MAJOR ARTICLE

Serum Immunoglobulin A Response to Human Papillomavirus Type 16 Virus–Like Particles in Human Immunodeficiency Virus (HIV)–Positive and High-Risk HIV–Negative Women

Raphael P. Viscidi,[1] Linda Ahdieh-Grant,[2] Michael F. Schneider,[2] Barbara Clayman,[1] L. Stewart Massad,[3] Kathryn M. Anastos,[4] Robert D. Burk,[5] Howard Minkoff,[6] Joel Palefsky,[7] Alexandra Levine,[8] and Howard Strickler[5]

[1]Johns Hopkins University School of Medicine, and [2]Bloomberg School of Public Health, Baltimore, Maryland; [3]Southern Illinois University School of Medicine, Springfield, Illinois; [4]Montefiore Medical Center, and [5]Albert Einstein College of Medicine, Bronx, and [6]Maimonides Medical Center, Brooklyn, New York; [7]University of California at San Francisco, San Francisco, and [8]University of Southern California School of Medicine, Los Angeles

Serum samples from 2008 human immunodeficiency virus (HIV)–positive and 551 HIV-negative women were tested for immunoglobulin A (IgA) to human papillomavirus (HPV) type 16 capsids. IgA seropositivity was lower than previously reported IgG seropositivity (7% vs. 51%), but, like IgG antibodies, HPV 16 IgA was associated with sexual behavior, cervicovaginal HPV 16 DNA, and cytological abnormalities. IgA seropositivity was higher in HIV-positive women than in HIV-negative women (7.7% vs. 4.9%; $P = .02$), but the association was lost after adjustment for HPV 16 cervicovaginal infection. IgA, but not IgG, seropositivity was associated with progression to high-grade cytological abnormalities (relative hazard [RH], 2.2 [95% confidence interval, 1.2–4.2]), raising the possibility that an IgA response to HPV 16, as described for other DNA viruses, may be a marker of persistent viral replication. The risk of incident infection with non–16-related HPV types was increased in IgA seropositive women (RH, 1.8 [95% confidence interval, 1.3–2.6]), compared with seronegative women (RH, 2.2 [95% confidence interval, 0.9–5.4]), but there was no difference in the risk of incident HPV 16 or HPV 16–related infections. This may be evidence of partial type-specific or clade-specific immunity conferred by seropositivity to HPV 16 capsids.

Serological assays for human papillomavirus (HPV) infection that are based on viruslike particles (VLPs) have been validated as type-specific markers of past and current HPV infection [1]. Most seroepidemiological studies of HPV have measured serum IgG responses. IgA is the second-most-prevalent antibody class in serum (for review, see [2]). The source of serum IgA is the bone marrow, in contradistinction to secretory IgA, which is locally produced at mucosal surfaces. The biological activities and physiologic function of serum IgA are poorly understood. For a number of infections, it has been demonstrated that the serum IgA response parallels that of serum IgG but peaks at a lower level and is shorter lasting [3–8]. For a number of DNA viruses, IgA responses are associated with persistent viral replication and reactivation of latent infection [9–13].

A few seroepidemiological studies of HPV have measured serum IgA to HPV capsids. Conflicting results have been reported regarding the determinants of IgA seropositivity. For example, the IgA response has been found to be either strongly [14] or weakly [15] associated with the IgG response. In one study IgA seroreactivity was associated with HPV DNA persistence

Received 11 February 2003; accepted 17 July 2003; electronically published 5 December 2003.

Presented in part: 20th International Papillomavirus Conference, Paris, France, 6–10 October 2002 (abstract P301).

Financial support: National Institutes of Health (grants AI-42058 [to R.P.V.], U01-AI-35004, U01-AI-31834, U01-AI-34994, AI-34989, U01-HD-32632, U01-AI-34993, U01-AI-42590, and CA85178); General Clinical Research Center at University of California, San Francisco (M01-RR00079 and M01-RR00083).

Reprints or correspondence: Dr. Raphael P. Viscidi, Johns Hopkins Hospital, Blalock Rm. 1105, 600 N. Wolfe St., Baltimore, MD 21287 (rviscid1@jhem.jhmi.edu).

The Journal of Infectious Diseases 2003;188:1834–44

0022-1899/2003/18812-0007$15.00

[16], but in another study it was associated with HPV clearance [17]. In a serosurvey of healthy women, serum IgA, but not serum IgG, to HPV type 16 capsids was correlated with the number of recent sexual partners [15]. Women with human immunodeficiency virus (HIV) infection have been the focus of 2 studies. In one study HPV 16 IgA seroprevalence was higher in HIV-negative women than in HIV-positive women [18], and in another study the combined IgA seroprevalence to 3 high-risk HPV types (i.e., 16, 18, and 31) was lower in HIV-negative women than in HIV-positive women [14]. The large size of our HIV study population and careful follow-up with testing for a wide variety of HPV DNA types may clarify the latter, conflicting findings.

The Women's Interagency HIV Study (WIHS) is a large prospective cohort study of HIV-positive women and high risk–matched HIV-negative women [19]. HIV-positive women have a high prevalence of HPV infection, report sexual behaviors that place them at risk for past and current HPV infection, and may, because of HIV-induced immunosuppression, have increased susceptibility to HPV infection. These characteristics make HIV-infected women a highly informative population for the study of humoral immune responses to HPV. It is also of interest, in and of itself, to assess the effects that HIV-induced immune suppression has on the ability to mount an HPV type–specific IgA response. We determined the prevalence of serum IgA reactivity to HPV 16 capsids in relation to HIV status, cervicovaginal HPV infection, and demographic and behavioral risk factors for HPV infection. In addition, we examined the predictive value that HPV 16 IgA seropositivity had for subsequent detection of HPV DNA in the genital tract and for development of high-grade cytological abnormalities. Because the serum samples had previously been tested for HPV 16 IgG seroreactivity [20], we also compared serum IgA responses and serum IgG responses.

SUBJECTS, MATERIALS, AND METHODS

Study population. Serological testing was done on enrollment serum samples obtained from 2008 HIV-positive women and 551 HIV-negative women enrolled in the WIHS study in Bronx/Manhattan, NY; Brooklyn, NY; Chicago, IL; Los Angeles, CA; San Francisco, CA; and Washington, DC. All women with an available serum sample obtained at the enrollment visit were included in the study. These 2559 women constituted 97% of the 2628 participants enrolled in the WIHS. The cohort characteristics, recruitment methods, and protocols of the WIHS are described elsewhere [19]. Informed consent was obtained from all subjects, and the human-subjects investigational committees from each participating institution approved the study. Subjects were evaluated at enrollment and at 6-month intervals. In brief, each woman underwent an extensive structured interview detailing her medical, behavioral, and psychosocial history, including queries about past and current sexual practices. The enrollment of HIV-positive women and HIV-negative women was conducted through similar means, and it successfully achieved comparability between the seropositive and seronegative cohorts, with regard to age, ethnicity, level of education, injection drug use, number of lifetime sexual partners, and enrollment site. Each woman received a complete physical and gynecologic examination and provided blood, a Papanicolaou (Pap) smear, and a cervicovaginal-lavage sample (by use of 10 mL of sterile saline) for detection of the presence of HPV DNA.

Cervicovaginal cells were tested for the presence of HPV DNA by polymerase chain reaction (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. The PCR methodology and the results of PCR testing for HPV in women in the WIHS have been the subject of a previous publication [21]. HPV genotyping was done by dot blot with type-specific probes for the following HPV types: 6, 11, 16, 18, 26, 31, 32, 33, 35, 39, 40, 45, 51–56, 58, 59, 61, 66, 68–70, 73, and 82–84. Cervicovaginal cells from 42 women who were found to be positive for HPV 16 by PCR were tested in the Hybrid Capture II assay, a semiquantitative HPV DNA assay using an HPV 16 type-specific probe. Specimens were considered to be positive if the chemiluminescent signal was at least the average of 3 positive controls consisting of 1.0 pg of HPV 16 DNA/mL.

All Pap smears were read at a central location by 2 cytotechnologists who used the Bethesda System for cervicovaginal cytologic diagnosis [22]. Any smear found to be abnormal by either technologist, including atypical cells of undetermined significance (ASCUS), as well as 10% of normal Pap smears, were reviewed by a cytopathologist. For analysis, diagnoses were grouped as "normal," "ASCUS," "low-grade squamous intraepithelial lesions" (LSIL), "high-grade squamous intraepithelial lesions" (HSIL), and "cancer."

HPV 16 VLP–based ELISA. HPV-like particles were prepared in *Trichoplusia ni* (High Five; Invitrogen) cells from a recombinant baculovirus expressing the L1 and L2 genes of HPV 16 and were purified by density-gradient ultracentrifugation and column-chromatography techniques described elsewhere [20]. The blocking solution and the diluent for serum samples and conjugate were prepared as described by Studenstsov et al. [23]. Wells of PolySorp microtiter plates (Nunc) were sensitized, overnight at 4°C, with 50 ng of VLP protein in PBS (pH 7.2), and were blocked, for 3 h at room temperature, with 0.5% (wt/vol) polyvinyl alcohol (PVA) (molecular weight [MW], 30,000–70,000; Sigma) in PBS (0.5% PVA). The blocking solution was replaced with PBS, and the plates were stored at −20°C until

use. Before use and after each incubation step, the plates were washed 4 times with PBS containing 0.05% (vol/vol) Tween 20 (Sigma), in an automatic plate washer (Skanwasher 300; Skatron). Serum samples diluted 1:100 in 0.5% PVA were left to react for 1 h at 37°C. Antigen-bound immunoglobulin was detected by peroxidase-conjugated goat antibodies against human IgA (Southern Biotechnology) that were diluted 1:2000 in 0.5% PVA, 0.025% Tween 20, 0.8% (wt/vol) polyvinylpyrrolidone (MW, 360,000; Sigma) in PBS. After 30 min 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). After 20 min, the reaction was stopped by addition of 1% dodecyl sulfate, and absorbance was measured at 405 nm, with a reference wavelength of 490 nm, in an automated microtiter plate reader (Molecular Devices). The cut point for seropositivity was determined on the basis of the reactivity of 106 plasma samples from self-reported virgins [24]. The mean and SD of optical density (OD) values for the virgin control subjects were calculated, and values greater than the mean plus 3 SD were excluded. The analysis was repeated on the remaining samples until no further OD values could be excluded on the basis of this criterion. A positive cut point of 3 SD above the mean of this distribution was chosen. A cut point of 2 SD above the mean was also explored, and all of the results presented below were consistent at the lower and higher cut points.

The specificity and sensitivity of the enzyme-labeled anti-IgA and anti-IgG conjugates were determined in an ELISA using wells coated with purified immunoglobulin protein. In wells coated, at 1000 ng/mL, with purified IgA, IgG, and IgM, the mean OD value of the anti-IgA conjugate was 1.657, 0.027, and 0.033, respectively. The detection limit for IgA was 10 ng/mL. The goat anti–human IgG conjugate (Zymed) used in a previously published study of IgG seroreactivity had a detection limit of 3.3 ng/mL. In wells coated, at 333 ng/mL, with purified IgA, IgG and IgM, the mean OD value of the anti-IgG conjugate was 0.034, 1.740, and 0.116, respectively. The serum samples had been tested for IgG reactivity to HPV 16 VLPs, as described elsewhere [20].

Statistical analysis. Contingency tables were constructed from categorical data, including HPV 16 IgA seropositivity and HPV 16 IgG seropositivity. To assess the association between HPV 16 seropositivity and other categorical variables, either the Pearson χ^2 test or, in the case of ordered categorical data, the Cochran-Armitage trend test, and logistic regression were used. To obtain results adjusted for possible confounding variables, multiple logistic regression was used.

The risk of incident HPV cervicovaginal infection was analyzed by using either baseline HPV 16 IgA serostatus or HPV 16 IgG serostatus as the covariate of interest. The a priori hypothesis was that seropositivity would be a marker of immune protection and would thus confer a reduced risk of type-specific incident infection. Cox proportional-hazards regression was used to calculate the relative hazard (RH) and 95% confidence interval (95%CI) for incident infections with HPV 16, HPV 16–related types (31, 33, 35, 52, and 58), and non–16-related HPV types (all other types that were identified by PCR as described above) associated with HPV 16 IgA seropositivity and HPV 16 IgG seropositivity, according to HIV status. An incident infection for a particular HPV DNA group was defined as the first occurrence of an HPV DNA type in the group during ~1.5 years of follow-up, when an infection with a HPV DNA type in the indicated group had not been present at the baseline visit; the date of incident infection was defined as the midpoint between the date of the last known infection-free study visit and the date of the study visit when any HPV DNA type in the indicated group was reported for the first time.

Clearance of HPV 16 was defined as any HPV 16 DNA–negative result among individuals who had prevalent HPV 16 DNA infection at baseline. A given individual who was HPV 16 infected at baseline subsequently contributed multiple HPV 16 measurements during follow-up visits at which clearance could be documented. Generalized estimating equations (GEEs) were used to calculate the odds ratio (OR) and 95%CI for clearance of HPV 16 associated with either HPV 16 IgA seropositivity or HPV IgG seropositivity, compared with the corresponding seronegativity. In the context of this analysis, GEEs can be viewed as a generalization of the logistic regression model [25]. The GEEs adjust the SE of the estimated OR for the correlation between repeated HPV 16 results for each participant.

Women with HSIL, squamous-cell carcinoma, or adenocarcinoma on Pap smear were categorized as HSIL positive. Cox proportional-hazards regression was used to examine the effect that the following independent variables had on the timing of an occurrence of HSIL positivity: HPV 16 IgA seropositivity, HPV 16 IgG seropositivity, HPV DNA at baseline, the use of highly active antiretroviral therapy (HAART), and CD4 cell count. HAART use and CD4 cell count were assessed as time-dependent covariates. HIV-positive women who had a normal, ASCUS, or LSIL Pap-smear result at baseline were included in the analysis. Statistical analyses were performed by use of SAS version 8.2.

RESULTS

Comparison of HPV 16 IgA seropositivity and HPV 16 IgG seropositivity. The seroprevalence of IgA reactivity to HPV 16 capsids was 7.7% (155/2008 women) in HIV-positive women and 4.9% (27/551 women) in HIV-negative women. With regard to IgA seroprevalence, the difference between HIV-positive women and HIV-negative women was statistically significant ($P = .02$). In HIV-positive women, there was no statistically

significant association between HPV 16 IgA seroprevalence and either baseline CD4 cell count ($P = .69$) or baseline HIV RNA copies/mL ($P = .55$). The serum samples had previously been tested for IgG reactivity to HPV 16 capsids [20]. Serum HPV 16 IgA reactivity was strongly associated with HPV 16 IgG reactivity ($P < .01$). Of the 182 IgA-seropositive women, 144 (79.1%) were IgG seropositive; of the 1250 IgG-seronegative women, only 38 (3.0%) were IgA seropositive (data not shown). The association between IgA seroreactivity and IgG seroreactivity was statistically significant both in HIV-positive women and in HIV-negative women ($P < .01$).

HPV 16 IgA seropositivity and demographic, social, and sexual characteristics of the study population. The association of HPV 16 IgA seropositivity and various demographic, social, and sexual characteristics of the study population, stratified by HIV serostatus, are shown in table 1. HPV 16 IgA seropositivity was not found to be significantly associated with ethnicity, marital status, age, recent injection drug use, current cigarette smoking, or use of oral contraceptives during the preceding 6 months. There was no significant association between age at first intercourse and HPV 16 IgA seropositivity. However, for HIV-positive women, HPV 16 IgA seropositivity was associated with 2 other measures of sexual behavior—lifetime number of male sex partners and number of male sex partners during the preceding 6 months; the HPV 16 IgA seroprevalence increased from 6.4%, in women who reported 0–2 lifetime male sex partners, to 9.7% and 9.5%, respectively, in those who reported 15–40 and >40 lifetime male sex partners ($P = .01$), and it was 12.4% in HIV-positive women who reported having had ≥ 3 male sex partners during the preceding 6 months, compared with 7.3% in those who reported having had ≤ 2 male sex partners during the preceding 6 months ($P = .04$). There was no significant association between HPV 16 IgA seropositivity and self-reported prior gonorrhea, syphilis, or vaginal herpes, or self-reported genital warts, but, in HIV-positive women, IgA was significantly associated with a history of having had an abnormal Pap smear (9.5% vs. 6.6%; $P = .02$). Among HIV-negative women, there were no statistically significant associations between HPV 16 IgA seropositivity and any of the variables listed in table 1; however, only 27 HIV-negative women were IgA seropositive.

HPV 16 IgA seropositivity and HPV cervicovaginal infection and disease. HIV-positive women and HIV-negative women whose cervicovaginal lavage specimens were HPV DNA negative by PCR had similar HPV 16 IgA seropositivity rates (4.7% and 4.3%, respectively; table 2). HPV 16 IgA seroprevalence was 3 times higher in HIV-positive women with detectable HPV 16 DNA in the genital tract than in those with no detectable HPV 16 DNA (12.8% vs. 4.7%; $P < .01$). Of 34 women who were positive for HPV 16 by PCR and who also tested positive in the HPV 16–specific Hybrid Capture II assay, 6 (18%) were HPV 16 IgA seropositive, compared with none of 8 women who were positive for HPV 16 by PCR but who tested negative for it in the Hybrid Capture II, and, for women with >100 pg of HPV 16 DNA/mL, HPV 16 IgA seroprevalence was 27% (3/11 women). Only 10 HIV-negative women had a HPV 16 cervicovaginal infection, and none of these women were HPV 16 IgA seropositive. In HIV-positive women, HPV 16 IgA seropositivity was higher in women who were positive for HPV DNA genotypes other than HPV 16, compared with women who had no HPV DNA (9.3% vs. 4.7%; $P < .01$). There was no significant difference, in HPV 16 IgA seroprevalence, between women infected with HPV 16–related types and those infected with other HPV types (9.0% vs. 10.4% and 4.8% vs. 5.6%, in HIV-positive women and HIV-negative women, respectively).

Among HIV-positive women, serum HPV 16 IgA was significantly associated with squamous intraepithelial lesions in women who were positive for HPV 16 DNA, but not in women who were negative for HPV DNA and who had other HPV types in the genital tract (table 3). Among women who were positive for HPV 16 DNA, the prevalence of HPV 16 IgA seropositivity was 24.2% in women with either LSIL or HSIL, compared with 5.5% in women with either normal or ASCUS Pap-smear results ($P = .02$). Among women who harbored other HPV types in the genital tract, HPV 16 IgA seropositivity was comparable in women who had LSIL/HSIL Pap smears and in those who had either normal or ASCUS Pap smears (10.3% vs. 8.8%; $P = .52$). As expected, cytological abnormalities were uncommon in women who were negative for HPV DNA ($n = 14$); none of these women were HPV 16 IgA seropositive. HIV-negative women were not analyzed, because only a small number had cytological abnormalities ($n = 17$) and/or HPV 16 infection ($n = 10$).

HPV 16 IgA seropositivity and HIV infection. Multivariate logistic regression was used to examine the association between HPV 16 IgA seropositivity and HIV serostatus. The model examined the association between HIV infection and HPV 16 IgA seropositivity after adjustment for (1) sexual behaviors, which are established risk factors for past exposure to HPV; (2) a history of an abnormal Pap smear, which, in this population, was the only other demographic characteristic that was significantly associated with seropositivity in univariate analyses; and (3) baseline HPV cervicovaginal infection and HPV-associated cytological abnormalities. HIV serostatus was significantly associated with HPV 16 IgA seropositivity in a univariate analysis (OR, 1.6 [95%CI, 1.1–2.5]). After adjustment for the aforementioned variables, the association lost statistical significance (OR, 1.5 [95%CI, 0.9–2.4]). In the adjusted model, the only variable that remained significantly associated with HPV 16 IgA seropositivity was the detection of any HPV DNA in the genital tract (OR, 1.8 [95%CI, 1.2–2.7]).

Predictive value of HPV 16 IgA seropositivity and of HPV

Table 1. Association between seropositivity and various demographic, social, and sexual characteristics of women in the Women's Interagency HIV Study cohort, stratified by human immunodeficiency virus (HIV) serostatus.

	HIV positive			HIV negative		
Characteristic	Total	No. (%) positive	*P*	Total	No. (%) positive	*P*
Race			.09			.93
African American	1114	90 (8.1)		292	15 (5.1)	
White	360	19 (5.3)		87	5 (5.8)	
Hispanic/Latina	479	45 (9.4)		153	7 (4.6)	
Marital status			.64			.81
Currently married	437	38 (8.7)		143	8 (5.6)	
Not currently married	655	48 (7.3)		129	5 (3.9)	
Never married	900	66 (7.3)		275	14 (5.1)	
Age, years			.25			.20
⩽30	394	36 (9.1)		166	5 (3.0)	
>30	1614	119 (7.4)		385	22 (5.7)	
Recent injection drug use			.67			.23
No	1798	137 (7.6)		480	26 (5.4)	
Yes	200	17 (8.5)		69	1 (1.5)	
Oral contraceptive use during past 6 months			.44			.71
No	1915	150 (7.8)		506	24 (4.7)	
Yes	91	5 (5.5)		44	3 (6.8)	
Current smoker			.24			.31
No	898	62 (6.9)		198	7 (3.5)	
Yes	1105	93 (8.4)		351	20 (5.7)	
Age at first intercourse, years			.24			.62
>16	705	47 (6.7)		194	10 (5.2)	
15–16	533	41 (7.7)		165	10 (6.1)	
⩽14	741	67 (9.0)		183	7 (3.8)	
Lifetime sex partners, no.			.01[a]			.16[a]
0–2	188	12 (6.4)		50	2 (4.0)	
3–7	526	29 (5.5)		147	11 (7.5)	
8–15	398	30 (7.5)		127	4 (3.2)	
16–40	310	30 (9.7)		102	8 (7.8)	
>40	537	51 (9.5)		114	1 (0.9)	
Male sex partners during past 6 months, no.			.04			1.00
0–2	1857	136 (7.3)		496	24 (4.8)	
⩾3	129	16 (12.4)		51	3 (5.9)	
Ever have abnormal pap smear			.02			.66
No	1126	74 (6.6)		376	20 (5.3)	
Yes	813	77 (9.5)		149	6 (4.0)	
Genital warts			.37			.50
No	1519	112 (7.4)		497	26 (5.2)	
Yes	475	41 (8.6)		48	1 (2.1)	
Other sexually transmitted infections[b]			.24			.15
No	875	60 (6.9)		355	14 (3.9)	
Yes	1110	93 (8.4)		189	13 (6.9)	

[a] Value shown is P_{trend}.
[b] Gonorrhea, syphilis, or vaginal herpes.

Table 2. Human papilloma virus (HPV)–16 IgA seropositivity and HPV cervicovaginal infection in the Women's Interagency HIV Study cohort, stratified by human immunodeficiency virus (HIV) serostatus.

	HIV positive			HIV negative		
Group	Total	No. (%) IgA positive	OR (95%CI)	Total	No. (%) IgA positive	OR (95%CI)
HPV negative	640	30 (4.7)	1	348	15 (4.3)	1
HPV 16	94	12 (12.8)	3.0 (1.5–6.0)	10	0 (0.0)	Not available
Other HPV	1049	98 (9.3)	2.1 (1.4–3.2)	143	7 (4.9)	1.1 (0.5–2.9)

NOTE. 95%CI, 95% confidence interval; OR, odds ratio.

[a] Polymerase chain-reaction results were not available for 275 women.

[b] Polymerase chain-reaction results were not available for 50 women.

IgG seropositivity, for subsequent HPV cervicovaginal infection. Baseline HPV 16 IgA serostatus was analyzed in relation to HPV DNA–test results, for up to 1.5 years of follow-up (through visit 4). Of the 2284 participants for whom baseline HPV 16 IgA data and baseline HPV DNA test results were available, 1959 (86%) were tested for HPV DNA at follow-up visit(s) 2, 3, and/or 4: for 1064 women, DNA results from 3 follow-up visits were available; for 570 women, DNA results from 2 follow-up visits were available; for 325 women, DNA results from 1 follow-up visit were available (average, 2.4 follow-up visits/person). There was no statistically significant difference, in the risk of incident cervicovaginal infections with either HPV 16 or an HPV 16–related type, between HPV 16 IgA–seropositive women and HPV 16 IgA–seronegative women, regardless of HIV serostatus (table 4). Among HIV-positive women, the proportion with incident HPV 16 infections was 3.5% and 3.7% in HPV 16 IgA-seropositive women and HPV 16 IgA-seronegative women, respectively, and the corresponding proportion of women with incident HPV 16–related infections was 10.2% and 13.1%. As expected, incident HPV infections were much less common in HIV-negative women than in HIV-positive women (1.8% vs. 3.7% and 4.4% vs. 13.1%, for HPV 16 and HPV 16–related types, respectively). No incident infections with either HPV 16 or HPV 16–related types occurred among HIV-negative women who were HPV 16 IgA seropositive; however, very few HIV-negative women included in this analysis were HPV 16 IgA seropositive ($n = 18$).

On the other hand, among HIV-positive women, HPV 16 IgA seropositivity was associated with a statistically significant increased risk of subsequent infection with non-16 HPV types (RH, 1.8 [95%CI, 1.3–2.6]). Among HIV-negative women, there was a non–statistically significant 2.2-fold increased risk of a non–16-related HPV infection in HPV 16 IgA–seropositive women, compared with HPV 16 IgA–seronegative women. Among HIV-positive women, the increased risk of incident infection with non–16-related HPV types was also observed in relation to baseline HPV 16 IgG seropositivity (RH, 1.3 [95%CI, 1.0–1.6]; $P = .05$) (data not shown). Incident infections with non–16-related HPV types were further analyzed in terms of phylogenetic grouping (table 5). To limit the number of clades and to increase statistical power, clades that were genetically related or that included viruses with similar phenotypes were combined. Among HIV-positive women and HIV-negative women, the risk of incident infections was generally increased in HPV 16 IgA–seropositive women, compared with HPV 16 IgA–seronegative women, regardless of phylogenetic grouping, and, among HIV-positive women, the difference was marginally significant both for viruses of the A7 group ($P = .06$) and for the nononcogenic viruses ($P = .08$).

Of the 94 HIV-positive women who were HPV 16 DNA positive at baseline, 76 (81%) had at least 1 follow-up visit with HPV DNA testing. These 76 women accounted for 181 follow-

Table 3. Human papilloma virus (HPV)–16 IgA seropositivity and pap smear results among human immunodeficiency virus–positive women in the Women's Interagency HIV Study cohort.

HPV group, Pap-smear result	Total	No. (%) positive	*P*
HPV 16			.03
Normal	30	3 (10.0)	
ASCUS	25	0 (0.0)	
LSIL	24	5 (20.8)	
High-grade HSIL	9	3 (33.3)	
No HPV			.94
Normal	515	25 (4.9)	
ASCUS	90	4 (4.4)	
LSIL	11	0 (0.0)	
High-grade HSIL	3	0 (0.0)	
Other HPV types			.85
Normal	545	47 (8.6)	
ASCUS	238	22 (9.2)	
LSIL	216	23 (10.7)	
High-grade HSIL	26	2 (7.7)	

Table 4. Risk of incident human papilloma virus (HPV) cervicovaginal infection, by baseline HPV 16 IgA status, stratified by human immunodeficiency virus (HIV) status.

HPV type, HPV 16 IgA status	HIV positive			HIV negative		
	Total	No. (%) PCR positive	RH (95%CI)	Total	No. (%) PCR positive	RH (95%CI)
Type 16						
Seronegative	1343	49 (3.7)	1	397	7 (1.8)	1
Seropositive	114	4 (3.5)	1.0 (0.4–2.7)	18	0 (0.0)	NA
16-Related types						
Seronegative	1203	157 (13.1)	1	389	17 (4.4)	1
Seropositive	98	10 (10.2)	0.8 (0.4–1.4)	17	0 (0.0)	NA
Non–16-related types						
Seronegative	891	276 (31.0)	1	368	52 (14.1)	1
Seropositive	71	34 (47.9)	1.8 (1.3–2.6)	18	5 (27.8)	2.2 (0.9–5.4)

NOTE. 95%CI, 95% confidence interval; PCR, polymerase chain reaction; RH, relative hazard.

up visits (average, 2.4 follow-up visits/person). When GEEs were used to allow for repeated observations, there was no significant difference between HPV 16 IgA–seropositive women and HPV 16 IgA–seronegative women, with regard to the odds of clearance of HPV 16 DNA from the lower genital tract (OR, 1.1 [95%CI, 0.3–3.7]). HIV-negative women were not analyzed because none of the 10 HPV 16 DNA–positive women were HPV 16 IgA seropositive. Among HIV-positive women, there was also no statistically significant difference between HPV 16 IgG–seropositive women and HPV 16 IgG–seronegative women, with regard to the odds of clearance of HPV 16 DNA from the lower genital tract (OR, 0.9 [95%CI, 0.4–1.9]) (data not shown). After adjustment for CD4 cell count, there was no significant association between either HPV 16 IgA seropositivity or HPV 16 IgG seropositivity and clearance of HPV 16 DNA.

Predictive value of HPV 16 IgA seropositivity and HPV IgG seropositivity, for cervicovaginal cytological progression. Baseline HPV 16 IgA serostatus was analyzed in relation to Pap-smear results for up to 6.5 years of follow-up (through visit 14) of 1660 HIV-infected women. The risk of HSIL or a higher grade of cytological abnormality ($HSIL^+$) during follow-up was analyzed by Cox proportional hazards regression. The plot of time to incident $HSIL^+$ on Pap smear shows that the cumulative incidence is <10%, for both the 129 HPV 16 IgA–seropositive women and the 1531 HPV 16 IgA–seronegative women (figure 1). The unadjusted analysis showed that baseline HPV 16 IgA serostatus was significantly associated with progression to $HSIL^+$ on Pap smear (RH, 2.2 [95%CI, 1.2–4.2]). In contrast to the results observed for HPV 16 IgA seropositivity, there was no statistically significant association between HPV 16 IgG seropositivity and time to incident $HSIL^+$ (RH, 1.2 [95%CI, 0.7–1.9]) (data not shown). After adjustment for CD4 cell count (time dependent), HPV 16 DNA (at baseline), and HAART (time dependent), the risk associated with HPV 16 IgA seropositivity was lost (RH, 1.6 [95%CI, 0.8–3.4]), among 1542 women on whom data were available. Adjustment for either any HPV DNA or for HPV DNA of the 13 oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, and 68) did not change the results of the analysis. Five HIV-negative women developed $HSIL^+$ during follow-up. None of these women were HPV 16 IgA seropositive.

DISCUSSION

We assessed epidemiological and virological determinants of serum IgA reactivity to HPV 16 capsids and, in addition, compared IgA seropositivity to previously reported IgG seropositivity, in both HIV-positive women and HIV-negative women enrolled in a prospective cohort study. Among all women, the HPV 16 IgA seroprevalence (7%) was ~7-fold lower than the previously reported HPV 16 IgG seroprevalence (51%), consistent with the 6-fold-lower serum concentration of IgA compared with IgG (2 mg/mL vs. 12 mg/mL) [26]. When a cutoff point defined as the mean + 2 SD, rather than 3 SD, of the negative controls was used, the HPV 16 IgA seroprevalence increased only marginally, to 9%. The lower IgA seroprevalence is unlikely to be due to differences in the sensitivities of the assays, because the same antigen and protocol and the same method of determining the cut point were used in both assays. In any case, the choice of cut point did not alter the results of the analysis. In addition, evaluation of the IgA conjugate showed that the results of our study were not due to cross-reactivity of the serodiagnostic reagents. When purified immunoglobulin protein was used as a reference standard, both the IgA conjugate and the IgG conjugate were found to be sensitive and specific for detection of their respective antibody class.

Serum HPV 16 IgA seropositivity was strongly associated with HPV 16 IgG seropositivity; 79% of IgA-seropositive sub-

Table 5. Risk of incident non–16-related human papilloma virus (HPV) cervicovaginal infection, by baseline HPV 16 IgA status, stratified by HPV clade and human immunodeficiency virus (HIV) status.

	HIV positive			HIV negative		
HPV clade(s)	Total	No. (%) PCR positive	RH (95%CI)	Total	No. (%) PCR positive	RH (95%CI)
A7[a]						
Seronegative	1189	166 (14.0)	1	390	22 (5.6)	1
Seropositive	102	21 (20.6)	1.6 (1.0–2.4)	18	1 (5.6)	1.0 (0.1–7.4)
A5 and A6[b]						
Seronegative	1179	166 (14.1)	1	393	17 (4.3)	1
Seropositive	99	18 (18.2)	1.3 (0.8–2.1)	18	2 (11.1)	2.7 (0.6–11.5)
A1, A3, A8, A10–A11[c]						
Seronegative	1104	241 (21.8)	1	388	27 (7.0)	1
Seropositive	88	26 (29.5)	1.4 (1.0–2.2)	18	3 (16.7)	2.5 (0.8–8.2)

NOTE. 95%CI, 95% confidence interval; PCR, polymerase chain reaction; RH, relative hazard.

[a] HPV types 18, 39, 45, 59, 68, and 70.

[b] HPV types 26, 51, 53, 56, 66, 69, and 82.

[c] Nononcogenic HPV types 6, 11, 32, 40, 54, 55, 61, 73, 83, and 84.

jects were IgG seropositive. In contrast, Wang et al. [15] found a weaker association between HPV 16 IgA reactivity and HPV 16 IgG reactivity, with ~50% of IgA-positive subjects being IgG positive. The discrepancy may be due to differences between the study populations. The women in our study reported many more lifetime sexual partners, which would increase their risk of past exposure to HPV, and the majority of women were HIV positive, which would increase the likelihood of persistent HPV infection. Both factors would contribute to a high IgG seroprevalence, as well as increase the likelihood of recent, active HPV 16 infection, which might correlate with IgA (see below).

The epidemiological and virological determinants of HPV 16 IgA seropositivity were, in general, similar to those which a previous study had found for HPV 16 IgG seropositivity [20]; that is, HPV 16 IgA seropositivity was significantly associated with recent and lifetime number of male sex partners, a history of having had an abnormal Pap smear, detection of HPV 16 DNA in the genital tract, and HPV-associated cytological abnormalities. These associations were observed in HIV-positive women but not in HIV-negative women, perhaps because of the lower HPV 16 IgA seroprevalence in HIV-negative women and the smaller number of these women included in the present study. The only other study to examine the association between HPV 16 IgA and sexual history was that by Wang et al. [15]; they reported that, in HIV-negative women, IgA reactivity was associated with having had $\geq$1 male sex partner during the past month, whereas there was no association between IgG reactivity and the number of recent male sex partners. Both IgG seropositivity and IgA seropositivity were associated with the number of lifetime partners, with IgG reactivity more strongly associated than IgA reactivity. In contrast, we found that both IgA seropositivity and IgG seropositivity were associated with the number of recent male sex partners. These differences might be explained by the differences between the study populations. The population that we studied was older and more sexually active and thus more likely to have had previous HPV infections. In addition, we defined "recent" as during the past 6 months rather than during the past month. Some women in our study may have had an anamnestic response to HPV, and all the women would have had more time, between infection and blood sampling, in which to develop an IgG response. Our finding of an association between serum IgA to HPV 16 capsids and detection of HPV 16 HPV DNA in the genital tract and HPV-associated cytological abnormalities is in agreement with the results of previous studies [15, 16, 27].

Moreover, we observed an association between HPV 16 IgA seropositivity and HPV 16 viral load, as estimated by a semiquantitative HPV 16 DNA–specific assay, the Hybrid Capture II test. Taken as a whole, our findings are consistent with HPV 16 IgA seropositivity being a type specific biomarker of both past and current HPV infection, with the probability that seropositivity is associated, to some degree, with the level of active viral replication, as reflected both by HPV 16 load and by the presence of cervical lesions.

One focus of our study was to explore the relationship between HIV infection and HPV 16 seroreactivity. Two previous studies examined serum IgA responses to HPV capsids in HIV-positive women [14, 18]. Petter et al. reported that 30% of HIV-positive women were IgA seropositive for HPV 16, 18, or 31 capsids, compared with 19% of HIV-negative women with moderate-to-severe cervical dysplasia and 8% of HIV-negative women with either a normal Pap smear or mild cytological

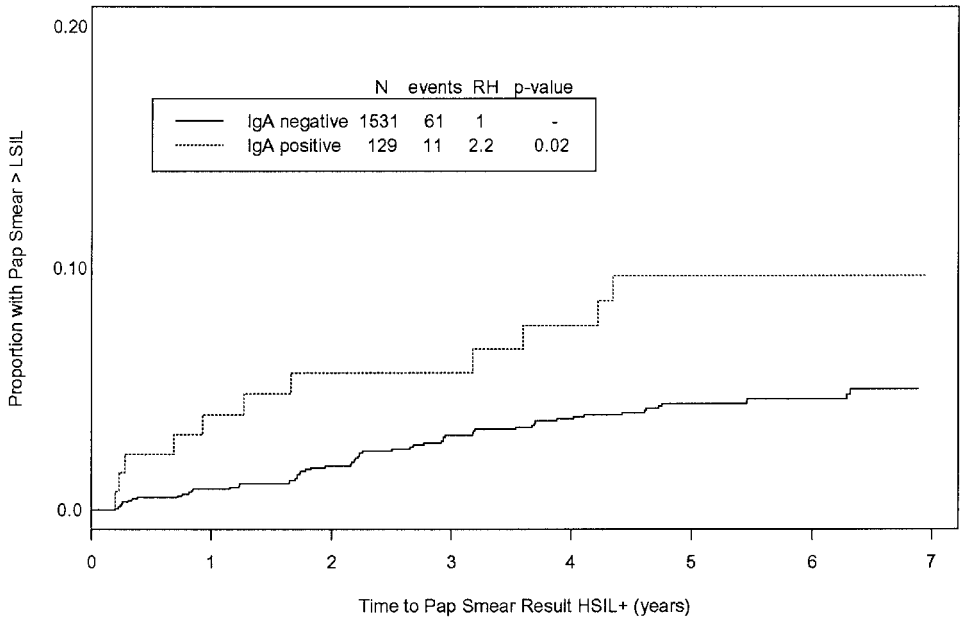

Figure 1. Cumulative incidence of a high-grade squamous intraepithelial lesion (HSIL$^+$) Pap-smear result, among 1660 women positive for human immunodeficiency virus, stratified by baseline seropositivity for human papilloma virus–16 IgA. LSIL, low-grade squamous intraepithelial lesion.

abnormalities. Of the HIV-positive women in the study by Petter et al., 30% had AIDS and 25% had moderate to severe cervical dysplasia. In contrast, Marias et al. [18] reported, in a study of female sex workers, a lower seroprevalence in HIV-positive women than in HIV-negative women: IgA reactivity to HPV 16 capsids was detected in 15% of HIV-positive women and in 40% of HIV-negative women. Neither the study by Petter et al. nor that by Marias et al. reported data on the specificity and sensitivity of the IgA serodiagnostic reagents used in the immunoassays. In agreement with the results reported by Petter et al., we found a higher HPV 16 IgA seroprevalence in HIV-positive women than in seronegative women; however, the seroprevalence found in our study was much lower than that found in either the study by Petter et al. or that by Marias et al. After adjustment for variables related to past exposure to HPV and baseline HPV infection, there was no significant association between HIV serostatus and HPV 16 IgA seropositivity. In the adjusted model, HPV 16 IgA seropositivity retained a significant association with the detection of HPV DNA in the genital tract. This finding suggests that the higher seroprevalence in HIV-positive women is explained by the higher prevalence of HPV infection in HIV-positive women, not by differences in past exposure to HPV. A direct effect of HIV-induced immune dysfunction on serum antibody levels cannot be excluded but seems unlikely, because there was no association between HPV 16 IgA seroprevalence and either CD4$^+$ cell count or HIV load. This finding is consistent with recent observations, in both the Women's Interagency HIV Study (WIHS) and the HIV Epidemiology Research Study (HERS), showing that, in HIV-positive women, HPV 16 is less strongly associated with CD4$^+$ T-cell levels than are most other HPV types [28].

Conflicting data have been reported on the relationship between the serum IgA response to HPV capsids and the course of HPV infection. In one study, serum IgA to HPV 16 was correlated with viral clearance [17], whereas in another study viral persistence was associated with serum HPV 16 IgA response that was earlier and more sustained than serum IgG response [16]. With regard to clearance of a prevalent HPV 16 cervicovaginal infection, we found no difference between HPV 16 IgA–seropositive women and HPV 16 IgA–seronegative women, although, because no HIV-negative women with a prevalent HPV 16 infection were HPV 16 IgA seropositive, our results are limited to HIV-positive women. Two limitations of our study are the short follow-up period (1.5 years) and the inclusion only of women with prevalent infection. In addition, among HIV-positive women, the distinction between clearance and intermittent detection of a persistent infection is problematic.

Although serum HPV 16 IgA reactivity had no prognostic value for subsequent detection of type-specific HPV DNA in the genital tract, serum HPV 16 IgA reactivity was predictive of the development of severe cytological abnormalities in women with either normal or low-grade abnormalities on a Pap smear at the time of serological testing; in contrast, there was a null association with serum IgG to HPV 16. Because the serum half-life of IgA is shorter than that of IgG [26], IgA responses are not as long-lasting as IgG responses [3–5]; and maintenance of a serum IgA response is believed to require repeated antigenic stimulation. For these reasons, virus-specific serum IgA antibodies

are considered to be a marker for active infection, frequent reactivation of a latent viral infection, and repeated viral infection; for example, patients with reactivated varicella-zoster infection exhibit significant titers of virus-specific serum IgA, whereas this marker is not usually found in healthy individuals with varicella-zoster seropositivity [9, 10]. In experimental bovine herpes-virus infection, reinfection or corticosteroid-induced reactivation produces a prompt but transient virus-specific serum IgA response [11]. The example most analogous to HPV-related cervical dysplasia is the association between IgA antibodies to Epstein-Barr virus (EBV) antigens and nasopharyngeal carcinoma [12]. In a recent population-based prospective cohort study from Taiwan, prediagnostic IgA antibodies to EBV were strongly predictive of nasopharyngeal carcinoma [13]. Persistent HPV infection is the single most important risk factor for cytological progression, and persistence, whether because of reactivation of endogenous infection or because of new exposures, would be expected to stimulate and maintain an HPV-specific serum IgA response. In contrast, IgG responses are long-lasting and thus cannot be used to distinguish between recent antigenic stimulation and a past infection. Because HPV DNA results are not yet available from later visits when many of the $HSIL^+$ Pap-smear results were observed, the proportion of disease associated with HPV 16 is unknown and the type-specific risk of $HSIL^+$ cannot be calculated.

An unexpected finding was the association between baseline HPV 16 IgA seropositivity—and, to a lesser extent, HPV 16 IgG seropositivity–and an increased risk of incident infections with non–16-related HPV types. There is no known biological mechanism that would account for seropositivity to one HPV type having a direct positive influence on the risk of infection with another type. Since HPV seropositivity is a marker of sexual behavior, which is the principal risk factor for acquisition of HPV, the greater risk of HPV infection in HPV-seropositive women compared with HPV-seronegative women may reflect a greater risk of exposure to HPV. Prior HPV infection may also increase the risk of reactivation of a latent infection. In contrast to non-16 HPV types, HPV 16 seropositivity was not associated with an increased risk of incident infection with either HPV 16 or HPV 16–related types. This may be evidence of partial type-specific or clade-specific immunity conferred by HPV 16 seropositivity, a conclusion that was also reached in a study of incident HPV infections in women of college age [29]. In that study, none of 12 women with persistent seropositivity to both HPV 16 IgG and HPV 16 IgA had incident infection with an HPV 16–related type.

References

1. Dillner J. The serological response to papillomaviruses. Semin Cancer Biol **1999**; 9:423–30.
2. Conley ME, Delacroix DL. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? Ann Intern Med **1987**; 106:892–9.
3. Welliver RC, Kaul TN, Putnam TI, et al. The antibody response to primary and secondary infection with respiratory syncytial virus: kinetics of class-specific responses. J Pediatr **1980**; 96:808–13.
4. Salonen EM, Hovi T, Meurman O, Vesikari T, Vaheri A. Kinetics of specific IgA, IgD, IgE, IgG, and IgM antibody responses in rubella. J Med Virol **1985**; 16:1–9.
5. Nikoskelainen J, Neel EU, Stevens DA. Epstein-Barr virus-specific serum immunoglobulin A as an acute-phase antibody in infectious mononucleosis. J Clin Microbiol **1979**; 10:75–9.
6. Nielsen SL, Sorensen I, Andersen HK. Kinetics of specific immunoglobulins M, E, A, and G in congenital, primary, and secondary cytomegalovirus infection studied by antibody-capture enzyme-linked immunosorbent assay. J Clin Microbiol **1988**; 26:654–61.
7. Rothbarth PH, Groen J, Bohnen AM, de Groot R, Osterhaus AD. Influenza virus serology—a comparative study. J Virol Methods **1999**; 78:163–9.
8. Koraka P, Suharti C, Setiati TE, et al. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. J Clin Microbiol **2001**; 39:4332–8.
9. Karner W, Bauer G. Activation of a varicella-zoster virus-specific IgA response during acute Epstein-Barr virus infection. J Med Virol **1994**; 44:258–62.
10. Wittek AE, Arvin AM, Koropchak CM. Serum immunoglobulin A antibody to varicella-zoster virus in subjects with primary varicella and herpes zoster infections and in immune subjects. J Clin Microbiol **1983**; 18:1146–9.
11. Madic J, Magdalena J, Quak J, van Oirschot JT. Isotype-specific antibody responses to bovine herpesvirus 1 in sera and mucosal secretions of calves after experimental reinfection and after reactivation. Vet Immunol Immunopathol **1995**; 47:81–92.
12. Henle G, Henle W. Epstein-Barr virus-specific IgA serum antibodies as an outstanding feature of nasopharyngeal carcinoma. Int J Cancer **1976**; 17:1–7.
13. Chien YC, Chen JY, Liu M, et al. Serologic markers of Epstein-Barr virus infection and nasopharyngeal carcinoma in Taiwanese men. N Engl J Med **2001**; 345:1877–82.
14. Petter A, Heim K, Guger M, et al. Specific serum IgG, IgM and IgA antibodies to human papillomavirus types 6, 11, 16, 18 and 31 virus-like particles in human immunodeficiency virus-seropositive women. J Gen Virol **2000**; 81:701–8.
15. Wang Z-H, Kjellberg L, Abdalla H, et al. Type specificity and significance of different isotypes of serum antibodies to human papillomavirus capsids. J Infect Dis **2000**; 181:456–62.
16. Sasagawa T, Yamazaki H, Dong YZ, et al. Immunoglobulin-A and -G responses against virus-like particles (VLP) of human papillomavirus type 16 in women with cervical cancer and cervical intra-epithelial lesions. Int J Cancer **1998**; 75:529–35.
17. Bontkes HJ, de Gruijl TD, Walboomers JM, et al. Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. II. Systemic but not local IgA responses correlate with clearance of HPV-16. J Gen Virol **1999**; 80:409–17.
18. Marais DJ, Vardas E, Ramjee G, et al. The impact of human immunodeficiency virus type 1 status on human papillomavirus (HPV) prevalence and HPV antibodies in serum and cervical secretions. J Infect Dis **2000**; 182:1239–42.
19. Barkan SE, Melnick SL, Preston-Martin S, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. Epidemiology **1998**; 9:117–25.
20. Viscidi RP, Ahdieh-Grant L, Clayman B, et al. Serum immunoglobulin G response to human papillomavirus type 16 virus-like particles in human immunodeficiency virus (HIV)–positive and risk-matched HIV-negative women. J Infect Dis **2003**; 187:194–205.
21. Palefsky JM, Minkoff H, Kalish LA, et al. Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-posi-

tive and high-risk HIV-negative women. J Natl Cancer Inst **1999**; 91: 226–36.
22. Kurman RJ, Henson DE, Herbst AL, Noller KL, Schiffman MH. Interim guidelines for management of abnormal cervical cytology. The 1992 National Cancer Institute Workshop. JAMA **1994**; 271:1866–9.
23. Studentsov YY, Schiffman M, Strickler HD, et al. Enhanced enzyme-linked immunosorbent assay for detection of antibodies to virus-like particles of human papillomavirus. J Clin Microbiol **2002**; 40:1755–60.
24. Herrero R, Schiffman MH, Bratti C, et al. Design and methods of a population-based natural history study of cervical neoplasia in a rural province of Costa Rica: the Guanacaste Project. Rev Panam Salud Publica **1997**; 1:362–75.
25. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics **1986**; 42:121–30.
26. Janeway CA, Travers P, Walport M, Shlomchik MJ. Structual variation in immunoglobulin constant regions. In: Janeway CA, Travers P, Walport M, Shlomchik MJ, eds. Immunobiology: the immune system in health and disease. 5th ed. New York: Garland Publishing, **2001**:141–51.
27. Strickler HD, Schiffman MH, Eklund C, et al. Evidence for at least two distinct groups of humoral immune reactions to papillomavirus antigens in women with squamous intraepithelial lesions. Cancer Epidemiol Biomarkers Prev **1997**; 6:183–8.
28. Strickler HD, Palefsky JM, Shah KV, et al. Human papillomavirus type 16 and immune status in human immunodeficiency virus–seropositive women. J Natl Cancer Inst **2003**; 95:1062–71.
29. Ho GYF, Studentsov Y, Hall CB, et al. Risk factors for subsequent cervicovaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. J Infect Dis **2002**; 186:737–42.