Increased HIV-1 Activity in Anal High-Grade Squamous Intraepithelial Lesions Compared With Unaffected Anal Mucosa in Men Who Have Sex With Men

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We studied 3 patients with focal intra-anal tissue high-grade squamous intraepithelial lesions (HSILs). All had increased human immunodeficiency virus type 1 (HIV-1) RNA and DNA in lesions compared with that in healthy mucosa. HIV-1 RNA and HIV-1 episomal DNA were indicative of ongoing viral replication, more so in anal HSILs.

Keywords: human immunodeficiency virus type 1; human papilloma virus; anal intraepithelial neoplasia; anal mucosa.

Successful human immunodeficiency virus type 1 (HIV-1) antiretroviral therapy (ART), with sustained undetectable plasma RNA load, does not completely suppress residual virus replication [1]. The continual reseeding of cellular reservoirs with virus, especially within multiple sequestered anatomical sites, has major implications for the eradication of HIV-1 infection. Coinfection with other pathogens will in all likelihood increase the viral reservoir dynamics as well as HIV-1 shedding at each specific site.

Among HIV-1–positive men who have sex with men (MSM), up to 95% have been reported with anal human papillomavirus (HPV) infection [2, 3]. Anal HPV infection is independently associated with HIV-1 acquisition [4]. Conversely, the rate of HPV diagnosis was shown to increase in a group of women from South Africa during their first year of HIV-1 infection, indicating mucosal immune dysfunction from the early stages of HIV-1 disease [5]. It has been shown that HIV-1–positive individuals are less prone to clear their HPV infection than are uninfected individuals [2]. Unlike most other opportunistic infections, the introduction of ART has not reduced the rates of diagnosed anal cancer [3]. Prolonged HPV infection of the anal mucosa will result in inflammation that will undoubtedly increase the numbers of immune cells carrying HIV-1 as well as uninfected cells with heightened susceptibility for infection. Here we aimed to address whether HPV can influence HIV-1 burden at the anal mucosa.

METHODS

This study was reviewed and approved by the Medical Ethical Committee of the Academic Medical Centre, University of Amsterdam, the Netherlands. After written consent, HIV-positive MSM were included with recently diagnosed focal tissue high-grade squamous intraepithelial lesions (HSILs) (anal intraepithelial neoplasia stage 3 [AIN3]) as described elsewhere [6]. Under high-resolution anoscopic vision, 2 adjacent biopsies were taken from a suspected HSIL lesion and 2 adjacent biopsies from unsuspicous normal-appearing anal mucosa. From each location, 1 biopsy was processed for histopathology to confirm HSIL and normal mucosa, respectively. The other 2 biopsies from respective locations were snap-frozen in liquid nitrogen and cryopreserved at −150°C. At time of sample collection, plasma HIV-1 RNA was measured using the Abbott m2000 Real-Time System (Abbott Molecular, Rockville, Maryland). CD4+ T-lymphocyte counts were determined via standard fluorescence-activated cell sorting, using commercially available fluorescent-labeled antibodies (Becton-Dickinson Immunocytometry). Additionally, blood was drawn on the same day the biopsy was performed using BD Vacutainer CPT (with sodium citrate) tubes. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood and cryopreserved (−150°C), as was 10 mL of plasma (stored at −80°C).

Thawed tissue was fully digested with proteinase K (Roche Applied Science) at 56°C overnight, by a nucleic acid isolation procedure using the QIAamp Viral RNA kit. The MACS cell separation system was used to isolate CD14+ monocytes and
CD4+ lymphocytes from PBMCs followed by nucleic acid isolation. Plasma was centrifuged (2 hours at 32 000 rpm) and the pellet resuspended in lysis buffer for subsequent nucleic acid isolation.

Nucleic acid detection was performed by an ultrasensitive protocol using highly conserved primers and probe located within the RUS long terminal repeat (LTR) region of the viral genome and which was performed using the Qiagen OneStep reverse transcription polymerase chain reaction (RT-PCR) kit according to the manufacturer’s instructions [7]. This assay detects all forms of viral RNA and DNA; however, a preamplification step with choice of specific oligonucleotide primers allows for differentiation between linear and circular viral forms as described [7]. Including a DNAse incubation step (Ambion TURBO DNase from Life Technologies) allowed us to differentiate between RNA alone or RNA/fully reverse transcribed DNA. Episomal HIV-1 DNA was amplified as previously described [7]. The tissue isolate input in the amplification reaction was expressed as micrograms of tissue with equal tissue input quantities utilized in all experiments.

RESULTS

Three individuals previously diagnosed with confirmed focal tissue HSIL disease (AIN3) by histopathology were included in the study. Biopsies were taken from both HSIL lesion as well as normal adjacent mucosa tissue with no signs of neoplasia and a normal differentiation pattern. All patients had undergone successful ART (Atripla, Truvada/atazanavir, and Truvada/atazanavir/ritonavir) for 1.3, 14.1, and 3.6 years as evidenced by continual undetectable plasma RNA loads. Their CD4+ cell counts had recovered to relatively high numbers (610, 580, and 718 cells/µL from nadir values of 180, 190, and 320 cells/µL, respectively). In addition, participants were negative for both anal chlamydia and gonorrhea and there were no clinical signs indicating an active herpes simplex virus infection.

Our sensitive PCR detected HIV-1 DNA in both the CD14+ and the CD4+ cell fractions, with the exception of the CD14+ cell fraction from patient 3 (data not shown). Viral RNA was then assayed in the plasma as an indication of de novo virus production. With our sensitive PCR assay we could detect fully reverse-transcribed viral DNA in all 3 study participants, indicating that the ultracentrifugation of the plasma had pelleted DNA-containing cell debris (Figure 1A). However, when the assay input was subjected to a DNAse 1 treatment prior to reverse transcription, the amplification revealed the presence of HIV-1 RNA in the plasma samples of patients 2 and 3 but not patient 1 (Figure 1A), indicating that de novo virus is indeed produced, and shed into the blood compartment.

Healthy and lesional mucosa samples were analyzed in parallel from all 3 study participants. Our choice of oligonucleotides (and depending on whether the sample was treated with DNAse or not) enabled us to detect viral RNA and DNA, or alternatively RNA alone (Figure 1B). All 3 study participants had HIV-1 RNA and DNA in both healthy and lesional mucosal tissues as shown by density quantification. The lesional HSIL biopsies from all subjects showed higher quantities of viral RNA than the neighboring healthy mucosa (Figure 1C). Additionally, the quantitative TaqMan assay identified higher levels of both HIV-1 RNA and DNA in lesional HSIL vs healthy mucosal tissue (Figure 1D).

The presence of HIV-1 RNA does not allow us to conclude whether the de novo–produced virus is generated from recently or historically quiescent infected cells, nor does it indicate the presence of infectious virus particles. We addressed this by testing for episomal HIV-1 DNA, which is indicative of recently infected cells. We found HIV-1 episomal DNA in the PBMCs from patients 1 and 2 for both the CD14+ and CD4+ cell fractions. Furthermore, from patient 2, who was diagnosed with HIV-1 in 1995, we found episomal DNA in the lesionsal HSIL biopsy whereas the healthy mucosa sample was negative (Figure 1E), indicating both increased viral activity and the presence of recently infected cells in the lesion.

DISCUSSION

We have shown in 3 patients heightened levels of HIV-1 RNA in HSIL intra-anal lesions in comparison to adjacent healthy tissue, indicating that HPV neoplastic lesions are associated with increased HIV-1 activity as shown in Figures 1C and 1D. The results shown in Figure 1C provide a snapshot of HIV-1 RNA after the final amplification whereas Figure 1D represents a more quantifiable qPCR output, which explains the apparent difference between the HIV-1 RNA levels in patient 1. The quantitative evaluation of RNA and DNA, both higher in the lesion, indicate higher HIV-1 infections in the lesion. This finding supports a previous study describing higher levels of HIV-1 being shed in the anal mucus of HIV-1/HPV–coinfected vs HIV-1–monoinfected individuals [5]. Local immune activation could cause this increased virus replication, or, alternatively, HIV-1–infected CD4+ T cells may be recruited to the lesional foci.

All study participants were under suppressive ART for as long as 15 years; however, the presence of HIV-1 RNA in HSIL mucosa clearly supports the concept that continuous virus replication is ongoing within the rectal mucosa, and more so in diseased tissue. The presence of HIV-1 RNA does not allow us to conclude whether the de novo–produced virus originates from recently or historically quiescently infected cells, nor does it indicate the presence of infectious virus particles. Reactivated long-lived HIV-1–infected reservoirs could be the source of virus, as could recently infected cells that ART failed to protect in the mucosal tissue. However, episomal
HIV-1 DNA (believed to have a short half-life suggesting recent infection [8]) was found both in peripheral CD4+ lymphocytes and CD14+ monocytes, suggesting that the de novo–produced HIV-1 particles may be infectious despite suppressive ART.

Gage et al have shown that supernatant from HPV-associated cervical cells could induce HIV-1 p24 and reduce interleukin (IL)-6 production, implying that HPV infection can affect HIV-1 pathogenesis [9]. Increased levels of immunoregulatory cytokines were also associated with HPV infection at the genital site, suggesting increased immune activation and inflammation in women coinfected with HPV/HIV [10]. Proinflammatory cytokines may play a key role in the HIV-1 life cycle, especially at the level of gene transcription, favoring the ability of HIV to establish latent reservoirs.

The 3 patients described here all showed increased HIV-1 activity in HSIL mucosa in comparison to nonlesional anal mucosa. Cell populations typically targeted by HIV-1 may be recruited to the infection site through alteration of the local cytokine/chemokine environment such as MIP-3a, IL-7, MIP-1B, IL-8, and RANTES, all of which are known to affect HIV-1 infection and replication [11]. It is also possible, however, that the lesion represents an anatomical site where drug concentrations are lower and where residual HIV-1 replication is increased. An increase in the concentrations of HIV-1 in HSIL mucosa will most likely lead to an increased transmission risk of HIV-1 via receptive anal intercourse.

Notes

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and J. D. V. performed experiments. G. P. wrote the manuscript, and all authors contributed to the final draft.

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