A Longitudinal Study of Human Papillomavirus DNA Detection in Human Immunodeficiency Virus Type 1–Seropositive and –Seronegative Women

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Cervicovaginal lavage samples from 124 human immunodeficiency virus type 1 (HIV-1)–seropositive and 126 HIV-1–seronegative women were collected monthly for 8 months and tested for human papillomavirus (HPV) DNA. The estimated prevalence of HPV was 42.8% in HIV-1–seropositive and 13.4% in –seronegative women ($P < .001$). There was no significant difference in HPV DNA detection in HIV-1–seropositive women with CD4 cell counts of $<300/mm^3$ (50% HPV-positive), $300–499/mm^3$ (36.4% HPV-positive), or $\geq 500/mm^3$ (40.5% HPV-positive). However, HIV-1–seropositive women who were more immunocompromised, as indicated by lower CD4 cell counts, were more likely to shed HPV persistently. The quantity of HPV DNA detected in cervicovaginal lavage samples was similar in HIV-1–seropositive and –seronegative women. This study further defined the characteristics of HPV infections in HIV-1–infected women.

Recent reports have provided compelling evidence of an association between human papillomavirus (HPV) and human immunodeficiency virus type 1 (HIV-1) in men and women (reviewed in [1, 2]). Both HPV infection and HPV-associated anogenital disease have been associated with HIV-1–induced immunosuppression in women [3]. However, these associations are still apparent in HIV-1–infected women who are asymptomatic [4], indicating that other factors in addition to HIV-1–induced systemic immunosuppression influence the association between HIV-1 and HPV.

Some studies have addressed possible mechanisms for the association of HIV-1 and HPV in asymptomatic HIV-1–infected women. Even though HIV-1–infected persons are susceptible to many agents that are not usually pathogenic in those who are seronegative, HIV-1–seropositive women are infected with the same HPV genotypes and sequence variants as HIV-1–seronegative women [5]. Molecular interactions between HPV and HIV-1 regulatory proteins can occur in vitro [6], raising the possibility that the two viruses may interact to affect HPV-associated pathogenesis. Similar effects of the HIV-1 regulatory protein, tat, have been shown with other viruses, such as JC virus [7].

At least two measurable properties of cervical HPV infection might be altered in HIV-1–infected persons: the duration of viral DNA shedding and the quantity shed. In this study, we measured these two properties of HPV infection and the estimated HPV prevalence over an 8-month period in a cohort of HIV-1–seropositive and –seronegative prostitutes in Kinshasa, Zaire.

Materials and Methods

Study population. Subjects were from a cohort of prostitutes recruited for study of sexually transmitted diseases and AIDS at the Prostitute Health Center in Kinshasa. HIV-1–seropositive and –seronegative prostitutes have attended the clinic since inception of the studies by Nzila et al. [8] and Laga et al. [9]. At present, this cohort consists of HIV-1–seropositive women, women who have seroconverted to HIV-1, and –seronegative women. The subjects in our prospective study were chosen from this well-defined cohort on the basis of reliable follow-up visits. The present study began in March and ended in October 1990.

Samples. Cervicovaginal lavage samples were taken from each woman at each monthly visit (maximum, 8). Cervicovaginal lavage was chosen since a comparison with cervical scrape showed that lavage was more sensitive for HPV detection [10]. Exfoliated cells were collected by centrifugation and suspended in 1 mL of 10 m*M* TRIS, 100 m*M* NaCl, 25 m*M* EDTA, and 0.5% SDS, pH 8.0.

CD4 cell counts. Complete blood cell counts were made with an automated counter (Coulter Electronics, Luton, UK). $CD4^+$ and $CD8^+$ lymphocytes were measured using commercial, dual-label monoclonal antibodies (Becton Dickinson Immunocytometry, San Jose, CA) and standard flow cytometric analysis (FACScan; Becton Dickinson). Peripheral blood lymphocytes

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This study was conducted with approval of the Ethical Committee, National AIDS Coordinating Committee, Ministry of Public Health, Zaire. Informed consent was obtained from all study participants. Human experimentation guidelines of the US Department of Health and Human Services were followed.

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were collected from women every 6 months as part of the protocol for the cohort study [9].

HPV DNA detection and quantitation. The Viratype (VT) assay (Digene Diagnostics, Silver Spring, MD) was chosen as the standard HPV DNA test because it has been standardized with respect to cervical disease. The VT assay detects three groups of HPV types (6/11, 16/18, and 31/33/35) and was done according to manufacturer's instructions.

HPV DNA was quantitated from 250 μL of cervicovaginal cells collected during the third month of this study using the Hybrid Capture System (Digene) with the RNA probe mixture to detect HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56. This assay was done according to manufacturer's instructions. The HPV DNA in each sample was quantitated by measuring chemoluminescence (in relative light units) with a luminometer and calculating the picograms of HPV DNA using the mean value from the 1 pg of HPV-positive DNA control provided with the assay.

To identify HPV types 45/51/52/56 (VT-negative, hybrid capture–positive), a conservative evaluation of the data collected during the third month of the study was used. A sample was considered positive only if it contained 1 pg of HPV DNA. VT-negative samples with ≥1 pg of HPV DNA detected by hybrid capture were considered positive for HPV types 45/51/52/56. The probability that this procedure would misclassify samples containing HPV type 16/18 or 31/33/35 as type 45/51/52/56-positive was low since the sensitivity of the VT assay should identify all specimens with ≥0.6 pg of HPV type 16/18 or 31/33/35 (Digene).

Statistical methods. Survivorship analysis examined the probability that a woman would have HPV DNA detected during the 8-month follow-up [11]. The detection rates of HPV were compared in HIV-1–seropositive and –seronegative women by calculating the Mantel-Haenszel weighted odds ratio and summary χ^2 statistic [12].

Results

HPV prevalence. We compared HPV prevalence rates in HIV-1–seropositive women (n = 86), women who converted to HIV-1 seropositivity (n = 38), and HIV-1–seronegative women (n = 126). HPV DNA detection rates by VT were similar in the seropositive and seroconverter groups (figure 1A). At enrollment, 26.9% of HIV-1–seropositive women, 23.7% of seroconverters, and 2.6% of seronegative women were HPV DNA–positive. During the second month, an additional 3.6% of HIV-1–seropositive women, 2.7% of seroconverters, and 3.8% of seronegative women were identified as HPV DNA–positive. The cumulative percentage of HIV-1–seropositive women identified as HPV DNA positive continued to rise an average of 2.7%/month for the next 6 months, that for seroconverters rose an average of 3.1%/month, and that for the seronegative group increased only 0.8%/month. Thus, there were no significant differences between HIV-1–seropositive and seroconverting women yet dramatic and significant differences between these subjects and those seronegative.

To determine if immune competence influenced the association between HPV and HIV-1, all HIV-1–seropositive women (including seroconverters) were grouped according to their mean CD4 cell count in 1990, and the estimated prevalence from life table analysis of any HPV type detected by VT was determined (figure 1B). Six (50%) of 12 with CD4 cell counts <300/mm^3, 8 (36.4%) of 22 with CD4 cell counts of 300–499/mm^3, and 32 (40.5%) of 79 with CD4 cell counts ≥500/mm^3 had HPV DNA detected. While there was a trend for women with CD4 cell counts <300/mm^3 to have a higher HPV prevalence, the difference between this group and those with >500 CD4 cells/mm^3 was not significant (P = .4).

Frequency of HPV DNA detection. Possible explanations for the increased association of HPV in HIV-1–infected women include a higher level of persistent infection with HPV or increased susceptibility to reinfection with HPV. To further explore the dynamics of HPV infection in this cohort, the frequency of persistent HPV DNA detection in 7 serial samples was determined in HIV-1–seropositive women who had both CD4 cell counts and at least 7 samples obtained during the 8-month period. HPV DNA was detected more frequently in serial samples from women with lower CD4 cell counts than from women with higher CD4 cell counts (P = .04; Figure 2). The median CD4 cell count for women with all 7 samples positive was 380/mm^3 compared with 660/mm^3 for women with only 1 of 7 samples positive.

HPV genotypes. Although more HIV-1–seropositive than –seronegative women had HPV (42.8% vs. 13.4% by the end of 8 months; P < .001), the distributions of HPV 6/11 and 16/18 were similar. Only HPV 31/33/35 was detected at greater frequency in seropositive women (30.2%) than in seronegative women (5.6%; P = .01).

To determine differences in the distribution of other HPV types, the results from the hybrid capture assay done during the third month of the study on 103 HIV-1–seropositive and 101 HIV-1–seronegative lavage samples were used to identify those HPV 45/51/52/56–positive. Twenty-four samples (23%) from HIV-1–seropositive women and 8 (8%) from –seronegative women were VT-negative and had ≥1 pg of HPV DNA detected and were thus considered positive for HPV 45/51/52/56 (P = .002). To compare this with VT data collected during this same month, the prevalence of HPV types 6/11, 16/18, and 31/33/35 was 36.2% in HIV-1–seropositive and 6.4% in –seronegative samples. Overall, the hybrid capture assay detected ≥0.4 pg of HPV DNA (types 16, 18, 31, 33, 35, 45, 51, 52, or 56) in 57.3% of samples from HIV-1–seropositive women (59/103) and in only 18.8% of samples from –seronegative women (19/101) (P < .001). Therefore, it was likely that our estimate of the prevalence of HPV types 45/51/52/56 was accurate.

Quantitation of HPV DNA. HIV-1–seropositive women may have more HPV DNA detected in cervicovaginal fluids, which would indicate more HPV replication. To address this

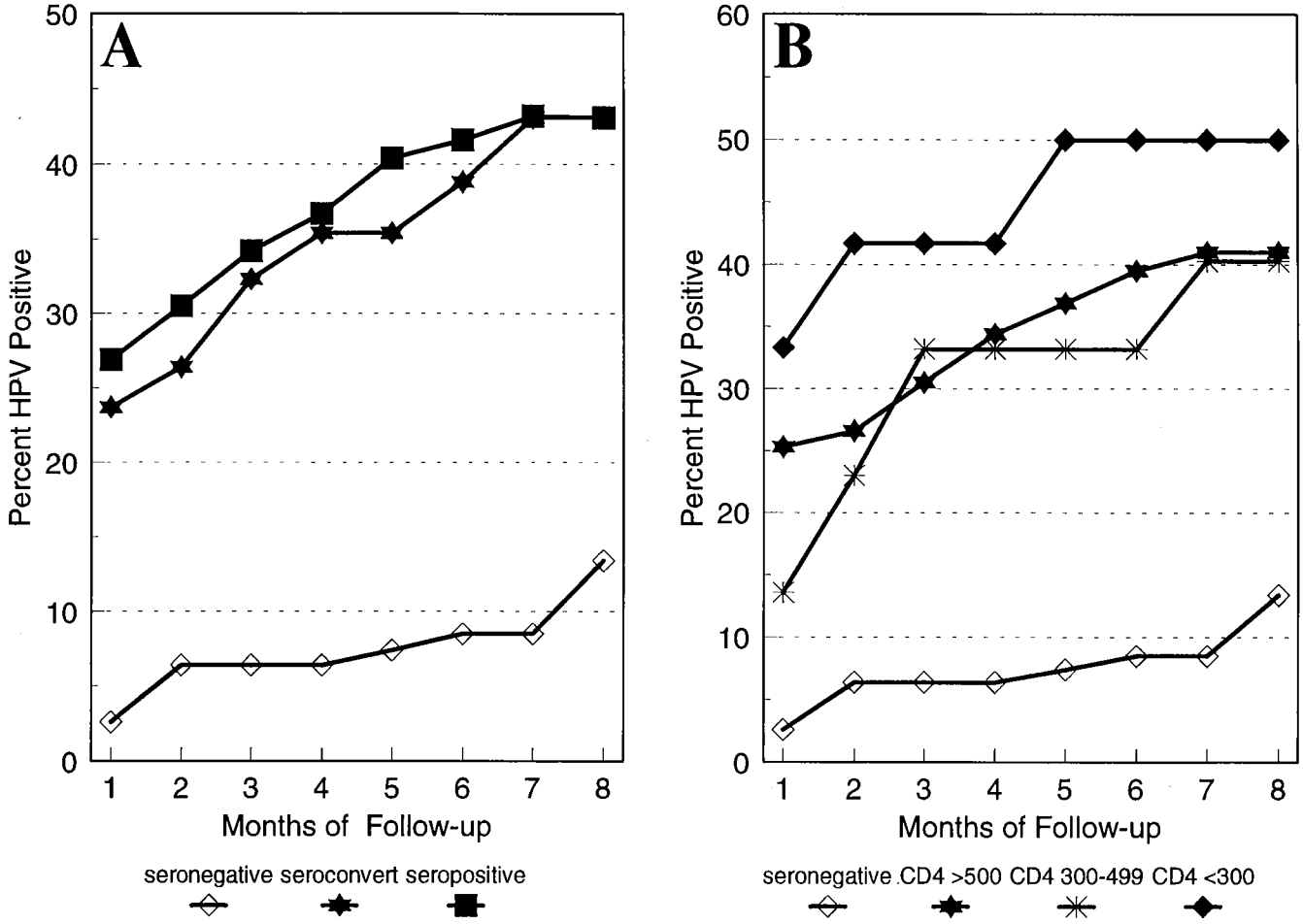

Figure 1. HPV detection by Viratype in HIV-1–seropositive, –seroconverter, and –seronegative prostitutes from Kinshasa, Zaire. Samples positive for any HPV DNA (types 6/11, 16/18, or 31/33/35) are shown. Women who dropped out from month to month are accounted for; thus, graph is representative of population sampled during particular month. **A**, By serostatus; **B**, by CD4 cell count/mm^3. Seronegative women were included without regard to CD4 cell count.

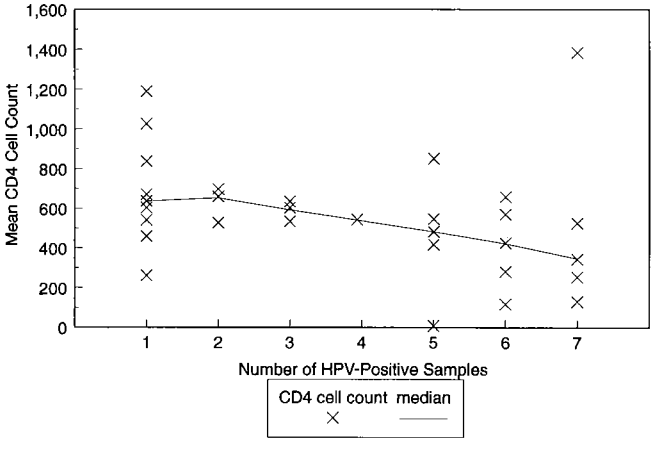

Figure 2. HPV persistence as shown by frequency of HPV DNA detection in HIV-1 seropositive women with at least 7 serial samples grouped by CD4 cell count/mm^3 (Spearman rank order correlation coefficient).

possibility, the quantity of HPV DNA in samples from HIV-1–seropositive women and –seronegative women was determined. No significant difference was detected: 49 of 103 HIV-1–seropositive women had positive samples (average, 24.9 pg of HPV DNA) and 13 of 101 –seronegative women had positive samples (average, 21.2 pg of HPV DNA).

Discussion

Our findings were in agreement with the initial case-control study of this population [4] and with studies of other populations showing that cervical HPV DNA detection was highly associated with HIV-1 seropositivity. Figure 1 illustrates the dramatic difference in HPV DNA detection among HIV-1–seropositive and –seronegative women during an extended follow-up period. This figure also shows that single time point detection rates of cervical HPV infection in HIV-1–seropositive women were insufficient for assessing the true

prevalence of HPV infection and that serial sampling of the cervix gave more accurate prevalence estimates for HPV status.

The prevalence of HPV in HIV-1–seronegative women was surprisingly low for this high-risk population but was consistent with other studies in this population and in lower-risk populations in Kinshasa [4, 9]. It may be that the VT assay was not sensitive enough to detect low-level infections that would be detected by polymerase chain reaction. This might indicate that these women have developed an immune response that keeps the virus in check.

Infection with HIV-1 disrupts immune regulation and ultimately results in severe immunosuppression. Some evidence indicates that immunosuppression is a mechanism for the HIV-1 and HPV association [3]. However, during the 8-month period of our study, most HIV-1–seropositive women were asymptomatic and had CD4 cell counts $>300/mm^3$, and still the association between HIV-1 and HPV was strong. In addition, women who had recently seroconverted to HIV-1 had an increased risk of HPV infection similar to that of women infected with HIV-1 for a longer time. We did demonstrate a trend of HPV persistence with decreasing CD4 cell counts, but the median CD4 cell count of $380/mm^3$ in women HPV-positive in all serial samples would not be categorized as severe immunosuppression or AIDS. Other mechanisms to explain the strong association between HIV-1 and HPV include virus-virus interactions [6] and the possibility that HIV-1 infection initially disrupts immune regulation without overt immunosuppression [13], resulting in increased HPV DNA replication.

No association between HIV-1 and specific HPV genotypes was observed. This confirmed previous observations in this population [5] and extends the observations to include statistically significant numbers of women and more genotypes. There was a high prevalence of HPV types 31/33/35 and mixed infections in this population. However, even though more women had HPV types 31/33/35, the risk of infection with any of the other detected HPV types was the same as that for HPV types 31/33/35.

The hybrid capture assay uses a mixture of probes to detect intermediate- and high-risk HPV genotypes. Using this expanded probe, we still found a strong association of intermediate- and high-risk HPV types (45/51/52/56) with HIV-1 infection. Interestingly, a large proportion of HPV infections detected in both the HIV-1–seropositive and –seronegative groups was types 45/51/52/56 (23% and 8%, respectively). Investigators in France also found that HPV types 45, 51, 52, and 56, in addition to HPV types 30, 39, and 61, were prevalent in women with low-grade cervical intraepithelial neoplasia [14]. In contrast, the prevalence of HPV types 45, 51, 52, and 56 was low in populations from the United States (only 10/500 women attending a sexually transmitted disease clinic) [15]. This observation demonstrated that HPV infection and associated anogenital disease in both HIV-1–seropositive and –seronegative women were associated with a heterogenous, pathologically diverse group of genital HPVs that may vary depending on the demographic characteristics and geographic location of the population under study.

The association between HPV and HIV-1 could not be explained by differences in the quantity of HPV DNA, since women infected with HIV-1 and seronegative women had similar quantities of HPV DNA types 16, 18, 31, 33, 35, 45, 51, 52, or 56. The comparison was only between women who had enough HPV DNA to detect by these assays and demonstrated that in any woman there was an average amount of HPV DNA detected. Thus, the spectrum of HPV infection as measured by quantity of HPV DNA was roughly the same between HIV-1–seropositive and –seronegative women.

This study identified significant differences in the detection of multiple genotypes of HPV DNA in HIV-1–seropositive and –seronegative women and demonstrated that these differences were not entirely due to HIV-1–induced immunosuppression as measured by CD4 cell counts. Any mechanism(s) seeking to explain the association between the two viruses must be consistent with these observations.

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Serum 2′-5′-Oligoadenylate Synthetase Levels and Clinical Response to Interferon-β Therapy in Women with Genital Human Papillomavirus Infection

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To investigate the activity of parenterally administered interferon-β (IFN-β) in women with genital human papillomavirus (HPV) infection, serum 2′-5′-oligoadenylate synthetase (2′-5′OAS), an enzyme induced by IFNs, was measured before, during, and after therapy. Serum levels of 2′-5′OAS significantly increased during therapy ($P = .005$). The patients classified as not responding to treatment at 3 months of follow-up had baseline levels of 2′-5′OAS significantly higher than those who responded to treatment ($P = .0011$). Differences between the IFN-β–treated group and untreated controls appeared at day 6 ($P = .0019$). 2′-5′OAS is a useful parameter in monitoring parenterally administered IFN-β. The data suggest that women with genital HPV infection and elevated baseline levels of 2′-5′OAS are unlikely to respond to therapy with IFN-β.

Genital infection due to human papillomavirus (HPV), clinical, subclinical, or latent, is one of the most frequently diagnosed sexually transmitted diseases [1]. The epidemiologic and clinical relevance of this infection can be found in the central role that HPV plays (certain strains in particular) in the genesis of squamous neoplasias of the lower genital tract of both men and women [1].

The therapeutic strategies most commonly used until now, based on the physical or chemical destruction of the viral lesions, have been marred by frequent persistence or relapse due to chronicity of the infection or by the presence of virus in normal appearing areas [2]. Therefore, there has been interest in drug therapies for HPV infection utilizing antiviral, immunoregulatory, and antiproliferative agents, such as interferons (IFNs), which may be able to eradicate the infection [3]. Several studies have used the fibroblast-derived IFN-β with different routes of administration and regimens [4]. However, the results of these studies could not be confirmed by others [5]. Therefore, a definitive drug therapy to eradicate HPV is not yet available.

IFN-β, unlike IFN-α, cannot be detected in the circulation after administration. Thus, it has not been possible to directly evaluate the kinetics of this drug or its bioavailability after intramuscular administration [3].

One mechanism of action of IFNs is that they inhibit effective translation of mRNA through the expression of the 2′-5′-oligoadenylate synthetase (2′-5′OAS) pathway [6]. 2′-5′OAS is induced by IFNs and activated by double-stranded RNA during viral replication, and this enzyme activates a ribonuclease that degrades viral mRNA and inhibits viral protein synthesis [6]. An increase in 2′-5′OAS has been observed during many viral [7] and autoimmune diseases [8].

IFN-β induces significant increases in 2′-5′OAS activity in monocytes and T and B lymphocytes, both in vivo and in vitro [9]. An increase in serum 2′-5′OAS levels has been observed in hairy cell leukemia and chronic B cell leukemias during chemotherapy with pentostatin. The median increase in 2′-5′OAS among responders was highly significant com-

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