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NOTES

Neisseria gonorrhoeae Induces Focal Polymerization of Actin in Primary Human Urethral Epithelium

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The pathogenic *Neisseria* species induce cytoskeletal reorganization in immortalized cell lines. In Chang conjunctival epithelium and T84 intestinal epithelium, focal cytoskeletal rearrangements in which bacteria contacted the epithelial surface were observed. We show that actin footprints are induced in gonococcus-challenged primary urethral epithelium. Moreover, the microbes induced microvillus extension from the epithelial cell surface. Our results indicate that formation of actin footprints is not an artifact of commonly used immortalized cell lines.

The genus *Neisseria* comprises gram-negative bacterial species that are found inhabiting mucosal surfaces exclusively within the human host (2, 4). These organisms invade neutrophils and squamous epithelia during natural and experimental infection (3, 6, 7). Grassmé et al. showed that gonococcal adherence to Chang conjunctival epithelium promoted transient cell surface focal actin polymerization at and around the *Neisseria gonorrhoeae*-Chang cell interface (5). These foci, termed actin footprints, also have been observed in T84 cells, an immortalized human intestinal epithelial cell line, challenged with *Neisseria meningitidis* (8, 9). The purpose of this study was to determine if transient focal actin polymerization occurs in gonococcus-challenged primary urethral epithelium in a manner similar to that observed in immortalized cell lines.

***N. gonorrhoeae* challenge.** Primary urethral squamous epithelium was cultured from human tissue explants in prostate epithelial growth medium (Clonetics Corp., Walkersville, Md.) on purified rat tail collagen for 3 to 4 weeks (6). The cells were subcultured onto collagen-coated glass coverslips in 24-well tissue culture dishes and allowed to grow to confluence. Prior to each experiment, the cells were given 1 ml of fresh prostate epithelial growth medium. *N. gonorrhoeae* organisms (strain FA1090, VP1, or 1291) expressing opacity-associated adhesin (Opa), pili, and *N*-acetyllactosamine-containing lipooligosaccharide were used in the challenge experiments (approximately 2×10^7 CFU per coverslip). *N. gonorrhoeae* VP1 was a gift from J. van Putten (Rocky Mountain National Laboratory, Hamilton, Mont.), and strain FA1090 was a gift from H. Seifert (Northwestern University, Chicago, Ill.). The urethral cells were challenged for 1 h prior to washing and fixation. The coverslips were washed twice with 1 ml of phosphate-buffered saline (PBS) and fixed in 2% paraformaldehyde in PBS for 30 min. The fixed cells were washed with PBS to remove the fixative and stored at 4°C in preparation for confocal microscopy and scanning electron microscopy (SEM).

Induction of actin footprints. For confocal microscopy analysis, the fixed cells on glass coverslips were permeabilized with 0.2% Triton X-100 in PBS, and cortical actin filaments were stained with rhodamine phalloidin conjugate (5 units/ml in PBS) as recommended by the manufacturer (Molecular Probes, Eugene, Oreg.). *N. gonorrhoeae* cells attached to or within the urethral cells were immunostained with the *N. gonorrhoeae*-specific mouse-derived monoclonal antibody 2C3 (anti-H.8 specific) (1) and goat anti-mouse immunoglobulin G-fluorescein isothiocyanate (FITC) conjugate as recommended by the manufacturer (Molecular Probes). The coverslips were mounted and visualized with a Bio-Rad MRC 1024 confocal scanning laser microscope (Bio-Rad, Hercules, Calif.) at the University of Iowa Central Microscopy Facility. Our data suggests that gonococci induce focal actin recruitment in these primary cells and that the foci are part of cytoskeleton structural changes within the epithelium.

Rhodamine phalloidin conjugate (red stain) demonstrated intense focal actin polymerization around bacteria that adhered to the epithelial surface (Fig. 1A). Where the FITC-labeled bacteria (green stain) are surrounded by actin (red stain), the fluorescence appears yellow-orange in color. Figure 1 comprises consecutive confocal images of the host cell apical surface (panel A) and the basolateral surface (panel B). These images show actin filament extension originating from the surface focal adhesins. Similar structures were not observed in control experiments in the absence of bacteria (data not shown). Thus, the actin footprints appear to be point adhesins where the ends of the actin filaments are attached to the cell surface.

Microvillus extension. For SEM, the fixed cells on glass coverslips were equilibrated in 0.1 M sodium cacodylate buffer, stained with 1% osmium tetroxide, and then washed and dried with a standard ethanol series. The samples were mounted on aluminum stubs, sputter coated, and viewed with a Hitachi S-4000 SEM (Hitachi, Mountain View, Calif.) at The University of Iowa Central Microscopy Facility.

Several reports in the literature have described host cytoskeletal reorganization in response to an invading pathogen (10–13). These cytoskeletal changes often accompany host cell surface changes including microvillus extension, pedestal for-

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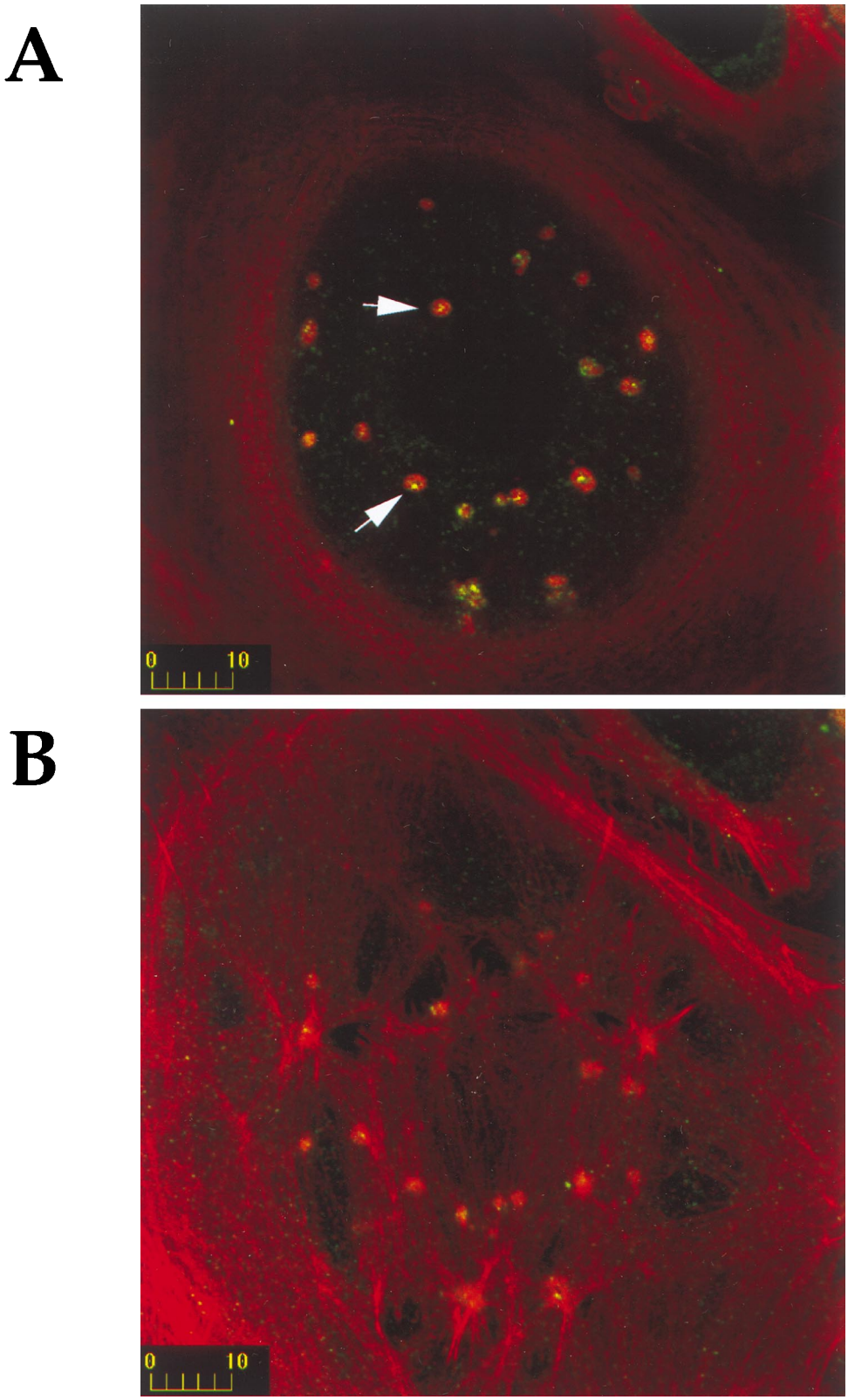

FIG. 1. Confocal microscopy images of gonococcal strain FA1090-challenged primary urethral cells. Polymerized actin filaments stained with rhodamine phalloidin conjugate fluoresce red, and *N. gonorrhoeae* stained with mouse monoclonal antibody 2C3 and goat anti-mouse FITC conjugate fluoresce green. Where the focal actin footprints (arrows) surround *N. gonorrhoeae* cells, the bacteria fluoresce yellow. Similar results were seen with strains 1291 and VP1 (data not shown). (A) Apical surface. (B) Basolateral surface. Final magnification, ×2,300. Bar, 10 μm.

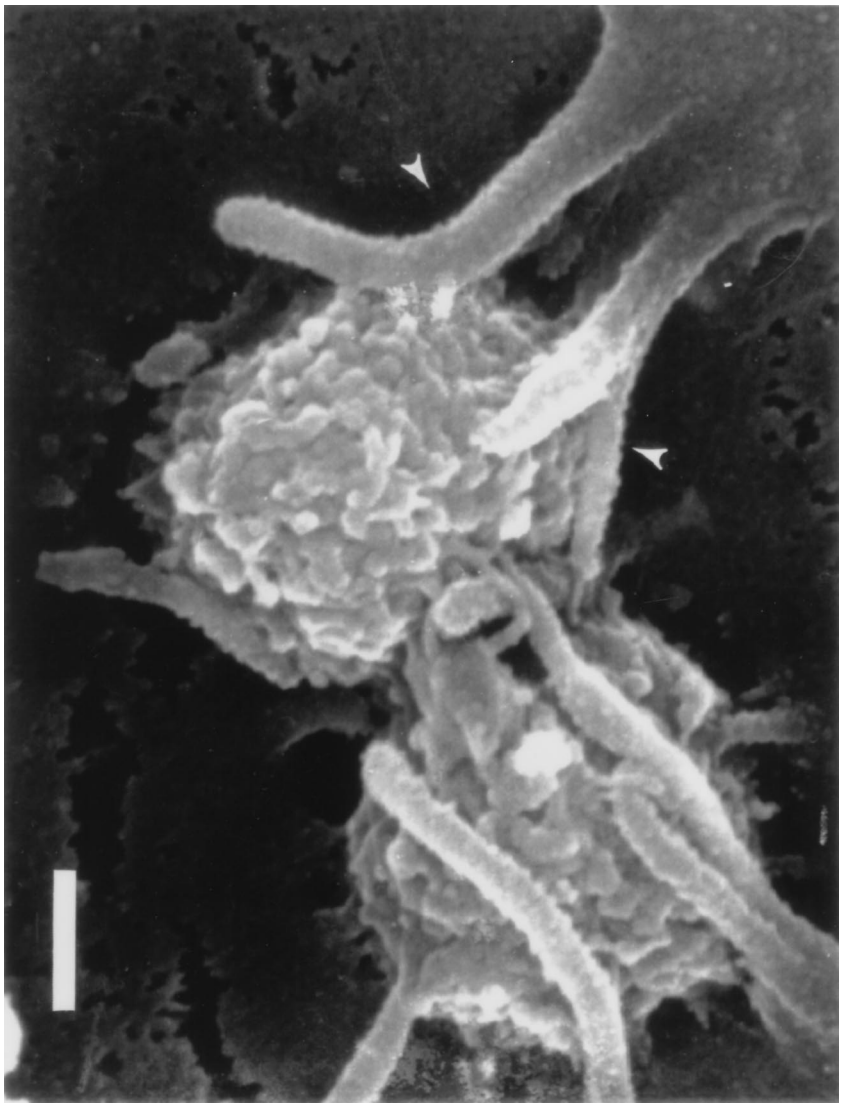

FIG. 2. Scanning electron micrograph of osmium tetroxide-stained FA1090-challenged primary urethral epithelium 30 min postchallenge. Note the microvillus extension toward the adherent bacteria and interaction of the host and *N. gonorrhoeae* membranes (arrows). Similar results were seen with strains 1291 and VP1 (data not shown). Final magnification, ×80,000. Bar, 0.25 μm.

mation, and/or membrane ruffling. SEM analysis of gonococcus-challenged epithelial cells shows microvillus extension and attachment to adherent bacteria, illustrating local changes in the host cell cytoskeleton within 1 h postchallenge (Fig. 2). It is well established that actin is instrumental in the formation of cell surface microvilli. Essentially identical results were observed with gonococcal strains VP1 and 1291. These results support our confocal microscopy data and show that *N. gonorrhoeae* induces peripheral cytoskeletal reorganization in primary urethral epithelium.

Summary. *N. meningitidis* and *N. gonorrhoeae* have been shown to induce the formation of actin footprints in immortalized cell lines (5, 8, 9). In this report we show that focal F-actin recruitment also occurs in primary human urethral epithelium and therefore is not an artifact of immortalized cells. Furthermore, focal actin adhesions appear to extend as radial actin filaments toward the basolateral surface. This overall cytoskeletal reorganization may have implications for *Neisseria* invasion and transcytosis during infection.

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