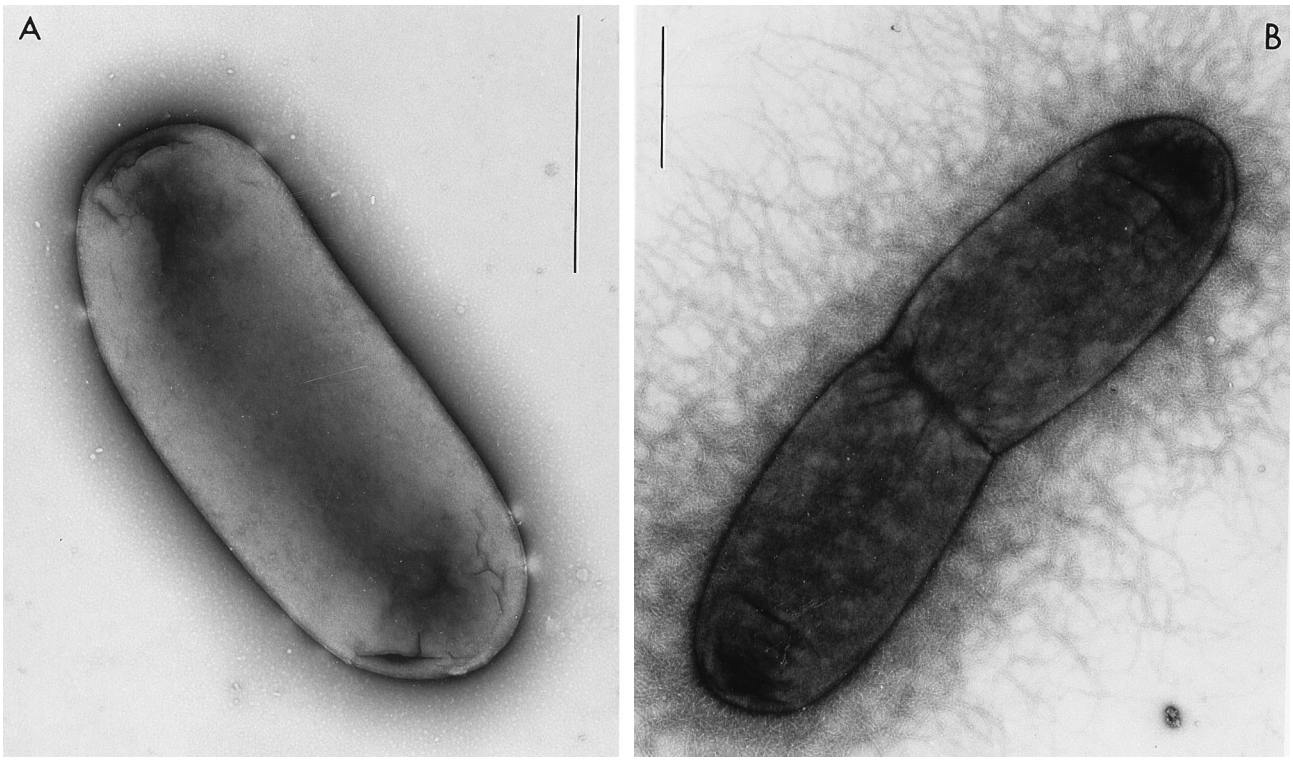

FIG. 3. Transmission electron micrograph of *E. coli* ORN172(pFB6) (A) and ORN172(pFB11) (B). Bars indicate 1 μm.

intestine. To this end, a 1:1 mixture of cultures from ORN172 and ORN172(pFB11) was used as an inoculum in the IOC. Larger numbers of ORN172(pFB11) than of ORN172 were recovered from villous intestine ($P < 0.05$) (Fig. 5). These data are consistent with a role of the *pef* operon in adhesion to the lining of the small intestine.

To confirm the results obtained with the IOC visually, we analyzed bacterial adhesion to thin sections from murine villous intestine with fluorescence microscopy. However, because of strong background binding, we were unable to observe differences in adhesion to these specimen between IR715 and AJB7 (data not shown). A possible reason for the observed strong binding of *S. typhimurium* may be the fact that this serotype expresses at least six different fimbriae (1). We therefore investigated adhesion mediated by PE fimbriae in a better-defined *E. coli* strain background. The nonfimbriated *E. coli* strain ORN172 bound poorly to sections of villous intestine (Fig. 6). In contrast, strain ORN172(pFB11) expressing PE fimbriae adhered in increased numbers to villous intestine. These data thus provide further evidence for a role of the *pef* operon in bacterial adhesion to murine small intestine.

Virulence of strains carrying *pefC* mutations in mice. The virulence of AJB7 in mice was compared with that of the wild type (IR715) by determining the LD_{50} after intragastric injection. The LD_{50} of AJB7 was found to be 1.4×10^6, while that of IR715 was 6×10^5. These data indicate that expression of PE fimbriae plays only a minor role, if any, during murine typhoid fever. This is consistent with earlier reports, in which plasmid cured derivatives of *S. typhimurium* could be complemented to nearly wild type virulence by introduction of the *spv* operon on a plasmid (12).

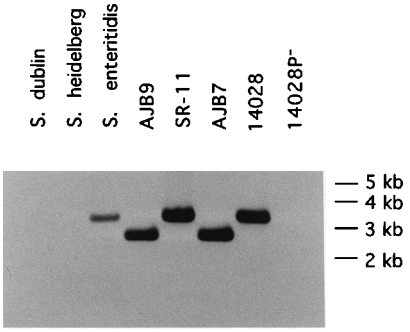

FIG. 4. Southern hybridizations of chromosomal DNA digested with *Hin*dIII-*Eco*RI with a *pefA* DNA probe. Positions of DNA fragments with known sizes are given at the right. Chromosomal DNA originated from strains indicated above the lanes.

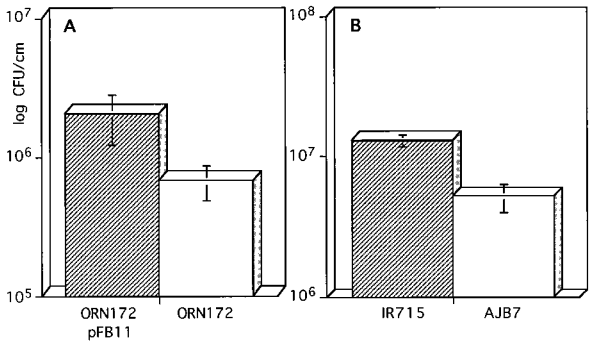

FIG. 5. Bacterial association with the intestinal wall after mixed infection with ORN172 and ORN172(pMS11) (A) or IR715 and AJB7 (B) in the IOC. Values are given as averages of bacterial numbers recovered ± standard error.

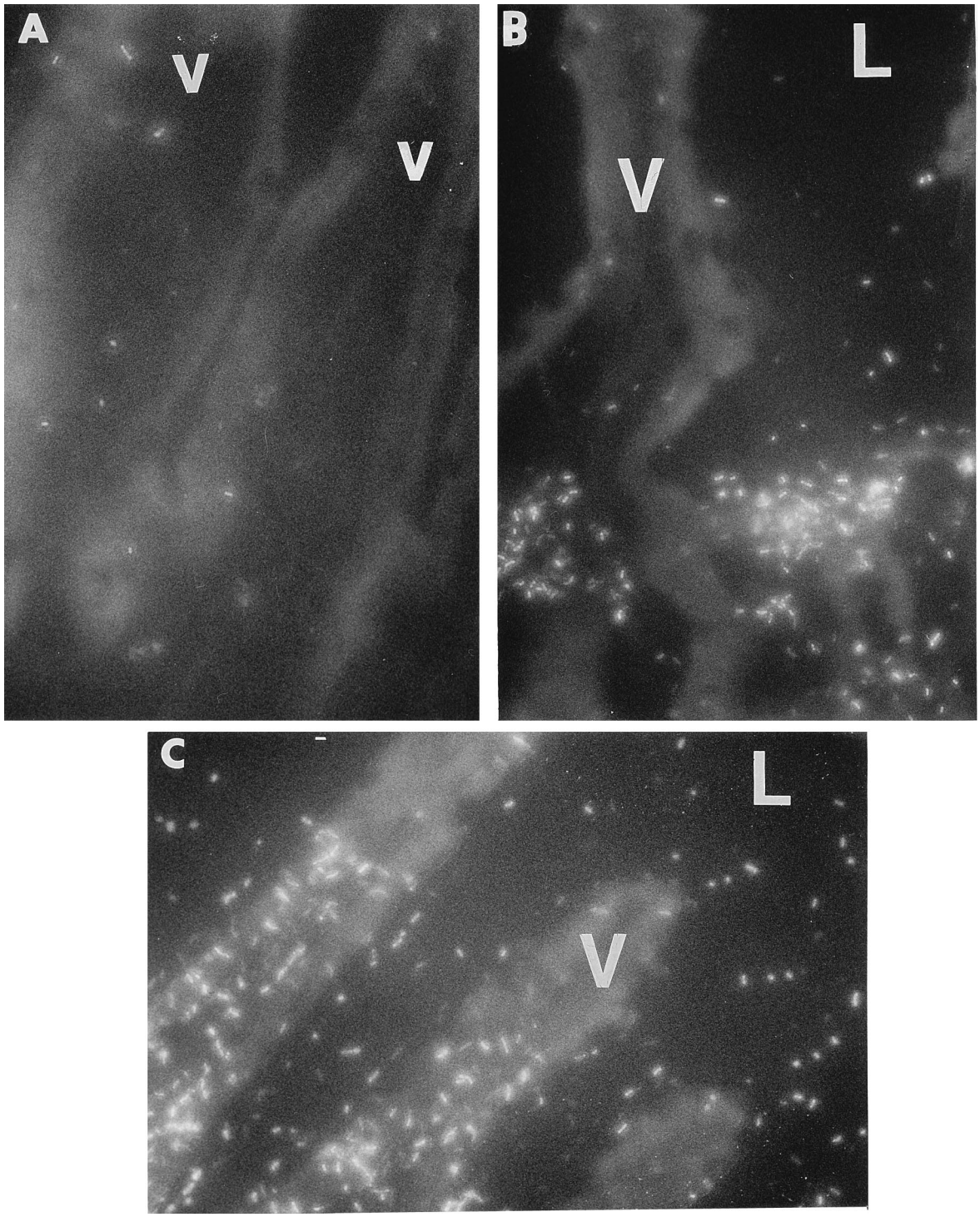

FIG. 6. Fluorescence micrographs of *E. coli* ORN172 (A) and ORN172(pFB11) (B and C) adhering to histological sections of murine small intestine. V, villus; L, intestinal lumen.

Role of PE fimbriae in induction of fluid accumulation in the infant mouse. The enteric fever caused by *S. typhimurium* in mice is thought to closely resemble human typhoid, a disease caused by *Salmonella typhi* in humans. Murine typhoid fever, however, does not mimic the acute gastroenteritis caused by *S. typhimurium* in humans (9). Instead, several cell culture and animal models have been used to study the various activities implicated in *S. typhimurium*-mediated diarrhea (10, 13, 23, 24, 28, 29, 31, 32, 37–39, 51). Since PE fimbriae mediate adhesion to the epithelial lining of the murine small intestine, we decided to undertake studies in mice. In a pilot experiment, fluid accumulation in mouse ligated ileal loops was monitored after injection of *S. typhimurium* wild-type strain IR715, a plasmidless derivative (14028P$^-$), or sterile PBS. While intestinal

TABLE 2. Fluid accumulation in infant suckling mice

Mice injected with:	Relevant genotype	Ratio[a]	Evaluation[b]
S. typhimurium 14028			
IR715	Wild type	0.137 ± 0.026	+
AJB7	*pefC*::Tet^r	0.087 ± 0.021	−
AJB7(p22.2)	*pefC*::Tet^r, *pefABCDE orf5* on plasmid	0.155 ± 0.008	+
14028P$^-$(pFB11)	*pef* operon on cosmid	0.117 ± 0.012	+
14028P$^-$(pFB6)	Truncated *pef* operon on cosmid	0.096 ± 0.011	−
14028rck	*rck*::Km^r	0.138 ± 0.013	+
S. typhimurium SR-11			
χ4252	Wild type	0.128 ± 0.015	+
χ4253	Δ*fim*	0.145 ± 0.019	+
AJB9	*pefC*::Tet^r	0.092 ± 0.025	−
E. coli			
ORN172	Nonpiliated	0.077 ± 0.009	−
ORN172(pFB11)	*pef* operon on cosmid	0.087 ± 0.011	−
PBS		0.096 ± 0.007	−

[a] Mean of the ratios between weight of mouse intestine and rest of the body from at least four different animals ± standard error.
[b] +, fluid accumulation, significantly greater than PBS control ($P < 0.025$); −, no fluid accumulation, not significantly greater than PBS control ($P > 0.1$).

loops infected with IR715 clearly showed fluid accumulation, 14028P$^-$ and the PBS control did not. We further studied the role of the *pef* operon in fluid accumulation in the infant suckling mouse assay (24). While the *S. typhimurium* wild-type strains IR715 and SR-11 caused significant fluid accumulation in infant mice compared with the PBS control ($P < 0.025$), infection with derivatives carrying a *pefC* mutation (AJB7 and AJB9) did not produce this effect (Table 2). In contrast, a mutation in *rck*, a gene located downstream of the *pef* operon, did not abolish fluid accumulation ($P < 0.005$) (Table 2). The *pefC* mutant AJB7 could be complemented for fluid accumulation by introduction of plasmid p22.2, containing the genes *pefBACD* and *orf5* ($P < 0.005$) (7). By transposon mutagenesis, all five genes present on plasmid p22.2 have been shown to be necessary for surface presentation of PE fimbriae (7). Furthermore, fluid accumulation could be observed after infection with a plasmidless *S. typhimurium* strain containing a cosmid carrying the entire *pef* operon (pFB11) but not with a strain containing a cosmid carrying a truncated *pef* promoter region (pFB6) (Table 2). Introduction of pFB11 into the *E. coli* ORN172, however, did not result in fluid accumulation. These results indicate that expression of the *pef* operon is necessary for, but is not the only factor involved in, fluid accumulation in infant mice. To determine the contribution of type 1 fimbriae to fluid accumulation, we tested an *S. typhimurium fim* mutant in the infant mouse assay. A deletion of the *S. typhimurium fim* operon did not decrease fluid accumulation.

DISCUSSION

In this report, we demonstrate that PE fimbriae mediate adhesion to murine small intestine. Mutational inactivation of *pefC* resulted in only a moderate decrease in mouse virulence of *S. typhimurium*, indicating that *pef*-mediated adhesion to murine small intestine is not essential for murine typhoid. Thus far, the contribution of two fimbrial adhesins during infection of mice has been studied by mutational analysis (2, 26). Loss of genes encoding type 1 fimbriae increases the virulence of *S. typhimurium* for mice about 10-fold (26). In contrast, LP fimbriae mediate adhesion to murine Peyer's patches and are necessary for full virulence in murine typhoid (2). These data therefore suggested that PE fimbriae serve a function distinct from that described for other *S. typhimurium* adhesins. Thus, bacterial adhesion can have different consequences during experimental infection of mice with *S. typhimurium.*

Using infant mice, we show that PE fimbriae are necessary for fluid accumulation mediated by *S. typhimurium*. However, although PE fimbriae mediated adhesion of *E. coli* to mouse small intestine, the *pef* operon did not mediate fluid accumulation. This result is consistent with the idea that the *pef* operon acts in concert with additional factors encoded on the *Salmonella* chromosome to cause fluid accumulation in the infant suckling mouse assay. Among the possible factors involved in fluid accumulation are several toxic activities found in *S. typhimurium* (3, 10, 13, 23, 24, 31, 32, 37–39, 51). However, these toxins have never been purified, and genes encoding these toxic activities have never been studied by mutational inactivation. A second possible diarrheagenic principle was suggested by Giannella and coworkers, who provided convincing evidence that inflammation contributes to fluid accumulation in rabbit ligated ileal loops (8, 9, 11). Recently, McCormick et al. established a cell culture model for the transepithelial migration of neutrophils induced by *S. typhimurium* (28, 29). Interestingly, the neutrophil transmigration response required adhesion of *S. typhimurium* to the epithelial apical membrane and subsequent reciprocal protein synthesis in both bacteria and epithelial cells. Adhesion of *E. coli* to epithelial cells did not result in transepithelial neutrophil migration. Thus, adhesins may act in concert with *S. typhimurium* factors, eliciting epithelial responses which lead to inflammation. These data are therefore in agreement with a role of PE fimbriae in fluid accumulation by mediating adhesion to murine intestinal epithelial cells.

Binding of fimbriae to receptors which are present only in certain species can contribute to determining the host range of enteric pathogens (5, 6, 16, 19, 22, 33, 47). Therefore, our results do not imply that PE fimbriae are necessary for diarrhea in humans. In fact, PE fimbriae may only mediate colonization of the mouse small intestine, and their contribution to fluid accumulation in infant mice does not allow conclusions as to their role in other host species. For example, we show that adhesion of *S. typhimurium* to the human intestinal epithelial cell line T84 is not mediated by PE fimbriae. McCormick et al. used polarized T84 cells in their model for induction of transepithelial migration of neutrophils by *S. typhimurium* (28). Thus, it is unlikely that PE fimbriae would contribute to transepithelial neutrophil migration in this model. Similarly, PE fimbriae do not contribute to fluid accumulation in rabbit ligated ileal loops (15). It is likely that adhesins other than PE fimbriae contribute to transepithelial neutrophil migration or

fluid accumulation in these models. The comparison with models for salmonellosis is further complicated by the observation that the mouse strain used can influence results obtained in the infant suckling mouse assay (42a). This may be the reason why studies using infant Swiss mice did not observe enteropathogenicity of *S. typhimurium* strains (20, 34). Therefore, the use of BALB/c mice may be recommended for future studies using this animal model.

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