Seroprevalence of Human Papillomavirus Types 6 and 16 Capsid Antibodies in Homosexual Men

Michael E. Hagensee,* Nancy Kiviat, Cathy W. Critchlow, Stephen E. Hawes, Jane Kuypers, Sarah Holte, and Denise A. Galloway

Human papillomavirus (HPV) has been implicated in the pathogenesis of anal carcinoma, which is increased in homosexual men. Little is known about the serologic response to HPV in normal or immunosuppressed men; therefore, HIV-infected and -uninfected homosexual men were screened for HPV-6 and -16 capsid antibodies. HIV-infected men had increased HPV DNA detection but did not significantly differ in the prevalence of HPV antibodies. HPV-6 DNA detection and the presence of anal warts were significantly correlated with serum antibody overall and in the HIV-infected subgroup. HPV-16 DNA detection was not significantly correlated with serum antibody overall or in either subgroup; however, HIV-infected men with high-grade anal squamous intraepithelial lesions were significantly more likely to have HPV-16 antibodies. HIV-infected men are able to generate an antibody response to HPV, and a lack of serum HPV antibodies cannot explain the increased HPV-associated disease seen in HIV-infected men.

Human papillomavirus (HPV) is the causative agent of genital warts and has been implicated in the pathogenesis of anal carcinomas [1, 2]. Epidemiologic studies have shown that homosexual men are at increased risk for infection with HPV, as manifested by genital warts, primarily of the anus [3–6], and anal carcinoma [4]. We have previously reported an increased risk for detection of HPV DNA and detection of high-grade anal squamous intraepithelial lesions (ASIL) in human immunodeficiency virus (HIV)–seropositive men, especially those with CD4 cell counts <500/mL [7].

Although unlikely to affect established ongoing productive infection, antibodies against HPV capsid proteins may be able to prevent reinfection or reexpression with the same or similar HPV type. This may be of particular importance for HIV-seropositive persons, most of whom have been previously exposed to HPV. Thus far, studies of serum antibody to HPV capsids have focused on HPV seroprevalence in women, with no data currently available on the serologic response in immunosuppressed persons. In women, many groups have observed an association between HPV-6 or -16 seropositivity and detection of HPV-6 or -16 DNA as well as clinical status (presence of genital warts for HPV-6 and presence of SIL for HPV-16) [8–14]. We undertook the present study to describe the type-specific serologic responses toward HPV capsid antigens in men with and without HIV infection.

Materials and Methods

Study subjects. Between October 1989 and December 1995, 895 homosexual or bisexual men aged 16–50 years were selected from persons presenting to the Seattle–King County Department of Public Health AIDS Prevention Project (Seattle) for HIV testing, counseling, and education. The demographic and behavioral characteristics, prevalence of HPV DNA, risk of developing high-grade ASIL, and relationships to HIV infection in this population have been previously reported [7]. Remaining sera were available for HPV serologic testing from 255 subjects (study group). These men differed from the 670 from whom sera were unavailable for testing in that a greater percentage of the study population was HIV-seropositive (60.4% vs. 54.8%), although this difference was not significant (P = .12). Mean age, number of sex partners, age at first rectal intercourse, and history of sexually transmitted diseases did not differ between those tested and not tested for serologic evidence of HPV infection. Furthermore, while the prevalence of anal HPV-16 was similar in the groups, the subjects tested in this study were more likely than those who were not tested to have anal HPV-6/11 DNA detected by polymerase chain reaction (PCR) (59% vs. 36%; P < .001).

Procedures and collection of specimen. Each subject underwent a standardized interview on past and current medical and sexual history, as well as a general physical examination, including a detailed genital and anoscopic examination with colposcopy. Specimens for cytology were collected with a dry cotton Dacron swab, placed on a glass slide, and fixed in 95% ethanol. A second sample was similarly collected for HPV DNA analysis and placed in transport media. Blood was drawn for HIV testing, lymphocyte subset analysis, and HPV serologic testing. Only a single blood
screening per patient was analyzed here; however, these patients have been followed longitudinally with multiple blood, DNA, and cytology samples.

**Classification of cytologic and histologic changes.** Smears were considered satisfactory if >200 easily visualized squamous cells were present and were classified according to the Bethesda recommendations (negative, atypia of uncertain significance, and low- and high-grade SIL) [15].

**Detection and typing of HPV DNA.** Samples were analyzed by PCR by use of HPV L1 consensus primers with subsequent classification of PCR products by HPV type–specific oligonucleotides, specifically an HPV-6/11 mix and a separate HPV-16 probe as described previously [7]. Primers PC04 and GH20 were used to prime the synthesis of a 256-bp fragment of the eukaryotic β-globin gene, and primers MY11 and MY09 were used to prime the synthesis of a 45-bp fragment of the HPV L1 gene. Each DNA sample template was amplified twice. Controls containing K562 cell line DNA and purified SiHa cell line DNA (HPV-16) were tested with each run of 22 patient samples. After amplification, PCR products underwent electrophoresis, staining with ethidium bromide, and observation under UV illumination. Amplified samples demonstrating the 250-bp β-globin fragment on a gel were considered to have sufficient numbers of eukaryotic cells present for analysis for HPV types by dot-blot hybridization with HPV-6/11 and -16 biotinylated probes by standard methods [7]. Consecutive HPV-6/11 or -16 DNA positivity was defined as being positive for the respective HPV DNA type at the same time as serologic testing and on the most recent HPV DNA test preceding the serologic test.

**HPV-6 and -16 capture ELISA.** The HPV-6 capture ELISA was performed as described by Carter et al. [8]. The amounts of monoclonal antibody and capsids used were predetermined by use of positive control sera to maximize the signal and minimize the background. After this determination, the amounts of these agents did not vary when test population sera were assayed. Briefly, the capture antibody was an anti–HPV-11 monoclonal antibody, H11b.2 (provided by N. Christensen, Pennsylvania State Medical Center, Hershey), which was diluted 1/800 in 0.1 M sodium carbonate (pH 9.5) buffer and allowed to attach overnight at room temperature to ELISA plates (Immulon 2; Dynatech Laboratories, Chantilly, VA). Plates were washed and then blocked with blotto (5% nonfat powdered milk, 0.15 M NaCl, 15 mM sodium azide) plus 0.05% Tween 20 for 1 h at room temperature. HPV-6b L1 capsids produced from recombinant vaccinia viruses were purified on cesium chloride gradients, diluted to 1/100 in PBS, added to the plates, and allowed to incubate at 37°C for 1 h. The plates were washed, human sera were diluted 1/100 in blotto and added to the plates, and plates were incubated at 37°C for 1 h. The plates were washed, incubated with a 1/1000 dilution of goat anti-human IgG alkaline phosphatase–conjugated antibody (Boehringer Mannheim, Indianapolis) for 1 h, washed, and developed. For each sample, the median of three wells without capsids was taken and subtracted from the median of three wells with capsids. The HPV-16 assay was performed as previously described by Carter et al. [14], using a monoclonal antibody to HPV-16, H16.V5 (provided by N. Christensen), at a 1/2000 dilution and vaccinia-produced HPV-16 L1 capsids that were cesium chloride–purified.

**Statistical methods.** Cutpoints for the ELISA were determined by two methods. The first assayed a group of 64 women with no previous sex partners and no evidence of genital HPV infection by DNA testing for serum IgG antibodies to HPV-6 and -16. Two standard deviations above the means of their response was used as the cutpoint (HPV-6, 0.877; HPV-16, 0.222) for positivity.

As an alternative, a cutpoint for classifying subjects as HPV-positive or -negative on the basis of their ELISA values was determined by use of a population-based method of maximum likelihood. This cutpoint was determined by assuming that the distribution of the observed log ELISA values was a mixture of two normal distributions and estimating the relevant parameters by the method of maximum likelihood [16]. By assuming that the log ELISA values for HPV-negative subjects followed the estimated normal distribution with the smaller mean and that the log ELISA values for HPV-positive subjects followed the estimated normal distribution with the larger mean, the cutpoint was chosen so that the sensitivity and specificity resulting from these two estimated distributions was equal. The histogram of log ELISA values for HPV-6, as well as the estimated normal distribution of these values for seropositive and seronegative subjects, is shown in figure 1. For HPV-6, a cutpoint of 0.830 was obtained that gave a sensitivity and specificity of 85%. (Sensitivity is defined as the percentage of subjects in the upper curve who have ELISA values to the right of the chosen cutpoint, whereas specificity is the percentage of men under the lower curve who have ELISA values to the left of the cutpoint.) For HPV-16, a cutpoint of 0.261 was obtained that gave a sensitivity and specificity of 75%. These cutpoints were very similar to those generated by assaying the sera from the sexually inexperienced women. By use of either cutpoint determination, no significant differences were noted in the data analysis. The data presented used the cutpoint determined from the sexually inexperienced women.

χ² values were calculated to assess the association between serology and other factors. Odds ratios (ORs) were calculated to assess the strength of these associations. When expected frequencies were <5, one-sided Fisher’s exact tests were performed. Mantel-Haenszel χ² and ORs were used to assess the relationship between serology and DNA detection by PCR when adjusting for either HIV status or CD4 cell count. Multivariate logistic regression was used to test for independent associations between demographic, clinical, and laboratory factors and HPV serology in both HIV-negative and -positive men. To assess which factors were independently associated with HPV serology, forward stepwise model-building methods, with an entry criterion of P < .1, were used. Four multivariate logistic regression models (one each for HIV-negative and -positive men for both HPV-6 and -16 serology) were built in this fashion.

For HIV-negative men, the factors included for evaluation in the stepwise forwards model building were age >35, recent receptive anal intercourse, age of first receptive intercourse, >50 lifetime sex partners, nonwhite race, history of intravenous drug use, history of anal warts, history of penile warts, history of gonococcal urethritis, persistent HPV detected by PCR, any HPV DNA detected by PCR, any anogenital dysplasia (ASIL) detected by cytology, warts detected on anal examination, low-grade anal cytology, and high-grade anal cytology. In HIV-positive men, we added CD4 cell count as a continuous variable, CD4 cell count <200, and CD4 cell count <500 to the list of potential independent risk factors.
Results

Clinical and laboratory evidence of HPV infection. As in the overall study population [7], HIV-positive subjects were more likely to have both HPV-6/11 DNA and HPV-16 detected (table 1) on either a single visit (HPV-6: OR, 1.4; 95% confidence interval [CI], 0.8–2.4; HPV-16: OR, 2.6; CI, 1.5–4.8) or consecutive (previous and current) visits (HPV-6: OR, 1.6; CI, 0.9–2.8; HPV-16: OR, 4.1; CI, 1.9–9.6). In addition, HIV-infected men were more likely than those without HIV infection to have anal warts present on examination (OR, 1.9; CI, 1.1–3.3) and to have low- (OR, 1.4; CI, 0.8–2.7) or high-grade ASIL on anal Pap smear (OR, 2.8; CI, 0.5–27.7). However, despite the fact that HIV-seropositive subjects were more likely to have anal HPV DNA detected, the prevalence of serum IgG antibodies to either HPV-6 (OR, 0.7; CI, 0.4–1.2) or HPV-16 (OR, 0.8; CI, 0.5–1.3) did not differ significantly among those with and without HIV infection. In addition, there was no difference in the average ELISA values for HPV-6 (P = .20) or -16 (P = .50) by HIV status.

Associations with HPV-6 seropositivity. Overall in the univariate analysis, HPV-6 seropositivity was associated (even after adjusting for detection of anal HPV-6 DNA) with the presence of anal warts (OR, 2.3; CI, 1.0–5.5) or genital warts (OR, 2.3; CI, 1.0–5.3) and age >35 (OR, 2.2; CI, 1.0–4.8). Other measures of anal HPV infection, including detection of anal HPV-6/11 DNA at one (OR, 1.7; CI, 0.7–4.0) or consecutive study visits (OR, 2.2; CI, 0.9–5.4) and the presence of anal warts on examination (OR, 1.9; CI, 0.7–4.6) were also more common among those with than among those without antibody to HPV-6 capsids. Among HIV-seropositive men (table 2), detection of anal HPV-6 DNA on one (OR, 2.9; CI, 1.3–6.3) and on consecutive visits (OR, 3.2; CI, 1.5–6.7) was associated with HPV-6 seropositivity. Furthermore, among HIV-seropositive men, those with anal warts present on examination were more likely to have antibodies to HPV-6 than were those without such findings (OR, 3.2; CI, 1.4–7.5). Mean CD4 cell count on the day of serologic testing was not associated with HPV-6 serology (P = .8). Seropositivity to HPV-6 was seen in 21 (29%) of 72 men with CD4 cell count >500/mL, 19 (37%) of 51 men with CD4 cell count between 200 and 500/mL, and 6 (24%) of 25 men with CD4 cell count <200/mL (P = .9).

A multivariate analysis was performed with the parameters outlined in Methods. For HIV-seronegative men, the multivariate analysis showed that a history of genital warts (OR, 3.2; CI, 1.3–8.5; P = .02) and a history of urethritis caused by gonorrhea (OR, 4.0; CI, 1.4–12.2; P = .01) were independently associated with antibodies to HPV-6. A trend was noted between the detection of HPV-6 DNA and antibodies to HPV-6 (OR, 2.1; CI, 0.8–5.7; P = .12). The optimal multivariate model for HIV-seropositive men demonstrated that anal warts seen on examination (OR, 2.9; CI, 1.1–8.5; P = .03) and the presence of HPV-6 DNA on consecutive visits (OR, 4.4; CI, 1.8–11.0; P = .001) were independently associated with antibodies to HPV-6.
Table 1. Clinical and laboratory evidence of HPV infection.

<table>
<thead>
<tr>
<th>HIV serologic status</th>
<th>Negative (n = 101)</th>
<th>Positive (n = 154)</th>
<th>P*</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV DNA by PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-6/11</td>
<td>50/92 (54)</td>
<td>91/145 (63)</td>
<td>.2</td>
<td>1.4 (0.8–2.4)</td>
</tr>
<tr>
<td>HPV-16</td>
<td>31/84 (37)</td>
<td>66/137 (48)</td>
<td>.10</td>
<td>1.6 (0.9–2.8)</td>
</tr>
<tr>
<td>Consecutive HPV-6/11</td>
<td>19/92 (21)</td>
<td>59/145 (41)</td>
<td>.001</td>
<td>2.6 (1.5–4.8)</td>
</tr>
<tr>
<td>Consecutive HPV-16</td>
<td>9/84 (11)</td>
<td>45/137 (33)</td>
<td>&lt;.001</td>
<td>4.1 (1.9–9.6)</td>
</tr>
<tr>
<td>Clinical evidence of HPV infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warts seen on examination</td>
<td>36/79 (46)</td>
<td>74/121 (61)</td>
<td>.03</td>
<td>1.9 (1.1–3.3)</td>
</tr>
<tr>
<td>Low-grade ASIL</td>
<td>26/85 (31)</td>
<td>54/144 (38)</td>
<td>.2</td>
<td>1.4 (0.8–2.7)</td>
</tr>
<tr>
<td>High-grade ASIL</td>
<td>2/85 (2)</td>
<td>8/144 (6)</td>
<td>.2</td>
<td>2.8 (0.5–27.7)</td>
</tr>
<tr>
<td>HPV antibody status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-6–seropositive</td>
<td>41/101 (41)</td>
<td>49/154 (32)</td>
<td>.15</td>
<td>0.7 (0.4–1.2)</td>
</tr>
<tr>
<td>HPV-6 mean ELISA value</td>
<td>0.81 ± 0.46</td>
<td>0.74 ± 0.40</td>
<td>.2</td>
<td></td>
</tr>
<tr>
<td>HPV-16–seropositive</td>
<td>48/101 (48)</td>
<td>65/154 (42)</td>
<td>.4</td>
<td>0.8 (0.5–1.3)</td>
</tr>
<tr>
<td>HPV-16 mean ELISA value</td>
<td>0.29 ± 0.34</td>
<td>0.26 ± 0.35</td>
<td>.5</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. positive/total (%) except where noted. OR, odds ratio; CI, confidence interval.
*Calculated by use of Mantel-Haenszel procedures.
1Status by polymerase chain reaction (PCR) at same visit as serologic testing and compared with no HPV-6/11 DNA detected plus or minus other HPV types.
2Status by PCR at same visit as serologic testing and compared with no HPV-16 DNA detected plus or minus other HPV types.
3Compared with negative pap smear diagnosis; ASIL, anal squamous intraepithelial lesions.
4Absorbance at 405 nm.

Associations with HPV-16 seropositivity. Overall, seropositivity to HPV-16 capsids was associated with age >35 (OR, 1.7; CI, 1.0–2.8; P = .04) and >50 lifetime sex partners (OR, 2.8; CI, 1.7–4.8; P < .001) but not with detection of anal HPV-16 DNA (P = .50) or the presence of low- (P = .6) or high-grade ASIL (P = .13, data not shown). In HIV-negative subjects, antibodies to HPV-16 were associated with age >35 (OR, 2.5; CI, 1.1–5.6; P = .03), >50 lifetime partners (OR, 4.8; CI, 1.8–9.5; P < .001), history of intravenous drug use (OR, 7.6; CI, 1.9–30.5; P = .004), and a history of gonococcal urethritis (OR, 2.7; CI, 1.1–6.5; P = .02). Among HIV-infected men, HPV-16–seropositive subjects were more likely to have had initial anal intercourse at an earlier age (P = .04) and to have >50 lifetime sex partners (OR, 2.3; CI, 1.2–4.5; P = .02) than were those who were without antibodies to HPV-16. In the univariate analysis for both HIV-seronegative and -seropositive subjects, there was no significant association between HPV-16–seropositive status and the presence of HPV-16 DNA (P = .8, P = .4, respectively), the presence of high-grade (P = .2, P = .3, respectively) or low-grade ASIL (P = .8, P = .6, respectively), or the average CD4 cell count (P = .9, P = .5, respectively). Seropositivity to HPV-16 was seen in 33 (46%) of 72 men with CD4 cell counts >500/mL, 22 (43%) of 51 men with CD4 cell counts between 200 and 500/mL, and 8 (32%) of 25 men with CD4 cell counts <200/mL (P = .3).

A multivariate analysis using a model as outlined in Methods demonstrated for HIV-seronegative men that >50 lifetime male sex partners (OR, 3.8; CI, 1.6–9.0; P = .002) was significantly associated with age >35 (OR, 2.0; CI, 0.8–4.7; P = .11) was suggestive of an independent association with antibodies to HPV-16. For HIV-seropositive men, age at first receptive intercourse (OR, 0.90; CI, 0.84–0.96; P = .003) and intravenous drug use (OR, 0.37; CI, 0.14–0.92; P = .04) were significantly associated with the lack of antibodies to HPV-16. Furthermore, a CD4 cell count <200 (OR, 0.43; CI, 0.15–1.1; P = .1) was suggestive of an independent association with the lack of antibodies to HPV-16. The presence of high-grade ASIL was independently associated with the presence of antibodies to HPV-16 (OR, 5.7; CI, 1.0–48.8; P = .07).

Discussion

In this study of men who have sex with men, we found that the presence of antibodies to HPV-6 was associated with clinical and laboratory evidence of HPV infection (presence of anal warts and the detection of anal HPV-6 DNA), while prevalence of antibodies to HPV-16 varied by demographic and behavioral characteristics (older age and number of lifetime sex partners). Interestingly, the prevalence of antibody to both high- and low-risk types of HPV infection (HPV-16 and -6, respectively) was similar among those with and without HIV infection and did
not significantly vary among HIV-seropositive men by CD4
cell count.

Cellular immune mechanisms are probably the most im-
portant means of controlling established HPV infection. Preva-
lence of HPV infection and severity of HPV-associated disease
is increased in patients with defects in cellular immunity, in-
cluding HIV infection [7, 17–24]. Furthermore, even though
disease caused by HPV is increased or more severe in the
HIV-infected patient, this study showed that the prevalence of
humoral antibodies to HPV-6 and -16 capsids is not influenced
by HIV status. In the multivariate analysis, an association of
a low CD4 cell count (<200) and the lack of serum HPV-16
antibodies was suggested, but the magnitude of this association
was small (2.5-fold) and not significant (P = .1). A larger study
may demonstrate a significant association between severely
immunosuppressed HIV-infected men and a lack of serum HPV
antibodies, but this would not explain the increase in HPV
DNA detection and HPV-associated disease seen at higher
(>200) CD4 cell counts [7]. It is unlikely that the presence of
HPV capsid antibodies in the serum have a major role in
controlling established infection. It is more likely that the de-
tected antibodies function to prevent reinfection or reexpression
with the same or a similar HPV type.

The strongest associations in both the univariate and multi-
variate analysis with HPV-6 seropositivity were the detection
of anal HPV-6/11 DNA at one or consecutive study visits and
the presence of genital warts. This is the first study to closely
examine the relationship between detection of HPV-6/11
DNA and seropositivity to HPV-6 in men, and it is believed
that a stronger relationship would be found if the HPV DNA
testing was restricted to detect only HPV-6. In addition, this
is the first demonstration in men of an association between
the presence of genital warts and seropositivity to HPV-6
capsids. We have previously screened for antibodies to
HPV-6 capsids in 293 men and 236 women enrolled in a case-
control study of genital warts [8] and showed that HPV-6
seropositivity was associated with warts only among women.
The men studied by Carter et al. [8] were predominately (96%)
heterosexual, had relatively few lifetime sex partners (mean,
~10), and most likely had penile rather than intraanal warts
[25]. A small study (n = 35) of patients attending a private
practice dermatology clinic [9] demonstrated that all 12
women with genital warts had antibodies to HPV-6 capsids,
whereas only 4 (16%) of 25 men were seropositive. This study
by Greer et al. [9] excluded patients who were immunosup-
pressed or had anal warts. The increased sexual activity of
the homosexual male population in this study or the location
of genital warts on a mucosal surface (anus) could explain
the positive association seen here between antibodies to HPV-6
capsids and the presence of genital warts.

<table>
<thead>
<tr>
<th>Table 2. Associations of HPV-6 L1 antibodies in human immunodeficiency virus (HIV)–positive and –negative men.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV serologic status</strong></td>
</tr>
<tr>
<td><strong>Negative (n = 101)</strong></td>
</tr>
<tr>
<td><strong>HPV-negative</strong> (n = 60)</td>
</tr>
<tr>
<td>Age &gt;35 years</td>
</tr>
<tr>
<td>Age at 1st receptive anal sex (years)</td>
</tr>
<tr>
<td>&gt;50 lifetime sexual partners</td>
</tr>
<tr>
<td>History of intravenous drug use</td>
</tr>
<tr>
<td>History of gonorrhea-urethritis</td>
</tr>
<tr>
<td>History of genital warts</td>
</tr>
<tr>
<td>Clinical and laboratory evidence of HPV infection</td>
</tr>
<tr>
<td>Anal warts seen on examination</td>
</tr>
<tr>
<td>ASIL–low-grade†</td>
</tr>
<tr>
<td>ASIL–high-grade†</td>
</tr>
<tr>
<td>HPV-6 DNA by PCR†</td>
</tr>
<tr>
<td>Consecutive HPV-6 by PCR†</td>
</tr>
<tr>
<td>Mean CD4 cell count/mL</td>
</tr>
<tr>
<td><strong>Positive (n = 154)</strong></td>
</tr>
<tr>
<td><strong>HPV-negative</strong> (n = 105)</td>
</tr>
<tr>
<td>Age &gt;35 years</td>
</tr>
<tr>
<td>Age at 1st receptive anal sex (years)</td>
</tr>
<tr>
<td>&gt;50 lifetime sexual partners</td>
</tr>
<tr>
<td>History of intravenous drug use</td>
</tr>
<tr>
<td>History of gonorrhea-urethritis</td>
</tr>
<tr>
<td>History of genital warts</td>
</tr>
</tbody>
</table>

NOTE. For discrete variables data are no. positive/total (%). For continuous variables, average is shown in units as noted. OR, odds ratio; CI, confidence
interval.
† Compared by Mantel-Haenszel procedures.
†‡ Status by polymerase chain reaction (PCR) at same visit as serologic testing and compared with no HPV-6/11 DNA detected plus or minus other HPV types.
§ Status by PCR at same visit as serologic testing and compared with no HPV-6/11 DNA detected plus or minus other HPV types.
** Calculated by use of Mantel-Haenszel procedures.
The associations between detection of anal HPV-6/11 DNA, the presence of genital warts, and HPV-6 capsid antibodies tended to be stronger in HIV-infected men. This is most likely due to the immunosuppressive effect of HIV that may allow more HPV-6 expression. However, it would be predicted that increasing immune suppression (lower CD4 cell counts) would lead to even stronger associations between the above parameters. This was not seen and may be due to the use of CD4 cell count as a surrogate of immune dysfunction. HIV load testing appears to be a better marker of HIV disease progression, and a stronger association between HIV load and HPV-6 seropositivity may exist.

In the univariate analysis, the presence of antibodies to HPV-16 capsid antigens was not associated with either HPV-16 DNA status or the presence of ASIL in either the HIV-seronegative or -seropositive subgroups. This is somewhat surprising, as numerous studies show an association in women between the presence of HPV-16 capsid antibodies and the detection of HPV-16 DNA [10–14], the presence of cervical dysplasia [10–14], and the presence of cervical cancer [13]. These studies examined seroprevalence to HPV-16 capsids in women with known HPV infection (HPV DNA–positive at the cervix), in women with HPV-related disease (cervical intraepithelial neoplasia or cervical cancer) [10–13], and in women initiating sexual activity [8]. The populations of women examined thus far are not comparable to the highly sexually active homosexual male population studied here. It would be interesting to study other male populations, perhaps with less extensive sexual histories, or men with anogenital malignancies for antibodies to HPV-16 capsids.

Only two studies have looked at the relationship of antibodies to HPV-16 capsids and anogenital malignancy in men. The study by Heino et al. [26] demonstrated a correlation between anal epidermoid cancer and HPV-16 antibodies but did not separate patients by gender. In a small case-control study of penile cancer [27], no HPV-16-seropositive subjects were found among either the patients with cancer or the controls. The multivariate analysis performed in this investigation did show an association between high-grade ASIL and HPV-16 antibodies. Perhaps if more subjects were studied, a stronger relationship between HPV-16 serology and anal dysplasia could have been detected. Finally, it may be that a cross-sectional approach as used in this study may miss either the development of an antibody response to a recent HPV infection or the fact that antibodies to HPV-16 capsids may have waned over time. A longitudinal study in men may help clarify this issue.

In summary, the risk factors for HPV-6 and -16 seroreactivity were studied in HIV-negative and -positive homosexual men. The most remarkable finding is that HIV infection was not significantly associated with HPV-6 or -16 seropositivity; therefore, a role for serum antibody in controlling existing HPV infection could not be demonstrated. The presence of HPV-6 capsid antibodies correlated with the detection of anal HPV-6/11 DNA and disease state (anal warts), whereas the presence of HPV-16 capsid antibodies did not.

Acknowledgments

We thank Neil Christensen (Pennsylvania State University, Hershey) for supplying the monoclonal antibodies to HPV-6 and -16 used in the ELISA and Kristin Robinson for technical assistance in the production of HPV-6 and -16 capsids.

References