Journal of Virological Methods 165 (2010) 186–197

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Development of an in vitro dual-chamber model of the female genital tract as a screening tool for epithelial toxicity

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Article history:
Received 30 April 2009
Received in revised form 22 January 2010
Accepted 26 January 2010
Available online 4 February 2010

Keywords:
HIV-1 transmission
Dual-chamber model
Female genital tract
Microbicides
Epithelial toxicity
Transmigration

A B S T R A C T

Heterosexual transmission of human immunodeficiency virus (HIV-1) is the predominant mode of infection worldwide. However, the early steps of transepithelial infection still need to be clarified. Using epithelial cells, originating from the female genital tract, and peripheral blood mononuclear cells as subepithelial target cells, an in vitro dual-chamber model of the female genital tract was developed. Remarkably, an intact layer of some cell types (HEC-1A, CaSki and Ect1) served as a protective barrier against cell-free but not against cell-associated HIV-1 that crossed the epithelial barrier through transmigration. Furthermore, dysfunctions of the epithelial layers were assessed by monitoring transepithelial electric resistance and transepithelial passage of FluoSpheres® and HIV-1 after treatment with nonoxynol-9 (N-9). Most of the functional assays showed dysfunction of the epithelial barrier at lower concentrations compared to a widely used colorimetric toxicity assay (WST-1). Finally, N-9 treatment caused a significant increase in the production of interleukin-8 (IL-8) and macrophage inflammatory protein-3α (MIP-3α) and a decrease of Secretory Leukocyte Protease Inhibitor (SLPI) and Monocyte Chemotactic Protein-1 (MCP-1) in this model. In conclusion, this model is a useful tool to (1) study HIV-1 transmission mechanisms and (2) evaluate epithelial toxicity of candidate microbicides.

1. Introduction

Women are particularly vulnerable to heterosexual transmission of HIV-1 due to both socio-economical and biological reasons (UNAIDS, 2008). Therefore, blocking HIV-1 transmission in the female genital tract is key to prevent infection. Because barrier methods, such as the male and female condom, are unfortunately not yet sufficiently accepted, efficacious vaccines and topical microbicides that interfere with viral transmission are high on the wish list (Blocker and Cohen, 2000; Cutler and Justman, 2008; Elias and Coggins, 1996). However, insufficient knowledge of cervico-vaginal physiology prevents rapid progress in this field. A simple and reproducible in vitro model of the female genital tract could constitute an important tool to study the biology of HIV transmission and to more efficiently guide new interventions.

The epithelium along the female genital tract differs in cellular organization: the vagina and ectocervix are composed of multilayered stratified epithelium, whereas the endocervix and uterus are covered with a single layer of columnar epithelial cells (Pudney et al., 2005). Possible early target cells of sexually acquired HIV-1 include intra-epithelial Langerhans cells and CD4+ T cells and subepithelial macrophages (MA), dendritic cells (DC) and CD4+ T cells (Poonia et al., 2006; Shen et al., 2009; Sugaya et al., 2004). There is little evidence of HIV-1 replication in epithelial cells in vivo (Dezzutti et al., 2001; Tan and Phillips, 1996). Infection of mucosal MA and T cells could result in local viral production, whereas the interaction of HIV-1 with Langerhans cells and interstitial dendritic cells may be more important with regard to successful spread of the virus (Li et al., 2009; Pope, 2003).

Male to female HIV-1 transmission occurs through contact of the female genital mucosa with infected semen. Semen of an infected subject contains cell-free and cell-associated virus, which may both

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doi:10.1016/j.jviromet.2010.01.018

contribute to infection. As yet, the relative contribution of cell-free and cell-associated virus to HIV transmission remains unknown (Coombs et al., 2003). Various transmission pathways have been proposed, including direct but transient HIV-1 infection of epithelial cells (Dezzutti et al., 2001; Furuta et al., 1994), transcytosis of HIV-1 through epithelial cells, paracellular transmigration of HIV-1-infected donor cells, uptake of HIV-1 by intra-epithelial Langerhans cells, or circumvention of the epithelial barrier via breaches in epithelial integrity (Shattock and Moore, 2003).

Microbicides are a potentially promising strategy to prevent sexual HIV-1 transmission. During the preclinical screening of candidate microbicides, compounds should be tested in suitable in vitro systems to select the most active and least toxic candidates before being further evaluated in animal models and clinical settings. The first product that was evaluated clinically contained the nonionic surfactant agent nonoxynol-9 (N-9) which had been used as the active component of spermicides for over 20 years. A large scale phase III clinical trial with N-9 showed an increased risk of HIV-1 acquisition and extensive in vitro testing revealed that N-9 caused epithelial disruption and induced serious inflammation at doses previously used in the clinical setting (Beer et al., 2006; Fichorova et al., 2001; Hillier et al., 2005; Van Damme et al., 2002).

Therefore, it is essential to select candidate microbicides that are active but that do not damage or interfere with the epithelial integrity and function. Rigorous preclinical testing in various in vitro assays covering cell toxicity, changes in epithelial integrity as well as immunological activation state of epithelial cells is required.

In the present study, a dual-chamber system is described as a model for heterosexual transmission. The model consists of a tight epithelial cell layer of vaginal, uterine cervical or endometrial origin, grown on a Transwell® inserts and a basal chamber with peripheral blood mononuclear cells (PBMCs). This novel dual-chamber model was used to study transmission of cell-free and cell-associated HIV across various female genital epithelial layers and it was validated, by assessing the effect of N-9 on epithelial integrity, as a sensitive model for preclinical safety evaluations.

2. Materials and methods

2.1. Peripheral blood mononuclear cells and epithelial cells

2.1.1. Preparation of mononuclear cells

HIV-seronegative buffy coats were obtained from the Blood Transfusion Center, Red Cross, University Hospital, Antwerp. Peripheral blood mononuclear cells (PBMCs) were isolated by Lymphoprep (Axis-Shield PoC AS, Oslo, Norway) density gradient centrifugation and stimulated with 2 μg/ml phytohemagglutinin (PHA) (Remel, Kent, United Kingdom) for 3 days and further maintained in RPMI 1640 – 2 mM L-glutamine medium supplemented with 10% fetal bovine serum (FBS) (Biochrom, Berlin, Germany), 10 mM HEPES, 1 ng/ml interleukin-2 (IL-2) (Gentaur, Brussels, Belgium), 100 U/ml penicillin and 100 μg/ml streptomycin (Bio-Whittaker, Verviers, Belgium), further referred to as IL-2 medium.

2.1.2. Epithelial cells

The human endocervical epithelial cell line ME-180 and uterine cell line HEC-1A were obtained from the American Type Culture Collection (ATCC-LGC Promochem, Teddington, UK). The ME-180 cell line originates from a metastatic cervical carcinoma and is known to carry human papilloma virus 39 (HPV-39) DNA (Reuter et al., 1991). HEC-1A is a human endometrial adenocarcinoma (Kuramoto et al., 1972). Ect1/E6E7, End1/E6E7 and VK2/E6E7 cells were kindly provided by Dr. R. Fichorova (Harvard Medical School, Boston, MA). End/E6E7, Ect1/E6E7 and VK2/E6E7 cells are derived from normal ectocervical, endocervical and vaginal epithelium respectively and immortalized through transduction with the human papilloma virus 16 (HPV-16) E6 and E7 genes (Fichorova et al., 1997). SiHa and CaSki cell lines were kindly provided by Dr. B. Pozzetto (University of Saint-Etienne, GIMAP, France). SiHa cells originate from a cervical carcinoma and have been reported to contain one to two copies of an integrated human papilloma virus 16 (HPV-16) genome per cell (Baker et al., 1987). CaSki is a cervical carcinoma cell line containing an integrated HPV-16 genome, about 600 copies per cell, as well as sequences related to HPV-18 (Adler et al., 1997).

ME-180 cells were cultured in RPMI 1640 – 2 mM L-glutamine medium – 10 mM HEPES supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and with 10% FBS, further referred to as RPMI medium. HEC-1A cells were cultured in Mc Coy's 5A modified medium (Invitrogen, Merelbeke, Belgium) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and with 10% FBS, further referred to as Mc Coy's medium. SiHa and CaSki cells were cultured in DMEM/F12 (Invitrogen) medium supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and with 10% FBS, further referred to as DMEM/F12 medium. Ect1/E6E7, End1/E6E7 and VK2/E6E7 cells were cultured in Keratinocyt-Serum Free Medium (Invitrogen) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), 0.05 mg/ml Bovine Pituitary Extract (BPE) (Invitrogen) and 0.1 ng/ml Epidermal Growth Factor (EGF) (Invitrogen), further referred to as K-SFM.

2.2. Viruses

2.2.1. Cell-free virus

The HIV-1 subtype B strain Ba-L (CCR5-tropic) (NIH AIDS Research & Reference Reagent program, Rockville, M.D.) and the HIV-1 subtype C VI829 (CCR5-tropic) patient isolate were used. A stock of cell-free virus was prepared by culturing HIV-1 Ba-L and VI829 in PHA/IL-2 stimulated HIV negative donor PBMCs. Cell-free virus from the same stock was used in all experiments.

2.2.2. Cell-associated virus

A stock of cell-associated virus was prepared by overnight incubation of freshly obtained HIV negative donor PBMCs with cell-free Ba-L at 10^{-2} multiplicity of infection (MOI) in RPMI medium without mitogen or cytokines. The next day, cells (further referred to as cell-associated virus) were collected, washed extensively and stored in liquid nitrogen. To eliminate inter-donor variability, cell-associated virus prepared from the same PBMCs was used in all experiments.

2.2.3. Infectious titers

The infectious titers of cell-free Ba-L and VI829 and cell-associated Ba-L were determined in PHA/IL-2 activated PBMCs from an HIV negative donor. The virus stock was serially diluted in sixfold replicates and then added to PHA/IL-2 activated HIV negative donor PBMCs. Following 7 days of incubation, supernatants were harvested and virus production was measured using an in-house p24 antigen capture ELISA (see below). Tissue culture dose for 50% infectivity ($TCID_{50}$) was calculated using the Reed and Muench method (Reed and Muench, 1938). 50% endpoint dilution calculation is considered a standard method for measuring the amount of infectious HIV. In this calculation method the number of p24 positive (p24+) and p24 negative wells at each dilution is compared and the dilution giving 50% infected wells is estimated. First, the proportionate distance (PD) of the 50% endpoint was calculated by a simple formula: PD = [(%p24+ wells at dilution next above 50%) – 50%]/[(%p24+ wells at next dilution above 50%) – (%p24+ wells at next dilution below 50%)]. Next, this PD was corrected by the dilution factor which is the logarithm of the dilution step employed. Then the 50% endpoint