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Campylobacter jejuni Mediated Disruption of Polarized Epithelial Monolayers is Cell-Type Specific, Time Dependent, and Correlates With Bacterial Invasion

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ABSTRACT: The precise mechanism by which the most common cause of bacterial enterocolitis in humans, *Campylobacter jejuni*, perturbs the intestinal mucosa remains elusive. To define effects of *C. jejuni* infection on mucosal permeability, Madin-Darby canine kidney (MDCK)-I and T84 cell monolayers were infected with *C. jejuni* for up to 48 h. All three tested *C. jejuni* strains caused a 73–78% reduction in transepithelial electrical resistance (TER) in intestinal (T84) cell monolayers, whereas only one strain slightly reduced TER of MDCK-I cells by 25% after 48 h infection. Infection with *C. jejuni* strains also caused a 2.3–4.5-fold increase in dextran permeability, but only in T84 cells. *C. jejuni* infection of monolayers also caused morphologic changes in desmosomes, observed by transmission electron microscopy. The cell-type specificity, demonstrated by increased T84 monolayer permeability, correlated with higher bacterial invasion into these cells, relative to MDCK-I cells. In T84 cells, invasion and bacterial translocation preceded barrier disruption and inhibition of *C. jejuni* invasion using a pharmacological inhibitor of phosphoinositide 3-kinase, reduced the drop in TER. These findings suggest that *C. jejuni* disruption of monolayers is mediated by invasion, provide new insights into *C. jejuni*-host epithelial barrier interactions, and offer potential mechanisms of intestinal injury and chronic immune stimulation. **(*Pediatr Res* 64: 599–604, 2008)**

Campylobacter jejuni is the leading cause of bacterial-induced enterocolitis across the globe (1,2). The burden of disease is considerable, especially among children, both in developing countries, where infection can result in life-threatening diarrhea, and in children from industrialized countries, who may be exposed to *C. jejuni* through travel, contaminated food, or contact with pets (3). Intestinal symptoms following infection with *C. jejuni* species are often self-limited, ranging from watery diarrhea to dysentery, although *C. jejuni* has also been implicated in chronic intestinal disorders, including postinfectious irritable bowel syndrome (4) and chronic inflammatory bowel diseases (IBD) (2,5,6). However, microaerophilic growth requirements and the paucity of suitable animal models limit current understanding of disease pathogenesis (7).

Several factors contributing to bacterial virulence have been identified to date, including adhesion, invasion, and the cytolethal-distending-toxin. Invasion is dependent on host microtubules (8), cytolethal-distending-toxin secretion (9), and the pVir type IV secretion system homologue (10). After invasion, *C. jejuni* manipulates host signaling to avoid lysosomal degradation (11). A recently described *C. jejuni* isolate, which causes diarrhea in humans and in a ferret model of infection, despite very low invasion rates *in vitro*, suggests involvement of other pathogenic mechanisms (12).

The intestinal barrier is comprised of a single layer of polarized epithelial cells serving to separate the luminal content, including microbes, from the underlying mucosa. The main physical component contributing to the barrier is the apical junctional complex, which includes highly regulated tight junctions and adherens junctions, bridging between epithelial cells and connecting the cytoskeleton (13). Breaches in the epithelial barrier integrity result in penetration of luminal antigens and microbes, which stimulate pro-inflammatory responses, leading to chronic intestinal and systemic diseases, including IBD (13). Pathogenic bacteria can disrupt the mucosal barrier by secretion of toxins, transfer of effector proteins into host cells, invasion of epithelial cells, translocation across the epithelial monolayer, or by disruption of intercellular junctions (14).

The aim of this study was to characterize the effects of three prototype *C. jejuni* strains on permeability by employing polarized epithelial monolayers with different characteristics. We demonstrate herein that *C. jejuni* infection causes a time-dependent increase in permeability, which correlates with the ability of *C. jejuni* to invade and disrupt epithelial cell monolayers.

METHODS

Epithelial cells in tissue culture. HEp-2 cells were cultured in Minimal Essential Medium (MEM), 15% heat-inactivated FCS, 0.1% sodium bicar-

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Abbreviations: EHEC, enterohemorrhagic *Escherichia coli*; **IBD,** inflammatory bowel diseases; **MDCK,** Madin-Darby canine kidney; **MOI,** multiplicity of infection; **PI3-K,** phosphoinositide 3-kinase; **TER,** transepithelial electrical resistance

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bonate, and 2% penicillin-streptomycin. Intestine 407 were cultivated in MEM, 10% FCS, and 2% penicillin-streptomycin. In contrast to HEp-2 and Intestine 407 cells, T84 and Madin-Darby canine kidney (MDCK)-I cells are polarized epithelial cells that form apical junctional complexes, resulting in high electrical resistance, useful for studying effects of bacteria on permeability (15,16). T84 human colon cancer epithelial cells were cultured in DMEM/F-12, 10% FCS, 2% penicillin-streptomycin, 2% sodium bicarbonate, and 0.6% L-glutamine. MDCK-I cells were grown in DMEM, 10% FCS, and 2% penicillin-streptomycin (All from GIBCO, Grand Island, NY). Cells were maintained in 25 cm^2-flasks (Corning Glass Works, Corning, NY) and then grown on six-well plates or 12-well Transwells (6.5 mm diameter; 0.4 or 3 μ pore size; 37°C; 5% CO_2; Corning).

Pharmacological inhibitors. To disrupt host microtubules, monolayers were pretreated for 1 h before infection with 5–50 μg/mL colchicine (Sigma Chemical Co., Saint Louis, MO). In experiments involving a phosphoinositide 3-kinase (PI3-K) inhibitor, monolayers were pretreated with 25–100 μM of either LY294002 or an inactive analog, LY303511 (Calbiochem, La Jolla, CA) for 1 h before and throughout the infection.

Bacterial strains. Three prototype *C. jejuni* strains were used, to represent a variety of potential virulence mechanisms. NCTC11168, the first sequenced *C. jejuni* strain (1); the invasive strain, ATCC81-176 (3); TGH9011 (ATCC43431), a clinical isolate characterized at the University of Toronto, and a noninvasive *flgF* isogenic mutant of TGH9011 (17). All strains were incubated in Mueller-Hinton broth and regrown on Columbia-blood-agar plates under microaerophilic conditions (48 h; 37°C).

Enterohemorrhagic *Escherichia coli* (EHEC), strain CL56 (serotype O157:H7) (18) was grown in Penassay-broth overnight, and regrown in 10:1 fresh Penassay-broth (3 h; 37°C). Multiplicity of infection (MOI) used for all experiments was 100:1, except for invasion assays (20:1).

Bacterial invasion and translocation. For invasion assays, epithelial cells were infected with *C. jejuni* strains. After various time points (1–48 h), wells were washed three times with PBS to remove nonadherent bacteria; fresh antibiotic-free cell culture medium was then added (2 h; 37°C) with or without gentamicin (Sandoz, Boucherville, Quebec, Canada). Gentamicin (100 μg/mL) effectively killed all bacteria (11). Cells were then washed and lysed with 0.1% Triton X-100 (15 min; 20°C; Sigma Chemical Co.). Aliquots (10 μL) were plated onto blood-agar plates in serial 10-fold dilutions and grown under microaerophilic conditions (48 h; 37°C). Bacteria recovered from wells with gentamicin represent only internalized organisms, whereas those present in wells without antibiotics represent both adherent and invasive bacteria. Invasion, as percentage of bacterial adhesion, which is an expression of the ability of adherent bacteria to invade cells (19), was calculated by a ratio of enumerated colony-forming units in each well. Bacterial translocation was measured by sampling 10 μL from the basal compartment of 3 μ-pore Transwells for determination of colony-forming units.

Measurement of transepithelial electrical resistance (TER) and macromolecular permeability. MDCK-I and T84 cells were plated onto Transwells (5×10^4 or 2×10^5 cells/well, respectively; 6.5 mm diameter; 0.4 μ-pore size; Corning) and grown until apical junctional complexes developed. Transwells were infected apically with either EHEC O157:H7 or *C. jejuni* strains (MOI: 100:1; 37°C; 5%CO_2). TER was measured prior to, 24 h, and 48 h after infection using a Millicell-ERS Voltmeter and chopstick electrodes (Millpore, Bedford, MA). TER of Transwells without cells was 32 $\Omega \cdot cm^2$.

Dextran flux was used to measure paracellular macromolecular permeability (20). After 48 h of infection, monolayers were washed four times with PBS, and infrared-labeled dextran (10 kD; 0.2 mL of 0.1 mg/mL in DMEM; Alexa-Fluor 647, Molecular Probes, Eugene, OR) was then inserted into the apical compartment of Transwells. After 5 h at 37°C, the basal compartment was sampled, diluted 1:20, and loaded into 96-well plates for infrared signal quantification using an imaging system at 700 nm (Odyssey, Licor, Rockford, IL). Integrated intensities were expressed relative to uninfected polarized monolayers.

Transmission electron microscopy. Confluent T84 Transwells were infected with *C. jejuni*, strain 81-176 (MOI: 100:1; 48 h; 37°C). Support membranes were washed, excised, and cells fixed in formaldehyde (4%) and glutaraldehyde (1%) in phosphate buffer, and postfixed in osmium tetroxide (1%; 2 h; 20°C). Specimens were then dehydrated, embedded, and stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy (JEM-1011, JEOL USA Corp., Peabody, MA) at 75 kV. Desmosome width was assessed by measuring spaces between adjacent membranes of well-oriented desmosomes.

Statistics. Results are expressed as means ± SEM. *N* represents the number of individual experiments. ANOVA and unpaired *t* test were conducted using InStat3 (GraphPad, San Diego, CA).

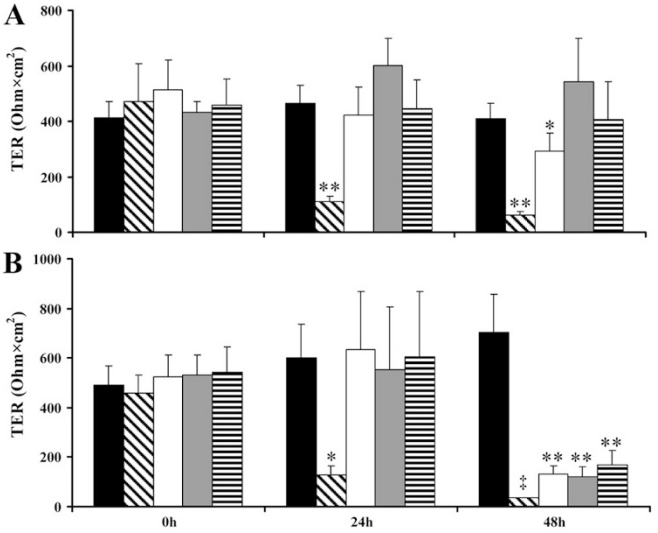

Figure 1. *C. jejuni* decreases TER of epithelial cell monolayers after 48 h of infection. Epithelial cells were infected with EHEC O157:H7 or *C. jejuni* (MOI 100:1; 37°C). Uninfected (■); EHEC (▨); *C. jejuni*, strains 9011 (□), 11168 (▩), and 81-176 (▤). *Panel A*, Only *C. jejuni*, strain 9011 significantly reduced TER by 24.7 ± 8.3% after 48 h in MDCK-I cells. *Panel B*, Infection of T84 cell monolayers with all *C. jejuni* strains caused a comparable decrease in TER, after 48 h (77.3 ± 8.3%, 78.0 ± 8.8%, and 72.8 ± 9.1% for strains 9011, 11168, and 81-176, relative to uninfected cells, respectively), but not after 24 h. EHEC O157:H7 caused a significant reduction in TER in MDCK-I (*Panel A*) and T84 (*Panel B*) cells at all time points tested. Mean ± SEM; $n = 4-6$; ANOVA: $*p < 0.05$, $**p < 0.01$, $‡p < 0.001$.

RESULTS

C. jejuni *decreases TER of T84 monolayers after 48 h.* In contrast to the rapid reduction in TER with EHEC O157:H7 infection, no reduction was demonstrated after 24 h of *C. jejuni* infection in both MDCK-I and T84 cells (Fig. 1*A* and *B*). However, a 25% reduction in TER was observed after 48 h infection with *C. jejuni*, strain 9011 in MDCK-I cells (Fig. 1*A*). In contrast to canine kidney-derived MDCK-I monolayers, which were less susceptible to *C. jejuni*-mediated barrier disruption, all three tested *C. jejuni* strains caused a comparable reduction of 73–78% in TER in human intestinal T84 cells after 48 h (Fig. 1*B*). Infection with a noninvasive *C. jejuni flgF* deficient mutant did not reduce TER ($n = 2$). Live bacteria were required, because there was no drop in TER with heat-inactivated ($n = 4$) or formaldehyde-fixed bacteria ($n = 2$). The effects were not due to the metabolic activity of bacteria on epithelial cells, because incubation with tissue culture medium corrected to pH 6 (the pH of medium after 48 h of infection; $n = 2$) and conditioned medium did not reduce TER ($n = 2$).

C. jejuni *infection increases macromolecular permeability of T84 monolayers.* Transcytosis of 10 kD dextran across monolayers supported the TER results. Consistent with previous reports (21), EHEC O157:H7 caused a dramatic increase in permeability to dextran in both epithelial cell types, indicating breakdown of the epithelial barrier. Infection with *C. jejuni* resulted in increased dextran permeability in T84 cells, but to a lesser degree relative to EHEC infection (Fig. 2).

Intercellular spaces of T84 monolayers are modified following* C. jejuni *infection. Transmission electron microscopy

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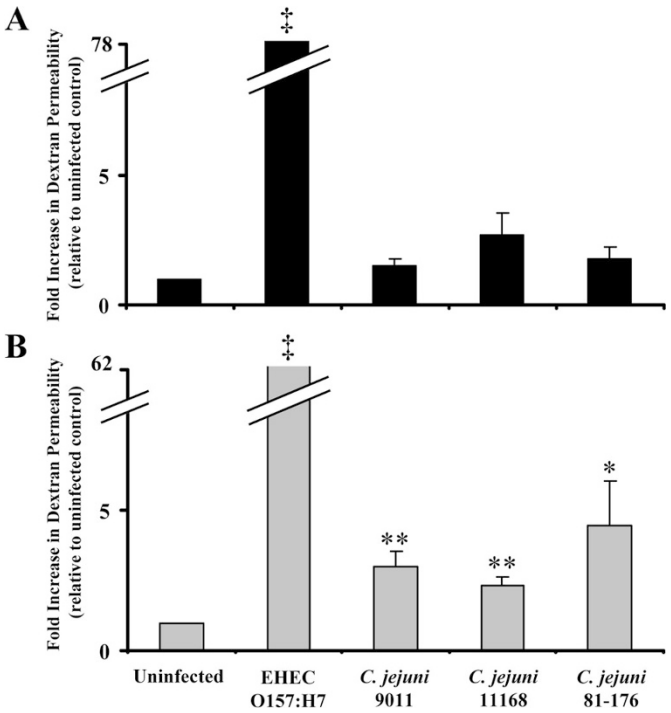

Figure 2. *C. jejuni* infection increases T84 cell monolayer permeability. After 48 h of infection of monolayers grown on Transwells, an infrared-labeled dextran (10 kD) probe was placed into the apical compartment and sampled 5 h later from the basal compartment. Samples were then diluted and the infrared signal was quantified. EHEC infection resulted in a 78- and 62-fold increase in permeability to dextran in MDCK-I (*Panel A*) and T84 (*Panel B*) cells, respectively. *C. jejuni* infection caused a 2.3–4.5-fold increase in permeability to dextran, relative to uninfected controls, respectively, but only in T84 cell monolayers (*Panel B*). Average dextran concentration in the basal compartment of uninfected wells was 8 ± 2 and 18 ± 3 ng/mL in MDCK-I and T84 Transwells, respectively. Mean ± SEM; $n = 4-5$; ANOVA: ‡$p < 0.001$; *t* test: *$p < 0.05$, **$p < 0.01$.

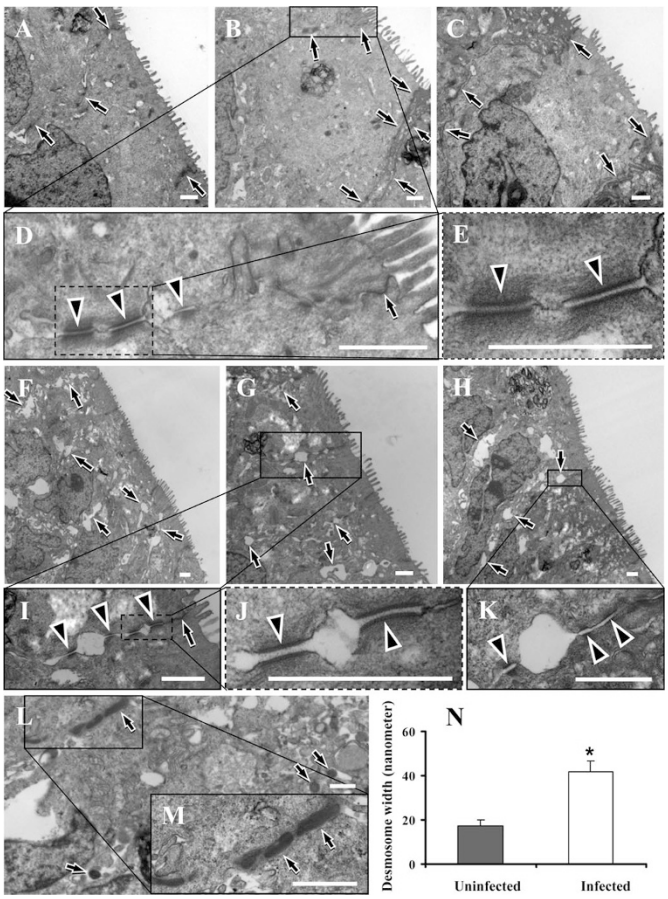

Figure 3. Bacterial disruption of intercellular junctional complexes. Confluent T84 monolayers were either left uninfected or infected with *C. jejuni*, strain 81-176 (MOI: 100:1; 48 h). *Panels A–C*, Uninfected T84 monolayers with normal intercellular junctions (*arrows*). *Panel D*, Higher magnification of apical junctional complexes of uninfected T84 monolayers [*Panel E*, Enlarged insert demonstrating normal desmosomes (*arrowheads*)]. *Panels F–H*, T84 monolayers after 48 h infection with *C. jejuni*, strain 81-176 showing multiple gaps between cells (*arrows*) with intact tight junctions. *Panels I, J* (*insert of Panel I*) and *K* demonstrate abnormal desmosomes with widened gaps in infected T84 monolayers (*arrowheads*), but normal tight junctions (*arrow in Panel I*). *Panels L* and *M*, invasive bacteria in infected T84 cells (*arrows*). *Panel N*, Desmosomes of infected T84 monolayers had double the width of uninfected monolayers. Thirteen and eleven desmosomes were measured for uninfected and infected monolayers, respectively (mean ± SEM; *t* test: *$p < 0.0001$). Measurement bar: 1 μ.

of infected T84 monolayers supported the findings observed by TER and dextran permeability. Relative to uninfected cells (Fig. 3*A–E*), *C. jejuni* infection disrupted intercellular spaces with the formation of larger gaps between adjacent T84 cells at 48 h (Fig. 3*F–K*), but not after 18 h infection (data not shown). Although the morphology of tight junctions remained unchanged (Fig. 3*F–I*), the intercellular portion of desmosomes was increased following infection, when compared with uninfected T84 monolayers (Fig. 3*I–K* and 3*D–E*, respectively; quantified in Fig. 3*N*). Invasive bacteria were detected in the cytosol of infected cells (Fig. 3*L* and *M*).

T84 cells are susceptible to* C. jejuni *invasion. To identify mechanisms responsible for the observed sensitivity of T84 monolayers to *Campylobacter*-induced barrier damage, the ability of *C. jejuni* to invade four epithelial cell lines was assessed. *C. jejuni*, strain 9011 was more invasive in T84 cells than in three other epithelial cell lines (Fig. 4), including both polarized (MDCK-I) and nonpolarized (HEp-2 and Intestine 407) cells, all previously used to assess microbial invasion (22,23). *C. jejuni*, strain 81-176 also showed increased invasion of T84 cells, whereas *C. jejuni*, strain 11168 demonstrated a trend for increased invasion of T84, relative to MDCK-I cells.

Epithelial cell invasion and bacterial translocation precede increased monolayer permeability. To determine a temporal connection between *C. jejuni* internalization and monolayer permeability, bacterial invasion, translocation across monolayers, and TER were assessed over 48 h in the same Transwells. Invasion and translocation of *C. jejuni*, strain 81-176 into T84 cells were apparent as of 1 and 3 h after infection, respectively, while TER remained at control levels until 48 h postinfection (Fig. 1). Taken together, these results indicate that *C. jejuni* first invades epithelial cells and then, similar to other enteropathogens (18), transcytoses across the monolayer without compromising intercellular junctions that are disrupted later during the infectious process.

C. jejuni were introduced directly into the basolateral compartment of Transwells (3 μ-pore size), to bypass the need for bacterial transcytosis via cellular invasion and translocation.

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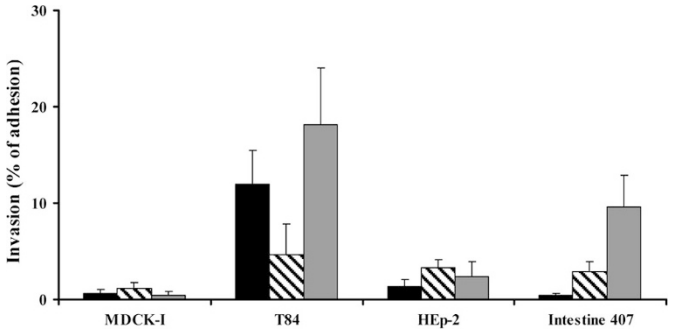

Figure 4. *C. jejuni* invasion into epithelial cells is strain- and cell type-specific. MDCK-I, T84, HEp-2, and Intestine 407 cells were grown to confluence in 24-mm wells and infected with prototype *C. jejuni* strains (MOI: 20:1; 4 h; 37°C) and then incubated with or without gentamicin (100 μg/mL; 2 h). Epithelial cells were then lysed in 0.1% Triton-X and plated in serial 10-fold dilutions. Invasion is presented as the percentage of bacterial adhesion. *C. jejuni*, strain 9011 (■) showed higher invasion efficiency into T84 cells (12 ± 3.5%) relative to the three other cell-lines (HEp-2: 1.4 ± 0.7%; MDCK-I: 0.6 ± 0.4%; Intestine 407: 0.4 ± 0.2%; $n = 4-5$; ANOVA: $p < 0.01$). *C. jejuni*, strain 81–167 (▤) also showed increased invasion into T84 cells (18.1 ± 5.9% *vs*. 0.4 ± 0.3%, in MDCK-I cells; $n = 4-7$; $p < 0.05$), and a trend for increased invasion of strain 11168 (▨) into T84 cells was demonstrated (4.7 ± 3.2% *vs*. 1.1 ± 0.6% in MDCK-I cells; $n = 4-5$; $p = 0.07$). In Intestine 407 cells *C. jejuni*, strain 81-176 was more invasive than strain 9011 ($n = 4-5$; *t* test: $p = 0.03$).

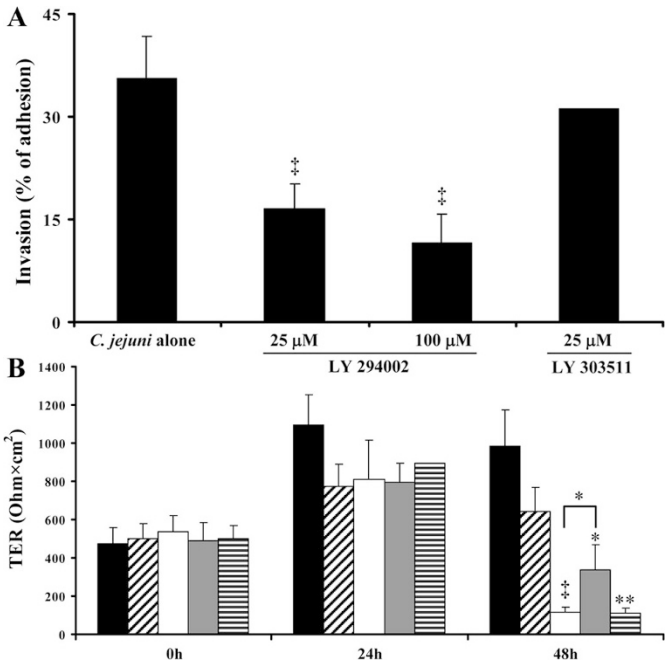

Figure 5. PI3-K inhibition decreases bacterial invasion and reduces *C. jejuni*-induced barrier disruption in T84 monolayers. T84 cells were pretreated with either the PI3-K inhibitor LY294002 or an inactive analog, LY303511 (1 h; 37°C) before infection with *C. jejuni*, strain 81-176. *Panel A*, Invasion into T84 epithelial cells, measured 4 h after infection, was inhibited by 53 ± 7.5% and 72 ± 3% with LY294002 pretreatment (25 and 100 μM, respectively), but not with the inactive analog (25 μM). *Panel B*, *C. jejuni*, strain 81-176, 48 h infection of T84 monolayers reduced TER without the PI3-K inhibitor (■) or with the inactive analog (▤) (11.3 ± 1.6% and 12.7 ± 2.4% of control, respectively), but to just 34.8 ± 10.9% of control values with the active inhibitor (▩). Uninfected (□); LY249002 alone (▨).

However, this approach did not enhance the drop in permeability (data not shown) because basolateral infection only shortened the process by the 3 h required for bacteria to translocate the monolayer, whereas TER is reduced later in the infectious process. Colchicine (5–50 μg/mL), reported to inhibit bacterial invasion in intestine 407 cells (8), did not reduce *C. jejuni* invasion of T84 cells (data not shown) and, therefore, was not useful for evaluating the association between invasion and permeability.

PI3-K inhibition reduces bacterial invasion and blocks the drop in TER in* C. jejuni-*infected T84 monolayers. Consistent with previous reports (24), there was reduced invasion of *C. jejuni*, strain 81-176 into T84 cells after pretreatment with the pharmacological inhibitor of PI3-K, LY294002. A higher concentration of the PI3-K inhibitor (100 μM) resulted in a larger reduction in bacterial invasion, whereas pretreatment with the inactive analog had no effect on microbial invasion (Fig. 5*A*). LY294002 pretreatment of polarized monolayers also attenuated the *C. jejuni*-induced drop in TER, thereby providing evidence that the effects of *C. jejuni* on epithelial barrier dysfunction were mediated by bacterial invasion. By contrast, pretreatment with the inactive analog had no effect on *C. jejuni*-induced drop in TER (Fig. 5*B*). Furthermore, the protective effect of LY294002 on *C. jejuni*-mediated barrier disruption may be underestimated, because LY294002 itself attenuated the increase in TER to 65.6 ± 16.6% of uninfected controls. The inability of the PI3-K inhibitor to completely protect against *C. jejuni*-mediated barrier disruption may be attributed to partial inhibition of bacterial invasion (Fig. 5*A*). Taken together, the findings indicate that *C. jejuni*-induced disruption of polarized monolayers is mediated by invasion of organisms into the cytoplasm of infected epithelial cells.

DISCUSSION

This study shows that *C. jejuni* infection of polarized epithelial monolayers induces cell-type specific alterations in polarized epithelial monolayers. The findings in the present study show that, similar to other intestinal pathogens (18,21), *C. jejuni* infection inflicts changes in epithelial barrier function, thereby leading to potential exposure of the subepithelial immune components to chronic antigenic stimulation. Such changes in epithelial permeability are well documented in humans with IBD (13), to which *C. jejuni* infection has been linked (2,5,6).

Other studies have shown increased permeability of human polarized intestinal cells (T84 and Caco-2) infected by *C. jejuni* (25–28). Similar to our findings, Chen *et al.* (25) observed a delayed effect of *C. jejuni* on T84 cells resulting in activation of innate immunity. In contrast, other studies using Caco-2 cells, which attain lower baseline TER, report a more variable drop in resistance (26,27). Such cell type-dependent variability prompted us to study the effects of *C. jejuni* infection in epithelial cells with different characteristics. We, therefore, used transformed human colonic T84 cells and canine kidney MDCK-I cells as model polarized epithelium, which both express mature apical junctional complex proteins and maintain cell polarity (29), and are used extensively to study host-microbial interactions (15,30).

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Lack of morphologic alterations of tight junctions of cells infected with *C. jejuni* is in keeping with previous observations regarding ZO-1 (25). Changes in desmosomes of infected T84 cells, observed by transmission electron microscopy, have not been previously described, and contrast the lack of effect of pathogenic *E. coli* and *Citrobacter rodentium* on desmosomes (31). Since desmosomes are involved in cell-cell contact and in anchoring the cytoskeleton, separation of desmosomes could contribute to epithelial barrier dysfunction. However, it remains unclear whether alterations in desmosomes observed in the present study are the reason for increased permeability (32). Further studies, focusing on the effects of bacterial infection on proteins involved in maintenance of desmosomes, such as desmoglein and desmoplakin, may provide additional insight.

Our findings clearly demonstrate increased vulnerability of human intestinal (T84) cells following *C. jejuni* challenge, relative to renal tubule-derived canine (MDCK-I) cells. The increased susceptibility of T84 cells was demonstrated by TER reduction and was also reflected by an increase in macromolecular permeability, relative to MDCK-I monolayers. There are several potential explanations for the differences observed. T84 cells produce large amounts of mucin, which act as a chemoattractant for *C. jejuni* (33). However, there was no increase in bacterial adherence to T84 cells (data not shown). Therefore, binding of *C. jejuni* to mucins is not likely to be responsible for the greater susceptibility of T84 cells to changes in barrier function. Another difference between the two polarized epithelial cell lines is in the expression of tight junction proteins. MDCK-I cells achieve high resistance, at least in part, due to the absence of claudin-2, which results in more 'leaky' junctions (34), whereas T84 cells express claudin-2 (35). Such differences could contribute to varying susceptibility to *C. jejuni*-induced barrier disruption. Interestingly, patients with IBD demonstrate a relative increase in the expression of claudin-2 and a decrease in other claudins, which correlates with increased gut permeability (36).

In addition to effects on permeability, the *C. jejuni* strains tested were also more effectively internalized into T84 cells than MDCK-I cells. Because the susceptibility of T84 cells to both barrier disruption and invasion by the three strains was comparable, the observed effects could represent a mechanism that is common to all *C. jejuni* strains. It is possible that bacterial invasion into polarized epithelial cells is dependent on the modification of junctional physiology and paracellular passage of the organism (37). However, if this were the case, a drop in TER might have been expected before bacterial invasion and translocation. In contrast, our findings indicate an opposite sequence of events, where bacteria enter into infected eukaryotic cells to induce changes in intercellular junctions by altering signal transduction pathways. A similar sequence of events has also been described for other invasive pathogens (38), but contrasts with *Helicobacter pylori* infection, which is reported to alter the physiology of apical junctional complexes without entering into the cytoplasm (39).

To determine whether effects on barrier function were dependent on bacterial internalization into the cytosol, invasion of *C. jejuni* was inhibited using a pharmacological inhibitor of PI3-K. As previously reported (24), this inhibitor reduced invasion of *C. jejuni*. The same inhibitor also prevents interferon-γ-induced barrier disruption, but not disruption by enteropathogenic *E. coli* (40), which argues against direct protection from barrier disruption induced by bacteria. The partial reduction in barrier disruption with inhibition of PI3-K suggests the involvement of additional mechanisms, such as displacement of occludin from lipid-rafts (25). Recent findings indicate that bacterial invasion is also reduced using inhibitors of Rho GTPases (41), which are involved in regulation of intercellular junctions (42).

Taken together, the results of the present study indicate that epithelial barrier disruption induced by *C. jejuni* infection depends on bacterial invasion. An improved understanding of the precise mechanisms leading to *C. jejuni*-induced diarrhea, intestinal injury, and chronic immune stimulation is essential for developing strategies to interrupt the infectious process and prevent its complications.

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