ORIGINAL ARTICLE VIROLOGY

Variability of human immunodeficiency virus-1 in the female genital reservoir during genital reactivation of herpes simplex virus type 2

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Abstract

Clinical and subclinical genital herpes simplex virus type 2 (HSV-2) reactivations have been associated with increases in human immunodeficiency virus (HIV)-1 genital shedding. Whether HSV-2 shedding contributes to the selection of specific genital HIV-1 variants remains unknown. We evaluated the genetic diversity of genital and blood HIV-1 RNA and DNA in 14 HIV-1/HSV-2-co-infected women, including seven with HSV-2 genital reactivation, and seven without as controls. HIV-1 DNA and HIV-1 RNA *env* V1–V3 sequences in paired blood and genital samples were compared. The HSV-2 selection pressure on HIV was estimated according to the number of synonymous substitutions (dS), the number of non-synonymous substitutions (dN) and the dS/dN ratio within HIV quasi-species. HIV-1 RNA levels in cervicovaginal secretions were higher in women with HSV-2 replication than in controls (p0.02). Plasma HIV-1 RNA and genital HIV-1 RNA and DNA were genetically compartmentalized. No differences in dS, dN and the dS/dN ratio were observed between the study groups for either genital HIV-1 RNA or plasma HIV-1 RNA. In contrast, dS and dN in genital HIV-1 DNA were significantly higher in patients with HSV-2 genital reactivation ($p < 0.01$ and $p < 0.05$, respectively). The mean of the dS/dN ratio in genital HIV-1 DNA was slightly higher in patients with HSV-2 genital replication, indicating a trend for purifying selection (p 0.056). HSV-2 increased the genetic diversity of genital HIV-1 DNA. These observations confirm molecular interactions between HSV-2 and HIV-1 at the genital tract level.

Keywords: Africa, genital herpes, *Herpes simplex* virus, HIV-1, HSV-2, phylogeny, variability
Original Submission: 27 January 2015; **Revised Submission:** 27 April 2015; **Accepted:** 11 May 2015
Editor: G. Antonelli
Article published online: XXX

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Introduction

The female genital tract constitutes an anatomical human immunodeficiency virus (HIV)-1 reservoir, in which there is active production of both cell-free HIV RNA and cell-associated HIV-

http://dx.doi.org/10.1016/j.cmi.2015.05.014

1 DNA [1], both of which could be sources for sexual transmission [2]. The cervicovaginal production of HIV is considered to be partly compartmentalized, at least transiently, in comparison with the virus population that replicates within the systemic compartment [3–7], and may be influenced by numerous local and systemic factors, notably the presence of other sexually transmitted infections and mucosal inflammation [1,6,8]. In particular, herpes simplex virus type 2 (HSV-2) may increase the infectiousness of HIV-1-infected individuals by increasing the frequency and quantity of HIV-1 genital shedding during clinical and subclinical herpetic episodes [9–13].

Both the quantity of HIV-1 in the genital tract and its diversity seem to play a role in sexual transmission. The heterosexual acquisition of HIV-1 infection is considered to be the result of selection of HIV-1 genetically restricted variants from the genital compartment of the HIV-1-infected index partner to both the genital compartment and systemic compartment of the newly infected partner [2,14,15].

In addition to its impact on genital HIV-1 replication, whether HSV-2 genital shedding may participate in the selection of specific HIV-1 variants within the female genital reservoir remains unknown. The aim of the present study was to evaluate the genetic diversity of HIV-1 cervicovaginal variants during episodes of HSV-2 genital reactivation in HIV-1/HSV-2-co-infected women.

Materials and methods

Participant recruitment and sample selection

The ANRS12-12 Study was an individually randomized, placebo-controlled trial of acyclovir (400 mg three times daily for 5 days) added to the standard antibiotic combination used for the syndromic management of genital ulcer disease (GUD). Trial participants were women presenting with GUD at sexually transmitted infection (STI) clinics in Accra and Kumasi (Republic of Ghana) and Bangui (Central African Republic). Full details of the procedures have been previously described [9,16]. The aetiology of GUD was determined by testing lesional swab specimens for HSV-2, *Haemophilus ducreyi*, *Treponema pallidum*, *Klebsiella granulomatis* and *Chlamydia trachomatis* lymphogranuloma venereum with molecular techniques, as previously described [9]. For this study, the samples were selected from the subgroup of women who were chronically co-infected with HIV-1 and HSV-2 as determined by serology, including seven with lesional (vulva or vagina) specimens and cervicovaginal secretions positive for HSV-2 DNA (group 1), and seven with lesional specimens negative for HSV-2 DNA and for other bacterial GUD aetiologies, and with cervicovaginal secretions also negative for HSV-2 DNA (group 2). Genital sampling was carried out when women were presenting with GUD at STI clinics. None of the women had ever received any antiretroviral treatment. Ethical approval was received from the research ethics committees of the Ministries of Health of Ghana and the Central African Republic, and the London School of Hygiene & Tropical Medicine. The ANRS12-12 trial was registered with the International Clinical Trials Registry (ClinicalTrials.gov number NCT00158483).

Blood and genital sample collection and laboratory analyses

At STI clinics, a standardized 60-s cervicovaginal lavage (CVL) fluid specimen was collected and immediately centrifuged 1,000 *g* for 15 min, and the cellular pellet and cell-free supernatants were aliquoted and stored frozen at −80°C until processing, as previously described [17].

CVL fluid supernatants were examined visually for the presence of blood, and tested for traces of haemoglobin with Hemastix dipsticks (Bayer Diagnostics, Puteaux, France). Samples containing haemoglobin and/or traces of contaminating semen, as determined by means of a Y chromosome PCR in DNA extracted from the cellular pellet of CVL fluid, were excluded [9]. HIV-1 RNA and HSV-2 DNA detection and quantification were performed in the cell-depleted fraction of the CVL fluid, and HIV-1 RNA detection and quantification were performed in the plasma by real-time RT-PCR, as previously described [17]. Viral quantification was expressed as copies/mL of collected sample, with a detection threshold of 250 copies/mL. All viral loads were $\log_{10}$-transformed for analyses.

PCR amplification and cloning of the HIV-1 V1–V3 env gene

Nucleic acids were extracted from 0.2 mL of plasma and from 0.2 mL of the acellular fraction of CVL fluid with the EasyMag System (bioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions. In addition, DNA was extracted from the pellet of centrifuged CVL fluid with the Qiagen DNA minikit (Qiagen Hamburg, Hilde, Germany). Extracted RNAs from plasma and cell-free CVL RNA were subjected to one-tube reverse transcription and further amplification by means of the Superscript one-step RT-PCR kit for long templates (Invitrogen, Groningen, The Netherlands), with E00 and ES8b as primers, allowing amplification of a region of gp120 including V1, V2, and V3 [15]. Extracted DNA from CVL pellets was subjected to the same V1–V3 *env* PCR. Products of the first PCR were then amplified by nested PCR with the primers E20 and E115 [15]. Cloning was carried out by means of the TOPO TA Cloning kit for sequencing (Invitrogen). Plasmids were extracted with the REAL prep 96 plasmid kit (Qiagen

Please cite this article in press as: LeGoff J, et al., Variability of human immunodeficiency virus-1 in the female genital reservoir during genital reactivation of herpes simplex virus type 2, Clinical Microbiology and Infection (2015), http://dx.doi.org/10.1016/j.cmi.2015.05.014