

Prevalence and Risk Factors for Human Papillomavirus Infection of the Anal Canal in Human Immunodeficiency Virus (HIV)-Positive and HIV-Negative Homosexual Men

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One of the groups at highest risk of anal cancer is homosexual and bisexual men. Like cervical cancer, anal cancer is associated with human papillomavirus (HPV) infection. Anal HPV infection was characterized in a study of 346 human immunodeficiency virus (HIV)-positive and 262 HIV-negative homosexual and bisexual men. Anal HPV DNA was detected in 93% of HIV-positive and 61% of HIV-negative men by polymerase chain reaction. The spectrum of HPV types was similar in HIV-positive and HIV-negative men, with HPV-16 the most common type. Infection with multiple HPV types was found in 73% of HIV-positive and 23% of HIV-negative men. Among HIV-positive men who were positive by hybrid capture for group B HPV types (16/18/31/33/35/39/45/51/52/56/58) or group A types (6/11/42/43/44), lower CD4 cell levels were associated with higher levels of group B DNA ($P = .004$) but not group A DNA. These data suggest increased replication of the more oncogenic HPV types with more advanced immunosuppression.

Several studies have shown the risk of anal cancer to be elevated among men with a history of homosexual or bisexual activity (hereafter called homosexual) [1–3]. The incidence of anal cancer among homosexual men has been estimated to be ~35/100,000 [2], which is several times higher than current rates of cervical cancer in women in the United States [4] and similar to rates of cervical cancer prior to the introduction of routine cervical cytology screening.

Recent reports have shown that human immunodeficiency virus (HIV)-positive homosexual men have higher rates of anal squamous intraepithelial lesions (ASIL), putative anal cancer precursors, than do HIV-negative men [5–10]. This suggests that HIV-positive men may be at higher risk of developing anal cancer than HIV-negative men. So far, data on the degree of excess incidence of anal cancer in HIV-positive homosexual compared with HIV-negative homosexual men are conflicting. Rabkin and Yellin [11] reported little increased risk of anal cancer among single, never-married men aged 25–44 years in the San Francisco Bay Area since the onset of the HIV epidemic. However, in a study linking HIV registries to the Surveillance, Epidemiology, and End Results database, Melbye et al. [12] reported an increase in the observed-to-expected ratio

of cases of anal cancer with increasing proximity to a diagnosis of AIDS.

Several lines of evidence point to an important role for human papillomavirus (HPV) infection in the pathogenesis of anal cancer and ASIL. HPV DNA has been detected in anal cancer and ASIL tissues [13–15]. Similar to their oncogenic risk in the cervix [16], most cases of anal high-grade squamous intraepithelial lesions and anal cancer contained HPV-16, whereas anal low-grade squamous intraepithelial lesions were more likely to have HPV types known to have a low risk of oncogenicity in the cervix, such as HPV-6 or -11. An understanding of anal HPV infection and risk factors for detection of anal HPV in HIV-positive and HIV-negative homosexual men is important, given the role of HPV infection in the pathogenesis of ASIL and anal cancer.

Previous studies have reported HIV-positive homosexual men to have a higher prevalence of anal HPV infection than do HIV-negative homosexual men [6–10, 17]. However, most of these studies were small and had limited information on risk factors for anal HPV infection. No studies to date have described the spectrum of HPV types detectable in the anal canal using a polymerase chain reaction (PCR)-based DNA detection method capable of detecting a wide variety of genital and nongenital HPV types, nor have they characterized the levels of HPV DNA in anal specimens and their relationship to HIV-related immunosuppression. The goal of our analyses was to characterize the prevalence of 29 different HPV types and a mixture of 10 additional types in the anal canal among HIV-positive and HIV-negative men. We examined the relationship between HPV DNA levels and HIV-related immunosuppression and characterized risk factors for detection of HPV DNA in these groups.

Materials and Methods

In total, 346 HIV-positive and 262 HIV-negative men were recruited between November 1991 and March 1994 from among

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The study protocol was approved by the institution's Committee on Human Research and informed consent was obtained from all men.

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homosexual and bisexual men in the San Francisco Men's Health Study (SFMHS) [18], in the San Francisco General Hospital Cohort Study (SFGH) [19], and at the University of California, San Francisco (UCSF), responding to newspaper advertisements. Subjects were interviewed using a comprehensive questionnaire regarding their medical history, including prescription drug use, x-ray exposure, hospitalizations, transfusions, history of sexually transmitted diseases, intestinal parasites, hepatitis, anal conditions such as fissures and fistulas, and symptoms related to HIV. Subjects were queried about sex practices, cigarette smoking, drinking, and recreational drug use.

HPV testing. To obtain a sample for HPV testing, a Dacron swab was inserted into the anal canal [20] and the swab placed into a tube containing 1 mL of Sample Transport Medium (Digene Diagnostics, Silver Spring, MD). The tubes were frozen at -70°C until processed for analysis. After defrosting, the tubes were heated to 56°C for 1 h to inactivate HIV.

Because of its high sensitivity, PCR was used in this study to detect low-level HPV infection. Since a positive PCR result does not discriminate between low-level and high-level infection, we also used the hybrid capture (HC) test (Digene), a non-amplification-based test that is not as sensitive as PCR. The quantity of HPV DNA in the specimen can be determined by measuring the HC relative light unit (RLU) ratio, computed by dividing the chemiluminescent signal of the specimen by that obtained with a control sample containing 10 pg/mL HPV-16 DNA.

Part (200 μL) of the specimen was used for PCR and the remainder was used for HC. PCR was performed using MY09/MY11 consensus HPV-L1 primers as well as primers for amplification of the human β -globin gene [21]. After 30 amplification cycles, 6 μL of amplification mixture was applied to a nylon membrane and probed with a biotin-labeled HPV-L1 consensus probe mixture consisting of full-length HPV-16, -18, and -51 genomic DNA [21]. A separate membrane was probed with a biotin-labeled probe to the human β -globin gene. Each specimen was also studied for the presence of specific HPV types by preparing membranes as described above with 6 μL of specimen. The membranes were studied with probes to 29 different HPV types: 6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 66, 68, 69, 70, 73, AE2, Pap 155, and Pap 291, as well as the following 10 HPV types together in a probe mixture: 2, 13, 34, 42, 57, 62, 64, 67, 72, and W13B. A sample was considered HPV-positive when it was positive with the consensus probes or with ≥ 1 probes for specific HPV types (only 1 specimen that was β -globin-positive and consensus-negative was positive for a specific HPV type). Specimens negative for β -globin gene amplification were excluded from analysis. Negative controls for each experiment consisted of amplification of solution containing all the above components except for sample DNA. Positive controls included amplification of cloned HPV DNA.

HC testing was conducted according to the manufacturer's recommendations. Two separate tests were performed on each anal specimen, consisting of 50 μL for the HPV types associated with a low risk of cervical cancer in probe group A (6/11/42/43/44) and 50 μL for detection of the HPV types associated with intermediate and high risk of cervical cancer in probe group B (16/18/31/33/35/39/45/51/52/56/58). Specimens with an RLU ratio >0.65 with probe group A mix were reprobed separately for HPV-6/11 and HPV-42/43/44. Specimens were considered positive for group

A if the RLU ratio for group A mix, HPV-6/11, or HPV-42/43/44 was >1.0 . Specimens with an RLU ratio >0.65 with probe group B mix were separately reprobed for HPV-16 alone, HPV-18/31/33/45/58, and HPV-35/39/51/52/56, subgroups that were selected according to their frequency of detection in cervical cancer tissues (HPV-16 had the highest frequency, HPV-18/31/33/45/58 were found in $>2\%$ of cervical cancers, and HPV-35/39/51/52/56 were found in $<2\%$ of cervical cancers [16]). Specimens were considered positive for group B if the RLU ratio for group B mix, HPV-16/18/31/33/45/58, or HPV-35/39/51/52/56 was >1.0 . All experiments were performed in triplicate, and the average of the three results was used to compute the RLU ratio.

Measurement of HIV status and CD4 cell levels. ELISA screening was used to determine HIV status in all men, and all positive ELISAs were confirmed with a Western blot assay. Different techniques were used to measure CD4 cell levels, and appropriate adjustments were made to correct for differences in these techniques, using calibration curves that compared the results of the different methods using the same samples. For HIV-positive men, CD4 cell levels were classified as $>500/\text{mm}^3$, $200\text{--}500/\text{mm}^3$, and $<200/\text{mm}^3$. After adjusting for differences in the techniques, 1 subject was moved from the $<200/\text{mm}^3$ category into the $200\text{--}500/\text{mm}^3$ category.

Statistical analysis. Potential risk factors for anal HPV infection that were examined included history of anal conditions (hemorrhoids, anal fissures or fistulas, anal infection or discharge, rectal itching or burning, constipation, and use of enemas or laxatives), history of sexually transmitted diseases (genital warts, syphilis, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, and herpes simplex virus), lifetime exposure to sexual activities (receptive anal intercourse, insertive anal intercourse, rimming, receptive fisting, and rectal use of recreational drugs), and substance use (lifetime smoking, lifetime alcohol consumption, and use in the previous 10 years of marijuana, cocaine, hallucinogens, "ecstasy," "poppers," "uppers," and "downers").

The presence or absence of the condition or behavior was used when practical to evaluate the association between HPV infection and potential risk factors. However, many of the behaviors were practiced by almost all of the men. Therefore, summary variables quantifying exposure to these factors were created to characterize lifetime exposure to the activity [22]. Different measures of duration and quantity of activity were used depending on the activity. To obtain exposure measures comparable for the different cohorts, cut points were chosen to create the summary variables so that the distributions of the exposure measures were similar for the HIV-positive subjects in each cohort. The derived variables were converted to scores of 0, 1, 2, and 3, with larger numbers representing higher levels of exposure. The scores were averaged to create a summary variable for each behavior for each individual.

The summary risk factor exposure categories thus created were used to divide study subjects into the following exposure groups: (1) never/rare: men who were exposed to the risk factor over a small portion of their lifetime and never or rarely engaged in the activity, (2) low exposure: men who exceeded the limits of the never/rare category in at least one aspect of the activity, (3) moderate exposure: men who engaged in the activity most of their lives for a moderate amount of time and may have occasionally engaged in it extensively, and (4) high exposure: men who engaged in the activity most of their lives and usually engaged in it extensively.

Because there were few HIV-negative men in the highest exposure category for most behaviors, the medium- and high-risk categories were grouped together to evaluate risk factors for HPV infection in HIV-negative men.

The SAS statistical package was used for data analysis. Analyses were conducted separately for HIV-positive and HIV-negative men. Among HIV-negative men, overall positivity for HPV by PCR and HC was used as the outcome variable. Relative risks (RR) and 95% confidence interval (CI) were calculated [23]. The Mantel-Haenszel procedure was used to compute adjusted RRs. For multilevel categoric variables, the Mantel-Haenszel test for trend was computed. Mean numbers of HPV types and mean RLU ratios were compared for various strata of potential risk factors by using analysis of variance. For dichotomous risk factors, CIs for differences between means were based on the *t* statistic. For multilevel risk factors, the least-significant difference was used to determine which means were different from each other [24]. The logarithmic transformation of the RLU ratio was done before analysis, and the means, differences in means, and CIs for the differences were transformed back to the original scale to obtain a normal distribution. This resulted in the expression of differences between groups as a ratio of the two means. For number of HPV types, a transformation was not necessary.

Results

In total, 608 men were eligible for this study; 346 were HIV-positive and 262 were HIV-negative. There were no differences between the HIV-positive and HIV-negative men or between the SFGH, UCSF, and SFMHS cohorts by race, ethnicity, or education. Overall, 91% of the subjects were Caucasian (not Hispanic), 7% were Hispanic, 2% were African-American, and 1% were other. Nine percent reported ≤ 12 years of education, 54% reported 13–16 years, and 36% reported > 16 years of education. The mean age of the HIV-positive men was 42 years (range, 24–64) and the mean age of the HIV-negative men was 45 years (range, 26–73). The UCSF cohort was slightly younger (mean age, 42; range, 24–73) and had a higher proportion of HIV-positive men (70%) than the SFMHS (mean age, 43; range, 31–63, 44% HIV-positive) and SFGH cohorts (mean age, 45; range, 28–68, 56% HIV-positive). Behavioral variables, such as smoking, alcohol consumption, recreational drug use, and sexual practices, were similar among the cohorts when stratified by HIV status. HIV-positive men reported significantly more receptive anal intercourse than the HIV-negative men ($P < .001$).

Results of HPV testing. Of the 601 men for whom a sample was available for analysis by PCR, 112 (19%) tested negative for β -globin and were excluded from analysis. The β -globin negativity rate varied little between the HIV-negative men (23%) and the HIV-positive men who had CD4 cell counts $> 500/\text{mm}^3$ (17%) or between 200 and $500/\text{mm}^3$ (19%). However, HIV-positive men with CD4 cell counts $< 200/\text{mm}^3$ were less likely (7%) to be β -globin negative.

Table 1 shows the number of men stratified by HIV status who were positive for specific HPV types by PCR. Among

HIV-positive men, 269 (93%) tested positive with the consensus HPV probes or ≥ 1 of the type-specific probes. Among the specific HPV types, HPV-16 was detected in 109 samples (38%) and was the single most common HPV type. Other common HPV types included 6, 18, 31, 53, and 70, each of which was found in at least 20% of the HIV-positive men.

Among HIV-negative men, 122 (61%) tested positive with the consensus HPV probes or ≥ 1 of the type-specific probes. As in the HIV-positive men, the most common HPV type in the HIV-negative men was HPV-16, which was found in 37 samples (19%). The next most common HPV types were 6, 31, 53, and 58, each of which was found in 7%–9% of the HIV-negative men. Of the HIV-negative men, 18 (9%) were positive with the consensus probes but were negative for the 29 specific and 10 grouped HPV types. Table 2 shows that the proportion of men positive for HPV by PCR was higher among HIV-positive men than among HIV-negative men. Among HIV-positive men, a high proportion were HPV-positive by PCR at all CD4 cell levels.

The number of HPV types detected by PCR was used as another measure of the amount of HPV infection in HIV-positive and HIV-negative men. Of the HIV-positive men, 212 (73%) had > 1 specific HPV type. In contrast, 46 (23%) of the HIV-negative men had > 1 HPV type. The mean number of HPV types among HIV-positive men who were positive for ≥ 1 HPV type did not differ significantly among those with different CD4 cell counts, with a mean of 3.4 types among those with counts $> 500/\text{mm}^3$, 3.3 types in those with counts of 200– $500/\text{mm}^3$, and 3.9 types in those with counts $< 200/\text{mm}^3$. The mean number of types among HIV-negative men who were positive for ≥ 1 HPV type was 1.9, significantly fewer than among the HIV-positive men ($P < .001$). We also determined whether there was a relationship between detection of particular HPV types in HIV-positive men and more advanced immunosuppression as measured by CD4 cell levels. In an examination of the association between CD4 cell counts and positivity for the 20 individual HPV types or groups detected in at least 20 HIV-positive men, types 18, 45, 53, and 59 showed an increasing prevalence with decreasing CD4 cell count, but none was statistically significant.

HC data were available for 600 of the 608 men in the study; 297 (87%) of the HIV-positive men and 97 (37%) of the HIV-negative men were positive for HPV by HC. Table 2 shows that the proportion of men positive for HPV by HC was higher among HIV-positive men than HIV-negative men. Similar to the results of PCR, among HIV-positive men, a high proportion were HPV-positive by HC at all CD4 cell levels, but the proportion was highest among those with the lowest CD4 cell level. Among the 268 HIV-positive men who were also positive for HPV DNA by PCR and who had HC results, 256 (96%) were HPV-positive by HC. Significantly fewer ($P < .001$) of the HIV-negative men who were positive for HPV DNA by PCR were also positive for HPV by HC (74/122, 61%).

Of the 340 HIV-positive men for whom HC results were available, 272 (80%) were positive for ≥ 1 group B types and

Table 1. No. of HIV-positive and HIV-negative homosexual men with anal infection by HPV type as measured by polymerase chain reaction (PCR).

HPV type using PCR	HIV positive (n = 289)			HIV negative (n = 200)		
	No.	% HPV ⁺ of total subjects*	% HPV ⁺ of all HPV ⁺ subjects [†]	No.	% HPV ⁺ of total subjects*	% HPV ⁺ of all HPV ⁺ subjects [†]
HPV-positive	269	93	100	122	61	100
6	58	20	22	14	7	11
11	43	15	16	12	6	10
16	109	38	41	37	19	30
18	80	28	30	5	3	4
26	7	2	3	0	0	0
31	72	25	27	16	8	13
32	15	5	6	3	2	2
33	34	12	13	9	5	7
35	13	5	5	2	1	2
39	19	7	7	3	2	2
40	4	1	1	1	1	1
45	33	11	12	7	4	6
51	12	4	4	4	2	3
52	40	14	15	7	4	6
53	75	26	28	16	8	13
54	21	7	8	5	3	4
55	15	5	6	6	3	5
56	18	6	7	6	3	5
58	52	18	19	17	9	14
59	23	8	9	2	1	2
61	42	15	16	12	6	10
66	27	9	10	2	1	2
68	26	9	10	6	3	5
69	23	8	9	6	3	5
70	63	22	23	8	4	7
73	18	6	7	3	2	2
AE2	7	2	3	1	1	1
AP155	28	10	10	6	3	5
AP291	18	6	7	4	2	3
Mix [‡]	36	12	13	5	3	4
Other [§]	23	8	9	18	9	15

* % given for each specific type represents proportion of all subjects.

[†] % given for each specific type represents proportion of all HPV-positive subjects.

[‡] Mix contains probes for HPV-2/13/34/42/57/62/64/67/72/W13B.

[§] Specimens positive with consensus probes but negative for 39 specific types.

213 (63%) were positive for infection with ≥ 1 group A types. When group B was separated into 2 groups containing types 18/31/33/45/58 and types 35/39/51/52/56 (based on their frequency of detection in cervical cancer), these were equally common with 194 subjects (57%) in each group. The least common group consisted of types 6/11 and was found in 126 subjects (37%). HPV-16 sought as a single type was found in 119 (35%) HIV-positive men. Of the 260 HIV-negative men for whom HC results were available, 75 (29%) were positive for ≥ 1 group B HPV types and 42 (16%) were positive for ≥ 1 group A HPV types. The most common specific group consisted of types 18/31/33/45/58 and was found in 39 subjects (15%). The least common group consisted of types 42/43/44

and was found in 15 subjects (6%). HPV-16 sought as a single type was found in 23 (9%) HIV-negative men.

The HC RLU ratio was used to measure the amount of HPV DNA in the specimens. Table 3 compares the levels of HPV DNA among HIV-positive and HIV-negative subjects who were HPV-positive by HC. Among those positive for group A, the mean group A RLU ratio was higher for the HIV-positive men than the HIV-negative men, but there was little difference by CD4 cell level among the HIV-positive men. Among men positive for group B, the mean group B ratio was higher among HIV-positive men than HIV-negative men, and among the HIV-positive men, there was an association between higher RLU ratios and lower CD4 cell levels ($P = .004$).

Table 2. No. of men positive for HPV and relative risks for HPV positivity using polymerase chain reaction (PCR) and hybrid capture (HC), stratified by HIV status and CD4 cell level.

HIV status and CD4 cells/mm ³	PCR ⁺ , no. (%)	PCR ⁻ , no.	RR (95% CI)	HC ⁺ , no. (%)	HC ⁻ , no.	RR (95% CI)
HIV-negative	122 (61)	78	1.0	97 (37)	163	1.0
HIV-positive*	269 (93)	20	1.5 (1.4–1.7)	297 (87)	43	2.3 (2.0–2.8)
CD4 >500	60 (86)	10	1.4 (1.2–1.6)	65 (77)	19	2.2 (1.8–2.7)
CD4 200–500	110 (96)	4	1.6 (1.4–1.8)	124 (89)	16	2.6 (2.1–3.1)
CD4 <200	99 (94)	6	1.6 (1.4–1.8)	106 (94)	7	2.7 (2.3–3.2)
<i>P</i> for trend [†]			.05			.001

NOTE. RR, relative risk; CI, confidence interval.
 * CD4 cell counts were not available for 3 HIV-positive men.
[†] Tests for trend were calculated on data for HIV-positive subjects only.

Risk factors for detection of HPV. Lifetime exposure to potential risk factors for detection of anal HPV DNA was evaluated for HIV-negative men. For HIV-positive men, risk factors for the presence or absence of HPV DNA could not be assessed because most were positive for HPV DNA by PCR or HC. Among the risk factors examined in univariate analyses for the HIV-negative men, the RRs for HPV infection detected by PCR were lifetime rectal drug use (RR, 1.4; 95% CI, 1.1–1.7), lifetime history of rectal discharge (RR, 1.3; 95% CI, 1.0–1.7), and lifetime level of receptive anal intercourse when compared with no receptive anal intercourse (low, RR, 1.3; 95% CI, 0.97–1.7; medium or high, RR, 1.5; 95% CI, 1.1–2.1; *P* for trend = .03). There was no confounding of the univariate relative risks by other factors and little change in the risk estimates. Other risk factors examined were not related

to HPV infection. The RRs for HPV DNA positivity by HC were similar to those by PCR.

Discussion

Compared with studies on cervical cancer [16], the number of studies of anal cancer and the number of anal cancer cases analyzed have been limited [15]. Furthermore, no studies have subjected anal cancer to techniques capable of detecting the wide number of HPV types reported in cervical cancer. Therefore, the true range of HPV types associated with anal cancer is not known. Likewise, no studies have characterized the range of HPV types detectable in the anal canal of men without anal lesions or with precancerous lesions. In this study, we used PCR to detect 29 individual HPV types as well as 10 grouped types and compared the distribution of HPV types in HIV-positive and HIV-negative men. Anal HPV infection detected by PCR was nearly universal (93%) among HIV-positive homosexual men, consistent with the results of an earlier study [8]. A large proportion of HIV-negative homosexual men were also HPV-positive by PCR (61%). The range of HPV types detected in the anal canal was wide and did not differ appreciably between HIV-positive and HIV-negative men. In both groups, the single most frequently detected type was HPV-16, the type most commonly detected in both cervical cancer [16] and anal cancer [15].

A large proportion of the men had HPV types typically categorized as medium to high risk, on the basis of the HPV types associated with cervical cancer [16]. It is not known if these HPV types confer a similar risk for anal cancer. Among the HPV types found relatively infrequently in cervical cancer but commonly in the anal canal in our study were types 53, 58, 61, and 70. HPV-70 is most closely related phylogenetically to HPV 39, which is considered an intermediate-risk HPV type [25]. Similarly, types 53, 58, and 61 are phylogenetically related to other intermediate-risk HPV types.

We also report detection of HPV-32 outside the oral cavity in a small proportion of both HIV-positive and HIV-negative

Table 3. Mean RLU among men positive for hybrid capture group A or group B HPV infection, stratified by HIV status and CD4 cell levels.

HIV status and CD4 cells/mm ³	No.	Mean RLU	Mean RLU of HIV ⁺ /mean RLU of HIV ⁻	95% CI
Group A*				
HIV-negative	42	5.6	1.0	—
HIV-positive [†]	213	13.3	2.4	1.4–4.0
CD4 >500	39	12.7	2.3	1.1–4.6
CD4 200–500	94	13.8	2.4	1.4–4.4
CD4 <200	79	12.9	2.3	1.3–4.2
Group B[‡]				
HIV-negative	75	4.5	1.0	—
HIV-positive [†]	272	17.5	4.0	2.7–5.4
CD4 >500	56	10.8	2.4	1.5–3.8
CD4 200–500	108	17.6	3.9	2.6–5.8
CD4 <200	106	23.4	5.1	3.4–7.7

NOTE. RLU, ratio of chemiluminescent signal in test sample divided by control sample that contains 10 pg/mL viral DNA. CI, confidence interval.
 * HPV-6/11/42/43/44.
[†] Some HIV-positive subjects did not have CD4 cell results available.
[‡] HPV-16/18/31/33/35/39/45/51/52/56/58.

men. HPV-32 is typically associated with an oral lesion known as focal epithelial hyperplasia (Heck's disease), a benign lesion of the oral cavity characterized by multiple nodular lesions histologically similar to flat warts. Detection of HPV-32 in anal specimens is consistent with the possibility of oral-anal transmission of HPV infection, perhaps through the sexual practice of oral-anal intercourse.

One of the most striking findings in this study was the frequent detection of multiple HPV types in a single sample. Of the HIV-positive men, 73% had >1 HPV type, and on average these men had >3 types. Detection of higher numbers of HPV types among the HIV-positive men than among HIV-negative men may reflect more reporting of receptive anal intercourse by HIV-positive men. Since it is not known when HPV was acquired by the men in this study, we do not know if differences between HIV-positive and HIV-negative men reflect in part the length of time infected with HPV prior to study entry. The differences between the groups may also reflect immunosuppression-mediated HPV DNA replication, since this may facilitate detection of HPV types that would otherwise be missed if present at levels below the threshold of sensitivity of PCR. However, since there was no association between number of HPV types and CD4 cell level among those who were HPV-positive, these data suggest that any influence of immunosuppression manifests relatively early in the course of HIV disease.

The role of multiple HPV types in the pathogenesis of ASIL and anal cancer is unclear, and it is unknown whether different HPV types interact to potentiate pathogenesis. However, detection of multiple HPV types was associated with concurrent anal lesions and progression of anal lesions to a higher grade over a 2-year follow-up period in both HIV-positive and HIV-negative men compared with detection of a single type or no type (Palefsky J, unpublished data).

HIV-positive men had higher mean RLU ratios for both group A and group B HPV types than did HIV-negative men, and this may reflect the higher average number of HPV types detected in the HIV-positive men. However, among HIV-positive men, lower CD4 cell counts were associated with higher group B DNA RLU ratios but not higher group A HPV DNA RLU ratios. Since the mean number of HPV types varied little by CD4 cell level, these data suggest that there may be selective replication of group B types as immunosuppression increases. Further, our data showed a nonsignificant trend toward increasing detection of 4 specific HPV types among HIV-positive men with lower CD4 cell levels: 18, 45, 53, and 59. This is of interest for two reasons: (1) In a study of cervicovaginal HPV infection in 1781 HIV-positive women enrolled in the Women's Interagency HIV Study, the same 4 types were significantly more common among the women with lower CD4 cell levels (Palefsky J, unpublished data), and (2) 3 of these types (18, 45, and 59) are closely related phylogenetically [25].

The mechanisms underlying the association between HIV-associated immunosuppression and higher levels of group B HPV DNA or increasing detection of particular HPV types

with decreasing CD4 cell levels are not known. Cell-mediated immune response may play a role, as demonstrated by lower rates of immune response to HPV antigens among women who were positive for HPV-16 with cervical lesions compared with women without cervical lesions [26, 27]. Therefore, higher levels of anal HPV DNA or replication of antigenically related HPV types may partially be due to loss of HPV-specific immunity subsequent to HIV-mediated immunosuppression. Direct interactions between HIV and HPV may also play a role, given evidence of transactivation of HPV early-region gene expression by the HIV-1 tat protein [28].

Among HIV-negative men, detection of anal HPV DNA was associated with anal activity such as receptive anal intercourse and recreational use of rectal drugs. These data suggest that among HIV-negative men, anal HPV infection is sexually acquired, similar to data showing that cervical HPV infection in women is sexually acquired [29–31]. Although risk factors for HPV positivity could not be assessed among HIV-positive men, it is likely that sexual activity plays a role in this group as it does in the HIV-negative men, given the generally high levels of sexual activity in HIV-positive men.

The results of this study should be interpreted with caution, since they were performed in highly sexually active urban populations with a mean age >40 years, and it is not known if such results are applicable to populations of homosexual men with different levels of sexual activity or at different ends of the age spectrum. In addition, many men in this study had multiple HPV types, and the number of HPV types was probably underestimated because probes were not available for all types. Finally, compared with many other studies, a high proportion of the samples were negative for β -globin. This probably represents the presence of unknown inhibitors in the anal specimens for the majority of these cases. The reason for the low β -globin negativity rate among the HIV-positive men with the lowest CD4 cell counts is not clear, but the high rate of negativity most likely did not affect our conclusions, since the specimens negative for β -globin were removed from the analyses and the remaining PCR data were stratified by HIV status and CD4 cell group.

Several studies have shown that anal HPV infection is an important risk factor for ASIL [7–9, 20], and our data are consistent with the higher rates of ASIL found among HIV-positive men [5–10]. The present study suggests that a substantial number of HIV-positive and HIV-negative homosexual men may be at risk for ASIL. Studies of change in HPV infection and levels of HPV DNA and their relationship to incident ASIL are in progress in this cohort.

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