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Polymer-Based Enzyme-Linked Immunosorbent Assay Using Human Papillomavirus Type 16 (HPV16) Virus-Like Particles Detects HPV16 Clade-Specific Serologic Responses

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Human papillomavirus type 16 (HPV16) virus-like particles (VLP) were used as antigen in a polymer enzyme-linked immunosorbent assay (ELISA) to measure antibodies to HPV capsid proteins. Serum samples from 575 college women, previously tested for the presence of cervicovaginal HPV DNA, were analyzed. The prevalences of anti-HPV16 VLP antibodies at baseline were 14.1% for immunoglobulin G (IgG) and 6.4% for IgA. The seroprevalences of IgG in women with cervicovaginal HPV16, HPV16-related types, and other HPV types were 55, 33, and 19%, respectively ($P < 0.001$), compared to the prevalence in women without an HPV infection (10%). HPV VLP IgA seropositivity was associated with high HPV16 VLP IgG optical density values. The seropositivity of IgG antibodies was independently associated with infection with HPV16 or HPV16-related types, increased number of lifetime male partners for vaginal sex, having sex with men ≥5 years older, history of abnormal PAP smear, older age, and living separately from parents. Use of HPV16 VLP polymer ELISA detects clade-specific responses and suggests an HPV16 VLP vaccine may have broader protection that initially anticipated.

Human papillomavirus (HPV) infection of the cervicovaginal area is the most common sexually transmitted disease in young adults and adolescents. Its prevalence in sexually active young women ranges from 20 to 46% in various countries (1, 4, 13, 17, 20, 23, 25). HPVs are classified based on their DNA sequence homology, and more than 100 genotypes have been characterized to date (10). Epidemiological studies indicate that HPV type 16 (HPV16) and, to a lesser extent, HPV18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66, and -68 play a central role in the development of cervical neoplasia and cervical carcinoma (3, 24). However, in the majority of immunocompetent individuals, HPV infection is normally asymptomatic and transient (13, 17, 26). Even in cases in which infection is associated with neoplasia, the lesions often regress, especially in cases of mild dysplasia in young women (12). In contrast, viral persistence is often associated with progression of lesions, which may ultimately develop into invasive cancer (18, 30). Why most individuals apparently clear the viral infection, whereas some fail to eradicate HPV is not well understood, but the increase in frequency of HPV and their associated lesions in immunosuppressed women implicate the immune system (28, 29).

Serological studies in HPV infection have been impeded by an inability to grow large amounts of HPV virions in the laboratory and the low sensitivity of antibody detection by using denatured recombinant HPV proteins (14). A major innovation in HPV serology was the use of recombinant DNA technology to produce HPV capsid proteins that self assembled into virus-like particles (VLPs) (16, 22, 33, 40). These methods produce conformationally intact HPV capsids in adequate amounts for use in enzyme-linked immunosorbent assays (ELISAs) (7, 21). Of major significance was the observation that antibodies to conformational epitopes on synthetically produced papillomavirus VLPs developed in response to type-specific infections and that such antibodies were neutralizing in cell culture systems and in an animal model (8, 9, 15, 31, 32, 38, 39).

In the present study, we utilize a newly developed HPV VLP ELISA protocol (34) to investigate risk factors for HPV16 VLP serum immunoglobulin G (IgG) and IgA antibody development and their association with concurrent cervicovaginal infection.

MATERIALS AND METHODS

Study population. Between September 1992 and March 1994, 608 female college students were invited to participate in a longitudinal study designed to investigate the natural history of cervicovaginal HPV infection, as previously reported (4). The study protocol was approved by the institutional review boards, and informed consent was obtained from all participants. Their mean age (± the standard deviation) was 20 ±3 years, and the racial-ethnic distribution was 57% white, 13% Hispanic, 12% black, 10% Asian, and 8% other. At baseline, 13% of the subjects denied having vaginal intercourse. Of the sexually active women, the median number of lifetime male sexual partners was three. The prevalence of HPV DNA at baseline was 26% (4).

Data collection. At baseline, subjects filled out a self-administrated questionnaire that obtained information on demographic background, sexual history, characteristics of sex partners, smoking history, recreational drug and alcohol use, oral contraceptive usage, and pertinent medical history. Pap smears were obtained and classified according to the 1988 Bethesda system (27). After the

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Pap smears were obtained, exfoliated cervicovaginal cells were obtained by lavage for HPV determination (5). In addition, 10 ml of blood was obtained at baseline.

HPV16 VLP ELISA. The ELISA protocol utilized 0.5% polyvinyl alcohol (PVA) as a blocking agent and 0.8% polyvinylpyrrolidone (PVP) as a secondary antibody adsorption enhancer, as previously described (34). Briefly, Polysorp C96 microtiter plates (Nalgene; Nunc, Inc., Naperville, Ill.) were coated with 50 ng of HPV16 VLPs/well, followed by incubation overnight at 4°C. Plates were blocked with 0.5% PVA 1× phosphate-buffered saline (PBS) for 3 h, and 100 μl of sample diluted 1:100 was added to each well, followed by incubation at 37°C for 1 h. Plates were washed, and 100 μl of goat anti-human IgG or IgA conjugated with horseradish peroxidase (HRP; Jackson Immunoresearch Laboratories, Inc., West Grove, Pa.) diluted 1:10,000 in 0.5% PVA–0.8% PVP–1× PBS was added to each well, followed by incubation at 37°C for 30 min. The plates were washed and 100 μl of ABTS [2,2′azinobis(3-ethylbenzthiazolinesulfonic acid)] peroxidase substrate (KPL, Inc., Gaithersburg, Md.) was added to each well, for up to 40 min. The optical density (OD) reading was made at 405 nm with a 490-nm filter as a reference. The interassay coefficients of variation for negative, intermediate-positive, and positive controls also used in a previous study (34) were 36, 14, and 10%, respectively, for the HPV16 VLP IgG assay and 41, 4, and 12%, respectively, for the IgA assay.

The OD cutpoints for seropositivity of the VLP16-IgG and VLP16-IgA ELISA assays were determined by receiver operating characteristic (ROC) analyses (41). The log-transformed OD values of 51 women with cervicovaginal lavages positive for HPV16 DNA, as determined by either PCR and/or Southern blot hybridization, were compared to those of 70 women who were HPV DNA negative and denied having vaginal sex. Since women with HPV16 infection may not have detectable antibodies, the seropositivity cutpoints were chosen to maximize the specificity among the virginal women. For the VLP16-IgG assay, the cutpoint of log OD of ≥ -0.79 (i.e., OD ≥ 0.16) gave a sensitivity of 47% and a specificity of 100%. For the VLP16-IgA assay, the cutpoint of log OD of ≥ -1.0 (i.e., OD ≥ 0.1) gave a sensitivity of 24% and a specificity of 100%. Serostatus was determined by applying the cutpoint to the mean log OD. The interassay reproducibility of the serostatus of the 575 baseline serum samples tested twice for VLP16-IgG was measured by using kappa statistics, with $\kappa = 0.92$ (95% confidence interval [CI] = 0.88 to 0.97). The reproducibility of the IgA assay was evaluated in 31 samples tested twice ($\kappa = 0.86$ [95% CI = 0.67 to 1.00]).

Secondary antibody evaluation. Goat anti-human IgG (Fcγ fragment specific) and goat anti-human IgA (α-chain specific) conjugated with HRP (Jackson Immunoresearch Laboratories, Inc.) were evaluated to determine their specificity by using purified human immunoglobulins. Purified human immunoglobulins (ChromePure Human IgG and ChromPure Human IgA; Jackson Immunoresearch Laboratories, Inc.) at 0.025 to 400 ng were diluted in carbonate buffer (pH 9.6), and 100 μl was applied in duplicate onto microtiter plates, followed by incubation overnight at room temperature. The wells were subsequently blocked with 0.5% PVA–1× PBS for 3 h at room temperature and then incubated with 100 μl of class specific HRP-conjugated secondary antibodies (1:10,000 or 8 ng/well) in 0.5% PVA–0.8% PVP–0.025% Tween 20–1× PBS for 30 min. After a washing step, 100 μl of ABTS-peroxidase substrate (KPL, Inc.) was added to each well and incubated for 1 h at room temperature, followed by the addition of 100 μl of stopping solution (KPL, Inc.). The plates were read at 405 nm, and the absorbance of the wells incubated with only carbonate buffer was subtracted. To test the assay sensitivity, a mouse monoclonal antibody (MAb), H16.V5 (a gift from Neil Christensen), that recognizes a conformational epitope on HPV16 virions and VLPs was utilized. It was protein G purified, and the concentration was determined by using the BCA protein assay kit (Pierce, Inc., Rockford, Ill.). Ten HPV16 DNA/ELISA-positive human sera were individually tested for their ability to block the interaction of H16.V5 MAb and the VLPs, as previously described (37). A strongly reactive sample (SRS-16; OD > 1.5), which showed complete reaction inhibition, was selected for further characterization. The H16.V5 MAb and the SRS-16 serum were serially diluted, 0.05 to 50 ng and 1:50 to 1:32,000, respectively, and tested in duplicate against 50 ng of HPV16 VLPs. The color development was terminated as soon as a human weakly reactive control serum sample reached an OD of 0.3.

Statistical analysis. Odds ratios were used to estimate the association between HPV16 VLP seropositivity and each of the potential categorical risk factors. In univariate analyses, the statistical significance of the association was assessed by Pearson χ^2 test. For ordinal risk factors, the Mantel-Haenszel χ^2 test for trend was used. Risk factors that were significant in univariate analyses were scrutinized in multivariate logistic regression analyses. Statistic analyses were done by using SAS software. The *P* values presented are two tailed.

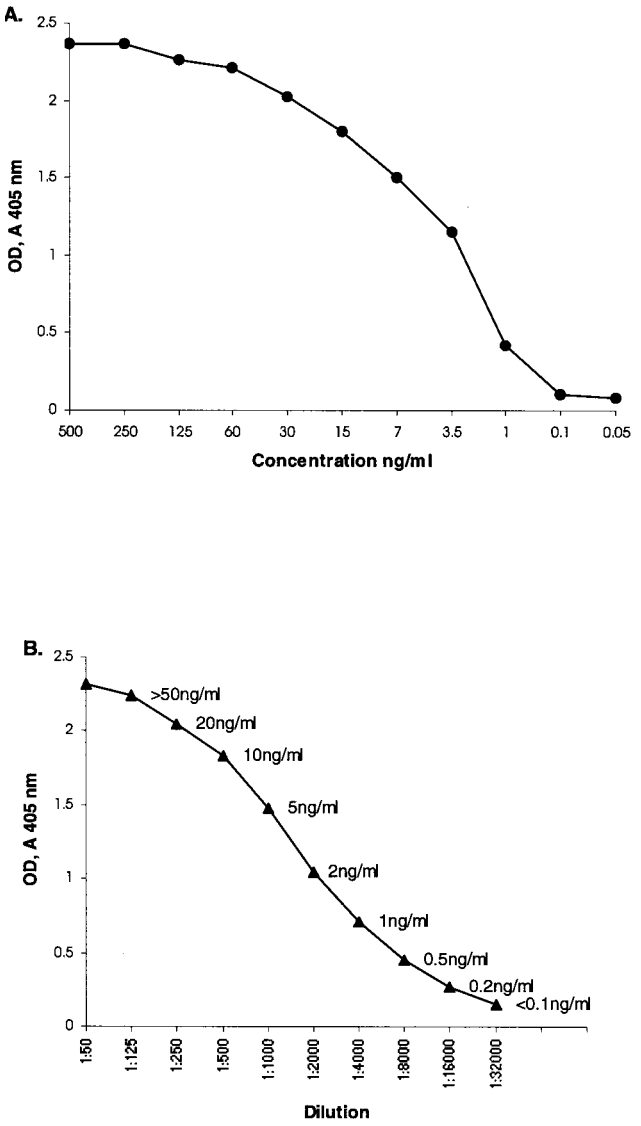

FIG. 1. (A) End point titration of HPV16 VLP conformation-specific H16.V5 MAb. (B) End point titration of an HPV16-seropositive sample (SRS-16). IgG concentrations shown on the curve were extrapolated from H16.V5 MAb reaction with the VLPs shown in panel A.

RESULTS

Sensitivity and specificity for detecting human IgG and IgA antibodies. The detection of different classes of antibodies reacting with immobilized VLPs was distinguished by specific secondary antibodies. Thus, we initially evaluated reagents for sensitivity and specificity to distinguish human IgG and IgA. HRP-conjugated anti-human IgG and IgA were tested by using purified human IgG and IgA. Reactivity was strongly class specific, and no cross-reactivity was observed with high IgG (400 ng/ml) or IgA (400 ng/ml) concentrations (data not shown). The sensitivity of HPV16 IgG-specific antibody detection was determined by endpoint dilution of purified H16.V5 monoclonal antibody (Fig. 1A). The lower limit of detection was ca. 0.1 ng/ml, which corresponded to the independently determined cutoff OD of 0.16. Concentrations between ca. 0.1

TABLE 1. Distribution of OD values among IgG- and IgA-seropositive samples

Immunoglobulin type (*n*)	OD value			
	Geometric mean (SD)	Median	Range	Interquartile range
IgG seropositive (81)	0.42 (2.24)	0.31	0.16–2.43	0.21–0.89
IgA seropositive (36)	0.23 (2.02)	0.18	0.10–1.22	0.13–0.35

and 50 ng/ml were linear with the OD values. ODs obtained with different concentrations of H16.V5 were compared to the ODs of a strongly reactive human serum sample SRS-16 (see Materials and Methods). The reciprocal dilution curve with defined concentrations of human serum is shown in Fig. 1B. The clinical sample showed an OD of 0.147 at a dilution of 1:32,000, which corresponded to a concentration of 0.1 ng of reactive antibodies/ml. This indicates that strongly reactive samples contain ca. 350-fold higher levels of specific antibodies than weakly reactive samples. These studies demonstrated that there was no cross-reactivity between IgG and IgA detection and delineate the linear range for antibody detection.

Antibody prevalence at baseline. The overall prevalence of anti-HPV16 VLP IgG and/or IgA antibodies at baseline was 16%. HPV16 VLP IgG and IgA antibodies were detected in 81 of 575 (14.1%) and 36 of 575 (6.2%) subjects, respectively. Based on the OD values, the majority of reactive serum samples displayed a low immunoreactivity to HPV16 VLPs (see Table 1), and the OD values for the IgA-seropositive samples were considerably lower than those of the IgG-seropositive samples.

There was a strong association between seropositivity of HPV16 VLP IgG and IgA: 69.4% of the samples positive for IgA were also positive for IgG, whereas only 10.4% of the samples negative for IgA were IgG positive ($P < 0.0001$). There was also a strong correlation between IgG OD values and IgA seropositivity. When IgG OD values were >0.3, 55% of the samples were seropositive for IgA, whereas at lower IgG positive OD values (0.16 to 0.3) only 7.3% were IgA seropositive ($P = 0.0001$).

Association between HPV16 VLP antibodies and HPV DNA status. There was a strong association between antibody detection and HPV DNA types detected in the cervicovaginal lavage (Table 2). Seroprevalence was highest among subjects who had HPV16 DNA, followed by those with HPV16-related types (HPV31, -33, -35, -52, and -58) and those with non-HPV16-related "other" types. Among women who were negative for HPV DNA, 9.4% and 3.9% were seropositive for HPV16 VLP IgG and IgA antibodies, respectively. Based on PCR and Southern blot results, HPV DNA viral load was classified as high (Southern blot positive with or without PCR positivity) or low (PCR positive, Southern blot negative). This classification was used to evaluate the relationship between viral load and HPV16 VLP seropositivity. Figure 2 shows that infection with a high viral load of HPV16-related types was associated with a similar proportion of IgG seropositivity as HPV16 DNA-positive women with either a high or a low viral load. In addition, 28.6% of the individuals with a high viral load of HPV16 or HPV16-related types were also seropositive for IgA compared to 10.5% of those with an infection of a low viral load ($P = 0.303$).

TABLE 2. Association between HPV DNA and HPV16 VLP ELISA IgG seropositivity

Patient status	No. of HPV16 VLP IgG-positive samples/total no. (%)[c]	No. of HPV16 VLP IgA-positive samples/total no. (%)[d]
HPV DNA negative		
No vaginal sex	1/42 (2.4)	0/42 (0.0)
Sexually active	38/373 (10.2)	16/373 (4.3)
HPV DNA positive		
Other types[a]	19/101 (18.8)	10/101 (9.9)
HPV16-related types[b]	6/18 (33.3)	2/18 (11.1)
HPV16	12/22 (54.6)	6/22 (27.3)

[a] "Other types" include HPV types exclusive of HPV16 and HPV16-related types.
[b] HPV16-related types include HPV31, -33, -35, -52, and -58.
[c] $P < 0.0001$.
[d] $P < 0.0001$.

Risk factors for HPV seropositivity. Results of the univariate analyses for risk factors associated with HPV16 VLP IgG seropositivity are shown in Table 3. Similar analyses were not done for IgA seropositivity due to the small numbers of seropositive subjects. The demographic characteristics significantly related to seropositivity included older age, year in college, being black, and living separately from parents. HPV16 VLP seropositivity was associated with various sexual behaviors, in-

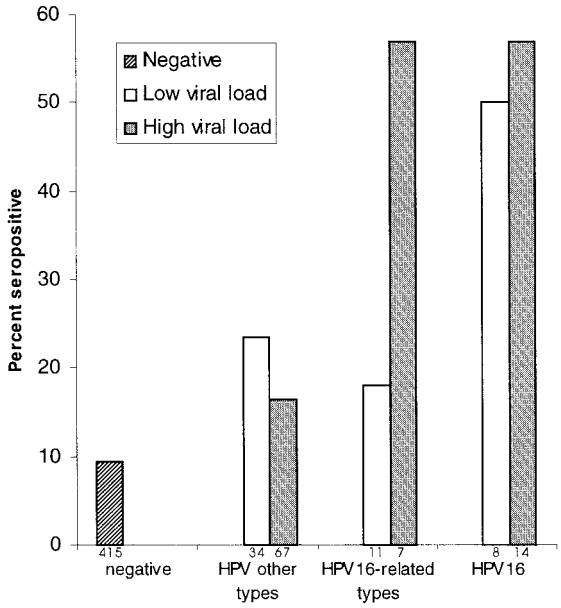

FIG. 2. Association between HPV16 VLP ELISA IgG seropositivity and HPV viral load. A high viral load was defined as HPV DNA positive by Southern blot with or without PCR, whereas a low viral load had HPV DNA detected only by PCR. Other HPV types and HPV16-related types are described in Table 2. The number of subjects in each group is shown below the graph.

TABLE 3. Univariate analyses of risk factors associated with HPV16 VLP IgG seropositivity

Risk factor	No. of HPV16 VLP-positive samples/total no. (%)	Odds ratio (95% CI)	*P*
Demographic factors			
Age (yr)			
≤20	55/461 (12)	1.00	
21–23	12/69 (17)	1.55 (0.78–3.08)	
≥24	14/45 (31)	3.34 (1.67–6.65)	<0.001[a]
Ethnicity			
White/other	51/427 (12)	1.00	
Hispanic	12/77 (16)	1.36 (0.69–2.69)	0.374
Black	18/71 (25)	2.50 (1.36–4.61)	0.003
Annual family income			
<$40,000	35/211 (17)	1.00	
≥$40,000	32/253 (13)	0.73 (0.43–1.22)	0.229
Yr in college			
1–2	57/467 (12)	1.00	
3–5	11/59 (19)	1.65 (0.81–3.36)	
Postgraduate	13/49 (27)	2.60 (1.30–5.19)	0.003[a]
Currently living with parents			
Yes	12/144 (8)	1.00	
No	69/431 (16)	2.10 (1.10–3.99)	0.022
Sexual behavioral factors			
Sexual experience			
Never had any type of sex	1/44 (2)	1.00	
Oral or anal sex	1/30 (3)	1.48 (0.09–24.67)	
Vaginal sex	79/501 (16)	8.05 (1.09–59.31)	0.004[a]
Age (yr) at first vaginal sex[b]			
>16	37/265 (14)	1.00	
≤16	42/236 (18)	1.33 (0.82–2.16)	0.240
No. of male vaginal sex partners in lifetime[b]			
1	11/133 (8)	1.00	
2	16/99 (16)	2.14 (0.94–4.84)	
3–4	13/128 (10)	1.25 (0.54–2.91)	
≥5	39/141 (28)	4.24 (2.07–8.70)	<0.001[a]
No. of male vaginal sex partners in last 6 mo[b]			
0	10/46 (22)	1.00	
1	44/299 (15)	0.62 (0.29–1.34)	
≥2	25/156 (16)	0.69 (0.30–1.56)	0.630[a]
Condom use in last 6 mo[b]			
Never/rarely/sometimes	40/230 (17)	1.00	
Most of the time	16/138 (12)	0.62 (0.33–1.16)	
All of the time	17/129 (13)	0.72 (0.39–1.33)	0.680
Ever douched after sex[c]			
Never	74/486 (15)	1.00	
Rarely or all the time	6/44 (14)	0.88 (0.36–2.15)	0.778
Had casual sex in last 6 mo[c]			
No	49/355 (14)	1.00	
Yes	31/176 (18)	1.34 (0.82–2.18)	0.248
No. of regular partners in last 6 mo[c]			
0	17/88 (19)	1.00	
1	46/365 (13)	0.60 (0.33–1.11)	
≥2	17/78 (22)	1.16 (0.55–2.47)	0.057
Maximum frequency of vaginal sex with regular partner in last 6 mo[d]			
0–5 times	9/80 (11)	1.00	
1–3 times/mo	14/93 (15)	1.40 (0.57–3.43)	
≥Once/wk	40/268 (15)	1.38 (0.64–2.99)	0.470[a]
Ever lived with a partner for >6 mo			
No	69/529 (13)	1.00	
Yes	12/45 (27)	2.42 (1.19–4.92)	0.012

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TABLE 3—*Continued*

Risk factor	No. of HPV16 VLP-positive samples/total no. (%)	Odds ratio (95% CI)	*P*
Before age 16 had sex with men ≥5 years older			
No	59/520 (11)	1.00	
Yes	22/55 (40)	5.2 (2.85–9.53)	<0.001
Ever had genital warts			
No	75/556 (13)	1.00	
Yes	6/19 (32)	2.96 (1.09–8.03)	0.026
Had non-HPV-related sexually transmitted disease			
No	64/503 (13)	1.00	
Yes	17/72 (24)	2.12 (1.16–3.88)	0.013
Last Pap smear			
Never had a Pap smear	19/222 (9)	1.00	
Normal	46/288 (16)	1.50 (0.90–2.50)	
Abnormal	8/18 (44)	8.55 (3.02–24.23)	<0.001[a]
Ever used oral contraceptive pills			
No	37/335 (11)	1.00	
Yes	44/239 (18)	1.82 (1.13–2.92)	0.013
Currently using oral contraceptive pills			
No	59/442 (13)	1.00	
Yes	21/131 (16)	1.24 (0.72–2.13)	0.437
Ever been pregnant			
No	61/512 (12)	1.00	
Yes	20/63 (32)	3.44 (1.90–6.23)	<0.001
Ever had an abortion			
No	64/516 (12)	1.00	
Yes	17/59 (29)	2.86 (1.54–5.32)	<0.001
Lifestyle factors			
Current smoker			
No	71/475 (15)	1.00	
Yes	10/99 (10)	0.64 (0.32–1.29)	0.208
Frequency of alcohol use in last 6 mo			
<1 time/wk	62/434 (14)	1.00	
≥1 time/wk	19/141 (13)	0.93 (0.54–1.62)	0.810
Frequency of drug use in last 6 mo			
None	53/400 (13)	1.00	
<1 time/mo	22/110 (20)	1.64 (0.94–2.83)	
≥1 time/mo	6/65 (9)	0.67 (0.27–1.62)	0.973[a]

[a] *P* value for trend.
[b] Among subjects who had had vaginal sex.
[c] Among subjects who had had any type of sex.
[d] Among subjects who had a regular partner in the last 6 months.

cluding experience with vaginal sex, cohabitation, an increased number of lifetime sexual partners for vaginal sex, a sexual relationship with men who were ≥5 years older than self, a history of pregnancy, abortions, the use of oral contraceptive pills, sexually transmitted diseases, and an abnormal Pap smear. IgG seropositivity did not show an association with recent sexual activities, such as the number of male partners for vaginal sex, the number of regular partners, and experience with casual sex.

In multivariate logistic regression analysis (Table 4), IgG seropositivity was independently associated with infection with HPV16 or HPV16-related types, an increased number of lifetime male partners for vaginal sex, having had sex with men who were ≥5 years older than self, a history of an abnormal Pap smear, older age, and living separately from parents.

DISCUSSION

We evaluated human IgG and IgA antibody responses to HPV16 capsid proteins among a cohort of college age women utilizing a newly developed polymer-based ELISA (34). The polymer-based ELISA detection limit for HPV16 VLP IgG antibodies was ca. 0.1 ng/ml. In agreement with other data (35), college age women were four times more likely to be seropositive for HPV16 infection (16%) than to have a detectable level of HPV16 DNA in cervicovaginal cells (3.6%) (4). This indicates a high level of exposure to HPV16 infection among sexually active individuals and self-clearance of infection in the majority of cases. As expected, most seropositive human sera had low HPV16 VLP antibody levels.

Two previously reported studies of HPV16 VLP serology (6,

TABLE 4. Multivariate logistic regression analysis of risk factors associated with HPV16 VLP IgG seropositivity

Risk factor	Adjusted odds ratio (95% CI)	*P*
HPV DNA type		
Negative	1.00	
Other type	1.45 (0.72–2.92)	0.305
HPV16-related type	5.38 (1.64–17.66)	0.006
HPV16	5.17 (1.70–15.67)	0.004
Male vaginal sex partners in lifetime (per partner increase)	1.11 (1.03–1.19)	0.005
Had sex with men ≥5 years older before age 16	5.22 (2.49–10.96)	<0.001
Last Pap smear was abnormal	4.26 (1.26–14.29)	0.020
Age (per year increase)	1.11 (1.02–1.22)	0.021
Currently living with parents	0.31 (0.13–0.72)	0.007

36) in young women were compared to the present study (Table 5). The polymer-based ELISA showed a twofold increase in specificity (19% versus 8.7% among HPV-negative patients) with simultaneous improvement in sensitivity (46% versus 52.4% among HPV16-positive patients) compared to a study using a standard HPV16 VLP ELISA test (34). In contrast, a study using an HPV16 VLP capture ELISA protocol (6) detected a seroprevalence among the HPV-negative and HPV16-positive groups similar to that reported here; however, the polymer-based ELISA showed a fourfold increase in the detection of antibodies among patients with other than HPV16 DNA (5.2% versus 23.8%). These discrepancies might be attributable to a number of factors: first, different systems for VLP production and antibody detection; second, conformational changes in VLP structure due to the absence of L2 in the VLPs used in the capture assay; third, different cutoff levels for ELISA interpretation; and fourth, potential differences in population composition, sex behavior, endemic-HPV patterns, etc. Due to the improved detection ability of α-HPV16 VLP-specific antibodies, the polymer-based ELISA detected a clade-specific response (i.e., clade A9–HPV16, -31, -33, -35, -52, and -58). The detection of HPV16-related infection independent of HPV16 exposure was supported by the viral-load data: when the viral load of HPV16-related types was high, seroreactivity with HPV16 VLPs was threefold more likely. This association disappeared with other HPV types, for which no difference in

TABLE 5. Comparison of HPV16 VLP serology in young women

Parameter	Variables and findings of:		
	Carter et al. (6)	Viscidi et al. (36)	Present study
Study population, U.S. State	Female university students, Washington	Female graduate and undergraduate students, Maryland	Female college students, New Jersey
No. of subjects at baseline	293	376	575
Mean age (yr [range])	19 (18–20)	22 (18–40)	20 ± 3[a]
HPV DNA data (no. [%])			
Negative	216 (74)	247 (66)	422 (73)
Positive	58 (20)	101 (27)	132 (23)
HPV16 DNA positive	19 (6)	28 (7)	21 (4)
ELISA procedure			
Type	Capture, H16.V5 MAb based	Direct	Direct
Antigen	HPV16 L1 VLP (produced by recombinant vaccinia virus, CsCl purified)	HPV16 L1/L2 VLP (produced by recombinant baculovirus, CsCl purified)	HPV16 L1/L2 VLP (produced by recombinant baculovirus, CsCl purified)
Antigen concn (ng/well), treatment	NA[b]	500, overnight at 4°C	50, overnight at 4°C
Blocking agent	1× PBS–0.05% Tween 20–0.5% nonfat milk	1× PBS–0.05% Tween 20–0.5% nonfat milk	1× PBS–0.5% PVA
Primary antibody dilution	1:100	1:10	1:100
Detection system	AP[c]–*p*-nitrophenyl phosphate disodium hexahydrate	HRP-ABTS-H_2O_2	HRP-ABTS-H_2O_2
Cutoff OD value	0.259	0.166	0.16
Subtracted value (definition)	OD of each tested serum reacted with no VLP well	OD of each tested serum reacted with VLP disrupted in carbonate buffer	OD of control human serum reacted with no VLP well (OD = 0.025 to 0.05)
ELISA results for IgG (%)			
Seroprevalence	9.5	24	14
HPV DNA negative	6.9	19	8.7
HPV DNA positive	5.2	30	23.8
HPV16 DNA positive	52.6	46	52.4

[a] Mean ± standard deviation.
[b] NA, not applicable.
[c] AP, alkaline phosphatase.

antibody detection was associated with viral load. Conversely, a high viral load with HPV16 was not significantly associated with increased IgG seropositivity (50% versus 57%) but led to higher IgG OD numbers, which were highly associated with IgA seropositivity. IgA OD values were also increased with a high viral load. A previous study in humans found systematic IgA, but not IgG or mucosal IgA, to be associated with clearance of HPV16 infection (2). Furthermore, in a prospective study, Ho et al. (19) reported on protection from an HPV16-related second infection in subjects seropositive for HPV16 VLP antibodies, a finding consistent with the detection of antibodies associated with concurrent HPV16-related A9 group infection.

Risk factors for HPV16 VLP ELISA positivity were evaluated against those previously determined for participants with baseline HPV DNA (4). Seropositivity was significantly associated with age, race, sexual activity, number of lifetime sexual partners, genital warts, and other sexually transmitted diseases, associations in agreement with those found in previous studies (11, 36); however, recent sexual exposure was not associated with seropositivity. This finding is consistent with the notion that antibodies require time from exposure for development.

In conclusion, clade-specific reactivity suggests VLP-based vaccines might have a broader protective effect than initially anticipated.

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