The Impact of Human Immunodeficiency Virus Type 1 Status on Human Papillomavirus (HPV) Prevalence and HPV Antibodies in Serum and Cervical Secretions

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Human immunodeficiency virus (HIV) type 1–infected (HIV-positive) and –uninfected (HIV-negative) sex workers were examined for the presence of cervical human papillomavirus (HPV) DNA. Cervicovaginal rinse and serum samples from these women were examined for IgG and IgA antibodies to HPV-16 virus-like particles (VLP-16) by ELISA. The HIV-positive women displayed a significantly higher prevalence of HPV DNA (40/47 [85%]) than did the HIV-negative women (22/52 [42%]; \( P = .00001 \)). Both HIV-positive and HIV-negative sex workers displayed a high seroprevalence rate for anti–VLP-16 IgG antibodies (27/40 [68%] and 30/43 [70%], respectively), but significantly fewer HIV-positive women than HIV-negative women had anti–VLP-16 serum IgA (6/40 [15%] vs. 17/43 [40%], respectively; \( P = .012 \)). Significantly more HIV-positive women than HIV-negative women had cervical anti–VLP-16 IgG antibodies (16/49 [33%] vs. 6/63 [10%], respectively; \( P = .002 \)) but not IgA antibodies (\( P = .3 \)).

Seroreponses to HPV-6 and HPV-16 virus-like particles (VLP-6 and VLP-16) have been reported in HIV-positive homosexual men [5], in whom the presence of anal warts corresponded with anti–VLP-6 antibodies and the anti–VLP-16 antibodies with high-grade anal intraepithelial lesions. There have been no reports, to our knowledge, on the prevalence of serum or cervical antibodies to HPV antigens in HIV-positive women. Populations at high risk of HPV infection have shown a high seroprevalence of anti–VLP-16 IgG antibodies, which was strongly associated with sexual behavior [6].

This study compared the cervical and systemic antibody responses to VLP-16 of HIV-1–seropositive (HIV-positive) and HIV-1–seronegative (HIV-negative) sex workers, at high risk of HPV infection, to investigate the nature of the immune response to HPV infection. The HPV-16 DNA status of the groups of women were compared and were related to their anti–VLP-16 systemic and cervical antibody responses.

Materials and Methods

Study population and sample collection. A joint initiative between the United Nations Programme on HIV/AIDS and the Medical Research Council of South Africa has funded an efficacy trial, on a microbicide to prevent HIV infection, among a group of female sex workers operating at truck stops in the KwaZulu/Natal midlands of South Africa [7]. A recombinant HIV-1/HIV-2 ELISA (Abbott, Abbott Park, IL) and the Vironostika HIV Uniform II Micro-ELISA 4 system (Organon Teknika, Boxtel, The Nether-
Figure 1. Box plot analysis of the IgA and IgG antibody optical density (OD) values (A 492) obtained for cervical lavage (CVL) specimens (A) and serum samples (B) of human immunodeficiency virus-positive (HIV+) and -negative (HIV-) women. Error bars represent the 10th and 90th percentiles. Median OD values are represented by the lines in the boxes. Mean CVL OD values were 0.08 for HIV+ IgG, 0.05 for HIV- IgG, 0.08 for HIV+ IgA, and 0.02 for HIV- IgA. Mean serum OD values were 0.63 for HIV+ IgG, 0.90 for HIV- IgG, 0.17 for HIV+ IgA, and 0.26 for HIV- IgA.

lands) found 50.3% of the women to be HIV positive. Samples from some of the women, obtained before commencement of the microbicide efficacy trial, were made available for use in the present study. Cervicovaginal rinse (CVR) specimens from 112 women and paired serum samples from 83 of the 112 women were collected during November and December 1998. Saline (5 mL) was inserted into the vaginal cavity, with a 1 min wait before »3.0–3.5 mL of CVR was retrieved with a syringe. CVR specimens and serum were stored at −70°C until required. Women with bloodstained CVR samples ( ) were excluded from the study. The demographic data of the excluded population did not vary significantly from those of the included population (data not shown).

Of the 83 paired CVR and serum samples, 40 were from HIV-positive women and 43 from HIV-negative women. Of the 29 CVR-only samples, 9 were from HIV-positive and 20 from HIV-negative women. The 112 women were 16–48 years old (mean age, 26 years), and all had >6 sex partners per week (mean, 18.6; range, 7–40). The frequency of condom use was very low (66% used condoms for <25% of sexual episodes). Most women had not been cytologically tested for cervical neoplasia. CD4+ cell counts were available for 11 HIV-positive women, 4 of whom had cell counts <500 × 10⁹/L but none of whom had counts <200 × 10⁹/L.

ELISAs and polymerase chain reaction (PCR) analyses. The VLP-16 ELISAs were conducted as described elsewhere [8], with modifications. HPV-16 VLPs used in the ELISAs were kindly supplied by MedImmune, Inc. (Gaithersburg, MD), and bovine papillomavirus VLPs were supplied by Dr. R. Rose (Rochester University, Rochester, NY). VLPs were used at a coating concentration of 2 µg/mL. The CVR samples were diluted 1:1, and serum was diluted 1:20. Serum ELISA cutoff values were estimated on the basis of children’s sera, as described elsewhere [8]. In the absence of control CVR specimens, cutoff levels were determined as described by Wang et al. [9]. PCR determinations on CVR samples were performed by amplification of the L1 region of HPV DNA with MY primers and an HPV-16–specific primer, as described elsewhere [8]. Protein assays were performed on CVR samples (bicinchoninic acid; Pierce, Rockford, IL), and total immunoglobulin estimations were performed, as described by Rudin et al. [10], using alpha-chain–specific goat anti-human IgA or gamma-chain–specific IgG (Dako, Carpinteria, CA) and, as standards, polyclonal human IgA or IgG (Dako). Bound total immunoglobulins were detected as described elsewhere [8], and the concentration of immunoglobulin in the diluted samples was estimated from the standard immunoglobulins. Multiple regression analysis was performed, to determine the impact of possible confounders (years as a sex worker, number of partners per day, condom use, gonorrhea infection, syphilis infection and HIV-1 seropositivity) on the significance of the different HPV DNA prevalence rates in the HIV-positive versus the HIV-negative women.

Results

CVR samples from 63 HIV-negative and 49 HIV-positive women were tested by ELISA for IgA and IgG antibodies to VLP-16. Figure 1A shows a boxplot analysis of the CVR optical density (OD) values obtained in the ELISAs. Anti–VLP-16 antibody levels in CVRs were low but were similar to the levels described by Wang et al. [9]. The mean OD values for CVR anti–VLP-16 IgA and IgG were not significantly different in the HIV-positive and HIV-negative women (P = .08 and P = .42, respectively; figure 1A). The effect of HIV-1 status on anti–VLP-16 antibodies in serum was tested in 40 HIV-positive and 43 HIV-negative individuals. Boxplots of the ELISA OD values obtained are shown in figure 1B. Mean OD values for
The aims of this study were to determine the prevalence of HPV infection in the sex workers and to evaluate the HPV-specific antibody responses systemically and locally in HIV-positive sex workers and compare them with the responses of HIV-negative sex workers. We hoped that this would give us insights into the response to HPV infection in immunocompetent individuals. The nature of the lifestyle of the sex workers studied, with 7–40 partners per week, would result in a high level of exposure to HPV. This was confirmed by the high rate of HPV infection found in the HIV-positive sex workers (85%) and is consistent with findings reported by others [1, 2]. Despite their high-risk lifestyle and large number of sex partners, only 42% of the HIV-negative sex workers, versus 85% of the HIV-positive women, were HPV DNA positive at the cervix. This could indicate that the immunocompetent, HIV-negative women were better able to clear the HPV infection than were the HIV-positive women. There were no significant differences in length of time as a sex worker, number of sex partners, or condom use between HIV-negative and HIV-positive women, so there was no definable difference in the rate of HPV exposure between the 2 groups.

More HIV-positive women than HIV-negative women were found to have mounted an anti–VLP-16 IgG response at the cervix (P = .002). When the presence of anti-HIV antibodies in cervical secretions of HIV-positive individuals is assessed, IgG antibodies have been shown to predominate over IgA [11], and this correlates with a normal or impaired anti-HIV IgA local response [12]. In the present study, anti–VLP-16 IgG antibodies were found to be more prevalent in CVRs of HIV-positive women, and both total IgA and IgG levels were higher in the CVRs of the HPV-positive group than in those of the HPV-negative group. HIV-positive women would have had HPV-16 infection longer than the HIV-negative group who were able to clear the infection and, therefore, would be more likely to produce anti–VLP-16 antibodies. Also, the local mucosal immune response in HPV-positive women is reportedly enhanced, in comparison with that of HIV-negative women [11]. It has been postulated that systemic polyclonal B-cell activation, which is characteristic of HIV-1 infection, could affect the secretory immune system at the cervix and increase local immunoglobulins [13].

### Table 1. A comparison of the total protein and total immunoglobulin levels and IgG and IgA responses to human papillomavirus–16 virus-like particles (VLP-16) in cervico-vaginal rinse (CVR) specimens and serum of human immunodeficiency virus (HIV)–positive and HIV-negative sex workers.

<table>
<thead>
<tr>
<th>Test</th>
<th>HIV positive</th>
<th>HIV negative</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVR total protein, μg/mL</td>
<td>286.3 ± 215.2 (37)</td>
<td>237 ± 284.6.4 (57)</td>
<td>—</td>
<td>.436</td>
</tr>
<tr>
<td>CVR total IgA, μg/mL</td>
<td>25.4 ± 20.3 (44)</td>
<td>14.6 ± 16.8.6 (61)</td>
<td>—</td>
<td>.008</td>
</tr>
<tr>
<td>CVR total IgG, μg/mL</td>
<td>128 ± 92.7 (46)</td>
<td>46.5 ± 49.9.6 (61)</td>
<td>—</td>
<td>.000</td>
</tr>
<tr>
<td>CVR VLP-16 IgA positive</td>
<td>11/49 (22)</td>
<td>9/63 (14)</td>
<td>1.74 (0.59–5.12)</td>
<td>.305</td>
</tr>
<tr>
<td>CVR VLP-16 IgG positive</td>
<td>16/49 (33)</td>
<td>6/63 (10)</td>
<td>4.16 (1.45–14.76)</td>
<td>.002</td>
</tr>
<tr>
<td>Serum VLP-16 IgA positive</td>
<td>6/40 (15)</td>
<td>17/43 (40)</td>
<td>0.27 (0.08–0.87)</td>
<td>.012</td>
</tr>
<tr>
<td>Serum VLP-16 IgG positive</td>
<td>27/40 (68)</td>
<td>30/43 (70)</td>
<td>0.9 (0.32–2.52)</td>
<td>.82</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD (n) or no./total (%). OR, odds ratio; CI, confidence interval.

* By t test for equality of means.
* By χ² analysis.

Discussion

The aims of this study were to determine the prevalence of HPV infection in the sex workers and to evaluate the HPV-specific antibody responses systemically and locally in HIV-negative women, but not for anti–VLP-16 IgA, were significantly higher in HIV-negative than in HIV-positive women (P = .04 and P = .26, respectively; figure 1B).

The percentage of women positive for anti–VLP-16 antibodies in CVRs and serum and the total IgA and IgG levels in CVRs are shown in table 1. There was a significant difference between the number of HIV-positive women and the number of HIV-negative women with CVR anti–VLP-16 IgG antibodies, but not IgA antibodies (P = .002 and P = .3, respectively). There was a significant difference between the number of HIV-negative women positive for anti–VLP-16 serum IgA and the number of HIV-positive women (17/43 vs. 6/40; P = .012). There was no significant difference between the number of women with anti–VLP-16 serum IgG antibodies in the HIV-positive group and HIV-negative group (27/40 vs. 30/43; P = .82). The total immunoglobulin levels (but not total protein) in CVRs were significantly higher in HIV-positive women than in HIV-negative women (table 1), the former being similar to levels described elsewhere for HIV-positive individuals and controls [11].

To investigate the effect of HIV-1 status on HPV infection, the presence of cervical HPV DNA was determined in 99 sex workers (47 HPV positive and 52 HIV negative). Forty (85%) of 47 HIV-positive women were HPV DNA positive, whereas 22 (42%) of 52 HIV-negative women were positive for HPV DNA. The difference was significant (P = .00001). Multiple regression analysis found that the only significant variable associated with HPV infection was HIV-1 seropositivity (P = .0347). This indicates that HIV-1 alone influences HPV infection, with or without the other factors. HPV-16–specific typing was available for 57 (38 HPV positive and 19 HIV negative) of the 62 HPV DNA–positive specimens, and 6 (10.5%) were HPV-16 positive (5 specimens from HIV-positive women and 1 specimen from an HIV-negative woman).
Both anti-HIV IgA and IgG antibodies have been shown to impair virus transmission across the cervicovaginal mucosa, with IgA being more efficient [14]. In the present study, more HIV-positive than HIV-negative women had CVR anti-VLP-16 antibodies (and total immunoglobulins) and, by inference, should also have an improved protection of the local mucosa against HPV infection. All the sex workers had similar levels of other sexually transmitted diseases and other vaginal infections, and thus local inflammation caused by these could not be a contributing factor. However, even though more HIV-positive women displayed local antibody responses, most of them were still becoming infected with HPV. The local HPV antibody response of HIV-positive women, therefore, does not appear to be relevant to their increased rate of HPV infection, but it may relate to some other aspect of their HIV-disregulated immune response.

The majority of the sex workers (HIV positive, 68%; HIV negative, 70%) had anti-VLP-16 serum IgG antibodies. Serum anti-VLP-16 IgG antibody responses relate to the number of sex partners an individual has [6]. All the women under study had 7–40 sex partners per week, with an insignificant difference between the average number of partners per week of the HIV-negative women (18.3 partners) and the HIV-positive women (18.7 partners). Their large number of sex partners probably accounts for the large percentage of HIV-positive and HIV-negative women with serum anti-VLP-16 IgG. Although only a few sex workers were HPV-16 DNA positive, the anti-VLP-16 serum IgG antibodies in 68%–70% of them could be indicative of previous infections.

Significantly more HIV-negative women than HIV-positive women were shown to have mounted anti-VLP-16 IgA serum antibody responses ($P = .012$). It would appear, therefore, that anti-HPV serum responses, and possibly the serum IgA response, could be indicative of the HIV-negative sex workers’ ability to clear the high level of HPV infection to which they were exposed, and not their local antibody responses. These women were able to clear HPV infection, with a resultant reduced local HPV DNA prevalence, in comparison with the HIV-positive women. Although it is unclear how systemic antibodies function to eliminate virus, anti-VLP-16 serum IgA levels have been shown to correlate with virus clearance in infected individuals [15]. We propose that the relatively few HIV-positive women with anti-VLP-16 serum IgA in the present study (6/40) could be indicative of an increased risk of cervical disease in these women. [15].

In conclusion, it would appear that, despite similar high levels of HPV-16 exposure, it is a serum IgA response that indicates the ability of the HIV-negative sex workers to prevent persistent HPV-16 infection.

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References