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Pathogenicity of the Diffusely Adhering Strain *Escherichia coli* C1845: F1845 Adhesin-Decay Accelerating Factor Interaction, Brush Border Microvillus Injury, and Actin Disassembly in Cultured Human Intestinal Epithelial Cells

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The diffusely adhering *Escherichia coli* strain C1845 harboring the fimbrial F1845 adhesin can infect cultured human intestinal epithelial cells. The mechanism by which *E. coli* C1845 induces diarrheal illness remains unknown. This study investigated the injuries of cultured human intestinal cells promoted by *E. coli* C1845. Membrane-associated decay accelerating factor was identified as the intestinal receptor for the F1845 fimbrial adhesin of the *E. coli* C1845 strain by using purified F1845 adhesin, antibody directed against the F1845 adhesin, and monoclonal antibodies directed against the decay accelerating factor. Using monolayers of Caco-2 cells apically infected with *E. coli* C1845 and examined by scanning and transmission electron microscopy, we observed that strain C1845 induced injury to microvilli (MV) characterized by elongation and nucleation of the MV. We observed that infection of T_{84} and Caco-2 cells by *E. coli* C1845 was followed by disassembly of the actin network in the apical and basal cell domains. MV injury was differentiation related: *E. coli* C1845 promoted MV injury only when the cells were fully differentiated. The disassembly of the actin network occurred in poorly differentiated and fully differentiated Caco-2 cells but not in undifferentiated cells. Moreover, apical actin disassembly was observed in fully differentiated Caco-2 cells infected with the laboratory strain *E. coli* HB101(pSSS1) expressing the F1845 adhesin. In conclusion, *E. coli* C1845 promotes MV lesion in human epithelial intestinal cells, resulting from disassembly of the actin network.

Escherichia coli diarrhea is an important cause of infantile morbidity and mortality in developing countries (45). The diffusely adhering *E. coli* (DAEC) strain C1845 (7, 8) attaches to cultured human intestinal cells (40) expressing binding sites for the fimbrial adhesive factor F1845 (38). Involvement of DAEC in diarrheal illness remains controversial. According to several researchers, DAEC strains are not associated with diarrheal disease (14) and are not significantly pathogenic for volunteers (71). In contrast, other investigators have found that DAEC strains are significantly associated with diarrheal disease (27, 33, 34). Yamamoto et al. (74, 75) have provided interesting novel information on the DAEC family by examining the adhesion of different pathogenic *E. coli* strains, such as enteropathogenic or related strains (type 1 and type 2), enteroaggregative strains (type 1 and type 2), and DAEC strains. They proposed a classification of DAEC into three groups, i.e., type 1, type 2, and type 3. The first and common characteristic of all these *E. coli* strains is the diffuse pattern of adhesion to HeLa and HEp-2 cells. The second characteristic is based on hybridization with DNA probes: DAEC types 1 and 2 are non-enteropathogenic *E. coli* (non-EPEC) serotypes and hybridize or do not hybridize, respectively, with the F1845 DNA probe (7). In contrast, DAEC type 3 does not hybridize with the F1845 DNA probe and expresses characteristics of class II EPEC (3, 4). Altogether, these results clearly indicate that in fact the DAEC group is a heterogeneous family of pathogenic *E. coli* strains producing diffuse adherence to eucaryotic cells.

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The present study focused on the infectious mechanisms of DAEC C1845. Studies of the development of bacterial intestinal pathogenicity require appropriate cellular models that closely mimic intestinal infection. Human colon adenocarcinoma cells (24), along with other adenocarcinoma cell lines, have retained the ability to differentiate in culture. These cell lines have been used by many groups to investigate a broad spectrum of intestinal and epithelial functions (47, 76). We and others have recently reported that these human cultured intestinal epithelial cells are useful tools with which to study in vitro attachment of enterovirulent *E. coli* to human enterocytic cells (15, 26, 37–40). To explore the cellular events occurring after interaction of DAEC C1845 with the intestinal brush border, we used a panel of cultured human intestinal cells.

MATERIALS AND METHODS

Cell lines and culture. The cultured human colonic adenocarcinoma Caco-2 cell line (24), which spontaneously differentiates in culture, was used (63). Caco-2 cells were routinely grown in Dulbecco modified Eagle's minimal essential medium (DMEM) (25 mM glucose) (Eurobio, Paris, France) supplemented with 20% fetal calf serum (Boehringer, Mannheim, Germany) and 1% nonessential amino acids. The cells were seeded in six-well tissue culture plates (Corning Glass Works, Corning, N.Y.) at a concentration of 10^5 cells per cm^2. For maintenance purposes, cells were passaged weekly by using 0.25% trypsin in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) containing 0.53 mM EDTA. The cells were maintained and all experiments were carried out at 37°C in a 10% CO_2–90% air atmosphere. Differentiated cells were used for adherence assays at late postconfluence, i.e., 15 days in culture. To study bacterial adherence and appearance of injury of microvilli (MV) with respect to time course in culture and thus cellular differentiation, Caco-2 cells were used between postconfluence (5 days), when differentiation starts, and late postconfluence (15 days), when differentiation is completed.

Human intestinal T_{84} cells (16, 49) were routinely grown in a 50-50 mixture of DMEM and Ham's F-12 medium supplemented with 2 mM glutamine, 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 1% nonessen-

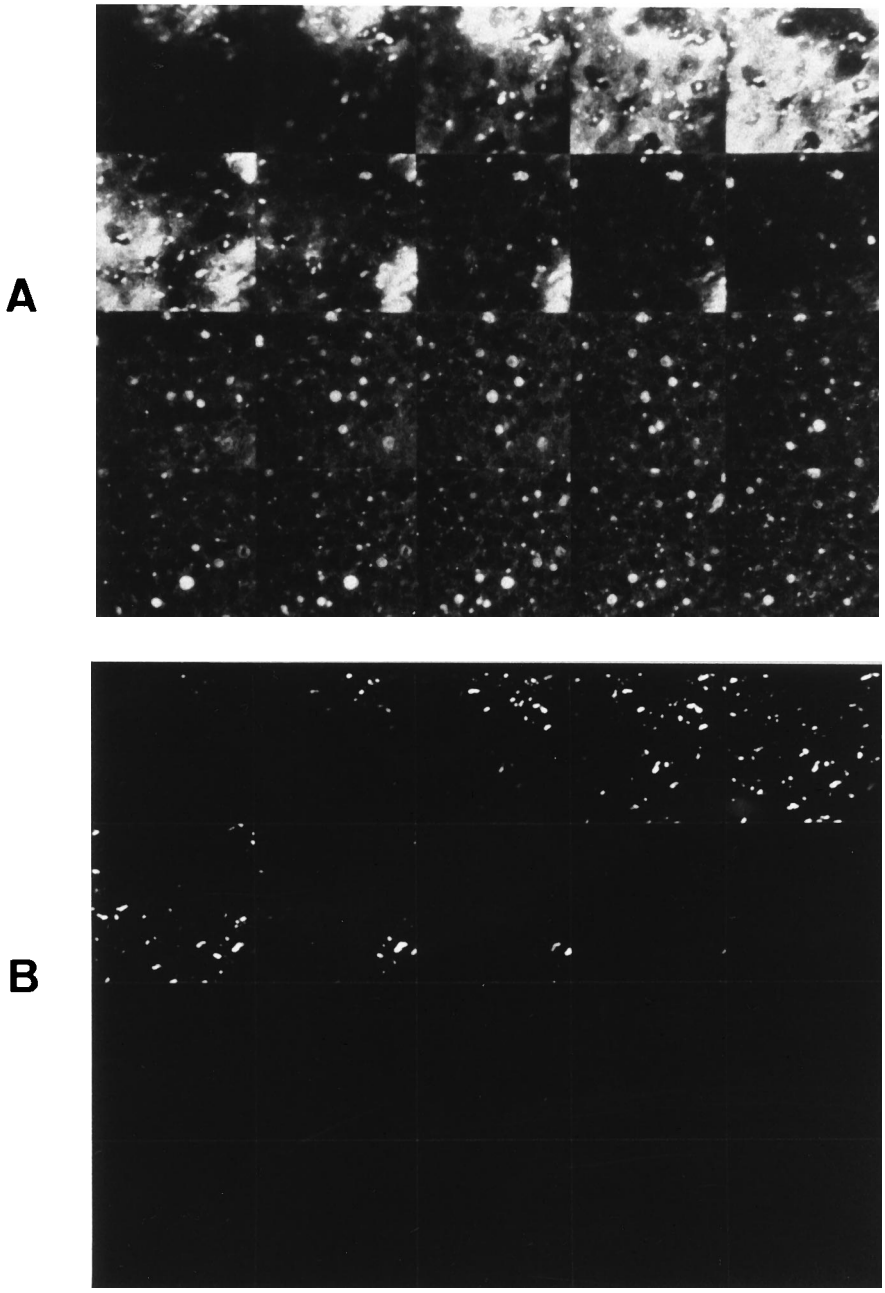

FIG. 1. Apical colocalization of *E. coli* C1845 and DAF in infected differentiated Caco-2 cells. Confocal micrographs (*x-y* orientation) are shown. (A) Localization of DAF, revealed by using anti-DAF MAb CY-DAF and fluorescein-labeled secondary antibody. Note the intense apical DAF immunoreactivity. Moreover, DAF-positive vesicles appeared intracellularly localized, suggesting that DAF could be sequestered in these vesicles for its transport to the apical domain. (B) C1845 bacteria, revealed by indirect immunofluorescence using rabbit anti-F1845 polyclonal antibody and rhodamine-labeled secondary antibody. Note that the bacteria are apically localized and colocalize with the apical DAF immunoreactivity observed in panel A.

tial amino acids, and 10% inactivated (30 min, 56°C) fetal calf serum (Boehringer) at 37°C in a 10% CO_2–90% air atmosphere. Cells were seeded at a concentration of $3 \times 10^5/cm^2$. Cells were used for adherence assays at late postconfluence, i.e., after 10 days.

Antibodies. Mouse monoclonal antibody (MAb) CY-DAF raised against human membrane-associated decay accelerating factor (DAF) was from Valbiotech (France). Ascites fluid containing antibody IF7 against DAF was from J. M. Bergelson (Dana-Farber Cancer Institute, Harvard Medical School, Boston, Mass.). MAb IA10 against DAF was from V. Nussenzweig (New York University Medical Center). Ascites fluid containing antibody HBB 2/614/88 against human sucrase-isomaltase (SI) was a gift from H. P. Hauri (Biocenter of the University of Basel, Basel, Switzerland). Ascites fluid containing antibody 4H3 against human dipeptidylpeptidase IV (DPP IV) was obtained from S. Maroux (Centre de Biochimie et de Biologie Moléculaire, Marseille, France). Polyclonal rabbit anti-F1845 antibody was obtained from S. S. Bilge (Washington University, Seattle) (8).

Bacterial strains and growth conditions. DAEC C1845 harboring the fimbrial F1845 adhesin (8) was stored in colonization factor antigen (CFA)-glycerin at −80°C. The strain was grown on CFA agar containing 1% Casamino Acids (Difco Laboratories, Detroit, Mich.), 0.15% yeast extract, 0.005% magnesium sulfate, and 0.0005% manganese chloride in 2% agar for 18 h at 37°C. The *E. coli* laboratory strain HB101 transformed with the pSSS1 plasmid producing F1845 adhesin (8) was grown at 37°C for 18 h on Luria agar.

Cell infection. A quantitative assay of binding of *E. coli* C1845 to Caco-2 cells was conducted with metabolically labeled bacteria. The *E. coli* was radiolabeled by the addition of [^{14}C]acetic acid (Amersham) (94 mCi/mmol; 100 μCi/10-ml

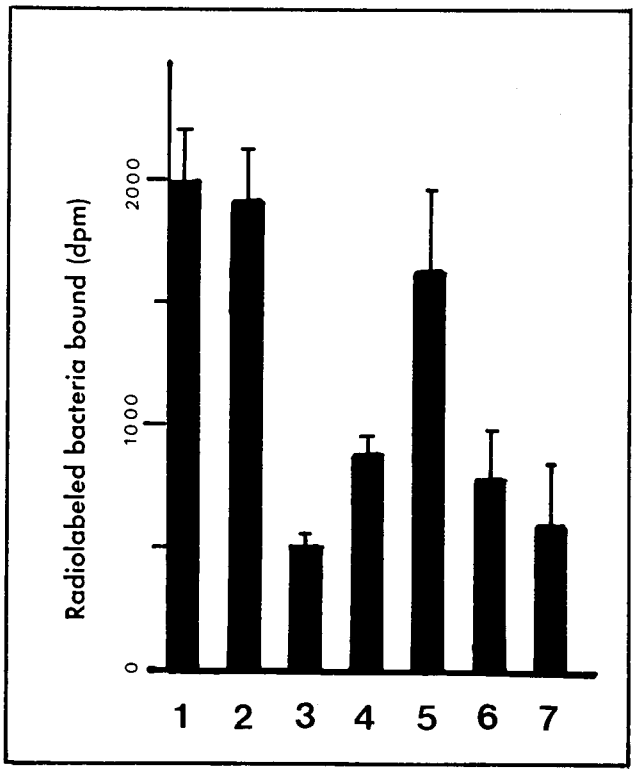

FIG. 2. DAF acts as a receptor for *E. coli* C1845 in cultured human intestinal Caco-2 cells. Lane 1, adhesion of *E. coli* C1845; lane 2, no inhibition of adhesion in the presence of chloramphenicol (20 μg/ml); lanes 3 and 4, inhibition of adhesion by anti-DAF MAbs CY-DAF and IF7, respectively; lane 5, no inhibition with anti-DAF MAb IA10; lane 6, inhibition of adhesion after preincubation of the cells with purified F1845 adhesive factor; and lane 7, inhibition of adhesion with polyclonal anti-F1845 antibody.

tube) in CFA broth, as previously reported (40). The cell monolayers were infected with radiolabeled bacteria (10^8 CFU/ml; 50,000 to 70,000 cpm) and incubated at 37°C in 10% CO_2–90% air for 3 h. The monolayers were then washed three times with sterile PBS. Adhering bacteria and intestinal cells were dissolved in a 0.2 N NaOH solution. The level of bacterial adhesion was evaluated by liquid scintillation counting. Each adherence assay was conducted in duplicate with three successive cell passages. Inhibition of adhesion was determined by using purified F1845 adhesin (500 μg/ml), rabbit polyclonal antibody directed against purified F1845 fimbrial adhesin (diluted 1:20 in PBS) (8), chloramphenicol (100 μg/ml), and anti-DAF MAbs CY-DAF, IF7, and IA10 (all diluted 1:20 in PBS).

Transmission and scanning electron microscopy. After the bacterial adhesion assay, the cells were fixed and treated. For scanning electron microscopy, cells were fixed with 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 1 h at room temperature, washed with phosphate buffer, postfixed for 30 min with 2% (wt/vol) OsO_4 in the same buffer, washed three times with the same buffer, and dehydrated in a graded series (30 to 100%) of ethanol. The cells were dried in a critical-point dryer (Balzers CPD030) and coated with gold. For transmission electron microscopy, samples embedded in Epon were reembedded in order to make sections perpendicular to the bottom of the flask. The specimens were then examined with a JEOL electron microscope.

Immunofluorescence. Monolayers of cells were prepared on glass coverslips which were placed in six-well tissue culture plates (Corning Glass Works). Adhering *E. coli* C1845 cells, differentiation-associated markers, and DAF were revealed by indirect immunofluorescence labeling of unpermeabilized cell layers as previously described (38, 39). Preparations were fixed for 10 min at room temperature in 3.5% paraformaldehyde in PBS. Cell monolayers were incubated with specific primary antibody for 45 min at room temperature, washed, and then incubated with the respective fluorescein-conjugated secondary antibody. Primary antibodies were diluted 1:20 to 1:100 in PBS (rabbit polyclonal anti-F1845 serum, 1:20; CY-DAF, 1:50; anti-SI antibody HBB 2/614/88, 1:200; and anti-DPP IV antibody 4H3, 1:50) in 2% bovine serum albumin–PBS. Secondary antibodies were either fluorescein- or rhodamine-conjugated goat anti-mouse immunoglobulin G from Immunotech (Luminy, France) or fluorescein-conjugated goat anti-rabbit immunoglobulin G from Institut Pasteur Productions (Paris, France), used at a dilution of 1:20 in 2% bovine serum albumin–PBS. No fluorescent staining was observed when nonimmune serum was used and when the primary antibody was omitted.

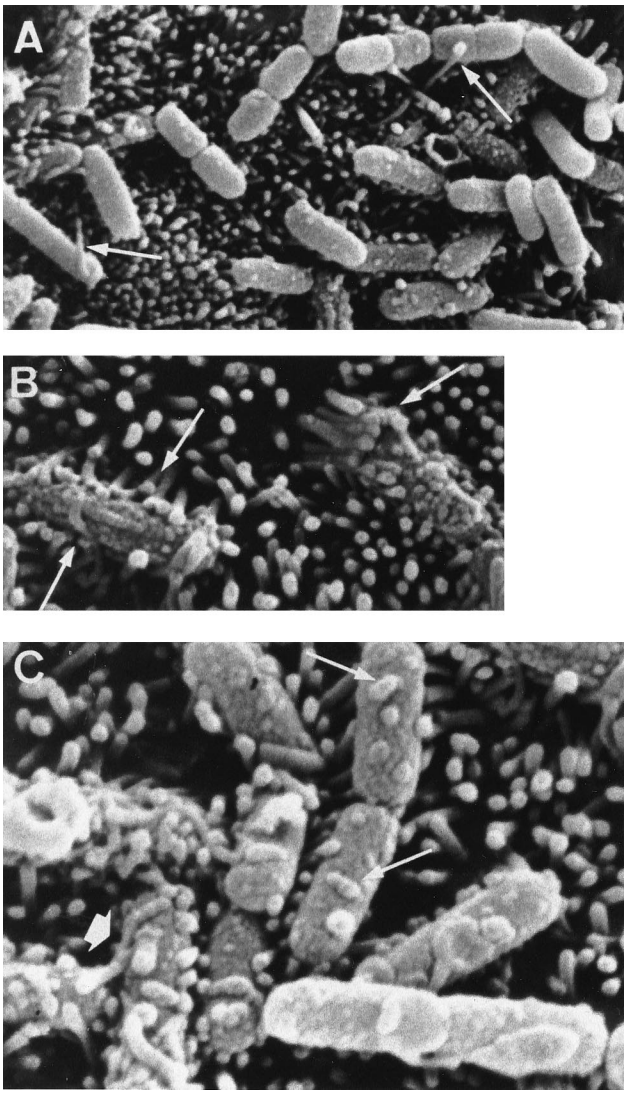

FIG. 3. Scanning electron micrographs of human enterocytic Caco-2 cells infected apically by *E. coli* C1845. Low and high magnifications of fully differentiated HT-29 $glc^{-/+}$ cells infected apically with *E. coli* C1845 show interaction of bacteria with the well-organized MV at different times of adhesion. (A) After 1 h of adhesion, bacterium-associated MV are elongated (arrows). (B) After 3 h of adhesion, bacteria are entrapped with numerous elongated MV (arrows). (C) High magnification shows detached tips of MV (fine arrows) and elongated MV (large arrow) attaching to the cell surface of adhering bacteria.

When apical and basolateral F-actin in polarized intestinal cells was to be visualized, coverslips were incubated with 0.2% Triton X-100 in PBS for 4 min before incubation with fluorescein-phalloidin (Molecular Probes, Junction City, Oreg.) for 45 min at 22°C, and the coverslips were then rewashed three times with PBS.

Specimens were examined by epifluorescence and by interference contrast microscopy using a Leitz Aristoplan microscope with epifluorescence. Images of some of the monolayers were also obtained by confocal laser scanning microscopy using a Bio-Rad MRC 1000 confocal system mounted on a Diaphot II Nikon microscope. Beginning at the apical membrane of the monolayers, optical sections were obtained every 1.6 μm. All photographs were taken with Kodak T-MAX 400 black and white film (Eastman Kodak Co., Rochester, N.Y.).

RESULTS

DAF acts as receptor for *E. coli* C1845 in human cultured intestinal Caco-2 cells. *E. coli* bearing F1845 fimbriae develops a system of adhesion to erythrocytes mediated by the Dr blood

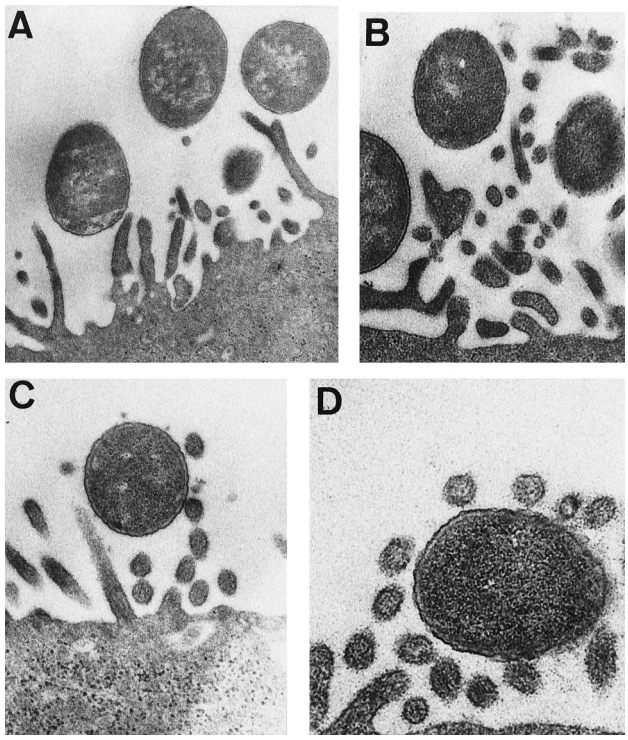

FIG. 4. Transmission electron micrographs of human enterocytic Caco-2 cells infected apically by *E. coli* C1845. (A) Association of a bacterium with multiple elongated MV; (B) disorganized MV around adhering bacteria; (C) elongated MV and vesiculated MV appear around adhering bacteria; (D) adhering bacteria associated with numerous vesiculated MV separated from the epithelial cell surface.

group antigen (57, 58). Nowicki et al. (56) have demonstrated that the Dr receptor is a part of the DAF (DAF-CD55) (55) by using CHO cells transfected with DAF cDNA and MAbs against DAF. We conducted experiments to determine the nature of the intestinal receptor involved in the adhesion of *E. coli* C1845 to Caco-2 cells (Fig. 1 and 2).

The localization of the DAEC C1845 bacteria and the DAF in infected Caco-2 cells was determined by indirect immunofluorescence followed by scanning laser confocal microscopy (Fig. 1). Labeling of DAF was done with MAb CY-DAF, and the adhering C1845 bacteria were revealed by using the rabbit polyclonal anti-F1845 antibody. DAF was localized at the apical domain of the Caco-2 cells, since intensive DAF immunoreactivity was observed at this domain. This intensive reactivity disappeared in intracellular sections, although several intracellular vesicles appear to contain DAF (Fig. 1A). The C1845 bacteria showed striking localization at the apical domain (Fig. 1B), and their binding colocalized with the apical DAF immunoreactivity.

Adhesion of *E. coli* C1845 to its intestinal receptor was investigated by using a quantitative bacterial adhesion inhibition assay with metabolically radiolabeled *E. coli* C1845, purified F1845 adhesin, polyclonal antibody directed against F1845 adhesin, chloramphenicol, and MAbs directed against DAF (Fig. 2). After incubation of the radiolabeled C1845 bacteria with the anti-F1845 antibody or after preincubation of the cell monolayer with the purified F1845 adhesin, the total binding of bacteria to Caco-2 cells was inhibited in a highly significant manner. In contrast, no inhibition was observed with chloramphenicol. We further examined the role of DAF in the adhesion of *E. coli* C1845 to Caco-2 cells. We conducted adhesion inhibition experiments with the anti-DAF MAbs CY-DAF, IF7, and IA10, previously used to identify DAF as a receptor for echovirus (5). Before the bacterial adhesion assay, the cell monolayers were preincubated for 1 h at 37°C with each anti-DAF antibody and then incubated with radiolabeled *E. coli* C1845. Anti-DAF MAbs CY-DAF and IF7 induced significant inhibition of the F1845-specific attachment of *E. coli* C1845 to Caco-2 cells. In contrast, no inhibition was obtained with anti-DAF MAb IA10, although this MAb recognizes a 70-kDa human DAF molecule as determined by Western blot (immunoblot) analysis (41). An identical negative result with MAb IA10 was reported for echovirus, which recognizes DAF as a receptor (5), suggesting that for both *E. coli* C1845 and echovirus, this MAb may recognize an epitope distant from their binding sites.

MV injury after attachment of *E. coli* C1845 to the brush border of cultured human intestinal Caco-2 cells. At late postconfluence, cultured intestinal epithelial Caco-2 cells express a typical well-organized intestinal brush border with highly ordered and dense MV, which carpet the apical surface. Observation by scanning electron microscopy of the Caco-2 cells infected with clinical *E. coli* isolate C1845 showed that after 1 h of adhesion, elongated MV appeared to rapidly associate with the adhering bacteria (Fig. 3A). After 3 h, the bacteria were trapped by numerous elongated MV at the sites of attachment (Fig. 3B). Under high magnification, the material associated with the adhering bacteria seemed to be the tips of the MV (Fig. 3C).

In order to determine whether the cellular material associated with the bacteria was effectively separated from the intestinal brush border, transmission electron microscopy was performed (Fig. 4). Contacts between bacteria and elongated MV were observed (Fig. 4A), and disorganization of the MV occurred (Fig. 4B). Concomitantly, vesiculated MV appeared around adhering bacteria (Fig. 4C), and the bacteria were entirely covered by numerous vesiculated MV separated from the epithelial cell surface (Fig. 4D).

The *E. coli* C1845-associated material was further identified by indirect immunofluorescence labeling. The MV of the Caco-2 brush border express the differentiation-associated hydrolase DPP IV (67). If separated MV remain associated with the adhering *E. coli* C1845 cells, they can be revealed by immunofluorescence labeling of the MV-associated DPP IV. After adhesion assay and washing to remove nonadhering bacteria, the C1845 bacteria adhering to Caco-2 cells were collected by lysing the infected-cell monolayer with H_2O. The recovered adhering bacteria were placed on a glass coverslip, and indirect immunofluorescence labeling of DPP IV was conducted (Fig. 5). A significant number of the recovered adhering C1845 bacteria appeared positively stained with DPP IV immunofluorescence, indicating that the *E. coli* C1845 cell-associated material is of MV origin. As a control, the same experiment was conducted with enterotoxigenic *E. coli* CFA/I, and no DPP IV immunoreactivity was observed for the removed adherent enterotoxigenic *E. coli* cells (not shown).

***E. coli* C1845 promotes alteration of the actin network in polarized epithelial intestinal T_{84} cells.** *E. coli* C1845 can colonize colonic cells (6). Cultured human intestinal epithelial T_{84} cells (16, 49) are considered a model of colonic crypt cells (2). We have previously reported that T_{84} cells can be apically infected by the DAEC C1845 strain, since they express DAF at their apical surface (6). The F-actin network of T_{84} cells has been well characterized (31, 48, 51, 68). Labeled by fluorescein-phalloidin, the control T_{84} cells revealed localization of F-actin at the apical cell surface and at the basolateral domain (Fig. 6A to C). The fine, flocculated actin centrally located

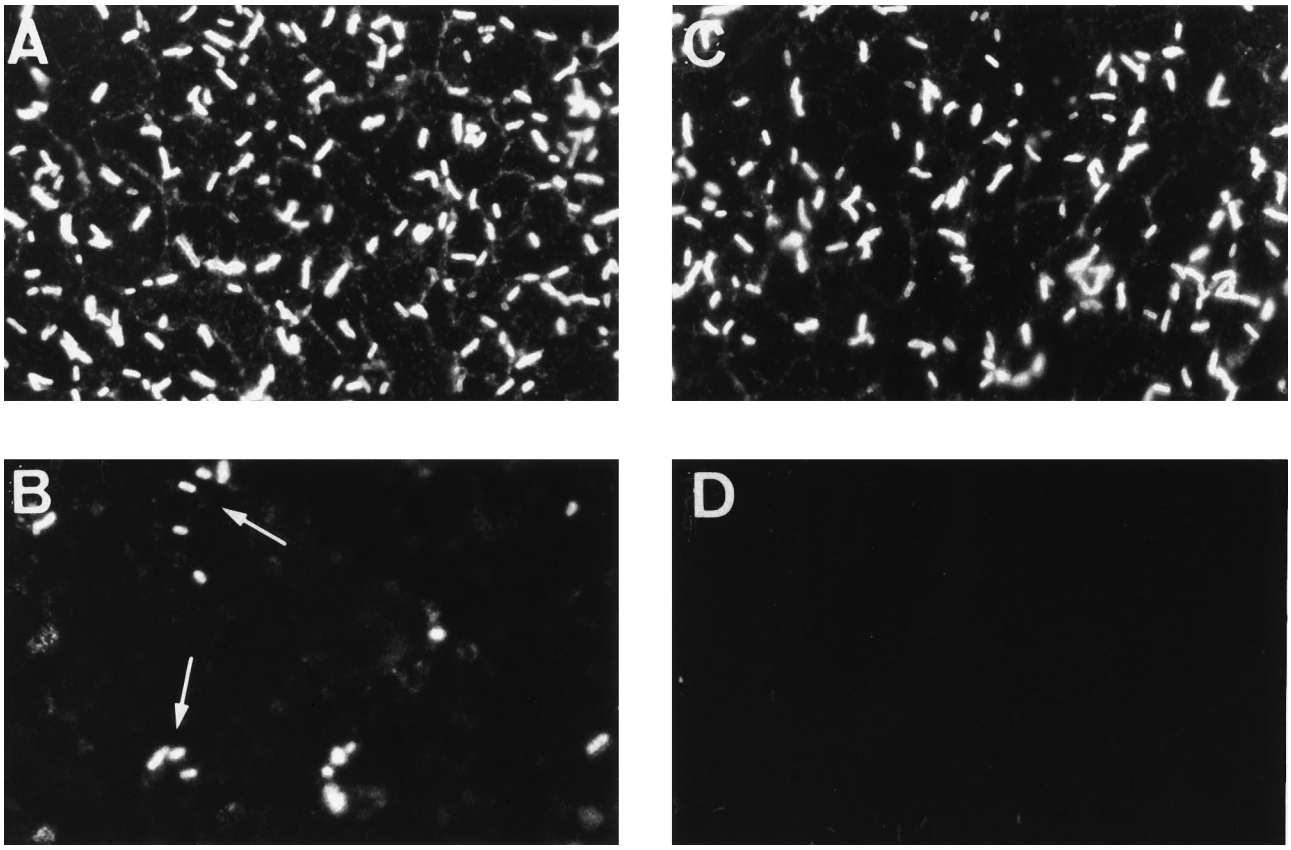

FIG. 5. Revelation by indirect immunofluorescence that the adhering *E. coli* C1845 cells recovered from the HT-29 $glc^{-/+}$ cells were labeled with DPP IV, a brush border-associated hydrolase. DAEC C1845 revealed with the polyclonal rabbit anti-F1845 antibody (7, 38) in an HT-29 $glc^{-/+}$ cell monolayer (A) and in control bacteria placed on a glass coverslip (C) and revelation of positive DPP IV immunofluorescence in C1845 cells recovered from the HT-29 $glc^{-/+}$ cells and placed on a glass coverslip (B) are shown. Note the absence of DPP IV immunoreactivity in the control *E. coli* C1845 placed on a glass coverslip (D).

in the cells represents MV-associated F-actin (Fig. 6B). The continuous beaded pattern at cell-to-cell contacts represents F-actin underlying the apical membrane. The randomly dispersed pattern of F-actin constitutes the basolateral cytoskeletal cortex (Fig. 6C).

Observation of the infected cells by interferential phase-contrast microscopy revealed that alteration of the cell surface occurred at 3 h postinfection (Fig. 6G) and that considerable cell rounding of some cells, to which bacteria adhered at 6 h postinfection (Fig. 6J), occurred. We examined the actin network of the T_{84} cells after infection with the DAEC C1845 strain. No change in the fine, flocculated apical F-actin was observed in the C1845-infected T_{84} cells at 1 h postinfection, whereas the perijunctional ring of actin appeared more marked (Fig. 6E). In contrast, at 3 h postinfection, the fine apical F-actin was replaced by the displacement of F-actin to the periphery of cells, resulting in central lucent zones (Fig. 6H). At 6 h postinfection, the apical F-actin alterations were accentuated and the perijunctional ring of actin appeared highly densified (Fig. 6K). Alteration of the basolateral cytoskeletal F-actin was also observed in infected cells. The finely dispersed pattern was not altered at 1 h postinfection (Fig. 6F); alteration commenced at 3 h postinfection, with the appearance of central clearing zones (Fig. 6I). At 6 h postinfection, the finely dispersed array of homogeneous F-actin filament disappeared and the size of the central clearing zones increased (Fig. 6L). In a blind review, 62 randomly obtained micrographs (16 micrographs for 1 h postinfection, 22 micrographs for 3 h postinfection, and 24 micrographs for 6 h postinfection) from 12 monolayers resulting from three experiments were examined. A total of (5 ± 1)%, (72 ± 7)%, and (90 ± 12)% of the *E. coli* C1845-infected cells after 1, 3, and 6 h postinfection, respectively, presented alteration of both apical and basolateral F-actin distribution.

Development of MV injury and F-actin disassembly as a function of intestinal epithelial cell differentiation. In the intestine, enterocytes differentiate along the crypt-villus axis (47). We took advantage of the Caco-2 cell subpopulation (24), which spontaneously differentiates in culture (63) and provides the best tool with which to study intestinal brush border assembly (60–62) and intestinal cell functions (76), to study the role of cellular differentiation in the development of MV injury and F-actin disassembly after C1845 cell infection. We have previously provided evidence that the DAEC strain C1845 can infect both undifferentiated and differentiated cultured human intestinal cells to the same degree (37, 38). This correlates well with the fact that DAF, which acts as a receptor for DAEC C1845, is perpetually expressed in Caco-2 cells (6).

The state of differentiation was measured by indirect immunofluorescence labeling of the brush border-associated hydrolase SI (67) (Fig. 7D to F). No immunodetectable SI was observed at day 5 (Fig. 7D). SI was distributed in a random fashion in occasional cells at day 10 (Fig. 7E). At day 15 (Fig. 7F), SI was detected more consistently and the mosaic pattern indicated a variable expression from cell to cell. Changes in MV distribution and density occurred in Caco-2 cells as a

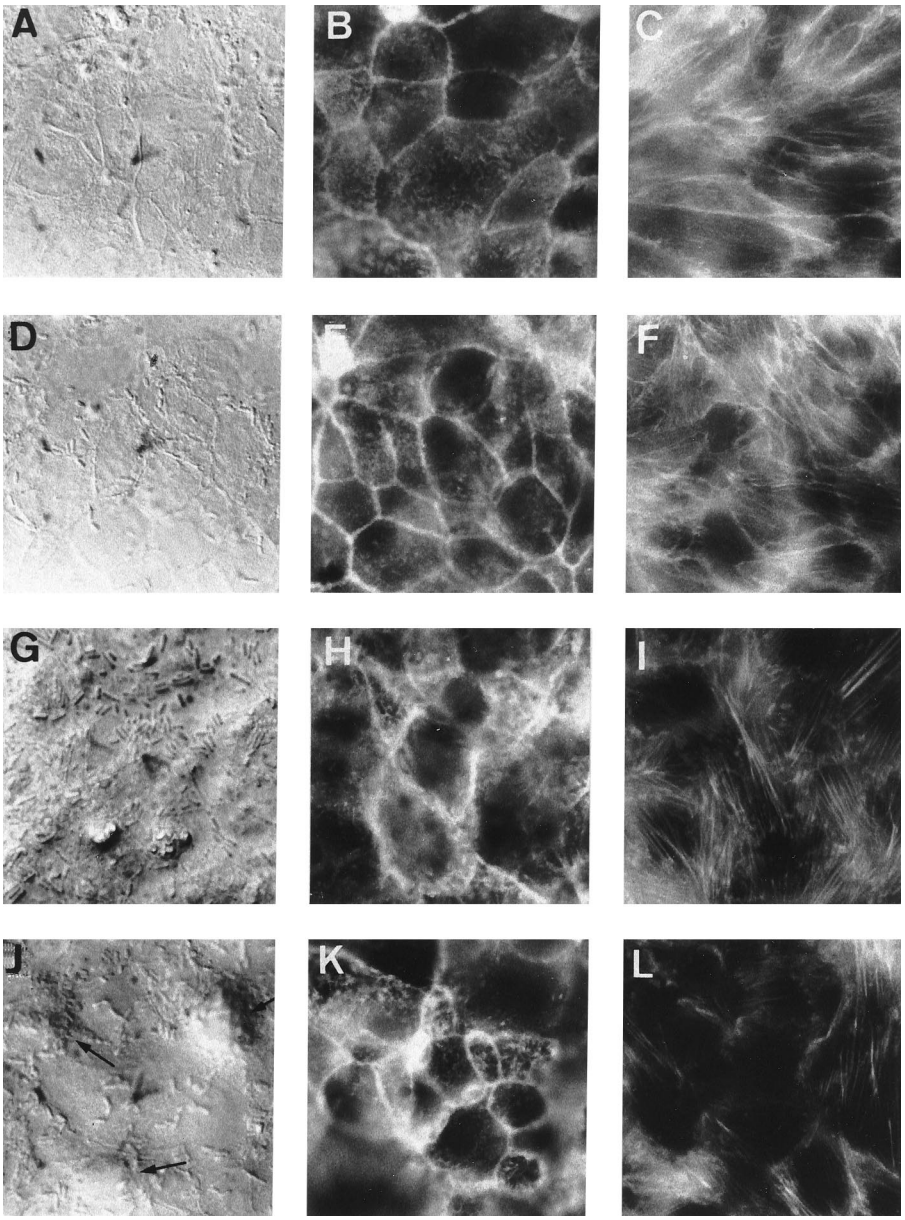

FIG. 6. Fluorescent localization of F-actin labeled with fluorescein-phalloidin in control T_{84} monolayers and in infected cells by DAEC C1845. Interference contrast micrographs show morphology of the cells (left panels) and F-actin in different optical planes of focus (central and right panels). In the central panels, the plane of focus is at the apical membrane and the perijunctional ring of actin. In the right panels, the plane of focus is at the basal pole of T_{84} cells. In control cells (A to C), the fine, flocculated actin centrally located in the cells in panel B represents MV-associated actin and the perijunctional ring of actin underlying the apical membrane. In panel C, the randomly dispersed pattern of F-actin represents the actin fibers composing the basolateral cytoskeletal cortex. The time course of the T_{84} cell infection by *E. coli* C1845 is shown (D to F, 1 h postinfection; G to I, 3 h postinfection; and J to L, 6 h postinfection). The distribution of F-actin in the apical and basolateral poles of the cells evolves during the time course of infection. In the apical pole, a densification of the perijunctional ring occurs, whereas the fine, flocculated actin in the centers of the cells is replaced by central clearing zones. The actin fibers composing the basolateral cytoskeletal cortex undergo changes characterized by the appearance of central clearing zones which increase in size during the course of infection. Note the morphological changes in infected cells characterized by undulations of the cell monolayer after 3 h postinfection (G) and cell rounding (arrows) after 6 h postinfection (J).

function of the number of days in culture. As observed by scanning electron microscopy, a striking change in the appearance of MV occurred. By day 5, occasional MV were present on the cell surface. Small cylindrical MV were present at day 10, and MV density increased further until day 15.

We examined the interaction of *E. coli* C1845 with Caco-2 MV as a function of cell differentiation by scanning electron microscopy (Fig. 7A to C). The C1845 bacteria appeared closely associated with MV, even when the cell surface expressed scattered MV (Fig. 7A and B). The bacteria associated with MV in fully differentiated Caco-2 cells at day 15 in culture (Fig. 7C) appeared covered by MV, and the MV appeared altered around adhering bacteria.

Disassembly of the Caco-2 actin network by DAEC C1845 was examined in Caco-2 cells as a function of cell differentiation (Fig. 8). In undifferentiated Caco-2 cells infected by *E. coli* C1845, no alteration of the actin network was observed (Fig. 8D). In contrast, the network of actin fibers in the recently

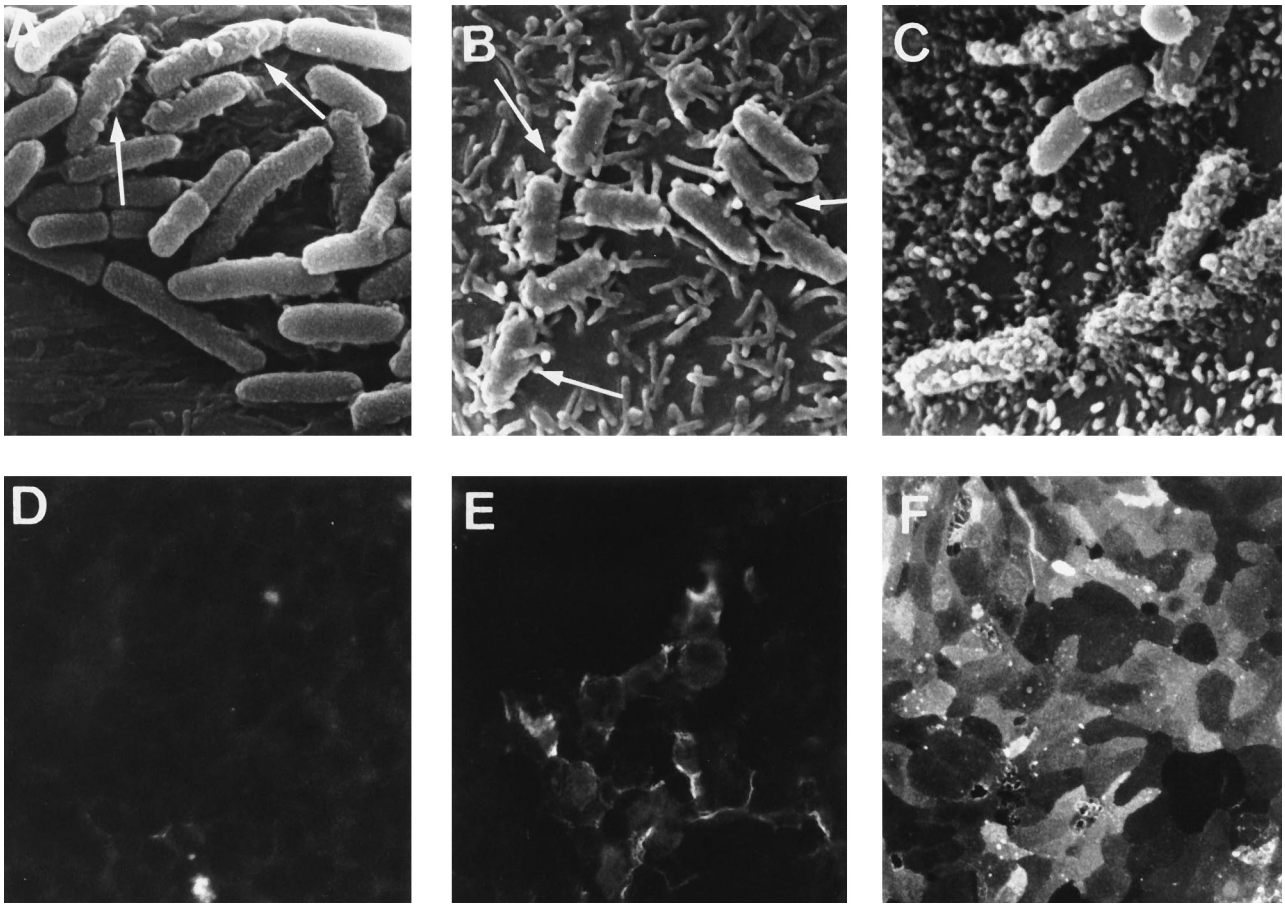

FIG. 7. Development of the MV lesion in human intestinal Caco-2 cells infected by *E. coli* C1845 as a function of cell differentiation. A series of high-magnification scanning electron micrographs of confluent Caco-2 cells at days 5 (A and D), 10 (B and E), and 15 (C and F) is shown. Expression of SI in unpermeabilized Caco-2 cells develops as a function of the number of days in culture. No immunodetectable SI is seen in confluent undifferentiated cells at day 5 (D). A faint, fuzzy apical SI staining is observed at day 10, at the beginning of the cell differentiation process (E). SI expression increases by day 15 (F), with the mosaic pattern characteristic of fully differentiated intestinal cells apparent. MV density steadily increases between days 5 (A), 10 (B), and 15 (C) in culture. *E. coli* C1845 adheres to undifferentiated (A), recently differentiated (B), and fully differentiated (C) cells. In panel A, the cell surface shows no organized MV embedded in the cell surface to which bacteria adhere (arrows). In panel B, the bacteria appear closely associated with the sparsely distributed MV (arrows). In panel C, adhering bacteria appear entirely covered by the MV material. MV around adhering bacteria appear shortened, and areas of cells are devoid of MV. The bacteria adhering to the undifferentiated (A) and recently differentiated (B) cells appear free of the bacterium-associated MV material.

differentiated infected cells (day 7) was dramatically altered, showing the appearance of a central clearing and disorganization of the perijunctional ring of actin (Fig. 8E). In the fully differentiated infected cells (day 15), peripherally relocated F-actin was observed with disruption of the actin fibers centrally located in the cells (Fig. 8F). As quantified in a blind review of randomly obtained micrographs (8 and 10 micrographs at 7 and 15 days in culture, respectively, from three experiments), (56 ± 10)% of the cells at 7 days in culture and (91 ± 12)% of the cells at 15 days in culture presented alteration of the basolateral actin network.

F1845 adhesin-DAF interaction in Caco-2 cells is followed by apical actin disassembly. To determine if the F-actin disassembly could be triggered directly by the F1845 adhesin-DAF interaction, we conducted additional experiments. For this purpose, we infected Caco-2 cells with the laboratory strain *E. coli* HB101(pSSS1) carrying the recombinant plasmid encoding the fimbrial F1845 adhesin (7, 8). Binding of this *E. coli* strain to Caco-2 cells was inhibited by anti-DAF MAb CY-DAF (data not shown). Apical F-actin distribution in the infected Caco-2 cells was examined (Fig. 9). The apical F-actin distribution represented by fine, flocculated MV-associated actin in control cells (Fig. 9A) was dramatically altered, showing relocalization of F-actin at the perijunctional ring with central clearing in infected cells (Fig. 9B).

DISCUSSION

In erythrocytes (57, 58) and in CHO-DAF$^+$ cells (56), *E. coli* C1845 bearing the F1845 fimbrial adhesin (7, 8) develops a system of adhesion which recognizes DAF as a receptor. We demonstrated that *E. coli* C1845 recognizes brush border-associated DAF as a receptor in the fully differentiated Caco-2 cells, since its binding was displaced by MAbs directed against DAF. We have recently reported that the expression of DAF is unrelated to the differentiation of the Caco-2 cells (6). This correlates well with previous observations that the F1845 binding site is expressed by both undifferentiated and differentiated cultured human intestinal cells (38) and that *E. coli* C1845 strongly binds to undifferentiated and differentiated cells (40).

We report here that the DAEC C1845 strain promotes MV injury after its close attachment to the brush border of cultured human intestinal epithelial cells. Intestinal brush border lesions are elicited by several enterovirulent microorganisms.

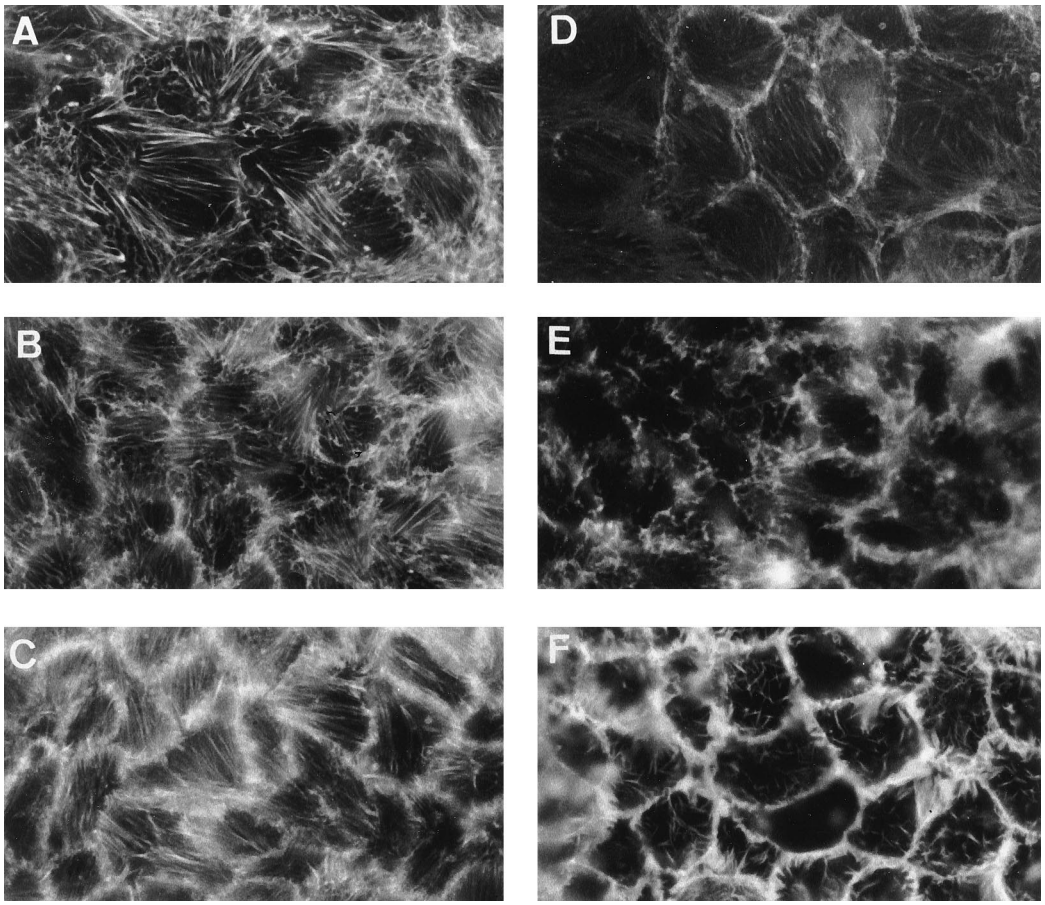

FIG. 8. Fluorescent localization of F-actin in Caco-2 cells as a function of cell differentiation, viewed en face, in control monolayers (left panels) and monolayers infected with *E. coli* C1845 (right panels). Confluent cells at 5 days in culture (A and D) and postconfluent cells at 7 (B and E) and at 15 (C and F) days in culture are shown. During the time course in culture of control Caco-2 cells, F-actin distribution undergoes a series of changes. In panel A, the large undifferentiated cells show dispersed random fibers of F-actin. In panel B, the size of the cells decreases, whereas the F-actin network densifies. In panel C, the fine organized pattern of random F-actin fibers is characteristic of differentiated epithelial intestinal cells. In infected undifferentiated confluent cells, no change in F-actin distribution is observed (D). In contrast, the F-actin distribution in the postconfluent (7 days in culture) infected cells appears dramatically altered. The perijunctional ring of actin is transiently disorganized, with disappearance of the actin fibers (E). In postconfluent cells at 15 days in culture, the randomly dispersed pattern of F-actin is totally disorganized, with fragmentation of the actin fiber in the centers of the cells apparent (F).

Enteroinvasive bacteria, such as *Salmonella* (32), *Yersinia* (32), *Shigella* (28), and *Listeria* (13) spp., also target the cytoskeleton proteins to develop their pathogenicity. For most of these pathogens, the cytoskeletal rearrangements are triggered by the stimulation of a signal transduction cascade (22). The attaching-effacing lesion elicited by EPEC (36, 43, 52) results from a cascade of cellular events (17) involving mobilization of actin (42) and stimulation of the inositol 1,4,5-triphosphate (IP_3) pathway (1, 19, 25, 65). In parallel, vesiculation of the MV after interaction of EPEC bacteria with the intestinal brush border has been reported (21). Several DAEC strains, such as the D2 strain, promote elongation of MV and elicit actin mobilization (75). In contrast, Dytoc et al. (20) recently demonstrated that *Helicobacter pylori* promotes the attaching-effacing lesion without actin mobilization and with an increase of IP_3. We have not observed the characteristic EPEC attaching-effacing lesion after attachment of *E. coli* C1845 to the intestinal brush border. We observed (i) multiple contacts between bacteria and numerous MV, (ii) elongation of the MV, and (iii) vesiculation of the MV tips, which were separated from the cells and which remained attached to the bacteria. Intestinal brush border MV lesions without bacterial infection have been also described. Matsudaira and Burgess (50) observed solation of core cytoskeletal components, vesiculation of the apical plasma membrane, and dramatic shortening of MV in brush border incubated in calcium. This could result from an increase in the cytoplasmic free Ca^{2+}, which would activate villin to depolymerize the actin core in the MV (10). Moreover, lectin interaction with the intestinal brush border induces MV injury, such as vesiculation and shortening of the MV (30, 73). In Caco-2 cells exposed to soybean agglutinin, reorganization of membrane and core actin filaments without changes in intracellular actin concentration and with shifts in the G-actin/F-actin ratio have been observed (18).

The mechanism by which *E. coli* C1845 induced the MV lesion remains unknown. The fact that C1845 interacted with DAF suggests that the F1845 adhesin-DAF interaction could be involved in the development of the MV lesion. DAF is present in cells lining the vascular compartment, and in these cells, DAF regulates the activity of both the classical and the alternative complement pathway C3 and C5 convertases (55). DAF is also present on epithelial cells lining extravascular compartments (9, 54, 66, 70); however, its function in this location remains unknown. We have postulated that the MV injuries promoted by the *E. coli* C1845-DAF interaction could result from a disorganization of the cytoskeletal actin network.

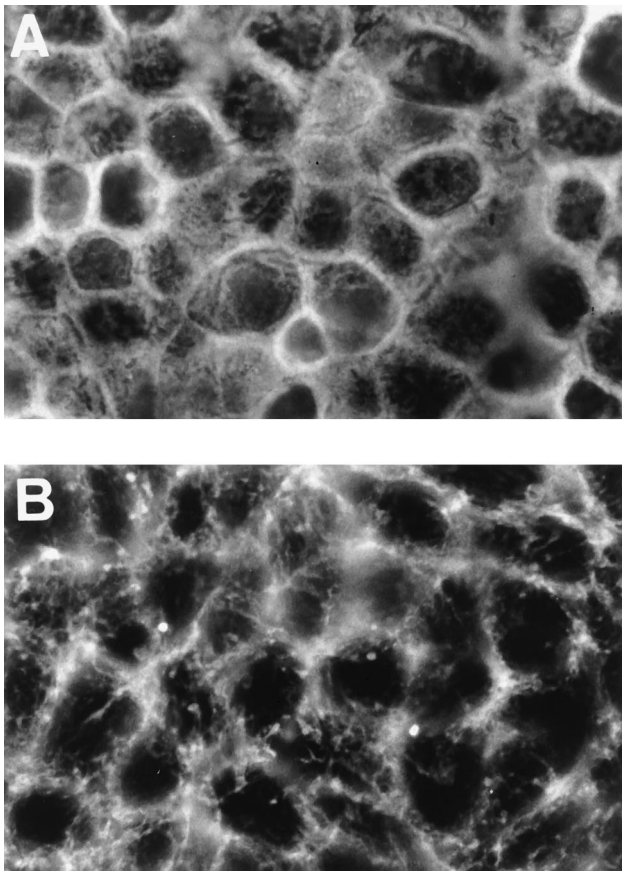

FIG. 9. Labeling of F-actin by fluorescein-phalloidin, showing apical F-actin disassembly in fully differentiated Caco-2 cells apically infected with the laboratory *E. coli* strain HB101(pSSS1) expressing the fimbrial F1845 adhesin. (A) Apical F-actin network in control cells. (B) Dramatic alteration of the distribution of F-actin in the apical pole of the infected Caco-2 cells, characterized by a densification of the perijunctional ring with central clearing zones replacing the fine, centrally located flocculated actin observed in control cells.

Indeed, in intestinal cells, brush border formation may be regulated by the state of actin assembly (46, 53, 60–62). We observed that *E. coli* C1845 promoted profound alteration of the apical and basolateral F-actin distribution. The DAF receptor for C1845 is a GPI-anchored protein (11) which transduces a signal via Ca^{2+} and IP_3 (69). It is tempting to speculate that the F1845 adhesin-DAF interaction could promote a transducible signal, which would induce a change in the F-actin distribution, resulting in MV lesion. Several lines of evidence have implicated the role of a second messenger system in the pathogenesis of cytoskeletal changes in polarized epithelial cells. It has been well documented that cytosolic calcium modulates the actin cytoskeleton in polarized intestinal epithelial cells (46, 53). Several reports document the mechanism by which cytoskeletal alteration could be induced. Actin disassembly accompanying MV lesion has been observed when intracellular calcium levels were kept elevated by modifying the extracellular Ca^{2+} concentration (10, 50) and by using Ca^{2+}-mobilizing hormones (29). Moreover, microfilaments can be altered by bacterial toxins (12, 31, 59) and by increasing intracellular calcium or by decreasing intracellular phosphatidylinositol-4,5-bisphosphate (PIP2) (35).

In conclusion, the results reported here provide novel data concerning the pathogenicity of the DAEC C1845 strain, i.e., that *E. coli* C1845 apical infection of the polarized intestinal epithelial cells induced a disturbance of the apical F-actin, promoting the MV lesion. By this pathogenic mechanism, it could disturb several epithelial functions and thus promote development of diarrhea. Indeed, in epithelial cells, polarization plays a fundamental role in vectorial movement of ions and macromolecules between biological compartments (44, 64). The apical membrane facing the external compartment and membrane proteins, such as enzymes and transport proteins, support specialized properties involved in absorption. In the basolateral domain facing the internal milieu, intrinsic and extrinsic membrane proteins, such as hormones and peptide receptors, participate in signal recognition and transduction involved in regulation of apical or basolateral systems. For example, the regulation of epithelial salt and water secretion may be influenced by interaction between calcium-binding cytoskeletal proteins and ion transport proteins (48, 68, 72). In polarized epithelial intestinal cells, the actin cytoskeleton and the associated proteins are essential for the maintenance of the structural organization of the cells, regulating the functionality of many membrane-associated components linked within the apical or basolateral membranes. Moreover, as recently reviewed, alteration in the actin cytoskeleton of epithelial cells is the basis of disease processes, such as secretory diarrhea (23).

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