Herpes simplex virus type 2 modulates the susceptibility of human bladder cells to uropathogenic bacteria

Received: 29 March 2001 / Accepted: 11 June 2001 / Published online: 15 August 2001
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Abstract The present study analyses the susceptibility of human bladder-derived cells (HT-1376) to the infection by herpes simplex virus type 2 (HSV-2) and Chlamydia trachomatis, as well as to the adhesiveness of uropathogenic bacteria. HT-1376 cells were efficiently infected by HSV-2 strain 333, as demonstrated by immunofluorescence staining of viral antigens, titration of cytopathic effect, and visualisation by transmission electron microscopy. This cell model was also prone to C. trachomatis (serovar E, Bour strain) replication and to the adherence of clinical uropathogenic isolates of Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris and Enterococcus faecalis. The pre-infection of HT-1376 cells with HSV-2 caused a tenfold increased adherence of an E. coli strain (U1), isolated from a patient affected by severe haemorrhagic cystitis, whereas in HSV-2 pre-infected cells the number of C. trachomatis inclusion bodies was significantly reduced. Our findings indicate that these cells are a suitable in vitro model for studying infection and super-infection of the lower urinary tract by viruses and bacteria.

Keywords Herpes simplex virus type 2 · Uropathogenic bacteria · Bladder cells · Co-infections

Introduction

It is well known that viral infections of mucosal barriers predispose the host to bacterial infections or lead to a

partial or complete inhibition of concomitant pathogens. Thus, viruses induce cellular modifications that may modulate the ability of other pathogens to adhere and invade epithelial cells, either producing more severe infections [34] or favouring the establishment of latent asymptomatic infections [5]. Co-infections and super-infections of urinary tract may often occur, so that multiple aetiology has been recognised. Bacteria cause most of these infections, whereas viral aetiology is seldom described. Identified viral agents include herpes simplex virus (HSV) that, besides genital mucosa, can infect urinary mucosa. In fact, HSV type 2 (HSV-2) has been recognised as a cause of urethritis [6, 7] and cystitis [22, 24, 35, 36]. The HSV-2 genome has also been detected in a high percentage of bladder cancer cells, raising the question whether such viral infection may contribute to the development and progression of urological malignancies in humans [14].

Among bacterial pathogens, Escherichia coli, Enterococcus faecalis, Proteus spp., and Pseudomonas aeruginosa are frequently involved in urinary tract infections, with E. coli being the species most commonly isolated [33]. The bacterial property mostly involved in the pathogenesis of lower urinary tract infections is the ability of the micro-organisms to adhere to urothelial cells. In E. coli this property is frequently mediated by filamentous surface adhesive organelles called type 1 pili [18], interacting with bladder mucosa through glycoproteins known as uropilakins [23].

Among intracellular bacteria Chlamydia trachomatis is also frequently recognised as the aetiological agent of symptomatic and asymptomatic urethral infections [1, 6].

Up to now, few data on bacterial colonisation of virus-infected urinary mucosa are available, since no suitable in vitro cell models, capable of mimicking the in vivo situation, have been investigated. In the present study we analysed in HT-1376 cells, a human bladder carcinoma cell line [27], the replication of HSV-2 and C. trachomatis and the adhesion ability of several uropathogenic bacterial strains belonging to Escherichia, Pseudomonas, Proteus, and Enterococcus genera. In
addition, to obtain further information on mixed viral-bacterial infections at the level of the urinary tract, the interactions between bacterial strains and virus-infected HT-1376 cells were studied. Results obtained showed that herpes virus infection may modulate the bacterial super-infection since the uropathogenic selected E. coli strain showed a significant increased adherence to HSV-2-infected HT-1376 cells, while the super-infection by C. trachomatis, serovar E, resulted in an inhibition of chlamydial and viral replication.

### Materials and methods

#### Cells

HT-1376 cells (a human bladder carcinoma cell line) were obtained from American Type Culture Collection. These adherent epithelial-like cells were from a Caucasian 58-year-old female [27]. Cells were cultured in minimal essential medium (MEM; Euroclone, UK), supplemented with 1.2 g/l NaHCO₃, 2 mM glutamine, 100 IU/ml penicillin, 0.1 mg/ml streptomycin and 10% heat-inactivated foetal calf serum (FCS, Flow Laboratories) in a 5% CO₂ incubator. HT-1376 cell viability was determined by the trypan blue (Sigma, Milan, Italy) dye exclusion method. Cell suspensions were stained and the percentage of viable cells determined using a haemocytometer.

#### Virus

HSV-2, strain 333, was propagated in Vero (African green monkey kidney) cells and the virus concentration was estimated by plaque assay as already described [21].

To determine viral antigen synthesis, HT-1376 cells, grown in 8-well microtissue chamber slides for 48 h in 5% CO₂, were washed and infected with HSV-2 at a multiplicity of 0.5, 1 or 2 plaque-forming units (PFU)/cell. After infection, cells were washed to remove non-adsorbed viral particles and fresh MEM was added to each well. After 22 h at 37°C the cells were washed in PBS, fixed in acetone at –20°C for 5 min, incubated with rabbit anti-HSV-2 antibodies (DAKO) for 45 min at 37°C and washed in PBS. Viral antigen synthesis was estimated as previously described by Marchetti et al. [21] using TRITC-conjugated anti-rabbit gamma-globulin antibodies (Sigma Chemical Company) and an UV Leitz microscope.

#### UV inactivation of virus

The virus stock solution was diluted 1:10, and an aliquot of 5 ml was irradiated by UV light from a UV lamp (0.67 mW/cm²) in a 50-mm Petri dish for 180 s at a distance of 120 mm. The preparation was inoculated to fresh cell cultures for control. No cytopathic effect was observed.

#### Bacteria

*E. coli*, *P. aeruginosa*, *Proteus vulgaris*, and *E. faecalis* were cultured in brain heart infusion broth (BHI, Oxoid, Basingstoke, UK) and subcultured on trypticase soy agar broth (TSA, Oxoid). All strains were isolated from patients affected by lower urinary tract infections.

For stock cultures *C. trachomatis*, serovar E, BOUR strain, was grown in HeLa 229 cell monolayers, in Eagle’s MEM supplemented with 0.5 μg/ml cycloheximide. Cells were infected with *C. trachomatis* suspensions that were centrifuged onto the surface of the cell monolayers at 1,800 g at 37°C for 1 h. After further incubation for 1 h at 37°C, the inoculum was discarded and replaced with fresh maintenance medium containing cycloheximide. After 72-h incubation at 37°C, infected cells were scraped off from the 75-cm² flasks and sonicated for 15 min in a sonic water bath. The sonicate was centrifuged at 400 g for 10 min at 4°C to remove cellular debris, and the supernatant was centrifuged at 12,000 g for 30 min at 4°C to pellet elementary bodies (EBs), as described previously [26]. The resulting EB pellet was further purified by Percoll (Sigma) density gradient centrifugation. Finally, purified EBs were suspended in sucrose phosphate glutamate, sonicated and stored in 1 ml aliquots at –70°C.

### C. trachomatis multiplication in HT-1376 cells

To analyse the multiplication of *C. trachomatis* in bladder-derived cells, HT-1376 cells (2×10⁵/vial), grown following the shell vial method [28], were infected at different time intervals with 1 ml of *C. trachomatis* suspensions containing 100–150 inclusion-forming units (IFUs). *C. trachomatis*-infected cells (48 or 72 h post infection) were stained with fluorescein-conjugated monoclonal antibody (Syva Microtrak) and microscopically controlled for the number of inclusions in the entire coverslip.

#### Bacterial adhesion and invasion assays

Before infection, bacteria were subcultured for 1 h at 37°C in BHI. For adherence assays, semi-confluent monolayers of HT-1376 cells (60,000 cells/ml), grown in 24 well Nunc culture dishes without antibiotics at 37°C in 5% CO₂, were infected at a multiplicity of infection (MOI) of 100 or 50 colony-forming units (CFU)/cell for 45 min at 37°C. After infection cell monolayers were carefully washed five times with MEM to remove unattached bacteria, lysed by the addition of cold 0.5% Triton X-100 and plated on TSA to determine the number of bound bacteria by performing CFU counts. Adherence was expressed as the percentage of the inoculated CFU that adhered to HT-1376 cells. In some experiments, after infection, monolayers were also washed five times with MEM, fixed in methanol, and stained by Giemsa stain to microscopically evaluate bacterial adhesion. For invasion assays, after infection, cells were washed five times with Eagle’s MEM. Fresh gentamicin-containing medium (1 ml, 50 μg/ml) was added to each well. At 2 h post infection, the cells were washed in MEM, trypsinised, lysed by addition of cold 0.5% Triton X-100, and plated on TSA to determine the number of viable intracellular bacteria.

#### Virus-bacteria mixed infections

The super-infection of virus-infected bladder cells was performed in HT-1376 cells grown in 24 well Nunc culture dishes (60,000 cells/well). After 48 hours incubation at 37°C in 5% CO₂ without antibiotics, cells were infected with HSV-2 (0.5 or 2 PFU/cell) for 1 h at 37°C. Non-adsorbed viral particles were then removed by washing with PBS, and fresh MEM without antibiotics was added for different time intervals before bacterial adhesion or invasion assays.

In virus-*C. trachomatis* co-infection assays, viral infection was performed in HT-1376 cells (2×10⁵/vial), grown following the shell vial method, at an MOI of 0.005 PFU/cell. At 24 h after viral infection, cells were super-infected with 1 ml of *C. trachomatis* suspensions containing 100–150 IFUs. After a further 48-h incubation at 37°C in 5% CO₂, the percentage of virus-infected cells and the number of chlamydial inclusions were both estimated by immunofluorescence assay.

#### Transmission electron microscopy

Infected (2 PFU/cell, 22 h) and mock-infected cell monolayers were fixed in 2.5% cacodylate-buffered (0.1 M, pH 7.2) glutaraldehyde for 1 h at room temperature and post-fixed in 1% OsO₄ for