

Ultrastructural Analysis of Primary Human Urethral Epithelial Cell Cultures Infected with *Neisseria gonorrhoeae*

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In men with gonococcal urethritis, the urethral epithelial cell is a site of infection. To study the pathogenesis of gonorrhea in this cell type, we have developed a method to culture primary human urethral epithelial cells obtained at the time of urologic surgery. Fluorescent analysis demonstrated that 100% of the cells stained for keratin. Microscopic analyses indicated that these epithelial cells arrayed in a pattern similar to that seen in urethral epithelium. Using immunoelectron and confocal microscopy, we compared the infection process seen in primary cells with events occurring during natural infection of the same cell type in men with gonococcal urethritis. Immunoelectron microscopy studies of cells infected with *Neisseria gonorrhoeae* 1291 Opa⁺ P⁺ showed adherence of organisms to the epithelial cell membrane, pedestal formation with evidence of intimate association between the gonococcal and the epithelial cell membranes, and intracellular gonococci present in vacuoles. Confocal studies of primary urethral epithelial cells showed actin polymerization upon infection. Polyclonal antibodies to the asialoglycoprotein receptor (ASGP-R) demonstrated the presence of this receptor on infected cells in the primary urethral cell culture. In situ hybridization using a fluorescent-labeled probe specific to the ASGP-R mRNA demonstrated this message in uninfected and infected cells. These features were identical to those seen in urethral epithelial cells in exudates from males with gonorrhea. Infection of primary urethral cells in culture mimics events seen in natural infection and will allow detailed molecular analysis of gonococcal pathogenesis in a human epithelial cell which is commonly infected.

The ability of gonococci to invade nonprofessional phagocytic cells has been recognized for many years (19, 24). The studies of McGee et al. (8) and Ward et al. (26) demonstrated that gonococci could invade fallopian tube epithelia. Ward and Watt (25) examined urethral scrapings from men with gonorrhea by electron microscopy and found that gonococci could adhere to epithelial cells of this mucosa. This adherence resembled the tight attachment that they had seen in fallopian tube epithelial cells just prior to engulfment of gonococci. Novotny et al. showed the association of gonococci with epithelial and mucous-secreting cells in infected exudates (9).

Recent studies demonstrated that urethral epithelial cells are invaded by *Neisseria gonorrhoeae* during gonococcal infection in men (1). Confocal microscopic analysis and immunoelectron microscopy of urethral exudates from males with gonococcal urethritis indicated that intracellular organisms are either in vacuoles or free in the cytoplasm of urethral epithelial cells. Studies with acridine orange demonstrated that the majority of organisms within these cells are viable. The polar nature of epithelial cell invasion, as demonstrated by image analysis and immunoelectron microscopy, suggested that these cells are invaded while still attached to the urethral mucosa. Based on these results and studies of experimental gonococcal infection in men (3, 15), it has been proposed that the primary site of infection in urethritis in males is the urethral epithelial cell. Multiple bacterial factors are involved in attachment and invasion. It is clear that the gonococcal pilus is a requirement for attachment (8). It appears that the Opa protein may also be an important attachment factor (23). The lipooligosaccharide (LOS) may also be a factor in the ability of the gonococcus to infect the urethral epithelium (16).

Four stages of infection can be proposed from these studies.

The first stage represents attachment and intimate association of the plasma membrane with the bacterial membrane. This is followed by the second stage, consisting of the uptake of the organisms by the epithelial cells and their incorporation into vacuoles. In the third stage of infection, the gonococci appear to replicate within these vacuoles. In the fourth stage of infection, organisms rupture from the cells into the urethral lumen, and infected epithelial cells are shed. These released organisms either infect other epithelial cells or appear in cultures of the urine. The development of a primary human urethral epithelial cell culture system would allow analysis of the molecular events in each stage of infection.

In this paper, we describe the development of a human primary urethral cell system and compare the ultrastructural events seen in the infection of the primary urethral epithelial cell line with those observed during gonococcal urethritis in men. The ability to culture human urethral epithelial cells from urethral samples obtained at surgery was accomplished by using modifications of the method developed by Reznikoff and coworkers (14). This primary urethral cell system allows at least two passages of viable cells to new culture plates or to 12-mm glass coverslips coated with bovine collagen before the epithelial cell growth is contaminated with growth of fibroblasts. Infection studies can be performed directly on these cells after they become confluent, and results can be analyzed by microscopy without disrupting the integrity of the epithelial layer. Generally, within 4 days of the first passage, confluent layers of urethral epithelial cells are observed on the coverslips. Studies comparing various exposure times of primary urethral epithelial cells to gonococci were performed. Immunoelectron microscopy studies showed the same pattern of invasion observed recently by our laboratory in exudates from infected patients, i.e., adherence of the organism to the epithelial cell membrane and pedestal formation with evidence of intimate membrane association of the gonococcus and the epithelial cell. These events were followed by intracellular localization.

In the past, the lack of an acceptable animal model and the cost and inconveniences associated with human volunteer stud-

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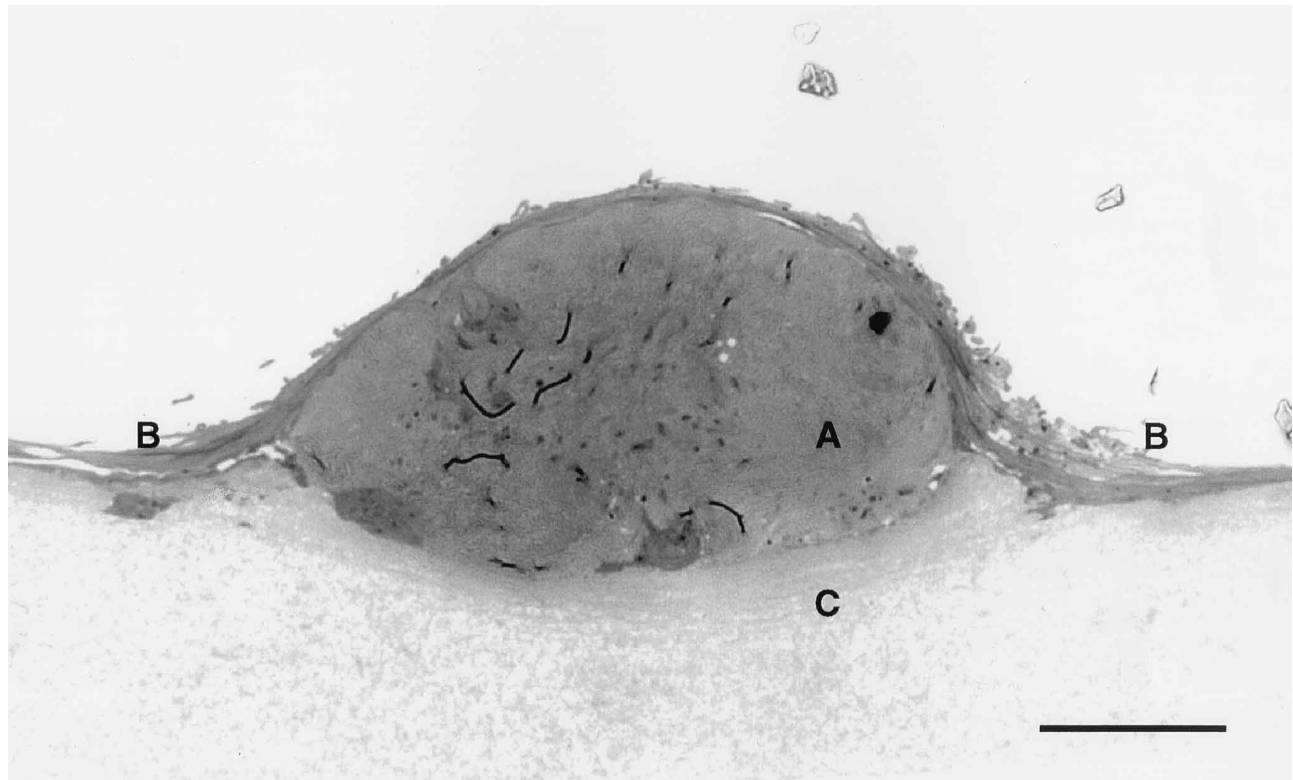


FIG. 1. One-micron-thick section of primary urethral epithelial cell growth and tissue explant (A) stained with toluidine blue. Epithelial cell growth (B) extends onto the rat collagen matrix (C) from the tissue specimen. Bar = 200 μ m.

ies have limited gonococcal pathogenesis studies. Studies with this system will allow the possibility of real-time molecular analysis of the bacterial and cellular events involved in gonococcal invasion of the urethral epithelium.

MATERIALS AND METHODS

Bacteria. *N. gonorrhoeae* 1291, P⁺ Opa⁺ was used in these infection studies of human primary urethral epithelial cell cultures. This strain was originally isolated from a male with gonococcal urethritis and has been previously utilized in cell invasion studies (11, 12). Cells were collected with a sterile swab from fresh overnight cultures on gonococcal medium-based agar and suspended in sterile phosphate-buffered saline (PBS). The cell concentration was adjusted to 50 Klett units ($\sim 10^8$ gonococci/ml), and an aliquot was diluted 1:10 in Ham's F12 (Gibco, Gaithersburg, Md.) without antibiotics for incubation with the epithelial cells.

Development of the primary epithelial cell culture. We developed the primary urethral epithelial cell system using tissue obtained from the membranous urethra of patients undergoing urologic surgery. Urethral tissue collected from surgery in sterile saline was cut into 4-mm² sections and placed epithelial side down on a rat-tail collagen matrix in polystyrene petri dishes (diameter, 35 or 60 mm). The explants were covered with filtered Dulbecco modified Eagle medium-Ham's F12 (1:1) containing 5% fetal calf serum (FCS), nonessential amino acids, penicillin (100 U/ml), streptomycin (1 mg/ml), and insulin (10 μ g/ml). After 24 h, the medium was changed to hormonally defined BEGM Bulletkit medium (Clonetics, San Diego, Calif.) containing an additional 2.5 μ g of Fungizone (Bristol-Myers Squibb, New York, N.Y.)/ml. Cells were allowed to grow undisturbed except for changes of the Bulletkit medium every 2 days. Cells were generally ready for passage or infection in 10 to 14 days. This primary urethral cell culture system allows at least two passages of viable cells to new collagen-coated petri dishes or to 12-mm glass coverslips coated with bovine collagen in 24-well cell culture dishes. Tissue samples from seven different male patients were used in this study.

Passage of cells. Cultures from the original tissue specimen were passaged by using a solution of 0.25% trypsin-0.1% EDTA diluted 1:1 in sterile PBS. Suspensions were rinsed with 1% FCS-Hank's balanced salt solution before resuspension and seeding in BEGM Bulletkit medium. For confocal microscopy, cell suspensions of 3×10^4 cells (70% viable by trypan blue exclusion staining) were seeded onto individual 12-mm coverslips in 24-well cell culture dishes.

Exudates from males with gonococcal urethritis. Three exudates from men with gonococcal urethritis were studied. The exudates were obtained from the sexually transmitted disease (STD) clinic at Boston City Hospital and from the STD clinic at the University of North Carolina. The samples were collected in 2% paraformal-

dehyde in PBS. The cells were sedimented after either 1/2 or 3 h of fixation, resuspended in PBS, and shipped to the University of Iowa on ice by overnight mail.

Infection studies. Infections were done on epithelial cells in 35- or 60-mm-diameter petri dishes or on 12-mm bovine collagen-coated glass coverslips in 24-well tissue culture plates. For electron microscopy, the medium covering the original cell cultures was replaced with medium containing 2×10^7 gonococci per 35-mm petri dish or 4×10^7 gonococci per 60-mm petri dish, ratios of 50 and 70 gonococci/epithelial cell, respectively. For confocal microscopy, 0.5 ml of cell culture medium containing 5×10^6 gonococci was placed over confluent epithelial cells on 12-mm coverslips. Urethral epithelial cells and gonococci were incubated together for the times noted below. For uninfected control studies, cells were incubated as long as infected cells but in the medium-buffer preparation described above without gonococci.

Electron microscopy. Following infection with gonococci, the samples were washed twice with 0.1 M sodium phosphate buffer and held at 4°C in 2.5% glutaraldehyde-0.1 M sodium phosphate buffer to fix the cells. Initial processing (buffer wash and ethanol dehydration) for embedment in acrylic resin was performed in the petri dish. This maintained the orientation of epithelial cell growth in relation to the original tissue specimen. Portions of the explant with the collagen matrix and an intact epithelial layer were excised for infiltration and embedment in medium-grade LRW resin (London Resin Company). Thick sections (1 μ m) were cut for light microscopy and stained briefly with toluidine blue. Thin sections (85 nm) were cut for immunoelectron microscopy studies and mounted on Formvar-coated single-slot nickel grids (1). Postembedment labeling was performed on epithelial cells infected with gonococci for 4, 24, and 48 h, by using either monoclonal antibody 2C3 or monoclonal antibody 6B4. Monoclonal antibody 2C3 (immunoglobulin G [IgG] isotype) is specific for the gonococcal surface protein H.8. Monoclonal antibody 6B4 (IgM isotype) is specific for the Gal β 1-4GlcNAc residues of the 1291 LOS molecule. Primary labeling of the samples was followed by secondary labeling with colloidal gold-conjugated murine anti-IgG antibodies (Auroprobe; Amersham Life Sciences, Arlington Heights, Ill.). The specimens were viewed with a transmission electron microscope (H-7000; Hitachi, Mountain View, Calif.) at 75 kV accelerating voltage, located in the University of Iowa Central Microscopy Research Facility.

Confocal microscopy. The Bio-Rad MRC 600 or the Bio-Rad MRC 1024 laser scanning confocal microscope at the University of Iowa Central Microscopy Research Facility was used for the following studies (1). For cytokeratin studies, urethral epithelial cells on 12-mm coverslips were washed twice with 50 mM HEPES-100 mM NaCl (pH 7.6), fixed overnight in 2% paraformaldehyde in the same buffer, and washed twice in PBS. Cell membranes were permeabilized for 15 min in 0.2% Triton X-100 in PBS. Blocking for 30 min in 5% goat serum-PBS was followed by a 2-h incubation in primary antibody, a commercially available murine monoclonal antibody to cytokeratin (Sigma, St. Louis, Mo.). This was

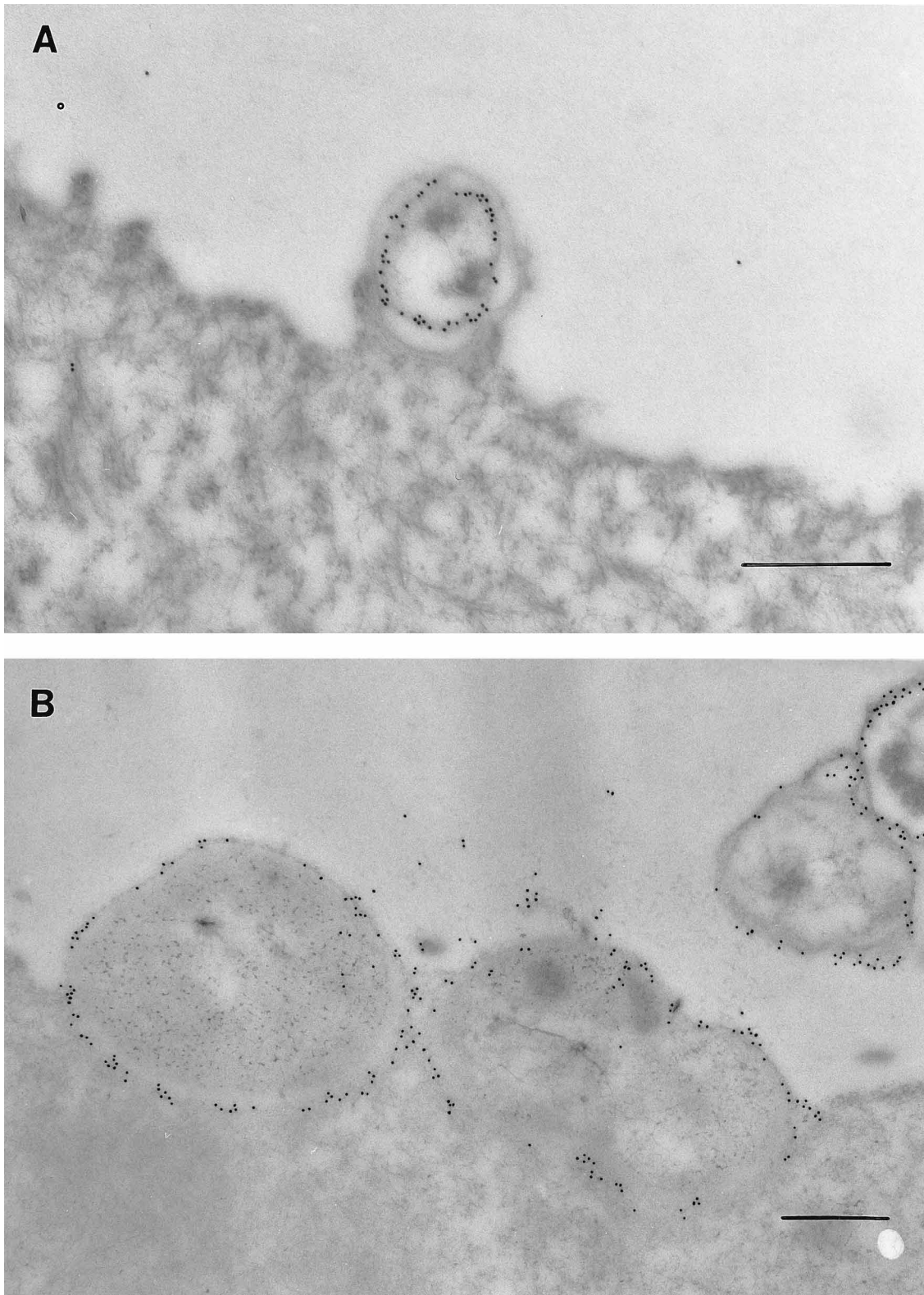


FIG. 2. (A) Immunoelectron micrograph of cell infected for 24 h, demonstrating pedestal formation as gonococci adhere to the epithelial cell surface. The gonococcus is circumferentially stained with monoclonal antibody 2C3, which binds to the outer membrane protein H.8. (B) Immunoelectron micrograph of cell infected for 4 h, demonstrating intimate association between the primary urethral epithelial cell plasma membrane and the gonococcal outer membrane during endocytosis. The gonococci are stained with monoclonal antibody 6B4, which binds to the Galβ1-4GlcNAc LOS epitope. Bar = 0.5 μm.

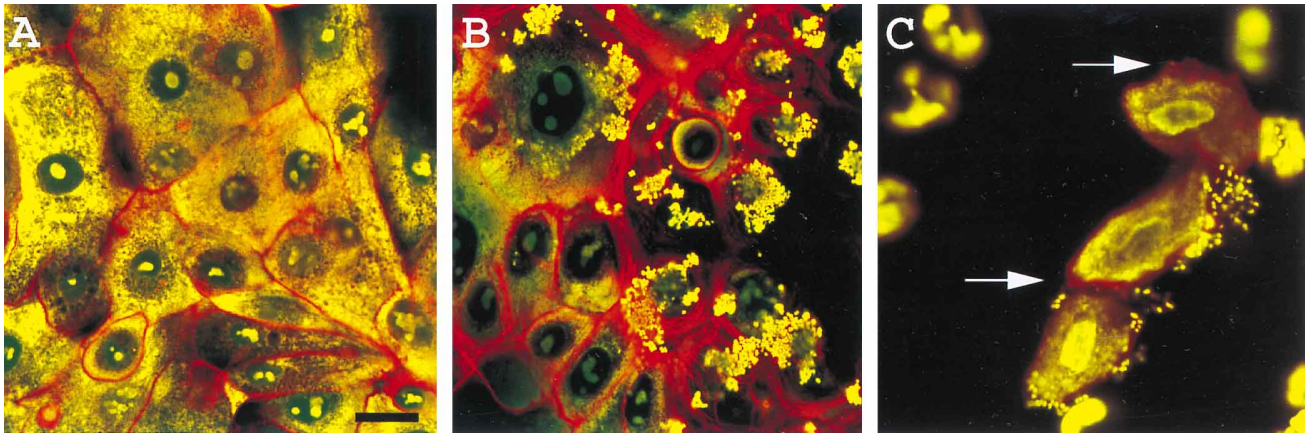


FIG. 3. Uninfected (A) and 4-h-infected (B) primary urethral epithelial cells and infected urethral epithelial cells from the exudate of a male with gonococcal urethritis (C) stained for actin (red) and nucleic acid (yellow). The uninfected primary cells were cultured the same amount of time as the infected primary cells. Note the thin rim of cortical actin in the uninfected cells. There is an increase in actin polymerization 4 h after infection. A band of cortical actin surrounds the epithelial cells in the exudate (arrows). Bar = 25 μ m.

followed by a 1-h incubation in secondary antibody, fluorescein-labeled anti-mouse IgG (Kierkegaard & Perry Laboratories, Inc.). After staining, the 12-mm coverslips were removed from the 24-well culture dishes, mounted face up on microscope slides with Vectashield mounting medium (Vector Laboratories, Inc.), covered with 22-mm square coverslips, and viewed by confocal microscopy.

Analysis for the asialoglycoprotein receptor (ASGP-R) was performed in a similar fashion. The primary antibody was either a rabbit polyclonal antiserum to ASGP-R (a gift from Milan Blake) or rabbit polyclonal antiserum, made in our laboratory, to recombinant ASGP-R lacking the membrane-spanning domain of the receptor. The secondary antibody was goat anti-rabbit IgG conjugated to the fluorophore Texas Red (Molecular Probes, Inc.).

Samples for actin studies (experimentally infected urethral epithelial cells and clinical exudates) were fixed for 30 min in 2% paraformaldehyde in HEPES-NaCl, washed, and permeabilized as described above. The samples were incubated for 30 min with rhodamine phalloidin (Molecular Probes, Inc.), which labels F-actin. Gonococci adherent to and inside of epithelial cells and nuclei of epithelial cells were localized with the fluorescent nucleic acid stain YOYO-1 (Molecular Probes, Inc.). After staining, the coverslips were mounted for confocal microscopy as described above.

In situ hybridization. Infected and uninfected primary urethral epithelial cells were washed in 150 mM sucrose and stained with 5 μ M Cell Tracker CM-Dil (Molecular Probes, Inc.). This is a cationic eukaryotic membrane tracer with excitation and emission wavelengths of 550 and 565 nm, respectively. The samples were then fixed for 30 min in 2% paraformaldehyde-PBS and hybridized overnight in a 37°C humid chamber to a digoxigenin-labeled oligonucleotide probe (3'TGATGCTCTGCCCGAAGTTCTTGA5') complementary to ASGP-R mRNA. A digoxigenin-labeled oligonucleotide probe complementary to the negative strand (5'CTTCAGCATCTGGACAATGAGGAG3') of ASGP-R DNA was used as a negative control. After being blocked for 5 min with 5% bovine serum albumin in 4 \times SSC (1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.2% Tween 20, the samples were incubated for 1 h at 37°C in fluorescein-labeled antidigoxigenin monoclonal antibody (Sigma). Following staining, the 12-mm coverslips were mounted as described above and viewed by confocal microscopy.

RESULTS

The primary urethral epithelial cells grew in culture under the conditions described. Within 3 to 5 days, epithelial cells could be seen extending from the surface of the tissue onto the collagen surface. By 2 weeks, organized confluent layers of epithelial cells could be found at distances of up to 2 cm from the tissue explant. The histological appearance of the epithelial tissue growing from the surgical sample was consistent with that of epithelial cells. The region closest to the surgical section showed stratification of cells. As cell growth extended onto the collagen layer from the tissue section, the degree of stratification decreased, and the layer continued as confluent epithelial cell growth (Fig. 1). Fluorescent antibody analysis using monoclonal antibodies specific for keratin and cytokeratin demonstrated that 100% of the cells stained for keratin, indicating that they were epithelial in nature (data not shown).

Immunoelectron microscopic analysis of gonococcal infection

of the urethral epithelial cells was performed at 4, 24, 48, 72, and 96 h. Studies of infection of the epithelial cell cultures from all seven donors showed similar findings. The cell layer was still intact after 96 h of infection, and viability counts indicated minimal cell death. The 4-h infection studies showed the entry events most clearly. Microvilli on the surfaces of the epithelial cells were elongated compared to those of uninfected cells (data not shown). Figure 2 shows gonococci entering an epithelial cell with intimate association of the gonococcal membrane and the epithelial cell plasma membrane. Participation of the epithelial cell in the invasion process is indicated by pedestal formation, which was frequently seen associated with gonococcal attachment and entry. This is indicative of the initiation of endocytosis and a concomitant increase in actin polymerization in the infected cells. Confocal studies of actin polymerization are shown in Fig. 3. These studies show rhodamine-labeled phalloidin binding to F-actin in infected and noninfected primary epithelial cells and in the epithelial cells from an exudate from a male with gonorrhea. There is an increase in actin polymerization after 4 h of infection. The distribution of the newly polymerized actin is primarily in the cortical region of the cell. A similar pattern of increased cortical actin polymerization can be seen in the urethral epithelial cells from an exudate of the patient with gonorrhea.

Figure 4 shows electron micrographs after 24 h of infection. The stratification of the epithelial cell layer can be observed. Gonococci were easily identifiable in vacuoles within the urethral epithelial cells. A group of organisms can be seen at the apical surfaces of the epithelial cells. Organisms could also be seen in the spaces between epithelial cells (data not shown). Initially, the organism-filled vacuoles were observed in close proximity to the apical layers of the epithelial cells. Transmigration of gonococci through the epithelial cell with release into the collagen layer was observed as infection progressed. Figure 5 shows a gonococcus being released from a vacuole at the basal surface of the cell. Numerous gonococci could be seen in the collagen matrix within 4 h after the initiation of infection (data not shown). Previous ultrastructural analysis of urethral epithelial cells in exudates from males with gonorrhea demonstrated the same series of events: pedestal formation, intimate association between the organism and the epithelial cell, entry into vacuoles, and exit of bacteria from the epithelial cells (1).

We have previously shown that the terminal galactose residue of the lacto-*N*-neotetraose of gonococcal LOS bound to the ASGP-R on HepG2 cells and that exposure to gonococci with

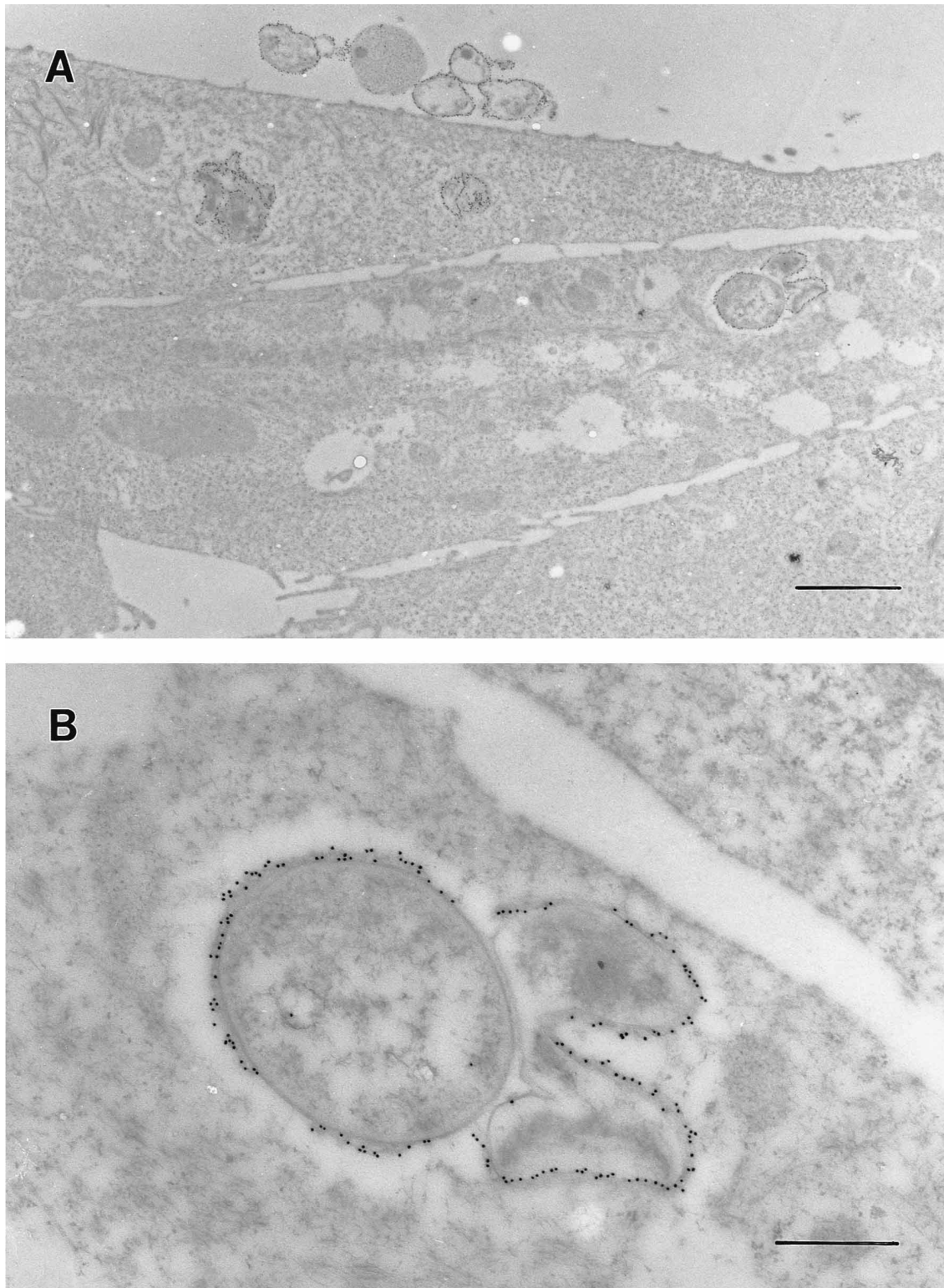


FIG. 4. (A) Immunoelectron micrograph of a 24-h-infected primary cell culture which demonstrates gonococci in intracellular vacuoles in stratified epithelial cells. Bar = 2 μ m. (B) Enlarged view of a vacuole containing gonococci. The gonococci are stained with an IgM-colloidal gold conjugate bound to monoclonal antibody 6B4. Bar = 0.5 μ m.

wild-type LOS increased expression of receptor mRNA and surface receptor expression (11, 12). Using an anti-ASGP-R antibody in fluorescent antibody studies, we have been able to demonstrate the presence of this receptor on infected urethral

epithelial cells from males with gonococcal urethritis (Fig. 6). Uninfected cells either did not stain or stained much less intensely than the infected epithelial cells. Analysis of the primary urethral epithelial cell culture indicated that the receptor is also present on

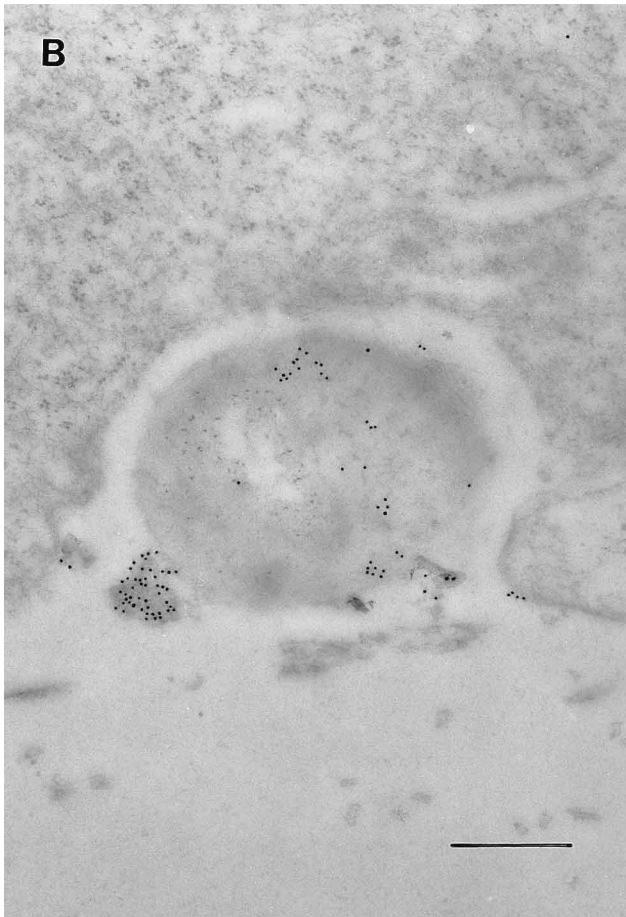


FIG. 5. (A) Electron micrograph of a gonococcus after 4 h of infection being exocytosed from the basal surface of an epithelial cell into the collagen matrix (arrow). A gonococcus is in the process of attachment at the apical surface of the epithelial cell (arrowhead). The gonococci are stained with IgM-colloidal gold conjugate bound to monoclonal antibody 6B4. Bar = 2 μ m. (B) Higher magnification of gonococcus shown in panel A at basolateral surface. Bar = 0.5 μ m.

these cells (Fig. 7). In situ hybridization studies using probes specific for mRNA of this receptor were positive in resting and infected urethral epithelial cells (data not shown).

DISCUSSION

The gonococcus is a strictly human pathogen, and animal models have not proven useful in studies of pathogenesis. For this reason, experimental human gonococcal infections have been undertaken to explore microbial factors related to pathogenesis. These studies have yielded important information about the course of infection, the human cytokine response, and organism phenotypes associated with infection (3, 13, 15, 16).

Recently, studies of exudates from males with gonococcal urethritis have indicated that the urethral squamous epithelial cell is the primary site of local urethral infection in men (1). A primary urethral epithelial cell line would allow real-time analysis of the biology of gonococcal infection in a biologically relevant cell system. During the past 10 years, an increased understanding of the factors required for the sustained in vitro growth of primary tissue cells has permitted the development of a number of human primary cell culture systems. Immortalized tissue culture cells are normally derived from malignant or virus-transformed cells. Alterations in membrane structure and cytoskeleton which affect normal biological functions can occur. Cell biologists have turned with increasing frequency to primary cell lines for study. In order to understand the biology of gonococcal invasion of the urethral epithelial cell, we have undertaken the development of a system to culture primary human urethral cells for the study of the factors involved in

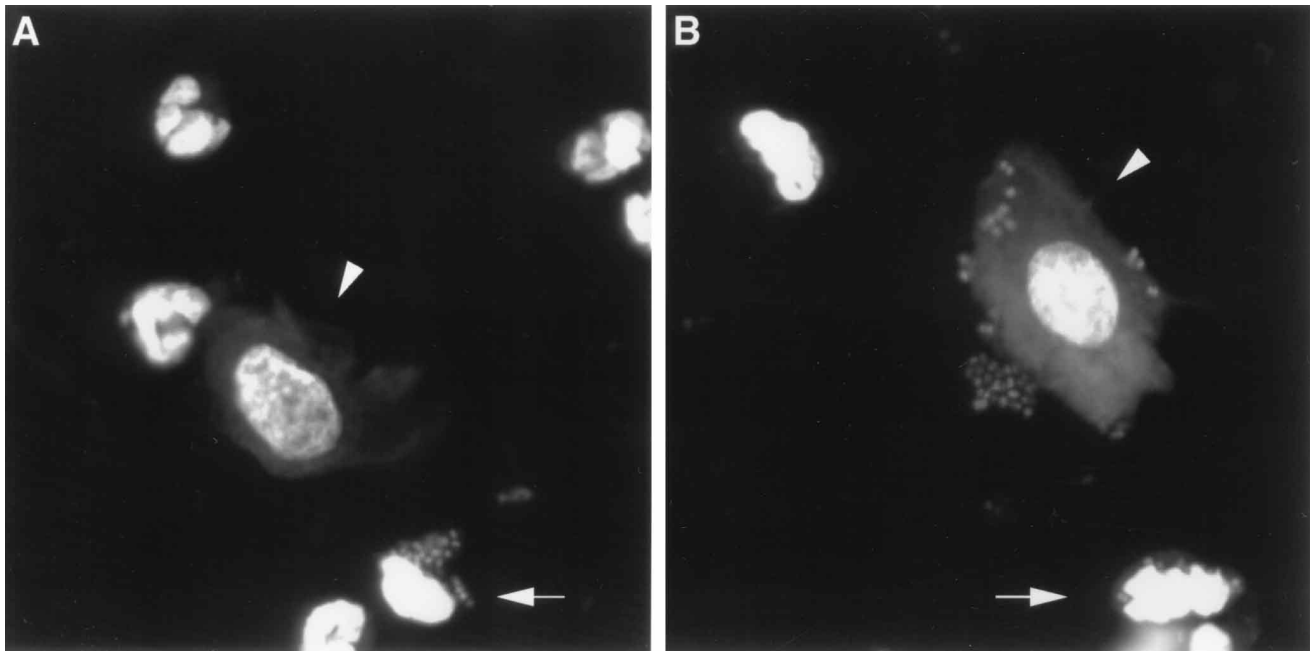


FIG. 6. Confocal analysis of urethral exudate of a male with gonococcal urethritis stained for ASGP-R and nucleic acid. The polyclonal rabbit anti-ASGP-R serum was used at a dilution of 1:1,000. The secondary antibody was a goat anti-rabbit IgG conjugated to Texas Red. Arrowheads, epithelial cells; arrows, neutrophils. (A) Uninfected epithelial cell above a neutrophil infected with gonococci. All the uninfected epithelial cells observed in the exudate were unstained by the ASGP-R antibody. The infected neutrophils did not stain with the anti-ASGP-R antibody. (B) Epithelial cells infected with gonococci in exudate from the same patient stained with the antibody to the ASGP-R.

gonococcal invasion. These cells can be grown in confluent monolayers with cell morphology and stratification similar to that seen in the urethral epithelium. They can be passaged and utilized for light, confocal, and electron microscopic analysis. Previous studies by Phillips and coworkers (10) utilized semen as a source for genital tract epithelial cells. Because of the complex mixture of cells in semen, these authors were not able to define the origin of these cells in the male genital tract.

Our studies have shown that at the electron microscopic level, the infection of the primary urethral epithelial cells mimics the process which has been described for infection of epithelial cells during gonococcal urethritis. We have demonstrated intimate association between the gonococcal and the epithelial cell membranes, endocytosis as exemplified by pedestal formation and F-actin polymerization, and incorporation of organ-

isms into vacuoles with transmigration through the epithelial cell. Grassmé et al. have also shown that there is a rearrangement of F-actin during gonococcal infection of Chang cells (5).

We have confirmed the presence of the ASGP-R on urethral epithelial cells in infected exudates and the presence of receptor mRNA in uninfected and infected human urethral epithelial cells in culture. We have demonstrated the presence of this receptor on the primary urethral epithelial cells during infection. These studies add further support to the hypothesis that this receptor is important to the pathogenesis of gonococcal infection.

Shaw and Falkow (18), van Putten and coworkers (21, 22), Chen et al. (2), and Tjia et al. (20) have shown that gonococci can invade tissue culture cell lines, including primary corneal cell tissue and Hec1B and Chang cells. van Putten has shown that sialylation of the LOS interfered with invasion of Chang

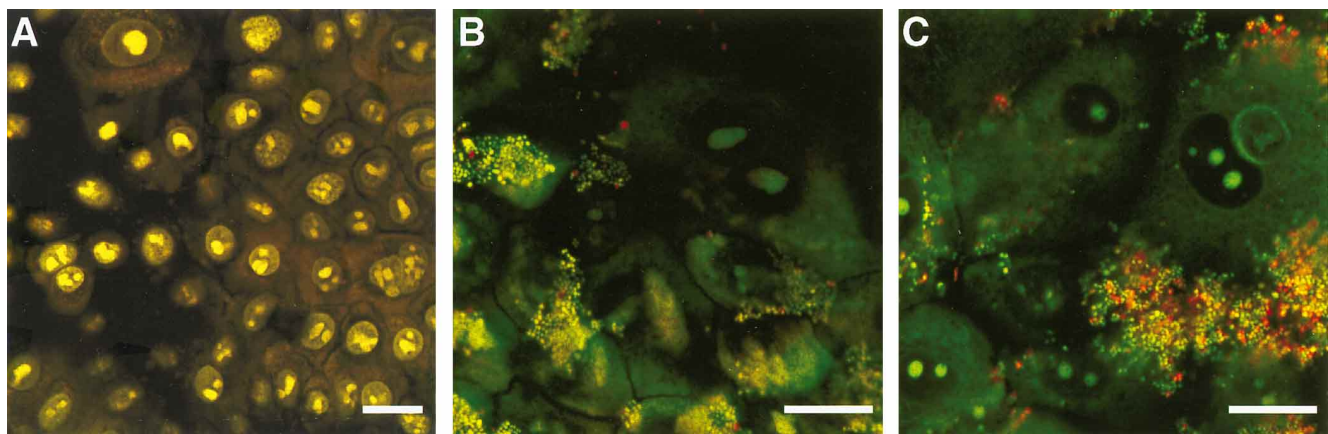


FIG. 7. Confocal analysis of primary urethral epithelial cells stained for ASGP-R (red) and nucleic acid (yellow) as in Fig. 6. (A) Uninfected cells; (B) 4-h-infected cells; (C) 24-h-infected cells. Note the lack of staining for ASGP-R in the uninfected cells and the increasing amounts of stain for ASGP-R with increased infection times. The uninfected cells were cultured for the same amount of time as the 4-h-infected cells. Bar = 25 μ m.

cells (21). Schwan et al. recently showed that gonococcal *rfaF* mutants which expressed an Rd₂ chemotype LOS and a genetically defined Opa protein could not invade Chang cells (17).

In vitro studies of invasion of various epithelial cell lines by *Shigella* and *Listeria* spp. have shown that this process involves multiple steps (4, 6). There appear to be a number of analogies, as well as a number of differences, between the results of these studies and what has been observed with *N. gonorrhoeae*. Phagocytosis by epithelial cells is induced by all of these organisms (4, 6). All enter vacuoles and, subsequently, *Shigella* and *Listeria* escape from the vacuole (4, 6). Our studies indicate that gonococci enter vacuoles. From our studies of human exudates and the primary urethral epithelial cells, it would appear that escape of gonococci from vacuoles within the cell is not a common event. We have seen vacuoles in the process of releasing gonococci from the cell surfaces in naturally infected epithelial cells (1) and in the primary urethral epithelial cell system. There appears to be transmigration of the organisms within vacuoles through the epithelial cell. McGee et al. demonstrated a similar event in their study of gonococcal infection of fallopian tube explants (7).

We have shown that entry occurs through the apical surface and discharge occurs through the basal surface of the cell. *Shigella* and *Listeria* free in the cytoplasm spread by the accumulation of actin filaments at one extremity of the bacterium (4, 6). *Shigella* and *Listeria* spread from cell to cell in finger-like protrusions which are phagocytosed by the adjacent cell. Eukaryotic cell and bacterial factors that interact to initiate and sustain these events have been found. We do not know the mechanism by which the gonococcus-infected vacuole transmigrates through the urethral epithelial cell or if the gonococcus can participate in the process.

The presence of ASGP-Rs on urethral epithelial cells may explain the role that the terminal galactose residue on the lacto-*N*-neotetraose on gonococcal LOS plays in the initiation of infection. This receptor is known to initiate endocytosis, and it could be one of the factors involved in the gonococcal invasion process. Porat and coworkers have shown that the galactose residues present on gonococcal LOS bind to Opa proteins on other gonococci in addition to at least two different galactose receptors on HepG2 cells (11, 12). One of these receptors is the ASGP-R. Studies by Schneider et al. (16) of experimental gonococcal infection in men have shown that efficiency of infection is enhanced if the terminal galactose is present and is not occluded by sialic acid. This would suggest that the interaction between this receptor and the LOS terminal galactose may be an important factor in addition to pili and the Opa protein in the invasion process.

Future studies in our laboratory with the primary human urethral epithelial cell system will investigate the role of the asialoglycoprotein in urethral cell infection. Efforts are also being made to immortalize this primary urethral epithelial cell system. This will enable us to compare the mechanisms of gonococcal invasion in a relevant primary cell system to a relevant cell line of the same tissue source.

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