Short Communication

Human Papillomavirus Capsid Antibody Response to Natural Infection and Risk of Subsequent HPV Infection in HIV-Positive and HIV-Negative Women

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Abstract

The association between seropositivity to virus-like particles (VLP) of human papillomavirus (HPV) types 16, 18, 31, 35, or 45 and subsequent cervical HPV infection was examined in 829 women with HIV and 413 risk-matched HIV-negative women. We found no statistically significant differences between HPV-seropositive and HPV-seronegative women in the risk of a new infection with the homologous HPV type, with the exception of a reduced risk of HPV 45 infections 4.5 years beyond the baseline serology measurement in HIV-positive women [hazard ratio, 0.21; 95% confidence interval (CI), 0.05-0.89]. Among HIV-negative women, HPV seropositivity was not associated with a statistically significant reduced risk of infections with related viruses in the HPV 16, HPV 18, or ''other'' HPV groups. Among HIV-positive women, HPV seropositivity was associated with a slightly increased risk of infection with group-related viruses, but the differences were only statistically significant for infection with HPV 16 group viruses (hazard ratio, 1.6; 95% CI, 1.1-2.3) in HPV 18-seropositive women and for infections with ''other'' HPV group viruses in HPV 31-seropositive women (hazard ratio, 1.45; 95% CI, 1.0-2.0). The lack of a protective immune effect from natural infection is most likely due to the low level of antibody elicited by natural HPV infection and/or the potential for reactivation of HPV, especially in HIV-positive women. (Cancer Epidemiol Biomarkers Prev 2005;14(1):283–8)

Introduction

Human papillomavirus (HPV) is the most common sexually transmitted viral infection in the world. An estimated 60% of sexually active women will become infected with a genital HPV in their lifetime (1–3). In the majority of women, the infection is asymptomatic and cleared within weeks to months (4, 5). More than 40 HPV types infect the genital tract. Whether the antibody response to one type of HPV confers protection against subsequent infection with the same or related types is unknown. For many viruses, the presence of serum neutralizing antibodies is a correlate of immune protection (6). HPV cannot be cultivated in tissue culture; therefore, true neutralization assays are not available. Surrogate neutralization assays with the use infectious pseudovirions have been developed but these methods are insensitive and inefficient for large-scale epidemiologic studies (7). Capsid proteins of HPV produced in insect cells self-assemble into virus-like particles (VLP; ref. 8). VLPs have many immunologic features of native virions including display of type-specific, surface-exposed neutralizing epitopes (8), and are suitable reagents for use in enzyme immunoassays (9). Among recipients of an HPV 16 L1 VLP vaccine, for example, the serum antibody titer measured by VLP-based enzyme immunoassay was highly correlated with the neutralizing antibody titer, thereby indicating the VLP-based enzyme immunoassay can serve as a surrogate marker for neutralizing antibodies (10).

Compared with HIV-negative women, HIV-infected women have a higher prevalence of cervical HPV infection (11) and are at increased risk of acquiring new HPV infections (12). Whether prior HPV type-specific immune status influences the risk of HPV infection in HIV-positive women is unknown. The HIV Epidemiology Research Study (HERS) is a large prospective cohort study of HIV-positive and risk-matched HIV-negative women recruited at multiple sites around the United States (13). The women in HERS have had HPV DNA measurements at approximately 6-month intervals for up to 6 years, providing a wealth of data on detection of type-specific HPV infection.

To assess whether anti-VLP antibodies are a marker of immune protection, we examined measures of baseline serum antibodies to VLPs of HPV types 16, 18, 31, 35, and 45 in 829 HIV-positive and 413 HIV-negative women enrolled in HERS and analyzed the association of seropositivity with risk of detection of HPV DNA during follow-up.

Methods

Study Population. The study population was drawn from HERS. These women were recruited at four sites: Baltimore, MD; Bronx, NY; Detroit, MI; and Providence, RI. The cohort characteristics, recruitment methods, and protocols of the

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HERS are described elsewhere (13). Subjects were evaluated at enrollment and at approximately 6-month intervals for up to 6 years. The enrollment of HIV-positive and HIV-negative women was conducted through similar means and successfully achieved comparability between the seropositive and seronegative cohorts according to age, race, ethnicity, level of education, injection drug use, number of lifetime sexual partners, and enrollment site. Each woman provided a blood sample and a cervicovaginal lavage sample for HPV DNA detection. All participants provided written informed consent and study protocols were reviewed and approved by the investigational committee of human subjects from each participating institution.

A total of 1310 women were enrolled in HERS. We excluded 56 women who lacked a baseline serum sample and 12 who had no HPV DNA data at any visit. Thus, our study cohort consisted of 1,242 women (829 HIV-positive and 413 HIV-negative women). The median follow-up time was 10 visits (interquartile range, 6-12 visits) for HIV-negative women and 10 visits (interquartile range, 5-12 visits) for HIV-positive women. Excluding visits of women lost to follow-up, 8.8% of HERS study visits were missed by the 1,242 subjects in the HPV analysis.

HPV DNA Testing. Cervical and vaginal secretions and exfoliated cells were collected by cervicovaginal lavage with 10 mL of sterile saline. Specimens were stored at −80°C until use. For HPV DNA testing, the lavage specimen was thawed and vortexed, and a 25-μL aliquot was removed and diluted with an equal volume of digestion solution to achieve a final concentration of 200 μg of proteinase K. Digestion was conducted at 55°C for 2 hours. A 10-μL aliquot of the digested material was tested for the presence of HPV DNA by PCR analysis with MY09/MY11 L1 consensus primers, including primer HMB01, and a control primer set (PC04/GH20), which simultaneously amplified a β-globin DNA fragment as described previously (11). HPV genotyping was achieved by dot blot with type-specific probes for the following HPV types: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, 73, 82, 83, and 84. We classified as untypable HPVs which were detected with the use of the generic probe but did not react with any of the type-specific probes; untypable HPVs were detected in 11% of women. Specimens negative for β-globin were excluded from analysis [169 specimens (1.7% of HPV records) from 147 women].

VLP-based Enzyme Immunoassays. HPV VLPs were prepared in *Trichoplusia ni* (High Five) cells (Invitrogen, Carlsbad, CA) from recombinant baculoviruses expressing the L1 and L2 genes of HPV type 16, 31, or 35 or the L1 gene only of HPV type 18 or 45 and purified by density gradient ultracentrifugation and column chromatography techniques as described previously (14). A single lot of VLPs of each HPV type was used for the study.

For the enzyme immunoassay, wells of PolySorp microtiter plates (Nunc, Naperville, IL) were sensitized overnight at 4°C with 50 ng of VLP protein in PBS (pH 7.2) and blocked for 3 hours at room temperature with 10% Superblock (Pierce, Rockford, IL) in PBS containing 0.05% (v/v) Tween 20 (PBS-T; Sigma, St. Louis, MO). The blocking solution was replaced with PBS and the plates were stored at −20°C until use. Before use and following each incubation step, the plates were washed four times with PBS-T in an automatic plate washer (Skanwasher 300, Skatron, Lier, Norway). Serum samples diluted 1:100 in PBS-T containing 10% Superblock were left to react for 1 hour at 37°C. Antigen-bound immunoglobulin was detected with peroxidase-conjugated goat antibodies against human immunoglobulin G, γ chain specific (Zymed, San Francisco, CA) diluted 1:4,000 in PBS containing 10% Superblock, 2.5% polyethylene glycol, molecular weight 20,000 (Sigma), and 0.5% Igepal CA-630 (Sigma). After 30 minutes at 37°C, color development was initiated by the addition of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution (Kirkegaard and Perry, Gaithersburg, MD). The reaction was stopped after 20 minutes by addition of 1% dodecyl sulfate and absorbance was measured at 405 nm (reference wavelength of 490 nm), in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA). Positive and negative control sera were included with each enzyme immunoassay run. The day-to-day coefficients of variation for the HPV 16, 18, 31, 35 and 45 assays were 27%, 35%, 23%, 25%, and 24%, respectively.

Statistical Analysis. Sera were dichotomized as antibody-positive and antibody-negative. The cutoff was calculated by comparing the distribution of values obtained for 100 self-reported virgins (15).

An iterative statistical approach was used that excluded outliers in the distribution of control sera until no remaining values were >3 SD above the mean absorbance value. The calculation was done for each VLP type and four to seven values were excluded for each type. The rationale for using the above procedure is that self-reports of sexual behavior may be inaccurate and some women in the control group may have been exposed to HPV. Seropositivity was defined as 5 SD above the mean absorbance obtained for the negative control sera (minus outliers). Samples with absorbance values greater than the mean plus 3 SD of the control samples and less than the positive cutpoint were considered indeterminate and were excluded from analyses that used serologic data. This exclusion minimizes misclassification of seroreactivity.

The rate of new type-specific HPV infection was computed for HIV-positive women and for HIV-negative women, after excluding those enrolled with a baseline type-specific HPV infection. This rate was defined as the number of women with a new infection during follow-up divided by the person-years of follow-up from baseline to the initial type-specific infection, or until censoring if an infection did not occur. All the study subjects were included in this analysis. Women who had a prior or interval hysterectomy were not excluded. For HIV-negative women, we have found that the prevalence of oncogenic HPV types does not differ between hysterectomized women and nonhysterectomized women (16).

The risk of new HPV cervical infection during follow-up was analyzed with the use of the baseline HPV serostatus of all women, excluding those with indeterminate serology results, and the baseline CD4 cell count of HIV-positive women as covariates. In addition, the number of male sexual partners in the last 6 months (self-report, categorized as 0, 1, or, ≥2), which was measured repeatedly at each study visit, was also considered as a time-varying covariate. To accomplish this, Cox proportional hazards regression with the use of the counting process style of input proposed by Terry and Therneau (17) was used to estimate the relative risk between the seronegative and seropositive groups with associated 95% confidence intervals (CI) for new infections with a specific HPV type or a group of HPV types. The resulting hazard ratios are thus adjusted for the covariates under consideration, namely baseline CD4 cell count for the HIV-positive women and number of male sexual partners in the last 6 months for both HIV-positive and HIV-negative women, the latter as a repeated measure being allowed to vary longitudinally over time. The analysis was done for HPV types 16, 18, 31, 35, or 45, and for the following HPV groups: HPV 16-related types (16, 31, 33, 35, 52, and 58), HPV 18-related types (18, 39, 45, 59, and 68), and other HPV types (6, 11, 26, 40, 42, 51, 53, 54, 55, 56, 57, 66, 73, 82, 83, 84, and nontypeables). Because of the low number of events, namely new HPV cervical infections, missing visits were excluded from consideration in modeling rather than attempting to perform adjustments through imputation or related methodology.

A new infection for a HPV type was defined as the first occurrence of that type from the second recorded visit and

Table 1. Type-specific HPV seroprevalence among women with determinate serology measurements by HIV serostatus, HERS

HPV type	HIV-negative		HIV-positive		*P*
	Total no.	No. (%) seropositive	Total no.	No. (%) seropositive	
16	397	188 (45.5)	788	429 (51.8)	0.021
18	385	147 (35.6)	731	431 (52.0)	<0.001
31	367	207 (50.1)	732	576 (69.5)	<0.001
35	364	145 (35.1)	708	349 (42.1)	<0.01
45	354	175 (42.4)	695	418 (50.4)	<0.001

beyond, where no infection of the type was present at the first or baseline visit. In preliminary analyses, we explored the use of a more stringent definition requiring no infection of the type at the first and second visits and found the stricter definition did not affect the outcome of the analyses. A persistent type-specific infection was defined as the detection of that HPV type on two or more visits. A second DNA-positive sample preceded by one or more negative DNA measurements was assumed to represent fluctuation in viral load below the PCR detection limit rather than reexposure to that type. A single intervening negative DNA measurement was observed with the following frequencies for each of the following type-specific persistent infections: 3 of 41 (7.3 %) for HPV 16, 0 of 31 (0%) for HPV 18, 7 of 22 (31.8%) for HPV 31, 3 of 30 (10%) for HPV 35, and 3 of 26 (11.5%) for HPV 45. A skip pattern of more than one negative DNA measurement between the first and second DNA-positive samples was observed with the following frequencies for each of the following type-specific persistent infections: 2 of 41 (4.9%) for HPV 16, 4 of 31 (12.9%) for HPV 18, 0 of 22 for HPV 31, 3 of 30 (10.0%) for HPV 35, and 5 of 26 (19.2%) for HPV 45. A new infection with a particular HPV group was defined as the first occurrence of any HPV type in the group from the second recorded visit and beyond, where no infection of any HPV type in the group was present at the baseline visit. Because time to infection was measured in terms of visits, we used Cox discrete-time hazard-rate model (18). The model was fit by maximum partial likelihood with the use of Proc Phreg in SAS (version 8.2). The assumption of the proportional hazards model was not satisfied (significance test not shown) for baseline HPV 45 antibody status and new infections with HPV 45 virus. In this instance we used a nonproportional Cox discrete-time hazard-rate model and calculated separate hazard ratios at each visit, where the antibody effect was allowed to interact linearly with time as neither the likelihood ratio test based on the log partial likelihood nor the Wald test for a quadratic term was significant (results not shown).

Results

Seroprevalence and Rates of New HPV Infections. Women with indeterminate serology measurements (absorbance values >3 SD and <5 SD above the mean of the reference negative control sera) were excluded from analyses that used serology data. The proportion of sera excluded was 5% for HPV 16, 19% for HPV 18, 11% for HPV 31, 14% for HPV 35, and 15% for HPV 45. The proportions were comparable across HIV serostatus groups. The large number of women with indeterminate serology measurements reflects the low levels of seroreactivity to HPV VLPs and the influence of small differences in the cutoff on the classification of many women. After these exclusions, the type-specific seroprevalence among HIV-negative women ranged from 35% to 50% and that among HIV-positive women ranged from 42% to 69% (Table 1). For each HPV type, the seroprevalence was significantly higher in HIV-positive than HIV-negative women.

The rate of new type-specific HPV infections in HIV-negative women ranged from 0.42 events (for HPV 35) to 1.23 events (for HPV 31) per 100 person-years (Table 2). Rates of new infections with HPV 16, 18, and 31 were approximately two to three times higher than those with HPV 35 and 45. For HIV-positive women, the rate ranged from 1.38 events (for HPV 31) to 2.27 events (for HPV 16) per 100 person-years, with little variation across types (Table 1). Infection rates for HPV 16, 18, 35, and 45 were two to four times higher in HIV-positive women than in HIV-negative women; the infection rate for HPV 31 infections was comparable in HIV-negative and HIV-positive women.

Anti-VLP Antibody Response and Risk of New Infection with the Homologous HPV Type. For HIV-negative women, we observed no significant differences in new infections with the homologous HPV type among women seropositive and seronegative for that type (Table 3). For example, of the 385 women who were HPV 16 DNA-negative at baseline, 7 (3.5%) of the HPV 16 seronegative women and 10 (5.4%) of the HPV 16-seropositive women had a new HPV 16 infection during the follow-up period. There were 85% and 60% reductions in risk of new HPV 18 and 45 infections, respectively, in women seropositive for the corresponding HPV type, but these reductions were not statistically significant and the number of infections was small (11 for HPV 18 and 7 for HPV 45). For HIV-positive women, there were also no significant differences in the risk of new

Table 2. Rates (per 100 person-years) of new type-specific HPV infection in HIV-negative and HIV-positive women who were HPV DNA-negative at baseline for the respective type, HERS

HIV status and HPV type	No. (%) incident cases observed	No. (%) who completed study follow-up without an event	No. (%) censored*	Person-years at risk	New infection rate/ 100 person-years
HIV-negative					
HPV 16	17 (4.4%)	159 (39.9%)	223 (55.9%)	1,609	1.06
HPV 18	15 (3.7%)	161 (39.6%)	231 (56.8%)	1,640	0.91
HPV 31	20 (5.0%)	155 (38.6%)	227 (56.5%)	1,625	1.23
HPV 35	7 (1.7%)	165 (40.3%)	237 (58.0%)	1,652	0.42
HPV 45	8 (2.0%)	167 (40.8%)	234 (57.2%)	1,652	0.48
HIV-positive					
HPV 16	69 (8.9%)	286 (36.8%)	423 (54.4%)	3,035	2.27
HPV 18	63 (8.1%)	296 (38.0%)	420 (53.9%)	3,035	
HPV 31	42 (5.4%)	301 (38.5%)	439 (56.1%)	3,048	1.38
HPV 35	48 (5.9%)	307 (37.8%)	458 (56.3%)	3,146	1.53
HPV 45	58 (7.4%)	304 (38.6%)	425 (54.0%)	3,065	1.89

*Women who did not experience an event and additionally did not complete 12 study visits, due either to dropping out or having incomplete HPV DNA data.

Table 3. Baseline type-specific HPV seropositivity and risk of new type-specific HPV infection over follow-up by HIV serostatus, HERS

HPV type and HPV serostatus*	HIV-negative			HIV-positive		
	Total no.†	No. (%) PCR positive‡	Relative risk (95% CI)§	Total no.	No. (%) PCR positive	Relative risk (95% CI)
Type 16						
Seronegative	201	7 (3.5%)		340	33 (9.7%)	
Seropositive	184	10 (5.4%)	1.68 (0.63, 4.44)	399	35 (8.8%)	0.78 (0.48, 1.28)
Type 18						
Seronegative	234	10 (4.3%)		289	27 (9.3%)	
Seropositive	145	1 (0.7%)	0.15 (0.02, 1.19)	401	28 (7.0%)	0.76 (0.44, 1.31)
Type 31						
Seronegative	159	10 (6.3%)		152	4 (2.6%)	
Seropositive	199	9 (4.5%)	0.78 (0.31, 1.93)	536	33 (6.2%)	2.10 (0.73, 6.00)
Type 35						
Seronegative	217	5 (2.3%)		355	16 (4.5%)	
Seropositive	143	2 (1.4%)	0.59 (0.12, 3.05)	338	24 (7.1%)	1.51 (0.80, 2.86)
Type 45						
Seronegative	179	5 (2.8%)		266	19 (7.1%)	
Seropositive	171	2 (1.2%)	0.40 (0.08, 2.06)	389	27 (6.9%)	2.46 (0.78, 7.81) if $t = 1$ 0.33 (0.11, 0.98) if $t = 10$¶ 0.26 (0.07, 0.93) if $t = 11$¶ 0.21 (0.05, 0.89) if $t = 12$¶

*Women with indeterminate serologic results were excluded from analysis.
† Women with an infection of the particular HPV type at the baseline visit were excluded from analysis.
‡ Women PCR-positive for homologous HPV type.
§Covariates in the Cox proportional hazards model included baseline serology status (binary, excluding indeterminate serology), time-varying number of male sexual partners in the last 6 months at each study visit, and baseline CD4 (for HIV-positive women only).
¶The proportional hazards assumption does not hold in this case. Therefore, antibody status was allowed to interact linearly with time (likelihood ratio test for an additional quadratic term was not significant), and a separate hazard ratio was calculated for each visit. Only hazard ratios for the first visit and for visits with a significant hazard ratio are shown.

infections with HPV 16, 18, 31, or 35 in women seropositive and seronegative for the corresponding HPV type (Table 3). The HPV 45 data did not satisfy the proportional hazards assumption of the Cox proportional hazards model, so for this case we calculated a separate hazard ratio for each visit. There was no significant difference in the risk of a new HPV 45 infection in HPV 45 seropositive and seronegative women during the first nine visits of follow-up. However, the risk of infection was significantly reduced for seropositive women at the 10th, 11th, and 12th visits (hazard ratio, 0.21; 95% CI, 0.05-0.89, $t = 12$).

We also examined the association of baseline seropositivity with a subsequent persistent HPV infection (defined as the detection of the same HPV type on two or more visits). For HIV-positive women, the risk of a persistent HPV infection among women seropositive for the measured VLP types did not differ significantly from the risk among women who were seronegative (data not shown). Very few events occurred among HIV-negative women and a hazard ratio either could not be computed or the precision in estimating it was low (data not shown).

Anti-VLP Antibody Response and Risk of New Infection with Genetically Related HPV Types. We also examined associations of type-specific seropositivity and risk of subsequent HPV group-specific infection. For HIV-negative women, the risk of group-specific infection did not differ significantly by seropositivity or seronegativity for HPV 16, 18, 31, 35, or 45 (Table 4). For each HPV antibody type, with the exception of HPV 16, the lowest risk was observed for viruses of the homologous HPV group. Among HIV-positive women only two significant differences in the risk for group-specific infection were noted. HPV 18-seropositive women were at greater risk than HPV 18-seronegative women of infection with HPV 16-related types (hazard ratio, 1.6; 95% CI, 1.1-2.3). Also, HPV 31-seropositive women were at greater risk than HPV 31-seronegative women of infection with ''other'' HPV types (hazard ratio, 1.5; 95% CI, 1.0-2.0).

Discussion

Our study of HIV-positive and HIV-negative women, who had a baseline serology measurement for five HPV types and a median follow-up of 47.8 months with HPV DNA testing every 6 months, failed to show that serum antibodies to HPV 16, 18, 31, 35, or 45 VLPs are associated with protection against detection of new infections with the homologous HPV type. We uncovered no evidence of protection for either transient or persistent HPV infection. The only exception to this generalization was that HIV-positive women who were HPV 45 seropositive had a significantly lower risk of a new HPV 45 infection at the 10th study visit 4.5 years after the baseline serology measurement. We also found no evidence for significant group-specific protection conferred by HPV seropositivity among HIV-negative women. Among HIV-positive women, the risk of a new group specific infection was generally higher in HPV-seropositive women than HPV-seronegative women but the increase was significant only for HPV 31-seropositive women who became infected with viruses in the ''other'' group and for HPV 18-seropositive women who became infected with viruses in the HPV 16 group. Owing to the women having serologic evidence of past exposure to HPV and being immunosuppressed from HIV infection, reactivation may in part explain the increased risk.

A recent study by Ho et al. (19) reported that a sustained high level of antibody to HPV 16 VLPs was associated with an approximately 50% reduced risk of subsequent infection with HPV 16 and its genetically related types. Our study differed in several respects from theirs: the women in HERS were older (mean age, 35 versus 20 years), more sexually experienced (mean lifetime number of sex partners, 10 versus 3), and HIV-positive. In the study of Ho et al., the significant correlate of protective immunity was seropositivity at two or more time points; in contrast, we defined serostatus by a one-time measurement of anti-VLP antibodies. In our previous study of women from Costa Rica, we found no reduction in the risk of

Table 4. Baseline type-specific HPV seropositivity and risk of new infection with HPV group viruses over follow-up by HIV serostatus, HERS

HPV serology/HPV group by PCR/HPV serostatus*	HIV-negative			HIV-positive		
	Total no.†	No. (%) PCR positive‡	Relative risk (95% CI)§	Total no.	No. (%) PCR positive	Relative risk (95% CI)
HPV 16						
16 Group						
Seronegative	193	29 (15.0%)	1	275	71 (25.8%)	
Seropositive	169	24 (14.2%)	1.02 (0.59,1.78)	308	95 (30.8%)	1.10 (0.80, 1.52)
18 Group						
Seronegative	200	21 (10.5%)		331	76 (23.0%)	
Seropositive	181	14 (7.7%)	0.77 (0.39, 1.53)	358	80 (22.4%)	0.86 (0.62, 1.19)
Other group						
Seronegative	167	75 (44.9%)		204	134 (65.7%)	
Seropositive	154	67 (43.5%)	0.97 (0.68, 1.38)	210	145 (69.1%)	1.01 (0.77, 1.31)
HPV 18						
16 Group						
Seronegative	220	32 (14.6%)		232	49 (21.1%)	
Seropositive	130	20 (15.4%)	0.97 (0.55 1.71)	311	98 (31.5%)	1.59 (1.11, 2.27)
18 Group						
Seronegative	228	23 (10.1%)		272	54 (19.9%)	
Seropositive	143	9 (6.3%)	0.58 (0.27, 1.27)	360	87 (24.2%)	1.21 (0.85, 1.72)
Other group						
Seronegative	188	79 (42.0%)		173	107 (61.9%)	
Seropositive	126	60 (47.6%)	1.16 (0.81, 1.66)	207	146 (70.5%)	1.28 (0.96, 1.69)
HPV 31						
16 Group						
Seronegative	148	29 (19.6%)		129	29 (22.5%)	
Seropositive	186	22 (11.8%)	0.61 (0.35, 1.07)	416	132 (31.7%)	1.25 (0.83, 1.90)
18 Group						
Seronegative	152	16 (10.5%)		142	31 (21.8%)	
Seropositive	201	18 (9.0%)	0.89 (0.45, 1.75)	486	118 (24.3%)	0.92 (0.61, 1.38)
Other group						
Seronegative	130	61 (46.9%)		91	52 (57.1%)	
Seropositive	171	76 (44.4%)	0.99 (0.69, 1.42)	294	213 (72.5%)	1.45 (1.04, 2.04)
HPV 35						
16 Group						
Seronegative	202	38 (18.8%)		282	74 (26.2%)	
Seropositive	127	15 (11.8%)	0.58 (0.31, 1.06)	244	76 (31.2%)	1.14 (0.82, 1.59)
18 Group						
Seronegative	211	20 (9.5%)		314	63 (20.1%)	
Seropositive	139	14 (10.1%)	1.06 (0.53, 2.12)	302	78 (25.8%)	1.25 (0.89, 1.76)
Other group						
Seronegative	171	69 (40.4%)		199	128 (64.3%)	
Seropositive	119	54 (45.4%)	1.03 (0.71, 1.52)	178	127 (71.4%)	1.07 (0.81, 1.43)
HPV 45						
16 Group						
Seronegative	164	21 (12.8%)		209	56 (26.8%)	
Seropositive	154	23 (14.9%)	1.18 (0.65, 2.15)	306	92 (30.1%)	1.16 (0.82, 1.64)
18 Group						
Seronegative	175	19 (10.9%)		242	54 (22.3%)	
Seropositive	166	12 (7.2%)	0.64 (0.31, 1.32)	358	84 (23.5%)	0.98 (0.69, 1.40)
Other group						
Seronegative	143	57 (39.9%)		154	100 (64.9%)	
Seropositive	146	64 (43.8%)	1.18 (0.81, 1.73)	211	149 (70.6%)	1.06 (0.79, 1.41)

*Women with indeterminate serologic results were excluded from analysis.
† Women with an infection of any HPV type in the group at the baseline visit were excluded from analysis.
‡ Women PCR-positive for viruses within the HPV group. HPV 16 group includes types 16, 31, 33, 35, 52, and 58; HPV 18 group includes types 18, 39, 45, 59, and 68; ''other'' HPV group includes types 6, 11, 26, 40, 42, 51, 53, 54, 55, 56, 66, 73, 82, 83, 84, and nontypeables.
§Covariates in the Cox proportional hazards model included baseline serology status (binary, excluding indeterminate serology), time-varying number of male sexual partners in the last 6 months at each study visit, and baseline CD4 (for HIV-positive women only).

new infections with homologous or genetically related HPV types among women seropositive for HPV 16, 18, or 31 (20).

There are two likely reasons anti-VLP antibodies were not markers of immune protection (despite the strong evidence for antibody mediated protection against HPV from animal studies and from a vaccine trial in humans). First is the low level of antibody elicited by natural HPV infection. In two studies that compared vaccinated women to HPV-exposed, unvaccinated women, the levels of antibody induced by HPV 16 infection were 50- to 60-fold lower than post-vaccination levels (10, 21). The second explanation, particularly for HIV-positive women, is that some of the new infections we detected may have been reactivation of a previous infection rather than an exogenously reacquired or true incident infection. Immunosuppressed patients, including those with HIV infection, have a high prevalence of HPV infection, which may in part be the result of reactivation of a latent infection (11, 22, 23). If some of the new infections in HIV-positive women were due to reactivation, then HPV-seropositive women would be expected to have a higher risk of subsequent HPV infection than HPV-seronegative women would. Among HIV-infected women we did see generally elevated risks for new HPV infections associated with HIV seropositivity, but only two comparisons were statistically significant and in both cases the increased risk of HPV infection was among women who were seropositive for a different HPV type.

Our study has a number of limitations. We measured serum antibodies once and thus could not examine the possibility that persistent seropositivity, as observed by Ho et al., is a marker of protection. Another limitation is the possibility that seropositive and seronegative women differ in the risk of exposure to HPV because of differences in sexual behavior: seroepidemiologic studies have shown that the overriding determinant of HPV seropositivity is past sexual activity (24, 25). Although we adjusted our analysis for number of recent sexual partners at each visit, this covariate does not capture information on male sexual behavior, which is an important determinant of risk of women for HPV infection (26). Even in a study as large as ours, the rates of type-specific HPV infection were small, which can lead to decreased power and potential type II errors.

The findings of our study should not be interpreted to mean that there is no naturally induced protective immunity to HPV. Serologic measurements may not identify all immune persons. Approximately 50% of HPV-infected women do not seroconvert but may nevertheless be protected against subsequent infection (27). These seronegative women may be protected as a result of being primed for a rapid anamnestic antibody response that clears HPV before infection reaches a level detectable by DNA-based methods. Serial serologic measurements taken at the time of a new infection may allow for the distinction between an anamnestic (rapid) response and a primary (delayed) response. Additionally, in some women the cellular arm of the immune system may mediate immune protection against subsequent infection with HPV by a more rapid clearance of infection. A possible implication of our study for vaccine development is that vaccine-induced immunity may wane over time if antibody levels decrease to those seen after natural infection.

Appendix The HER Study Group

The HER Study group consists of Robert S. Klein, M.D., Ellie Schoenbaum, M.D., Julia Arnsten, M.D., M.Ph., Robert D. Burk, M.D., Chee Jen Chang, Ph.D., Penelope Demas, Ph.D., and Andrea Howard, M.D., M.Sc., from Montefiore Medical Center and the Albert Einstein College of Medicine; Paula Schuman, M.D., and Jack Sobel, M.D., from the Wayne State University School of Medicine; Anne Rompalo, M.D., David Vlahov, Ph.D., and David Celentano, Ph.D., from the Johns Hopkins University School of Medicine; Charles Carpenter, M.D., and Kenneth Mayer, M.D., from the Brown University School of Medicine; Ann Duerr, M.D., Lytt I. Gardner, Ph.D., Scott Holmberg, M.D., Denise Jamieson, M.D., Jan Moore, Ph.D., Ruby Phelps, B.S., Dawn Smith, M.D., and Dora Warren, Ph.D., from the Centers for Disease Control and Prevention; and Katherine Davenny, Ph.D., from the National Institute of Drug Abuse.

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