INFECTION AND IMMUNITY, June 1992, p. 2541–2543
0019-9567/92/062541-03$02.00/0

Vol. 60, No. 6

# NOTES

## Cytoskeletal Composition of Attaching and Effacing Lesions Associated with Enteropathogenic *Escherichia coli* Adherence to HeLa Cells

B. BRETT FINLAY,[1]* ILAN ROSENSHINE,[1] MICHAEL S. DONNENBERG,[2] AND JAMES B. KAPER[3]

*Biotechnology Laboratory and Departments of Biochemistry and Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5,[1] and Division of Infectious Diseases[2] and Center for Vaccine Development,[3] Department of Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201*

Received 31 December 1991/Accepted 2 March 1992

**The cytoskeletal lesions associated with enteropathogenic *Escherichia coli* adhering to cultured HeLa epithelial cells were examined by immunofluorescence microscopy. The microfilament-associated proteins actin, α-actinin, talin, and ezrin were localized with adherent enteropathogenic *E. coli*, whereas tropomyosin, keratin and vimentin (intermediate filaments), tubulin (microtubules), and vinculin were not localized. These cytoskeletal structures differed significantly from those associated with *Salmonella typhimurium* internalization (invasion).**

Enteropathogenic *Escherichia coli* (EPEC) have the capacity to cause aggregation of host cell actin after adherence to epithelial cells and formation of microcolonies containing several adherent bacteria (7). This actin rearrangement is associated with the formation of attaching and effacing loci on the host epithelial cell surface (9) and is characterized by effacement of microvilli and intimate adherence between the bacterium and the host membrane, often with formation of a pedestal-like structure upon which the bacterium lies.

It has been reported that *Salmonella* species cause cytoskeletal rearrangements when entering cultured epithelial cells (4). These rearrangements include concentration of polymerized actin and other cytoskeletal proteins and are associated with invading bacteria. In an effort to further define the cytoskeletal components associated with EPEC adherence and to compare the cytoskeletal rearrangements that occur between these two organisms, we used immunofluorescence microscopy to examine HeLa cells infected with EPEC.

Pathogenic EPEC (strain E2348/69) (8) organisms were centrifuged onto cultured HeLa epithelial cells that were previously seeded on glass coverslips as described elsewhere (4). After incubation for 3 h at 37°C, infected monolayers were washed with phosphate-buffered saline, fixed with paraformaldehyde, and permeabilized with Triton X-100 (4). Rhodamine phalloidin (Molecular Probes, Eugene, Oreg.) was used to stain polymerized actin filaments. Polyclonal anti-α-actinin, monoclonal anti-α-actinin, polyclonal anti-talin, polyclonal anti-tropomyosin, monoclonal anti-tropomyosin, two different monoclonal anti-tubulin, monoclonal anti-keratin, monoclonal anti-vimentin, and monoclonal anti-vinculin antibodies were used as described elsewhere (4). Monoclonal anti-talin antibodies were obtained from Sigma (St. Louis, Mo.) and used at a dilution of 1/50. Polyclonal anti-ezrin antibodies, a gift from J. Krieg, Salk Institute, San Diego, Calif., were used at a dilution of 1/50. Primary antibodies were incubated with fixed permeabilized cells for 1 h and then labeled with fluorescein- or rhodamine-conjugated antibodies (4).

Polymerized actin accumulated under EPEC that were in intimate contact with HeLa cells (Fig. 1a and b), in agreement with a published report (7). This accumulation was tightly localized under the bacteria, outlining the adherent organisms. Actin accumulation did not occur under all adherent EPEC but was concentrated at the center of the groups of adherent EPEC. Very few lesions were seen within the first 2 h after EPEC addition, but by 3 h several were visible and these increased at later times.

The actin rearrangement caused by EPEC differed significantly in formation kinetics and structure from that caused by *Salmonella typhimurium* invasion of HeLa cells. With *S. typhimurium*, this rearrangement occurs soon after bacterial addition (within 30 min) and the actin returns to its normal distribution by 1 h (4). The actin accumulation caused by *S. typhimurium* is much larger (zones of accumulation approximately 5 to 7 μm in diameter) and less dense than that found with EPEC. With *S. typhimurium*, individual stringlike structures were visible (Fig. 1c and d), whereas the tight profile seen with EPEC was the same shape and size as the bacterium (1- to 3-μm zones).

α-Actinin, a molecule involved in cross-linking actin filaments, accumulated in a manner similar to that of actin in EPEC-infected cells, forming tight profiles associated with EPEC (Fig. 1e and f). Normal focal contacts and stress fibers were also visible in focal planes of the ventral cell surface below the bacteria. We also examined the localization of two eucaryotic proteins, talin and ezrin, that are thought to be involved in linking actin filaments to transmembrane receptors (1, 6). Antibodies to both talin and ezrin showed staining patterns similar to that seen with α-actinin and actin, accumulating at sites of EPEC adherence and outlining individual

* Corresponding author.

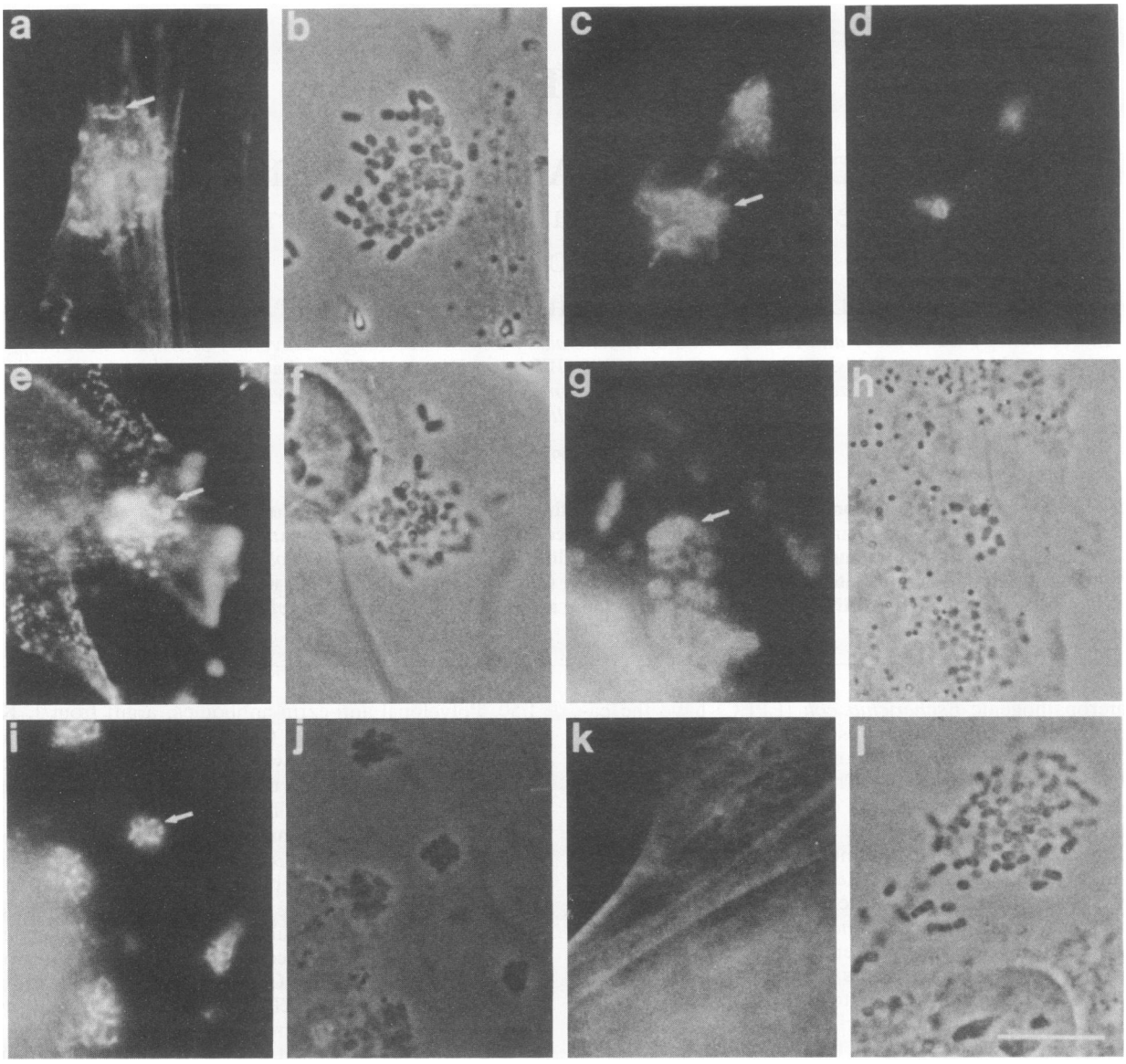


FIG. 1. Indirect immunofluorescence micrographs of HeLa cells infected with EPEC for 3 h (a, b, e through l) or *S. typhimurium* for 30 min (c, d) and then stained with phalloidin (a, c), anti-α-actinin (e), anti-talin (g), anti-ezrin (i), or anti-tropomyosin (k) antibodies. Corresponding phase-contrast micrographs are shown for EPEC (b, f, h, j, l), whereas the *S. typhimurium* micrographs were labeled with anti-LPS and a fluorescein secondary antibody (d) (4). The arrows illustrate rearranged cytoskeleton associated with bacteria. Bar, 10 μm.

organisms (Fig. 1g through j). Talin also stained the HeLa cell focal contacts, although this was found in ventral focal planes beneath the EPEC.

Several other stained cytoskeletal components did not exhibit rearrangement in response to EPEC adherence. Tropomyosin, a molecule involved in actin-myosin-mediated movement (10), occasionally showed a slight shadow accumulation beneath EPEC but normally was not rearranged around EPEC adherence sites when stained with either of two different anti-tropomyosin antibodies (Fig. 1k and l). Stress fibers stained normally with these anti-tropomyosin antibodies. It has been reported that colchicine, a drug that affects host microtubules, blocks EPEC internalization into host cells (3, 5). However, when HeLa cells were stained with two different monoclonal antibodies to tubulin, no rearrangement in microtubule structure was visible in EPEC-infected cells (data not shown). Similarily, intermediate filaments stained with anti-keratin or anti-vimentin antibodies were not affected by EPEC (data not shown). Vinculin, a focal contact protein possibly involved in linking actin filaments to membrane receptors at these sites, was also unaffected by EPEC adherence (data not shown).

We have shown here that several cytoskeletal components colocalize with adherent EPEC. Accumulation of talin and ezrin indicates that linkages may be formed between a transmembrane receptor that the bacterium adheres to and the cytoskeleton and that these molecules may be involved in establishing this bridge.

A summary of the cytoskeletal proteins that accumulate at attaching and effacing lesions caused by EPEC is given in

TABLE 1. Cytoskeletal rearrangements associated with EPEC or *S. typhimurium* infection of HeLa epithelial cells

| Cytoskeletal component | Cytoskeletal rearrangement with: | |
|---|---|---|
| | EPEC | *S. typhimurium* |
| Actin | + | + |
| α-Actinin | + | + |
| Talin | + | + |
| Ezrin | + | +[a] |
| Tropomyosin | − | + |
| Tubulin | − | + |
| Keratin | − | − |
| Vimentin | − | − |
| Vinculin | − | − |

[a] Unpublished data.

Table 1. For comparative purposes, a similar listing was compiled for the rearrangements associated with *S. typhimurium* invasion. As discussed above, these cytoskeletal structures differ in their morphological appearance and kinetics of formation. Both pathogens cause rearrangements of host actin, α-actinin, talin, and ezrin. However, in contrast to *S. typhimurium*-mediated accumulations, EPEC-associated lesions contain little, if any, tropomyosin or tubulin. Neither organism significantly affects intermediate filament structure or vinculin distribution.

EPEC has the capacity to enter epithelial cells (2). However, the lack of tropomyosin accumulation with EPEC suggests that the cytoskeletal accumulation caused by EPEC may be involved in forming a relatively inert, stable cytoskeletal structure that the bacterium rests on, rather than promoting an actin-myosin-tropomyosin-mediated event involved in mechanical bacterial uptake (10). *S. typhimurium* invades HeLa cells quickly (within 30 min) and efficiently (4), whereas EPEC internalization occurs approximately 3 h after infection (2). Thus, it appears that the cytoskeletal rearrangements triggered by *S. typhimurium* are designed to efficiently internalize the bacterium, with a subsequent return to the normal cytoskeletal distribution, whereas those caused by EPEC form a stable structure that remains for several hours at the center of localized bacterial adherence.

The differences in cytoskeletal rearrangements caused by EPEC and invading *S. typhimurium* may be mediated by the host cell receptors used by these bacteria or by other mechanisms such as triggering different signal transduction pathways. In conclusion, it appears that pathogenic bacteria have developed several strategies to rearrange and exploit the host cell cytoskeleton to their advantage.

We thank K. Burridge for providing the anti-talin antibodies and J. Krieg for providing the anti-ezrin antibodies.

This work was supported by an International Research Scholars Award from the Howard Hughes Medical Institute to B.B.F. and by Public Health Service grants AI32074 and AI21657 from the National Institutes of Health to M.S.D. and J.B.K., respectively. I.R. is the recipient of a long-term fellowship from the European Molecular Biology Organization.

## REFERENCES

1. **Burridge, K., and K. Fath.** 1989. Focal contacts: transmembrane links between the extracellular matrix and the cytoskeleton. BioEssays **10:**104–108.
2. **Donnenberg, M. S., R. A. Donohue, and G. T. Keusch.** 1989. Epithelial cell invasion: an overlooked property of enteropathogenic *Escherichia coli* (EPEC) associated with the EPEC adherence factor. J. Infect. Dis. **160:**452–459.
3. **Donnenberg, M. S., R. A. Donohue, and G. T. Keusch.** 1990. A comparison of HEp-2 cell invasion by enteropathogenic and enteroinvasive *Escherichia coli*. FEMS Microbiol. Lett. **57:**83–86.
4. **Finlay, B. B., S. Ruschkowski, and S. Dedhar.** 1991. Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial cells. J. Cell Sci. **99:**283–296.
5. **Francis, C. L., A. E. Jerse, J. B. Kaper, and S. Falkow.** 1991. Characterization of interactions of enteropathogenic *Escherichia coli* O127:H6 with mammalian cells in vitro. J. Infect. Dis. **164:**693–703.
6. **Gould, K. L., J. A. Cooper, A. Bretscher, and T. Hunter.** 1986. The protein-tyrosine kinase substrate, p81, is homologous to a chicken microvillar core protein. J. Cell Biol. **102:**660–669.
7. **Knutton, S., T. Baldwin, P. H. Williams, and A. S. McNeish.** 1989. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect. Immun. **57:** 1290–1298.
8. **Levine, M. M., J. P. Nataro, H. Karch, M. N. Baldini, J. B. Kaper, R. E. Black, M. L. Clements, and A. D. O'Brien.** 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. J. Infect. Dis. **152:**550–559.
9. **Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Giannella.** 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. Infect. Immun. **41:**1340–1351.
10. **Stossel, T. P.** 1978. Contractile proteins in cell structure and function. Annu. Rev. Med. **29:**427–457.