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A dual chamber model of female cervical mucosa for the study of HIV transmission and for the evaluation of candidate HIV microbicides

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Abstract

A dual chamber system was established to model heterosexual HIV transmission. Cell-associated, but not cell-free HIV, added to a confluent layer of cervical epithelial cells in the apical chamber, reproducibly infected monocyte-derived dendritic cells (MO-DC) and $CD4^+$ T cells in the basal compartment. Only minimal epithelial transmigration of HIV-infected mononuclear cells (HIV-PBMCs) was observed. Most evidence points to transepithelial migration of virus, released from HIV-PBMCs after their activation by epithelial cells.

We used this model for evaluation of the therapeutic index of various potentially preventive antiviral compounds, including non-nucleoside reverse transcriptase inhibitors (NNRTIs, including UC781 and various diaryltriazines and diarylpyrimidines), poly-anionic entry inhibitors (including PRO2000, cellulose sulphate, dextrane sulphate 5000 and polystyrene sulphonate) and the fusion inhibitor T-20. The epithelium was pre-treated with compound and incubated with HIV-PBMCs for 24 h. Afterwards the apical chamber was removed and MO-DC/$CD4^+$ T cell co-cultures were further cultured without compound. NNRTIs, including a TMC120 gel, blocked infection of the sub-epithelial targets at sub-micromolar concentrations. Polyanionic entry inhibitors (up to 100 μg/ml) and T-20 (up to 449 μg/ml) failed to inhibit transmission. Moreover, whereas the NNRTIs used interfered with epithelial integrity with cervical epithelium only at very high concentrations, the evaluated entry inhibitors showed toxicity at concentrations that did not prevent infection.

Keywords: Microbicides; Sexual HIV transmission; NNRTI; Entry inhibitors; Dendritic cells; In vitro model

1. Introduction

The HIV/AIDS pandemic continues its spread, with over 40 million people already infected and over 10,000 new infections per day according to UNAIDS estimates (UNAIDS Global Fact Sheet, 2005). More than 90% of new infections are acquired through sexual HIV transmission. Since prophylactic anti-HIV vaccination will not be available in the near future, there is an urgent need for effective HIV prevention strategies. Especially in developing countries, women and girls are more vulnerable to HIV infection for several reasons: (1) inadequate knowledge about HIV transmission and AIDS, (2) inherent higher biological susceptibility to transmission, (3) insufficient access to HIV prevention services, (4) inability to negotiate condom-use and safe sex and (5) lack of female-controlled HIV prevention methods (Blocker and Cohen, 2000; Elias and Coggins, 1996; Mayer and Anderson, 1995; Padian et al., 1997).

For all these reasons, the development of potent and safe intravaginal/intrarectal topical formulations of anti-HIV agents, referred to as HIV microbicides, which are able to block sexual HIV transmission has become a major priority in HIV research. In order to evaluate candidate microbicides in a pre-clinical stage, several in vitro and ex vivo models are currently being explored, including cell suspensions, tissue explant systems and

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a SCID mouse model (Collins et al., 2000; Di Fabio et al., 2003; Greenhead et al., 2000; Palacio et al., 1994; Shattock and Moore, 2003; Veazey et al., 2003).

In vivo, the lower female genital tract is comprised of different anatomical regions, including the vaginal mucosa, the ectocervix and the endocervix (Pudney et al., 2005). While the stratified squamous epithelium of the vagina and ectocervix constitutes a barrier to infection of subepithelial target cells, the single columnar epithelium of the endocervix is inherently more vulnerable (Greenhead et al., 2000). Especially, the squamocolumnar junction or cervical transformation zone, where the monolayered epithelium of the endocervix abruptly changes into the multilayered epithelium of the ectocervix, is believed to be most susceptible to HIV infection as it is also the main target of infection with the human papilloma virus and the major inductive and effector site for cell-mediated immunity in the lower female tract (Pudney et al., 2005). Moreover, this transformation zone harbours the highest concentration of lymphocytes and antigen-presenting cells of the genital tract, the latter including $CD1a^+$ dendritic cells (Pudney et al., 2005).

Combined evidence from in vitro and in vivo studies suggests that dendritic cells, either intra-epithelial Langerhans cells or sub-epithelial (interstitial) dendritic cells are very early targets for sexual HIV transmission (Hu et al., 2000; Kawamura et al., 2000; Sugaya et al., 2004). Interstitial dendritic cells and $CD4^+$ T lymphocytes are major targets for HIV infection, after the virus has passed the epithelium (Miller et al., 1992; Spira et al., 1996). Moreover, dendritic cells (DCs) have a key role in the induction of immune-responses (Banchereau and Steinman, 1998; Servet et al., 2002). Clearly, candidate microbicides should be able to block infection in DC/$CD4^+$ T cell co-cultures, but they should neither interfere with the integrity of the epithelial layer, nor with physiological DC-T cell interactions, responsible for induction of anti-HIV immune responses.

Several compounds, acting at different stages of the retroviral cycle, are being considered for development as microbicides. These include acid-buffering agents (e.g. Buffergel), polyanionic entry inhibitors (e.g. PRO2000, cellulose sulphate, etc.), surfactants (e.g. C32G) and reverse transcriptase inhibitors (e.g. PMPA, UC-781, TMC120) (D'Cruz and Uckun, 2004; McCormack et al., 2001; Turpin, 2002).

We developed a dual chamber in vitro model in which the apical chamber contains a confluent layer of ME-180 cervical epithelial cells and the basal chamber consists of co-cultures of monocyte-derived dendritic cells (MO-DC) and $CD4^+$ T cells. As such, we closely mimic the site where sexual HIV transmission is most likely to occur. The dual chamber in vitro model was used for the evaluation of a representative series of HIV entry inhibitors and non-nucleoside reverse transcriptase inhibitors (NNRTI), some of which are currently being evaluated in clinical trials. The evaluated NNRTIs included a series of highly potent compounds, belonging to the family of diaryltriazine (DATA) and diarylpyrimidine (DAPY) analogues (Andries et al., 2004; Janssen et al., 2005; Ludovici et al., 2001a,b). Additionally, a gel formulation with the DAPY NNRTI TMC120 (R147681) was evaluated.

2. Materials and methods

2.1. Epithelial and mononuclear cells

The human cervical epithelial cell line ME-180 was obtained from the American Type Culture Collection (ATCC-LGC Promochem, Teddington, UK) and cultured in RPMI-1640 culture medium (Bio-Whittaker, Verviers, Belgium), supplemented with 10% bovine fetal calf serum (FCS) (Biochrom, Berlin, Germany), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Roche Diagnostics, Mannheim, Germany), further referred to as complete medium.

Monocytes and lymphocytes were separated from donor buffy-coat peripheral blood mononuclear cells (PBMCs) by counter-flow elutriation. Monocyte-derived dendritic cells (MO-DC) were differentiated from the monocyte-containing fractions and $CD4^+$ T cells were purified from the lymphocyte fractions, according to previously described methods (Van Herrewege et al., 2002).

2.2. Evaluation of epithelial confluence by confocal microscopy and flow cytometry

The polycarbonate membrane of the apical chamber of a dual chamber Transwell system (pore size 3 μm and diameter 6.5 mm) (Corning Costar Corp., MA) was coated with 200 ng/ml of laminin (Sigma–Aldrich, St. Louis, Missouri) and air-dried. Five hundred thousand ME-180 cells in 100 μl were seeded in the apical chamber, whereas the basal chamber contained 900 μl of complete medium. The ME-180 cells were cultured in this system either for 1 day or for 3 days (to constitute, respectively, a non-confluent and a confluent layer). To evaluate epithelial confluence and the preservation of the tight intercellular contacts between the epithelial cells (further referred to as 'epithelial integrity'), the apical chamber was removed and the cells were fixed using 4% formaldehyde (VWR, Heverlee, Belgium)/250 mM Hepes (Cambrex, MD) for 10 min. Cells were rinsed twice in phosphate buffer saline (PBS, 20 mM, pH 7.4) and quenched in 50 mM NH_4Cl (UCB, Brussels, Belgium) in PBS for 10 min. Cells were subsequently rinsed twice in PBS and incubated with Alexa Fluor® 594 phalloidin (Molecular Probes, Leiden, The Netherlands), a red fluorescent dye for labelling F-actin, for 30 min at 37 °C (absorption and fluorescence emission maxima 590, respectively, 617 nm). The probe was diluted 1/5, in Tris–HCl (Dako, Glostrup, Denmark). As nuclear counterstain, DAPI (Molecular Probes, Leiden, The Netherlands) was used at a concentration of 1/5000 (absorption and fluorescence emission maxima 358, respectively, 461 nm). After 2 min, the membranes with the cells were washed, removed from the apical chamber and mounted on microscope slides with Vectashield (Vector laboratories, Orton Southgate, Peterborough, UK). Samples were analysed using a Bio-Rad Radiance 2100 Blue Diode CLSM system with a 60× plan apo oil immersion objective. Emission filters used were HQ442/45 for DAPI and E570LP for Alexa 594. Images were taken with Bio-Rad Lasersharp 2000 software (Bio-Rad, Hampstead, Hertfordshire, UK) using a sequential scanning method and processed with ImageJ (Ras-