Methods 55 (2011) 114–121

Contents lists available at SciVerse ScienceDirect

Methods

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

Review Article

Primary human epithelial cell culture system for studying interactions between female upper genital tract and sexually transmitted viruses, HSV-2 and HIV-1

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ARTICLE INFO

Article history:
Available online 4 October 2011

Keywords:
Sexually transmitted infection
Female reproductive tract
Ex-vivo primary culture
Polarized epithelial cells
Trans-epithelial resistance
HIV-1

ABSTRACT

Evidence from clinical and epidemiological studies indicates that women are disproportionately susceptible to sexually transmitted viral infections. To understand the underlying biological basis for this increased susceptibility, more studies are needed to examine the acute events in the female reproductive tract following exposure to viruses during sexual transmission. The epithelial lining of the female reproductive tract is the primary barrier that sexually transmitted viruses, such as HIV-1 and HSV-2 need to infect or traverse, in order to initiate and establish productive infection. We have established an ex-vivo primary culture system to grow genital epithelial cells from upper reproductive tract tissues of women. Using these cultures, we have extensively examined the interactions between epithelial cells of the female genital tract and HSV-2 and HIV-1. In this review, we describe in detail the experimental protocol to grow these cultures, monitor their differentiation and inoculate with HSV-2 and HIV-1. Prospective use of these cultures to re-create the microenvironment in the reproductive tract is discussed.

1. Introduction

Sexually transmitted infections (STIs) are among the most prevalent infectious diseases worldwide and are a major cause of morbidity and mortality. While bacterial STIs such as chlamydia and gonorrhea are curable, viral STIs such as genital herpes, HIV and HPV cause incurable lifelong infections. In 2008, a bulletin from the World Health Organization estimated that 536 million people aged 15–49 are infected with HSV-2, the virus that causes genital herpes [1]. In addition, 23.6 million people in this age group become newly infected with HSV-2 every year. The prevalence rates of HSV-2 infection are higher in women than men, with the lowest prevalence rates being 13% among West European men and the highest prevalence rates being 70% among sub-Saharan African women [1]. Similar to HSV-2 infections, women are also more likely to become infected with HIV. Since the 1980s, HIV has shifted from a disease caused predominantly by use of shared hypodermic needles and male–male contact to a disease caused by heterosexual transmission. In fact, recent estimates have found that 30–40% of annual worldwide HIV infections occur through heterosexual transmission in the female reproductive tract [2,3]. Currently, in sub-Saharan Africa, 57% of all people infected with HIV are women and girls, and 76% of young people (aged 15–24) living with HIV are female [4]. These and other epidemiological data from bacterial STIs, such as chlamydia and gonorrhea, have consistently found that women have higher prevalence rates compared to men [5].

2. Discrete morphological characteristics of female genital epithelial cells

One of the important considerations for understanding the heterosexual transmission of viruses in female genital tract is the morphological and functional distinctions between the epithelial cells that line the different compartments of the female reproductive tract. These epithelial cells are the first cells that sexually transmitted viruses encounter, therefore their physical and functional characteristics are important determinants in the outcome. The lower reproductive tract in women is composed of ecto-cervix and the vaginal tract. The mucosal lining in these compartments consists of stratified squamous epithelium that can be more than 25 cell layers thick [6]. In contrast, the upper reproductive tract, made up of the endocervix and endometrium, is composed of a single layer of columnar epithelium that rest on a continuous, thin basement membrane [7]. The columnar epithelium is characterized by the presence of tight junctions between cells that makes it impermeable to entry of any large molecules and particulate matter,

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doi:10.1016/j.ymeth.2011.09.022

including pathogens. In comparison, the upper layers of the stratified squamous epithelium, that from the lining of the lower genital tract, have been shown to lack tight junctions and are relatively permeable to large molecular weight soluble mediators [8]. While the multiple layers in the lower genital epithelium may provide a better mechanical protection against viral invasion than the single layer columnar epithelium that lines the upper reproductive tract, at the same time, the greater surface area of the vaginal wall and ectocervix, arguably allows greater access for virus entry, particularly when breaches occur in the epithelium, such as during sexual intercourse [3,9,10].

3. Interactions of the genital epithelium with sexually transmitted viruses

While all sexually transmitted viruses have to cross the obstacle of the female genital epithelium to cause a productive infection in the host, their specific interactions with the epithelial lining in the genital tract are quite different. Herpes simplex virus type 2 (HSV-2) directly infects genital epithelium and undergoes replication within the cells [11]. It then infects adjacent epithelial cells and other cell types located under the epithelium, subsequently infecting peripheral nerves where it can become latent. The latent virus re-activates from time to time to replicate, the lining is shed in the genital tract secretions leading to further transmission. The consequence of direct infection and replication in the genital epithelium is evident in the efficiency of transmission of HSV-2. In N. America, roughly one in every 4 or 5 sexually active adults is infected by HSV-2 [12], while in Sub-Saharan Africa roughly 50–70% of the population is infected. Compared to HSV-2, the interaction of HIV-1 with the genital epithelium is still not completely understood [7,13]. Unlike HSV-2, it has a comparatively poor rate of transmission (1:200 to 1:1000 for each exposure) [14]. This makes the likelihood of productive infection in genital epithelium unlikely. However, studies done in this area have been far from conclusive. Early *in vitro* studies indicated that genital tract epithelial cell lines could be infected by HIV [15]. X4-tropic strain of HIV (T-tropic) was shown to replicate in cultured human primary uterine cells, however, R5-tropic strain (macrophage-tropic) was taken up and released from the cells, unmodified [16]. Over the years, the demonstration of alternative cellular receptors, such as GalCer, C-type lectins such as DC-SIGN, mannose receptors, proteoglycans such as heparin sulfate and syndecan that bind to HIV have raised the possibility that virus could get into mucosal epithelial cells using these alternative receptors [15,17–20]. More recently, the organ culture models of intestine, tonsil and cervix have been able to add relevant information regarding HIV transmission across epithelium [21–23]. These studies indicate that HIV-1 could possibly bind to epithelial cells via β-1 integrin and penetrate the ectocervical epithelial cell surface. Additionally, the main target of HIV-1 replication appears to be primarily the Langerhans and T cells underlying the epithelium. The overall view regarding HIV-1 infection is that it does not infect the epithelium *per se*, but is able to cross the mucosal epithelium to infect immune cells, including CD4 + DCs and T cells in the lamina propria of the mucosa. However, the debate regarding early events in HIV-1 transmission in the genital tract seems to be far from over. A recent study reported inability to detect translocation of HIV-1 across a reconstructed human vaginal mucosa. Furthermore, presence of Langerhans cells in this model did not increase HIV-1 transmission [24].

4. Immune functions of genital epithelial cells

One of the important reasons for understanding interactions of sexually transmitted viruses with genital epithelial cell is that these cells are dynamically active. As the primary cells that form the barrier between the external environment and the female genital tract, they play a critical role as the first responders to any incoming pathogen. They perform the dual function of responding directly to the pathogen as well as relaying signals to activate other innate and adaptive cells of the immune system. Genital epithelial cells (GECs) perform both these tasks very efficiently. They secrete a variety of anti-microbial factors constitutively and upon induction. Both upper and lower genital tract epithelial cells express a discrete range of TLRs [25–28]which allows them to recognize a wide array of pathogens and respond by production of cytokines and chemokines [29–31], which in turn attract other immune cells, including neutrophils and dendritic cells (DCs) to the genital tract. There is also evidence that GECs condition DCs to initiate adaptive immune responses [32].

Among the anti-microbial peptides secreted by GECs, many are produced constitutively and others are induced or upregulated upon exposure to stimuli. Studies done in *in vitro* culture systems indicate that some of these, including secretory leukocyte protease inhibitor (SLPI), lactoferrin, beta defensin and trappin-2/elafin have anti-HIV properties. The anti-leukoprotease SLPI has been shown to be secreted by ECs both in the human cervix and endometrium and suggested to have anti-HIV activity [33–35]. Epithelial cells of the genital mucosa also produce β-defensins, a family of small cationic peptides, shown to have significant antimicrobial effects [36]. More recently, the serine protease inhibitor, trappin-2/elafin has been shown to be secreted by GECs and implicated in anti-HIV activity at mucosal surfaces [37,38].

GECs also express a rich array of pattern recognition receptors. In particular, expression of, and activation by Toll-like receptors (**TLRs**) in GECs has been well described. Vaginal and cervical epithelial cells and cell lines express TLRs 1, 2, 3, 5, and 6, while primary endocervical ECs express TLRs 1, 2, 3 and 6 allowing them to sense both bacterial and viral pathogenic motifs and rapidly relay messages to other innate and adaptive cells should a pathogenic breach occur (reviewed in [30]). Primary human endometrial ECs express an even broader array of TLRs 1–9, indicating the potential of upper reproductive tract to respond to a wide range of pathogens. TLR mediated activation leads to production of chemokines and cytokines, including interleukin (IL)-6, IL-8, stromal cell derived factor (SDF)-1, the β-chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, and regulated upon activation, normal T-cell expressed and secreted (RANTES) [25,27,39–41].

In addition to the pro-inflammatory cytokines and chemokines, GECs are also capable of producing Type I Interferon (IFN), mainly IFN-β. Type I interferons (IFN-α, -β) impede the HIV replication cycle through numerous mechanisms, including induction of the antiviral molecule apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (**APOBEC3G**) [42,43] and 2′,5′-oligoadenylate synthetase (2′,5′-OAS), which activates a latent endo-ribonuclease, RNase L that inhibits the replication of HIV-1 [44,45]. Protein kinase R (**PKR**), inducible nitric oxide synthase (**iNOS**), myxovirus (**Mx**)-family proteins and the 9–27 protein are other interferon-inducible proteins shown to have anti-HIV properties [46]. Schaefer et al. reported the upregulation of the message levels for IFN-β, MyxA and OAS in cultures of primary endometrial ECs following exposure to TLR 3 ligand Poly I:C [47]. Since then, other studies have measured production of IFN-β in cervical and cervicovaginal cells following activation by TLR ligands or virus exposure [29,48].

5. Overview of the ex-vivo primary genital epithelial cell system

We have used an ex-vivo endometrial epithelial cell model to examine the interaction of sexually transmitted viruses, HSV-2