Specific serum IgG, IgM and IgA antibodies to human papillomavirus types 6, 11, 16, 18 and 31 virus-like particles in human immunodeficiency virus-seropositive women

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To evaluate the humoral immune response to human papillomavirus (HPV) in women infected with human immunodeficiency virus (HIV), serum samples of 83 HIV-positive individuals were analysed by ELISA for specific antibodies of the isotypes IgG, IgA and IgM recognizing HPV-6, -11, -16, -18 and -31 L1 virus-like particles (VLPs). Papillomavirus-related lesions were present in 30 of 83 HIV-positive women. Twenty-one women (25%) presented with high-/intermediate-grade anogenital squamous intraepithelial lesions. PCR analysis and sequencing for HPV typing was done from biopsy specimens of 18 women; PCR-positive results were obtained in 90% of cases. In addition, HPV DNA hybrid capture assays were performed from cervical swabs of 58 HIV-positive women, 53% of whom had a positive result for high-risk HPV. Overall, positive IgG reactivity to HPV-6/-11 and HPV-16/-18/-31 was seen in 19%/31% and 49%/30%/24% of HIV-positive women, respectively. HPV-seropositivity was even higher than in 48 HIV-negative cervical intraepithelial neoplasia/cancer patients with percentages as follows: 8%/2% and 31%/15%/15%. This difference was significant for HPV-16 (P = 0.046). IgA responses were comparable to IgG. IgM responses were low. The extraordinarily high rate of antibodies to the capsid protein L1 of high-risk HPVs (HPV-16, -18 and/or -31) in 58% of HIV-positive women compared to 19% (P = 0.00001) of 102 healthy HIV-negative control women suggests a high lifetime cumulative exposure to HPV and increased expression of capsid proteins due to cellular immunodeficiency in HIV-infected women.

Introduction

The association of human papillomaviruses (HPV) and genital diseases is well established (Ho et al., 1998; Maiman et al., 1997; Nonnenmacher et al., 1995; Palefsky et al., 1998). Mucosal low-risk HPV types (HPV-6 and -11) are the causative agents of benign condylomata acuminata (Nouvo et al., 1991; Reid et al., 1987); in contrast, high-risk HPV types (HPV-16, -18 and -31) have been implicated in the pathogenesis of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), anal intraepithelial neoplasia (AIN) and anogenital cancer (Pfister et al., 1987). HIV-infected persons are five times as likely to develop HPV-related diseases, such as CIN or AIN, as compared to HIV-negative individuals (Laga et al., 1992; La Ruche et al., 1998; Palefsky et al., 1998).

Several studies have shown that HPV is mainly a transient cervicovaginal infection in immunocompetent individuals with a median duration of 8 months (Ho et al., 1998). Indirect evidence suggests that primarily impaired cellular immune responses facilitate the development of persistent HPV infection and consecutive malignant transformation (Nakagawa et al., 1997; Shamanin et al., 1996). Early, but not
self-limited HPV infection with persistent virus shedding, as detected in a high proportion of cervicovaginal lavage specimens with HPV PCR, is considered to be critical for the development of intraepithelial neoplasia in women infected with human immunodeficiency virus (HIV) (Sun et al., 1997).

DNA detection assays such as PCR for HPV are expensive and time-consuming. Moreover, these methods are not sufficient to exclude prior HPV exposure. In latency, viral DNA could persist in clinically unsuspicious regions from various anatomical areas where specimens for screening are not routinely obtained. Serological HPV assays could supply additional information to identify persons at risk of HPV-related cancers. Papillomavirus L1 capsid proteins that self-assemble into virus-like particles (VLPs) are effective antigens for serological studies and can be used to detect type-specific humoral immune responses in patient sera (Heim et al., 1995; Kirnbauer et al., 1992). It is important to note that the recognized antibodies directed against VLPs are thought to neutralize infectious viruses in model systems (Christensen et al., 1994). However, neutralizing antibodies do not induce regression of established infection in animal models.

So far, studies on serum antibodies to HPV capsids have focused on HPV seroprevalence in immunocompetent women (Carter et al., 1996; Heim et al., 1995; Kirnbauer et al., 1994; Nonnenmacher et al., 1995) and HIV-infected men (Hagenesse et al., 1997) indicating no lack of humoral immunity in the latter patient group. We are not aware of any study analysing HPV-specific antibodies in immunosuppressed HIV-positive women. The aim of our study was to evaluate humoral (IgG, IgA and IgM) responses to intact HPV L1 VLPs of HPV-6, -11, -16, -18 and -31 in women infected with HIV.

Methods

■ Patients and specimens. The AIDS Outpatient Clinic at the University of Innsbruck is the only primary care and referral centre for patients with HIV/AIDS in the Austrian Tyrol. Nearly all of the patients with AIDS (> 95%) reported to the health authorities are in treatment at this AIDS Outpatient Clinic. Eighty of 102 women known to be HIV-infected (aged 22–72 years, median 37) were regularly seen at our gynaecological outpatient department. Gynaecological data (between October 1987 and April 1998) were available from these women and all of them were included in our analysis. Each subject underwent routine gynaecological examinations, including colposcopy and cervical smears for cytology, at intervals of at least 3–12 months. Cytological classification followed the Munich classification system (i.e. Papinicolaou (Pap) IIID, signs of mild or moderate dysplasia, CIN I or CIN II; Pap IV, signs of severe dysplasia or carcinoma in situ, CIN III). Hybrid capture HPV DNA data for high- and low-risk HPV DNAs were obtained in 58 cases. Eighteen patients received surgical treatment at our clinic. PCR analysis and sequencing for HPV typing was done with formalin-fixed paraffin-embedded tissues from all 18 surgically treated patients.

■ Control subjects. Sera from 102 female patients (aged 22–67 years, median 35), randomly chosen from HIV-negative women visiting the gynaecological outpatient clinic representing a cross-section of women attending a university hospital in Austria, with no bias towards social or economic status were analysed. The reasons for consulting the clinic were routine gynaecological examinations, e.g. to receive oral contraceptives. Patients with drug abuse, malignancies, pregnancies, pelvic inflammatory disease, pathological PAP smears and existing or previous HPV-related diseases (e.g. genital warts, CIN, VIN) were excluded. In 36 of these women, an HPV PCR test (genital swab) was performed and was negative. An additional control group with HPV-associated diseases consisted of 48 non-pregnant, HIV-negative women (aged 20–72 years, median 32.5) with intermediate- or high-grade CIN, including 14 patients with CIN III and six cases with invasive cervical cancers. The majority of these patients were high-risk HPV DNA-positive.

■ HIV serology. Sera were screened for HIV antibodies using the Enzygnost Anti-HIV-1/2 ELISA (Behring) and confirmed by Western Blot (Du Pont or Biotechnologies) in at least two different samples.

■ Sampling. Serum samples for HPV analysis obtained from all women were frozen, preserved at −80 °C and used later for HPV serology.

■ HPV DNA typing and HPV PCR analysis. Nucleic acid hybridization tests (Digene Diagnostics) were performed from cervical or vulvar smears according to the manufacturer’s instructions for the presence of HPV-6/-11/-42/-43/-44 (low-risk) and HPV-16/-18/-31/-33/-35/-45/-51/-52/-56 (high-risk) DNA in cervical and vulvar smears. PCR was performed as follows. Formalin-fixed paraffin-embedded tissues were processed with the QiAamp Tissue kit (Qiagen) according to the manufacturer’s instructions. Purified total cellular DNA (10 µl) was employed in each PCR reaction (25 ng/µl). Cervical scrapes of patients were processed as described by Van den Brule et al. (1990). GP5+ and GP6+ general primer PCR and TS6, TS11, TS16, TS18 and TS31 type-specific PCRs were then performed as previously described (Van den Brule et al., 1990; De Roda Husman et al., 1995). Samples negative for GP5+ and GP6+ were additionally analysed with a more sensitive nested PCR for the detection of genital HPVs. Degenerate primers A5 (5’ TAT TTY SCT WCT CCT AGT GG 3’, HPV-16 L1 gene nt 871–900) and A10 (5’ CTK CCC AAC GGA WAY TGR TC 3’, HPV-16 L1 gene nt 1397–1378) were used for first-step PCR. A6 (5’ GCM CAG GGM CAY AAT GG 3’, HPV-16 L1 gene nt 946–965) and A8 (5’ CAA ART GCC ART CYT CCA A 3’, HPV-16 L1 gene nt 1213–1196) were used for second-step PCR. A6 and A10 are modified My11 and My09 primers, respectively (Bauer et al., 1991). First-step amplification reactions were performed in 50 µl containing 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 5 mM MgCl2, 0.05% (w/v) gelatin, 200 µM of each dNTP (Boehringer Mannheim), 2.5 units Taq DNA polymerase (Pharmacia), and primers A5 and A10 (0.5 µM each). Second-step amplification reactions were performed in 50 µl containing 50 mM KCl, 10 mM Tris–HCl (pH 9.0), 1.5 mM MgCl2, 200 µM of each dNTP, 2.5 units Taq DNA polymerase, and primers A6 and A8 (0.5 µM each). Three microlitres from the first-step PCR was used as input for the second-step PCR. Cycling conditions for first-step PCR were 95 °C for 3 min followed by five cycles of 95 °C for 45 s, 50 °C for 45 s and 72 °C for 1 min, followed by 30 cycles of 95 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min. Cycling conditions for second-step PCR were 95 °C for 3 min, followed by 35 cycles of 95 °C for 45 s, 56 °C for 45 s and 72 °C for 1 min. The size of the second-step PCR product (A6/A8) was 271 bp. PCR products (5 µl) were separated on 3% agarose gels and visualized by ethidium bromide staining. To avoid PCR contamination, the suggestions of Kwok (1990) were diligently considered. Five negative controls (water instead of patient samples and patient samples known to be HPV-negative) were included in each PCR run. HPV typing was either performed by type-specific PCRs (Van den Brule et al., 1990) or by direct sequencing of PCR products and comparison of the obtained sequences with an HPV
database (Myers, 1997). Direct sequence analysis of purified PCR products (QIAquick PCR purification kit; Qiagen) was performed with an ABI Prism 377 DNA sequencing using the Taq FS BigDye-Terminator cycle sequencing method (PE Applied Biosystems). Depending on the PCR product to be sequenced, A6 or GP5 + was used as sequencing primer.

**Preparation of baculovirus-expressed VLPs.** Recombinant baculoviruses expressing HPV-6 L1 (a gift of A. B. Jenson, Georgetown University, Washington, DC, USA; HPV-11, -16, -18, -31 and bovine papillomavirus (BPV)-1 L1 protein-expressing baculoviruses (provided by J. T. Schiller, National Institute of Health, Bethesda, MD, USA) were produced as previously described (Kimbauer et al., 1992; Christensen et al., 1994). The method of VLP purification has also been described previously (Christensen et al., 1994).

**MAbs.** Type-specific MAbs (H6.B10, H11.B2, H16.V5, H18.R5, H31.A6 and B1.A1) recognizing surface conformational epitopes on intact HPV-6, -11, -16, -18, -31 and BPV-1 L1 VLPs as control antigens, respectively, were generated as described previously (Christensen et al., 1996a, b). They were used as positive controls in serological assays.

**HPV ELISA.** HPV-6, -11, -16, -18, -31 and BPV-1 L1 VLPs were used as antigens in direct ELISAs. The method was previously described (Kimbauer et al., 1992; Christensen et al., 1994). Briefly, VLPs diluted in PBS (1 µg VLP protein in 100 µl PBS) were attached to wells of round-bottom ELISA plates (Nunc Brand Products and Petra Plastic 96 rounded wells γ-sterilized Typ F, ELISA 11041 E) by incubating 90 µl VLP solution per well at room temperature for 45 min. VLP-free PBS-incubated wells were used as negative control wells. All plates were washed three times with PBS (SLT Labinstruments 812 SW2) and were incubated with 200 µl milk buffer (1 g Fixmich Instant + 20 ml Dulbecco’s PBS) per well for 30 min (room temperature). Patient sera were heat-inactivated at 56 °C for 30 min and used at a final serum dilution of 1:100. Each patient’s serum (25 µl) and positive and negative controls were tested in duplicate wells and incubated for 1 h and then washed again three times. MAbs (100 µl) corresponding to VLP types (positive control wells) were diluted with 5% milk buffer (final dilution of 1:200). Subsequently, the wells were incubated with goat anti-human IgG (1:10,000), IgA (1:2000), IgM (1:2000) and RαM (1:1000) antibodies (Sigma). After incubation for 30 min (room temperature), the wells were washed three times and substrate (Tris buffer with p-nitrophenylphosphate tablets) was added. Absorbances at 405 nm were read on a microplate reader (SLT Labinstruments 400 ATC; Software Easy-Fit) 30, 60 and 90 min after adding substrate. Means and SD of duplicate wells were calculated as final A₄₅₀ values for each serum sample.

**Statistical methods.** In all experiments, patient and control sera were analysed together. Means and SDs of ELISA absorbency values of the control group were used statistically to define positive and negative patient sera for each target antigen (HPV-6, -11, -16, -18, -31 and BPV-1) and serum antibody isotype (IgG, IgA and IgM). Individual values in the control group exceeding the mean + (3 × SD) were excluded and the remaining readings were averaged again until no further values exceeded the recalculated mean + (3 × SD). Samples with values greater than the recalculated final mean + (3 × SD) were designated positive for reactivity to the appropriate antigen in the current experiment. For scalar variables, the non parametric Mann–Whitney U test was used for statistical analysis, because group A₄₅₀ values had an asymmetric distribution; Fisher’s exact test was used for categorical variables. P < 0.05 (two-tailed) was considered statistically significant. All calculations were done with SPSS Counting Software (SPSS) 7.0 for Windows 95.

**Results**

**Patient characteristics**

Patient characteristics are given in Table 1. Twenty-five (30%) of the 83 HIV-positive women had AIDS and one woman died as a consequence of cervical cancer. Of the HIV-infected women, 55% had a history of antiretroviral therapy; however, 24% of these were protease inhibitor-naive. HPV-related diseases were seen in 30 of 83 HIV-infected women (36%). In 21 women (25%), intermediate- or high-grade CIN and/or VIN was diagnosed. The median CD4 cell count at the time-point of first detection of HPV-related lesions (253 per μl; range 45–697) was lower than the median CD4 count on the day when the serum sample for HPV serology was taken (331 per μl; range 5–1075), but this difference was not significant (P = 0.5).

**HPV DNA analysis**

Data on cervical and vulvar smears of 58 HPV-infected women, routinely analysed for the presence of HPV DNA by nucleic acid hybridization tests (Digene), were available at different times (at least one up to eight samples per woman). Cumulative prevalences were as follows. Thirty-one (53%) of the 58 women with hybridization data were positive for high-risk HPV DNA.
### Table 2. Results of HPV DNA analysis in comparison with IgG and IgM antibody responses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HPV type(s) detected by PCR</th>
<th>Serotype (IgG)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paraffin Date</td>
<td>Before PCR*</td>
</tr>
<tr>
<td>CIN III</td>
<td>Mixed (6, 18, 31) Apr 97</td>
<td>11, 16, 31 (IgM 6, 11)</td>
</tr>
<tr>
<td>Condylomata acuminata</td>
<td>52 Aug 97</td>
<td>6, 11, 16, 31 (IgM same types)</td>
</tr>
<tr>
<td>CIN III</td>
<td>58 Apr 97</td>
<td>11, 16, 31</td>
</tr>
<tr>
<td>CIN III</td>
<td>16 Jun 96</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>CIN II</td>
<td>58 Jan 97</td>
<td>11, 16, 31</td>
</tr>
<tr>
<td>CIN III</td>
<td>39 Aug 97</td>
<td>11, 16, 31</td>
</tr>
<tr>
<td>CIN III</td>
<td>51 Jun 97</td>
<td>11, 31 (IgM 11)</td>
</tr>
<tr>
<td>CIN III</td>
<td>16 Sep 97</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>VIN III</td>
<td>31 Oct 97</td>
<td>11, 16, 31</td>
</tr>
<tr>
<td>CIN II</td>
<td>16 Feb 97</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>CIN II</td>
<td>34 Feb 97</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>CIN III</td>
<td>51 Jun 97</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>CIN III</td>
<td>16 Jul 95</td>
<td>11, 16, 31</td>
</tr>
<tr>
<td>Cancer</td>
<td>45 Jan 97</td>
<td>18, 31 (IgM 6, 11)</td>
</tr>
<tr>
<td>CIN II</td>
<td>31 Sep 97</td>
<td>6, 31, 16, 31</td>
</tr>
<tr>
<td>Pap IV</td>
<td>ND 74 Sep 97</td>
<td>16, 31, 16, 31</td>
</tr>
<tr>
<td>Condylomata acuminata</td>
<td>ND 16 Apr 98</td>
<td>6, 11, 16, 31</td>
</tr>
<tr>
<td>CIN II</td>
<td>— Nov 96</td>
<td>18, 31, 16, 31</td>
</tr>
<tr>
<td>Condylomata acuminata</td>
<td>— Dec 94</td>
<td>—</td>
</tr>
</tbody>
</table>

* 9–12 months. IgM-negative except where specified.

**nd:** Not done; **—:** negative.

risk HPV DNA and twenty-two (38%) were positive for low-risk HPV DNA. Women with a positive nucleic acid hybridization test for high-risk HPV types remained positive in consecutive tests (mean observation period 35 months) with the exception of four women. One of them became spontaneously negative and three became negative during the follow-up after surgery of HPV-related lesions.

HPV PCR data were obtained from 20 HIV-positive women (Table 2). Paraffin-embedded material was used from all 18 women presenting with HPV-related lesions requiring surgical treatment. Cervical scrapes were obtained from seven women. In five of them, an overlap with paraffin material allowed analyses of smears and tissue in the same patient at two time-points. PCR results were identical in corresponding paraffin and smear samples. Samples of 18 out of 20 patients (90%) were PCR-positive for HPV. Eleven cases (55%) were positive for high-risk HPV DNA. HPV-16 and related types (HPV-31, -33, -35 and -58) were found in 10 women (50%); HPV-18 and the related HPV-45 were in one patient each. HPV-34, -40 and -74 were detected in three samples; all of them represent extremely rare HPV types. HPV-52, which is common in China, and HPV-39, which is almost entirely confined to Central and South America, were found once each. More than one HPV type (HPV-6, -18 and -31) was seen in paraffin-embedded material of one woman. In one sample, the HPV type could not be determined with PCR analysis.

PCR was performed in 45 of 48 HIV-negative, CIN/cancer control patients and revealed HPV DNA in 38 cases (84%). High-risk HPV DNA was detected in 33 cases (73%) and low-risk HPV DNA was found in eight cases (17%) with double infection in three individuals. Typing by sequencing demonstrated HPV-16 DNA in 17 patients (37%). HPV-35 was found in three cases, and HPV-52 and -58 were observed in two cases each. HPV-6, -33, -34, -51, -56 and -73 were each found once and, in the remaining cases, the HPV type was not further determined.

**HPV ELISA: comparison of HIV-positive and HIV-negative women**

HIV-positive women were significantly more likely than HIV-negative women to have detectable levels of antibodies...
Table 3. Frequency of HPV-seropositive samples

Percentages of HPV antibody-positive patient sera are given. High-risk: HPV-16, -18 and/or -31. Low-risk: HPV-6 and/or -11. HIV-positive and HIV-negative women were compared (P values calculated by use of two-tailed Fisher’s exact test).

<table>
<thead>
<tr>
<th></th>
<th>HIV-positive (%; n = 83)</th>
<th>CIN/cancer (%; n = 48)</th>
<th>P value</th>
<th>No lesions (%; n = 102)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG high-risk</td>
<td>58</td>
<td>46</td>
<td>0.02</td>
<td>19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgG low-risk</td>
<td>36</td>
<td>8</td>
<td>0.001</td>
<td>18</td>
<td>0.007</td>
</tr>
<tr>
<td>IgA high-risk</td>
<td>30</td>
<td>19</td>
<td>0.02</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>IgA low-risk</td>
<td>34</td>
<td>8</td>
<td>0.001</td>
<td>7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IgM high-risk</td>
<td>12</td>
<td>4</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>IgM low-risk</td>
<td>15</td>
<td>0</td>
<td>—</td>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

Fig. 1. Serum IgG immune responses to HPV L1 VLPs in four different patient groups. Each bar represents the percentage of HPV antibody-positive sera. Black bar, HIV-positive, all patients (n = 83); dark grey bar, HIV-positive, CIN/cancer (n = 21); light grey bar, HIV-negative, CIN/cancer (n = 48); white bar, HIV-negative controls (n = 102). P < 0.01 (two-tailed Fisher’s exact test) for HPV-16, -18, -31 and -11 when HIV-negative women (black bars) were compared with healthy HIV-negative women (white bars). P < 0.05 for HPV-16 and -11 when HIV-positive women (black bars) were compared with HIV-negative, CIN/cancer women (light grey bars).

HPV ELISA: analyses within the HIV-positive cohort

The detection of high-risk HPV IgG antibodies was more common (though not statistically significant) in HIV-infected patients with clinically evident HPV-related diseases compared with women without genitoanal HPV lesions. An obvious trend (P = 0.07) was seen for HPV-16 antibodies which were more likely detected in patients with HPV-related lesions. Interestingly, women with a history of one or more positive HPV DNA hybrid capture result were not more likely to have any HPV antibodies detected than women with negative HPV DNA tests. We found an antibody detection rate of 83% in 18 HIV-positive, HPV DNA-positive women with HPV-associated diseases which was also not significantly higher than that seen in 21 HIV-positive, HPV DNA-negative women without HPV-related disease (66%, P = 0.17). Mean CD4 cell counts on the day of serological testing had no influence on HPV IgG, IgA or IgM antibody responses, as had prior antiretroviral therapy, clinical category of HIV disease or history of drug abuse.

HPV ELISA: type-specificity

Serotype and sequencing data corresponded most obviously when analysing HPV-16. For example, four of six HPV-16 PCR-positive, HIV-positive women (66%) were also HPV-16 IgG seropositive; the two others revealed serotype 18 or 31 (Table 2). Similarly, nine of 15 HPV-16 PCR-positive, HIV-negative CIN/cancer patients were also HPV-16 IgG seropositive (60%). In five of these nine sera, in addition, HPV-16 IgA antibodies were detected. Antibodies to HPV types other than HPV-16 were rarely detected in this subgroup of HPV-16 PCR-positive patients: HPV-18, -31 and -11 antibodies were present in two patients each (13%), and HPV-6 antibodies were detected once. In contrast, HPV-16 antibodies were seen only in four of 14 HIV-positive, HPV-16 DNA-negative women (28%) and in four of 30 HIV-negative, HPV-
16 DNA-negative CIN/cancer patients (13%). IgG, IgA and IgM reactivity to more than one high- or low-risk HPV type was not more likely in HIV-infected women compared to HIV-negative controls with or without HPV-associated diseases, indicating that the specificity of the serum ELISA test was similar in these two patient groups. Double IgG or IgA reactivity (defined as one serum reactive to two different HPV types) was seen preferentially within the groups of high- or low-risk HPV types in both HIV-positive and -negative patients. For example, in HIV-positive women, 42 sera were IgG-reactive to either HPV-6 or HPV-11, and 29% were found to be positive for both HPV-6 and -11. In HIV-negative women, this percentage was 31%. Double reactivity was less common ($P = 0.069$ in the two-tailed Fisher’s exact test) between the two groups of high- or low-risk HPV types. When, for example, IgG-6 (low-risk type) and IgG-16 (high-risk type) positivity was analysed, the percentage of double reactivity was only 12% in HIV-positive and 11% in HIV-negative sera. These trends were seen with all the VLP types. IgM reactivity was found to be far less type-specific; e.g., IgM-positive sera reacted in 44% with both HPV-6 and -16; in comparison, this percentage is much higher than observed and mentioned above with IgG and identical HPV-6 and -16 VLPs (12%).

**HPV ELISA: antibody reactivity over time**

The time-course of seroreactivity was retrospectively analysed in 29 HIV-infected women with a diagnosis of HPV-related lesions. The mean observation period was 35 months (range 3–76). Twenty-three women (79%) were seropositive to any high-risk HPV IgG at baseline; 17 of these remained positive in consecutive tests. Of six women (21%) seronegative for all high-risk HPV IgG antibodies at baseline, five had no history of HPV-related lesions at that time. These five women remained negative in consecutive tests, although lesions developed. The results for low-risk HPV IgG VLPs were as follows. Ten women (34%) were seropositive at baseline; seven of these remained seropositive throughout the study. Seronegativity at baseline was seen in 19 women (66%) and remained negative in consecutive tests; seroconversion was only observed in one exception. Appearance of HPV-related lesions or treatment actions (e.g. surgical excision, laser therapy) seemed to have no influence on serological status.

**Discussion**

Despite partial restoration of immune functions as a result of highly active antiretroviral therapy (Heard et al., 1998), HPV-associated malignancies may become a major problem in the future, because of rising life expectancy in HIV-positive patients (Palefsky et al., 1998). A significantly higher prevalence of HPV DNA in HIV-positive cases compared to HIV-negative controls has been described (Laga et al., 1992; Wright et al., 1994), but not confirmed by others (Braun et al., 1994; Cappiello et al., 1997). It was also reported that HPV DNA prevalence was largely independent from the level of immunosuppression (Cappiello et al., 1997). It was therefore proposed that HIV induces CIN by increasing the transforming activity of oncogenic HPV types (Buonaguro et al., 1994; Cappiello et al., 1997). However, there is little evidence in vivo to support such a model of association. Prolonged exposure to HPV proteins could be an alternative mechanism. This hypothesis is suggested by results of repetitive HPV DNA testing of cervicovaginal lavage specimens in HIV-infected women (Sun et al., 1997). Immunosuppression may promote a high rate of persistent shedding of HPV particles.

The present study is, to the best of our knowledge, the first published analysis of the specific humoral immune response against HPV particles in HIV-infected women. Our clinical and DNA data confirm a high prevalence of HPV-related lesions in HIV-infected women in the previously documented range (La Ruche et al., 1998). In addition, we have shown that HIV-infected patients are able to generate a vigorous anti-HPV serological response. Published results available for HIV-negative individuals reveal, in general, that high seropositivity rates are associated with clinically evident HPV lesions and/or detectable HPV DNA. Wideroff et al. (1995) reported the detection of HPV-16 VLP antibodies in 31% of patients with low-grade and in 52% of patients with high-grade cervical lesions. Kimmbauer et al. (1994) found 59% seropositivity with HPV-16 VLPs in patients with HPV-16 DNA PCR-positive lesions. In line with these published results, we found 60% seropositivity with HPV-16 VLPs in HPV-16 PCR-positive, HIV-negative women and 66% seropositivity in HPV-16 PCR-positive, HIV-positive women. Even in HIV-positive women without clinically evident HPV lesions there was, compared to healthy HIV-negative controls, a highly increased seropositivity rate with high-risk HPV VLPs (47% versus 19%; $P = 0.0001$). The data remained statistically significant ($P = 0.0002$) when woman with a history of drug abuse, a well-known single risk factor for sexually transmitted diseases (Fenaughty & Fisher, 1998; Siegal et al., 1996), were excluded as a confounding factor. The HPV seropositivity rate in the HIV-positive cohort was even higher ($P = 0.046$ for HPV-16 IgG) than in the control group of HIV-negative women with HPV-associated diseases. Since prolonged exposure to viral antigens is thought to be a primary determinant of a detectable antibody response our data suggest that HIV-positive women are intensely exposed to HPV proteins throughout the natural course of their HIV infection. Cellular immunosuppression could facilitate the occurrence of complete life-cycles of HPV and subsequent capsid antibody induction.

Nearly half of the women were seropositive for more than one HPV type. This could reflect cross-reactivity or infection with multiple HPV types. At least in part, it seems to be likely that the data indicate infection with multiple HPV types in HIV-positive and HIV-negative individuals, since we have demonstrated previously that type-specific IgG antibodies...
exist, which discriminate even the closely related HPV-6 and -11 (Heim et al., 1995). The role of HPV IgA and IgM isotypes in clinical practice remains to be determined in future studies. The isotype IgA is an immunoglobulin predominantly produced in submucosal lymphoid tissues and is expected to be highest in cervicovaginal secretions. However, even in serum, the prevalence of HPV IgA antibodies was relatively high in comparison to IgM antibodies. The frequency and type specificity of the latter isotype was generally low, and therefore there was no evidence of a time correlation between HPV IgM seroconversion and recent HPV infection, as is known from other infections.

Detectable HPV antibodies did not correlate strictly with HPV DNA results. One must consider that antibody responses also reveal past exposure to virus. It is also possible that, with increased time of HIV infection, there is a decrease in the responsiveness to the more recent HPV infections. Serotype and HPV type responsible for the disease at the date of seroconversion is therefore of importance to be determined.

In summary, we conclude that the high prevalence of HPV antibodies reflects a high lifetime cumulative exposure to HPV associated with clinical disease in HIV-positive women. This may be due to high-risk sexual behaviour and defects in the cellular control of HPV. However, even in immunosuppressed HIV-positive women, a humoral immune response to HPV is clearly detectable and the test has turned out to be as sensitive in HIV-positive women as in the general population. The humoral immune response to HPV may be due to high-risk sexual behaviour and defects in the immune response to HPV, as is known from other infections.

Serology may therefore not be identical.

In summary, we conclude that the high prevalence of HPV antibodies reflects a high lifetime cumulative exposure to HPV associated with clinical disease in HIV-positive women. This may be due to high-risk sexual behaviour and defects in the cellular control of HPV. However, even in immunosuppressed HIV-positive women, a humoral immune response to HPV is clearly detectable and the test has turned out to be as sensitive in HIV-infected individuals as has been shown previously for immunocompetent individuals (Carter et al., 1996; Heim et al., 1995; Kirnbauer et al., 1994). Therefore, HIV-infected patients may be candidates for future prophylactic vaccination strategies. The impact of HPV serology on diagnostic strategies in HIV-infected individuals remains to be determined.

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References


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