INFECTION AND IMMUNITY, July 1993, p. 2755–2762
0019-9567/93/072755-08$02.00/0

Vol. 61, No. 7

Enteropathogenic *Escherichia coli* Decreases the Transepithelial Electrical Resistance of Polarized Epithelial Monolayers

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Received 2 October 1992/Accepted 2 April 1993

The mechanisms whereby enteropathogenic *Escherichia coli* (EPEC) causes diarrhea remain undefined. We found that EPEC caused a decrease in transepithelial electrical resistance across polarized monolayers of Caco-2 and MDCK epithelial cells. This occurred approximately 6 to 10 h after bacterial addition and was reversible if the monolayers were treated with tetracycline or gentamicin. Although significant alterations in host actin occurred beneath adherent EPEC, actin filaments supporting tight junctions were not noticeably affected in the epithelial cells, nor was the distribution of ZO-1, a tight junction protein. Despite the decrease in transepithelial electrical resistance, EPEC did not cause an increase in [^{3}H]inulin penetration across MDCK monolayers. Unlike in the parental strain, mutations in any loci involved in adherence or formation of attaching and effacing lesions were unable to cause a decrease in transepithelial resistance. These data indicate that EPEC causes a decrease in transepithelial electrical resistance by disrupting a transcellular (intracellular) pathway rather than by disrupting intercellular tight junctions (paracellular) and that these disruptions occur only when attaching and effacing lesions are formed.

Enteropathogenic *Escherichia coli* (EPEC) is a leading cause of infantile diarrhea in developing nations (17, 24). Despite its prevalence, EPEC virulence factors involved in diarrhea are not well understood. Unlike enterotoxigenic *E. coli*, no toxin secretion is associated with EPEC pathogenicity (27). Instead, EPEC pathogenicity involves interactions of the bacteria with its target epithelial cells to cause attaching and effacing (A/E) lesions.

EPEC infection of epithelial cells progresses in three steps: (i) initial (localized) adherence, (ii) intimate adherence, and (iii) transduction of signals by the extracellular bacteria to the host cytoplasm (reviewed in reference 6). Initial adherence is mediated by a plasmid-encoded bundle-forming pilus (12) and possibly other factors (4). However, plasmid-cured strains still form a small number of A/E lesions (15), although they adhere much less frequently than parental strains do. Intimin, the product of the *eaeA* locus, is needed for intimate attachment to occur, and mutants with mutations in this locus adhere at normal levels but do not form intimate attachments (2, 13, 14). Mutants with mutations in other loci (class IV [*cfm*] mutants) are unable to transduce signals to the host epithelial cell, although they adhere intimately to the host cell surface at normal levels (2, 25). These signals include induction of tyrosine phosphorylation of a 90-kDa epithelial protein (25). Once the appropriate signals have been transduced, intimin participates in induction of assembly of cytoskeletal structures in the epithelial cells just beneath the attached bacteria to form cuplike pedestals on which the bacteria rest (15). These structures are composed of cytoskeletal elements such as actin filaments, α-actinin, myosin light chain, ezrin, and talin (11, 15, 22). The abilities of EPEC to induce its own internalization by normally nonphagocytic epithelial cells (3) and to cause localized degeneration of the host brush border microvilli (16) are probably consequences of this cytoskeletal rearrangement.

Currently, only mutants with lesions in the *eaeA* locus have been tested in human volunteers for diarrhea production. These mutants caused less diarrhea in human volunteers, but residual diarrhea often remained (7). In this communication we report that EPEC causes a decrease in transepithelial electrical resistance across monolayers of Caco-2 and MDCK polarized epithelial cells and that this effect is mediated by viable bacteria. The data presented here indicate that the loss in electrical resistance is due to alterations in an epithelial transcellular pathway rather than to disruption of tight junctions.

MATERIALS AND METHODS

Bacterial strains, media, and tissue culture. Bacterial strains used in this study are described in Table 1. Bacteria were stored in Luria-Bertani broth plus 15% glycerol at −70°C and grown in Luria broth overnight at 37°C without shaking or on agar plates. Polarized monolayers of MDCK and Caco-2 cells were grown as described elsewhere (9, 10).

Transepithelial electrical resistance. Transepithelial electrical resistance measurements of Caco-2 and MDCK monolayers grown in Transwell filter units (no. 3415; Costar) were made with a Millicell-ERS (Millipore) apparatus as described elsewhere (9, 10). Transepithelial resistance (ohm-square centimeters) was calculated by multiplying the measured electrical resistance by the area of the filter (0.33 cm^2).

Quantitation of bacterial penetration across polarized MDCK monolayers. Bacterial penetration was quantitated as described elsewhere (9, 10). We added 1.8×10^7 parental EPEC (streptomycin-resistant) and/or 10-5-1(1) (*eaeA*::Tn*phoA*; neomycin-resistant) cells to the apical surface of polarized Caco-2 monolayers grown in Transwell filter units. At 1-h intervals the filter units were transferred to fresh medium, and appropriate dilutions of the residual basolateral

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TABLE 1. Bacterial strains used in this study

Strain	Characteristics	Source or reference
E. coli		
E2348/69	Parental EPEC, Adherence$^+$, Invasion$^+$	18
CVD206	*eaeA* deletion mutant of E2348/69, Adherence$^+$, Intimate adherence$^-$, Invasion$^-$	5
JPN15	Plasmid$^-$, Adherence$^-$, Localized adherence$^-$, Invasion$^-$	13
14-2-1(1)	Class IV Tn*PhoA*, Adherence$^+$, Invasion$^-$, signal transduction affected	2
10-5-1(1)	*eaeA*::Tn*PhoA*, Adherence$^+$, Intimate adherence$^-$, Invasion$^-$	2
21-2-2(1)	Class III Tn*PhoA*, Adherence$^-$, Invasion$^-$	2[a]
K802	Noninvasive laboratory strain	ATCC[b]
S. typhimurium		
SL1344	Virulent, depolarizes epithelial monolayers	9

[a] 21-2-2(1) has been reclassified as a class III mutant after further characterization (1a).
[b] ATCC, American Type Culture Collection.

medium were plated onto streptomycin and neomycin agar plates.

Immunofluorescence microscopy. MDCK monolayers were grown in Transwell filter units until polarized (5 days) and then apically infected with 1.8×10^7 bacteria. After the transepithelial electrical resistances were measured, the samples were washed twice with phosphate-buffered saline (PBS) and fixed with cold (4°C) 2% paraformaldehyde for 45 min. The cells were permeabilized with 0.1% Triton X-100–PBS for 10 min and then washed twice with PBS. Fluorescein-phalloidin (diluted 1/20 in PBS) (Molecular Probes) was used to stain polymerized actin by being added to both apical and basolateral surfaces and incubated for 30 min at 23°C. Tight junctions were stained with an antibody directed against ZO-1 (Chemicon, Temecula, Calif.) by using standard procedures. Filters were excised, placed in mounting medium (Sigma) on a glass slide, and sealed with nail polish under a coverslip. Labeled filters were observed by using a Zeiss Axioskop fluorescence microscope or a Bio-Rad MRC-600 confocal laser scanning microscope. Confocal sections were usually taken at 1-μm intervals.

Scanning electron microscopy. Polarized MDCK monolayers grown in Transwell filter units were apically infected with 1.8×10^7 bacteria (a multiplicity of infection of approximately 100) and incubated for 12 h. After the transepithelial electrical resistance was measured, the monolayers were washed three times with PBS and fixed in cold 2% glutaraldehyde–PBS for 16 h at 4°C. The samples were then washed with PBS, postfixed with 1% OsO_4 for 1 h, and dehydrated with a graded series of alcohols. Following dehydration in a critical-point drying apparatus, samples were coated with gold and examined with a Hitachi S-4100 high-resolution scanning electron microscope.

Measurement of [^{3}H]inulin penetration. Polarized monolayers of filter-grown MDCK cells were infected with EPEC for 15 h as described above. After measuring electrical resistance and washing to remove bacteria, we added 175,000 cpm of [^{3}H]inulin (molecular weight, 5,200; Amersham) in 200 μl of tissue culture fluid to the apical surface. At 1-h intervals, 100 μl (10%) of the basolateral fluid was removed and placed in 10 ml of scintillation fluid (Beckman) prior to counting.

Quantitation of bacterial invasion. Polarized Caco-2 cells were infected with 1.8×10^7 parental EPEC cells and incubated. At 1 h prior to harvesting, monolayers were washed and gentamicin (100 μg/ml) was added. Monolayers were lysed and bacterial titers were determined as described elsewhere (25).

Regeneration of monolayer electrical resistance. Polarized Caco-2 monolayers grown in filter units were apically infected with parental EPEC (1.8×10^7 bacteria) and incubated for 12 h. At this point, and every 24 h thereafter, the transepithelial electrical resistance was measured and both apical and basal media of the Transwell units were replaced with fresh medium containing gentamicin (100 μg/ml for 2 h and then 10 μg/ml for 22 h) and/or tetracycline (15 μg/ml).

RESULTS

EPEC causes a decrease in Caco-2 and MDCK polarized-monolayer transepithelial electrical resistance. Monolayers of polarized MDCK (Madin-Darby canine kidney) and Caco-2 (human intestinal) epithelial cells were grown to confluency on 3.0-μm-pore-size filters. Uninfected Caco-2 monolayers had a normal transepithelial resistance of ~140 to 200 Ω cm^2, whereas uninfected MDCK monolayers typically had a transepithelial resistance of ~1,000 Ω cm^2 (Fig. 1). To determine the effect of the parental EPEC strain on transepithelial electrical resistance, MDCK and Caco-2 samples were apically infected with the parental strain or mutant CVD206 (the *eaeA* deletion mutant) and transepithelial resistance was measured at different times (Fig. 1). With both MDCK and Caco-2 polarized epithelial monolayers, the parental strain caused a large decrease (120 Ω cm^2 for Caco-2, 925 Ω cm^2 for MDCK) in the transepithelial resistance (Fig. 1). This decrease was first noticeable approximately 7 h postinfection. In contrast, the transepithelial resistance of the MDCK monolayers infected with CVD206 remained at uninfected levels (Fig. 1A) whereas the resistance actually increased when Caco-2 cells were infected with this mutant (Fig. 1B).

EPEC penetrates polarized MDCK monolayers. MDCK monolayers grown on filter units were apically infected with the parental strain, noninvasive mutant 10-5-1(1) (a Tn*phoA* *eaeA* mutant), or an equal mix of both. The rate of bacterial penetration per hour across the monolayer was determined (Fig. 2). The parental strain was first detectable in the basolateral fluid 11 h after bacterial addition, whereas the *eaeA* mutant first appeared 15 h after infection. The parental strain did not affect the penetration rate of the *eaeA* mutant, since the number of 10-5-1(1) bacteria penetrating the MDCK monolayer was approximately the same in the presence or absence of the parental strain (Fig. 2). Similarly, addition of the *eaeA* mutant had no effect on wild-type penetration rates. These results indicate that EPEC can penetrate through polarized MDCK monolayers. They also suggest that the EPEC-induced decrease in monolayer electrical resistance was not due to large intercellular spaces, since this should increase the penetration rates of the *eaeA* mutant.

EPEC infection of MDCK monolayers does not extensively alter actin filament morphology or disrupt ZO-1 in tight junctions. Tight junctions (zona occludens) are supported by polymerized actin filaments, and disruption of these actin filaments destroys tight junctions, thereby decreasing transepithelial electrical resistance (reference 19 and references therein). We used immunofluorescence microscopy to examine whether EPEC infection affected the actin filament

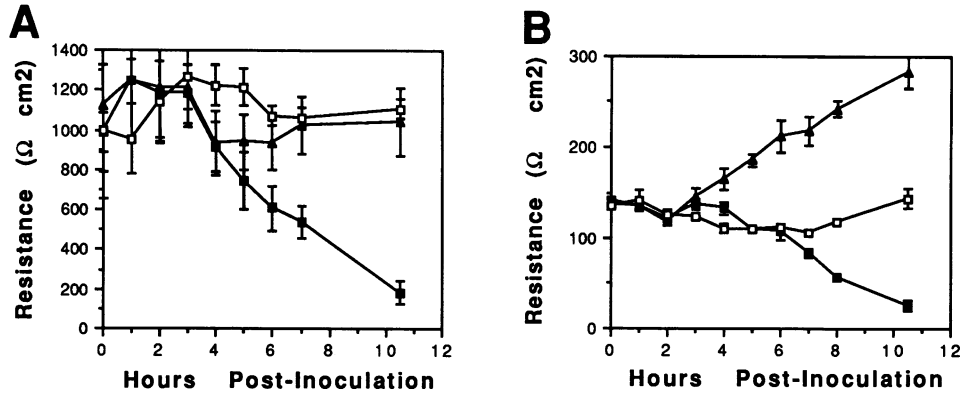

FIG. 1. Transepithelial electrical resistance of polarized epithelial monolayers infected with EPEC E2348/69 (parental strain) (■) or CVD206 (*eaeA* deletion) (▲) or left uninfected (□). We added 1.8×10^7 bacteria to the apical surface of polarized MDCK (A) or Caco-2 (B) monolayers. Each value is the average resistance for four filters and is representative of one of three experiments. Error bars show the standard deviations.

morphology near the tight junctions. Polarized MDCK monolayers were infected with EPEC for 12 h (such that they had low electrical resistance values) and were then stained with fluorescein-phalloidin, which stains polymerized actin filaments. Figure 3A and B illustrate two focal planes of infected MDCK monolayers stained with phalloidin. One is a focal plane of the apical part of the monolayer (Fig. 3B), and the actin accumulation that underlies adherent EPEC is visible. This staining often outlines the shape of single adherent bacteria. The other is a focal plane of the central part of the same epithelial monolayer (Fig. 3A), illustrating a honeycomb staining pattern characteristic of actin belts that are associated with tight junction formation.

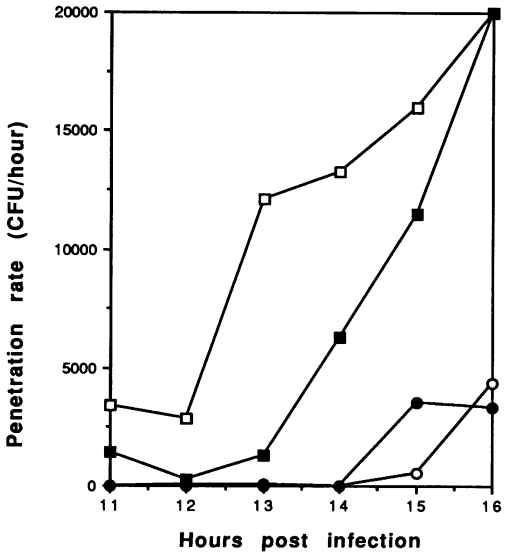

FIG. 2. Quantitation of bacterial penetration across MDCK monolayers. A total of 1.8×10^7 parental EPEC organisms, noninvasive strain 10-5-1(1) (an *eaeA* Tn*PhoA* mutant) organisms, or a mixture of both was added to the apical surface of MDCK monolayers, and the basolateral medium was harvested at 1-h intervals and plated on selective agar. Symbols: □, parental EPEC; ○, 10-5-1(1); ■, parental EPEC coinfected with 10-5-1(1); ●, 10-5-1(1) coinfected with parental EPEC. Values are the averages for two filters and are representative of one of four experiments.

Even when EPEC bacteria were lying on top of intercellular junctions, no extensive alterations were visible in the underlying actin belts (arrow). Therefore, EPEC does not appear to significantly disrupt the actin filaments involved in tight-junction formation.

This finding was further confirmed by indirect immunofluorescence microscopy of the tight junctions with anti-ZO-1 antibodies. No disruptions were found in any tight junctions in either uninfected monolayers or those which had been infected with EPEC for 15 h and showed a decrease in resistance (Fig. 3C and D).

Scanning electron microscopy also revealed that there were no obvious spaces between MDCK cells. Figure 4 illustrates an infected MDCK monolayer which had a low electrical resistance 12 h after EPEC addition. Adherent EPEC cells are visible, as are obvious distortions and shortening of the MDCK microvilli. However, there were no apparent spaces in the intercellular region between MDCK cells, again indicating that the decrease in electrical resistance is the result of a subtle effect on the monolayer rather than formation of large spaces between epithelial cells.

EPEC infection of MDCK monolayers does not elicit transepithelial fluxes of [^{3}H]inulin. To further examine the effect of EPEC on tight junctions, MDCK monolayers were infected with EPEC for 15 h (such that resistance was lowered) and then [^{3}H]inulin (an inert compound with a molecular weight of 5,200) was added to the apical surface. Inulin flux was measured by assaying radioactivity that penetrated the monolayers to the basolateral medium.

As expected, [^{3}H]inulin did not penetrate uninfected MDCK monolayers which had a high electrical resistance (Table 2), MDCK monolayers infected with noninvasive *E. coli* K802, or an EPEC mutant which does not affect electrical resistance (14-2-1 [see below]). However, in EPEC-infected monolayers which exhibited a decreased electrical resistance, [^{3}H]inulin penetration was also blocked (Table 2). In contrast, *Salmonella typhimurium*, which kills the MDCK cells after 15 h of infection (10), caused a significant [^{3}H]inulin flux within 1 h of inulin addition (Table 2).

Noninvasive EPEC mutants do not decrease Caco-2 and MDCK transepithelial electrical resistance. Polarized Caco-2 monolayers were infected for 12 h with parental EPEC or various EPEC mutants that are deficient or attenuated in

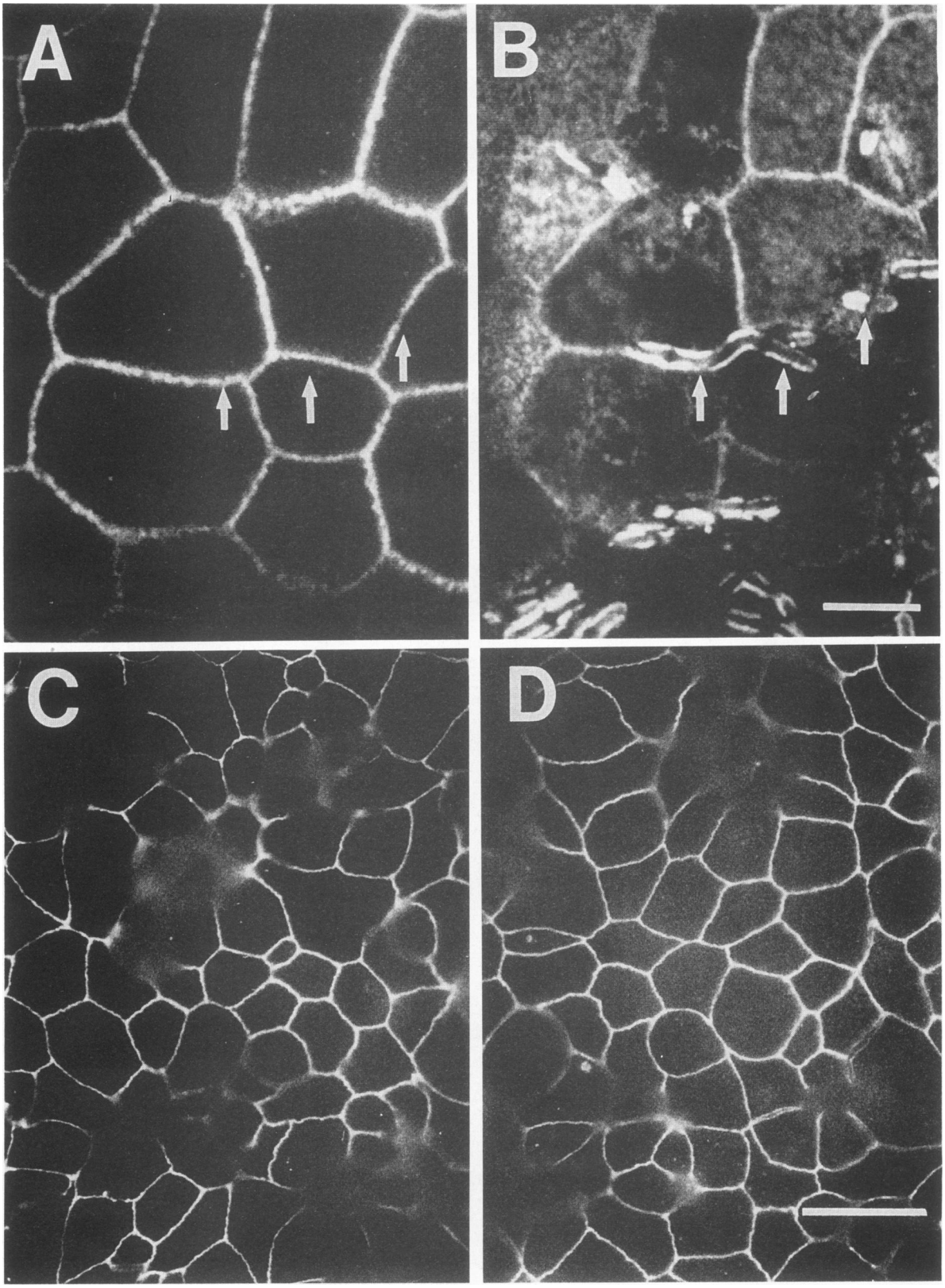

FIG. 3. Fluorescence microscopy of MDCK monolayers labeled with fluorescein-phalloidin (A and B) or anti-ZO-1 (C and D). Polarized monolayers were infected for 12 h with EPEC E2348/69 and then stained with fluorescein-phalloidin. Two focal planes are presented: a central focal plane corresponding to the midportion of the cells, illustrating the actin belts at the perimeter of the epithelial cells supporting tight junctions (panel A), and an apical focal plane (approximately 4 μm above panel A) illustrating the condensed actin beneath individual adherent bacteria (panel B). Although some of the bacteria lie directly above intercellular junctions (arrows), midsection actin filaments are not significantly disrupted. Bar (panels A and B), 5 μm; the depth of the images is approximately 1 μm. MDCK monolayers infected for 15 h (panel C) or uninfected control monolayers (panel D) were labeled with anti-ZO-1 to stain tight junctions. We could not detect any difference between the two monolayers. Bar (panels C and D), 20 μm. The slight blurring of the fluorescent images in all four frames was due to areas out of the focal plane resulting from surface undulations of the monolayer and do not represent any discontinuity of the actin or the ZO-1 belts.

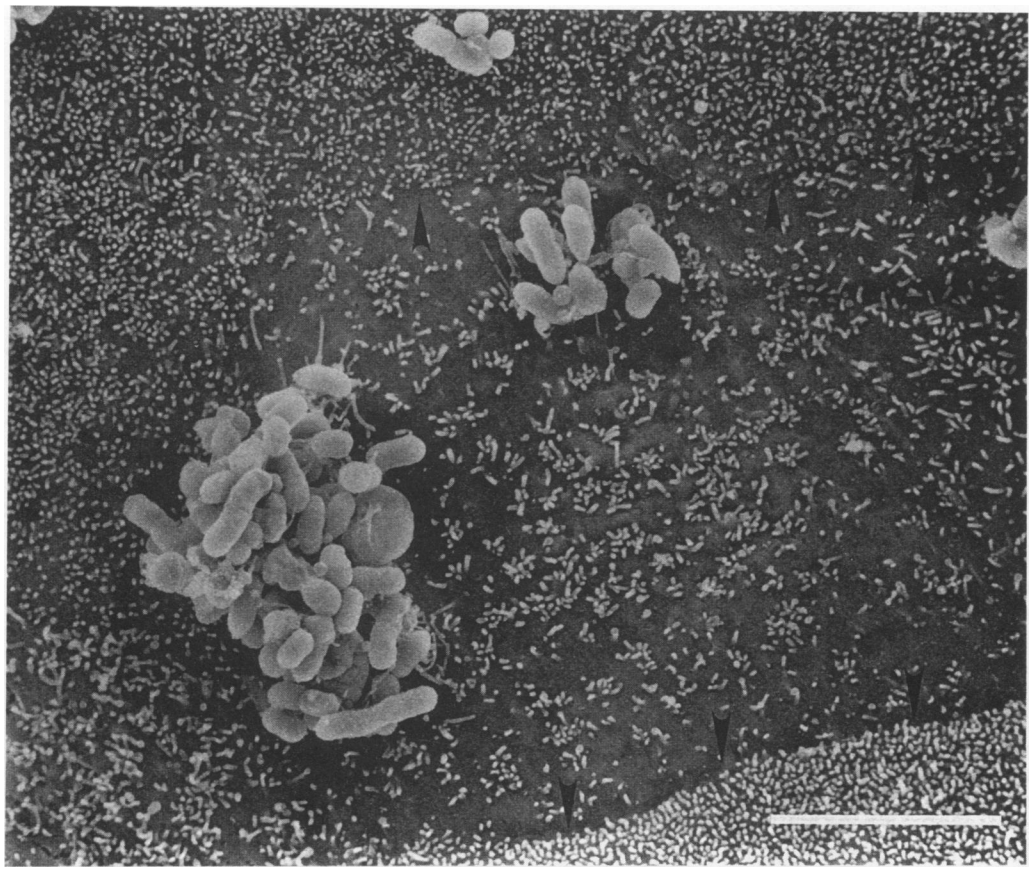

FIG. 4. Scanning electron micrograph of confluent MDCK monolayers apically infected for 12 h with EPEC 2348/69. Microcolonies of EPEC are visible attached to the surface of an isolated cell with the characteristic denuding of microvilli, while neighboring MDCK cells appear unaffected. The intercellular junctions (arrowheads) around the infected cell are not significantly disrupted. Bar, 5 μm.

their ability to induce cytoskeletal rearrangement and to invade (2). At 12 h after bacterial addition, the transepithelial resistance of Caco-2 monolayers infected with EPEC decreased by more than 100 Ω cm^2, while the resistance values of monolayers infected with either of the noninvasive strains remained at levels equivalent to those of uninfected monolayers (Table 3). Similar results were obtained when MDCK cells were used instead of Caco-2 cells (Table 3).

We often observed increases in the electrical resistance of Caco-2 monolayers when these monolayers were infected with EPEC mutants (Fig. 1B; Table 3). These increases appear to be nonspecific for various *E. coli* strains, since other nonpathogenic *E. coli* strains such as HB101 and DH5α also cause similar rises in electrical resistance values (data not shown).

Antibiotics regenerate transepithelial electrical resistance after EPEC infection. The inability of any of the A/E mutants to decrease the electrical resistance of infected monolayers despite their normal levels of adherence indicates that formation of fully developed A/E lesions is needed to cause this effect. Alternatively, it is possible that EPEC causes this effect from within the infected cells and therefore that adherent noninvasive mutants would not be able to reduce the electrical resistance of the monolayers. To examine these possibilities, we determined whether the decrease in electrical resistance caused by EPEC was reversible by the

TABLE 2. [^{3}H]inulin penetration of polarized MDCK monolayers after bacterial infection for 15 h

Strain	Electrical resistance[a] (Ω cm^2)	[^{3}H]inulin penetration[b] (cpm) after:			
		1 h	2 h	3 h	4 h
None	1,682 ± 134	10 ± 19	3 ± 2	22 ± 6	32 ± 10
EPEC E2348/69	528 ± 125	44 ± 12	65 ± 11	105 ± 16	126 ± 42
EPEC 14-2-1(1) (class IV)	1,497 ± 18	19 ± 4	36 ± 11	44 ± 11	55 ± 6
E. coli K802	1,922 ± 42	12 ± 4	34 ± 10	50 ± 8	85 ± 34
S. typhimurium	23 ± 28	1,182 ± 364	1,861 ± 419	2,680 ± 374	3,541 ± 482

[a] Electrical resistance measured 15 h after bacterial addition. The resistance prior to infection for all filters was between 1,400 and 2,000 Ω cm^2. Values are the means ± standard deviations for three filters and are representative of one of three experiments.

[b] Numbers are counts per minute obtained from assaying 100 μl (1/10 of total volume) of basolateral fluid.

TABLE 3. Transepithelial electrical resistance of polarized MDCK and Caco-2 monolayers infected with various EPEC strains

EPEC strain	Transepithelial electrical resistance[a] (Ω cm²) of:			
	Caco-2 monolayers		MDCK monolayers	
	0 h[b]	12 h	0 h	12 h
None	194 ± 7	185 ± 5	1,205 ± 170	1,092 ± 85
E2348/69	192 ± 4	45 ± 6	1,438 ± 70	665 ± 104
CVD206	201 ± 15	283 ± 19	1,264 ± 88	1,110 ± 112
JPN15	193 ± 54	236 ± 73	1,298 ± 57	1,212 ± 130
10-5-1(1)	193 ± 13	277 ± 6		
14-2-1(1)	195 ± 5	221 ± 4		
21-2-2(1)	193 ± 10	246 ± 13		

[a] Values are means ± standard deviations for four filters and are representative of one of three experiments.
[b] Time postinoculation.

addition of tetracycline (which affects both intracellular and extracellular bacteria) or gentamicin (which kills only extracellular bacteria).

We first determined the time required for EPEC to enter polarized epithelial cells. The number of intracellular bacteria increased steadily for 9 h after infection and was maximal about 12 h after infection (Fig. 5). Infection for longer than 12 h did not increase the number of intracellular bacteria (data not shown). Therefore, polarized Caco-2 monolayers were infected with EPEC for 12 h (Fig. 6, day 0.5 point). At this time, tetracycline, gentamicin, or a combination of the two was added to the monolayers and maintained for various times. Without addition of antibiotics, the transepithelial electrical resistance remained low in samples infected with EPEC (Fig. 6). In contrast, the electrical resistance of all the treated (tetracycline, gentamicin, and tetracycline-gentamicin) monolayers increased after antibiotic addition, and by 4 days the transepithelial electrical resistance had completely recovered. By day 5 the electrical resistance of the treated monolayers was about 100 Ω cm² higher than the electrical resistance of the uninfected monolayers. This increase in

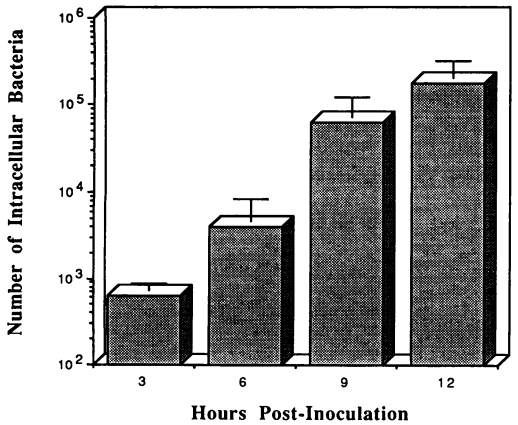

FIG. 5. Invasion of polarized Caco-2 monolayers by EPEC 2348/69. The number of intracellular bacteria was quantitated as described in Materials and Methods. There were approximately 2 × 10⁵ Caco-2 cells per filter. Values are the averages for three filters and are representative of one of three experiments. Error bars show the standard deviations.

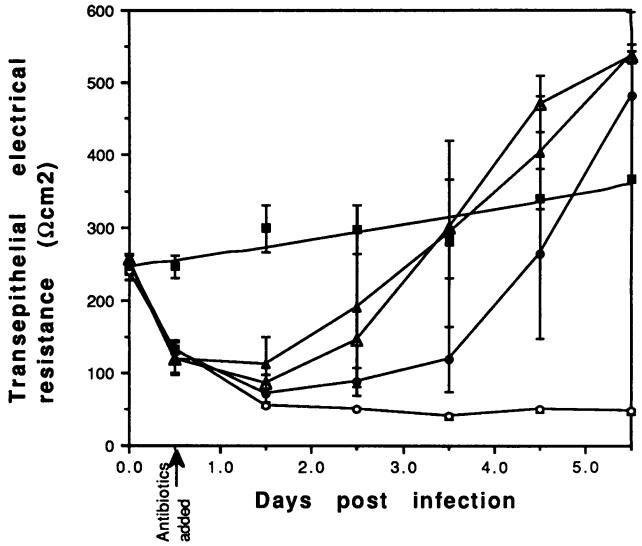

FIG. 6. Regeneration of Caco-2 electrical resistance after antibiotic addition. Polarized Caco-2 monolayers were infected with EPEC 2348/69 for 12 h, and then gentamicin and/or tetracycline was added and the cells were maintained as described in Materials and Methods. Symbols: ■, uninfected monolayers; ○, infected monolayers with no antibiotics added; ●, infected monolayers with tetracycline added; △, infected monolayers with gentamicin added; ▲, infected monolayers with tetracycline and gentamicin added. Values represent the averages for four filters and are representative of one of three experiments.

electrical resistance is comparable to that observed with monolayers infected with noninvasive *E. coli* (Fig. 1B).

DISCUSSION

Monolayers of cultured polarized epithelial cells grown on filters were used to demonstrate that on interaction with the apical surface of the epithelial cell, EPEC caused decreases in electrical resistance across polarized epithelial cell monolayers. The role of increased transcellular permeability in EPEC-induced diarrhea remains speculative, but an association between increased permeability and diarrhea has been noted for other diseases (19, 20). It is conceivable that these increases also occur in vivo and lead to electrolyte imbalance and diarrhea. This system was also used to show that, in vitro, EPEC penetrates polarized epithelial monolayers in the apical to basolateral direction. Similar results with some EPEC strains were also found in vivo with a piglet model system (26, 27) and in tissues of human infants (8). Therefore, the ability to penetrate to the basal side of the intestinal epithelia also may play a role in EPEC pathogenicity.

It has recently been shown that energy depletion of MDCK cells abolishes the gate function of their tight junctions, leading to an inulin flux (21). The lack of an inulin flux through EPEC-infected MDCK monolayers would suggest that after 15 h of infection the cells are still alive and energetically active. This is in contrast to the recently reported killing effect of EPEC on unpolarized HEp-2 epithelial cells (1). The lack of inulin penetration also suggests that the damage to intercellular tight junctions is minimal. This concept is supported by both electron and immunofluorescence microscopy studies and by the bacterial penetration studies. Moreover, the infected monolayers retain considerable transepithelial electrical resistance (approximately

50 Ω cm^2 for Caco-2 cells), indicating that the tight junctions are still intact. Collectively, these data suggest that EPEC does not disrupt the tight junctions, but, rather, they indicate that EPEC affects some transcellular pathway. In this regard, we found that 20 μM bumetanide (Sigma), an inhibitor of transcellular electrogenic Cl^- secretion (23), had no effect on the ability of EPEC to reduce resistance (data not shown). However, EPEC may alter any one of many ionic balances, possibly by opening an ion channel or affecting ion transport.

We have tested the ability of noninvasive EPEC mutants to reduce the transepithelial electrical resistance. Although two classes of these mutants adhere at normal levels, they are deficient in different functions that are needed to cause efficient A/E and invasion. The class IV mutant 14-2-1(1) has lost its ability to induce the host tyrosine protein kinase activity that is needed to induce cytoskeletal rearrangement. CVD206 and 10-5-1(1) carry a defective *eaeA* gene and therefore cannot attach intimately to the epithelial cell surface and do not cause mature A/E lesions. JPN15 is cured of the EPEC large plasmid. This plasmid encodes the bundle-forming pilus necessary for initial adherence to the host cell surface and a positive regulatory factor that increases *eaeA* expression (13). Loss of this plasmid results in low levels of adherence, formation of only a few A/E lesions, and low efficiency of invasion and host tyrosine protein kinase activation. Like JPN15, the class III mutant 21-2-2(1) forms only a small number of A/E lesions, and its invasion and ability to induce host tyrosine protein kinase are attenuated by its lack of adherence. In contrast to the parental EPEC strain, none of the above mutants reduced the transepithelial electrical resistance of infected monolayers. These results suggest that either (i) the reduction of the transepithelial electrical resistance is linked with formation of fully developed A/E lesions or (ii) efficient invasion is important since only intracellular bacteria (i.e., the parental strain) reduce the transepithelial electrical resistance. Since monolayers treated with gentamicin, which preferentially kills extracellular but not intracellular bacteria, regained their transepithelial electrical resistance, this would suggest that intracellular bacteria do not play an important role in reducing the transepithelial electrical resistance.

It has previously been reported that *Salmonella* species cause a rapid and complete loss of transepithelial electrical resistance in MDCK and Caco-2 polarized monolayers (9, 10). The effects reported here for EPEC differ significantly from those caused by *Salmonella* species. The electrical resistance losses caused by *Salmonella* species are complete by 2 h with Caco-2 monolayers and by 4 h with MDCK cells. In contrast, decreases caused by EPEC are much slower, with maximal effects observed approximately 12 h after bacterial addition. *Salmonella* species also cause a complete loss in electrical resistance, whereas disruptions caused by EPEC, although large, are never complete. Instead, EPEC-infected monolayers maintain 25 to 50% of their original resistance. *S. typhimurium* also caused a [^{3}H]inulin flux across monolayers, but EPEC did not. These comparisons emphasize the differences between these two enteric pathogens in their mechanisms of epithelial disruption, since *S. typhimurium* disrupts tight junctions whereas EPEC affects a transcellular pathway.

In conclusion, EPEC disrupts an undefined transcellular pathway in epithelial monolayers, leading to a decrease in transepithelial electrical resistance, which may be involved in diarrhea. This effect requires several bacterial loci, including those which participate in localized adherence, formation of A/E lesions, and invasion. As the contributions of each of these loci to EPEC interactions with epithelial cells become better defined, insights into the mechanisms of how EPEC affects transcellular permeability will be possible.

ACKNOWLEDGMENTS

We thank James Madara for helpful discussions.

C.C. was supported by an NSERC Summer Studentship, and I.R. was supported by a long-term fellowship from the European Molecular Biology Organization (EMBO) and a Canadian Gastroenterology Fellowship. This work was supported by a Howard Hughes International Research Scholar Award to B.B.F.

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