Natural History of Human Papillomavirus Type 16 Virus-Like Particle Antibodies in Young Women

Gloria Y. F. Ho,[1] Yevgeniy Y. Studentsov,[2] Robert Bierman,[4] and Robert D. Burk[2,3]

Departments of [1]Epidemiology and Population Health, [2]Microbiology and Immunology, [3]Pediatrics, and Obstetrics and Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, New York, and [4]Rutgers University Student Health Service, New Brunswick, New Jersey

Abstract

Immunization with a vaccine of human papillomavirus (HPV) type 16 virus-like particles (VLPs) can reduce incidence of HPV-16 infection and its related cervical intraepithelial neoplasia. However, development of detectable antibodies to VLPs does not always occur after natural HPV infection. This study examined prospectively for seroconversion and duration of antibodies to HPV-16 VLPs and their associated host and viral factors. Six-hundred eight subjects were tested for HPV DNA biannually and for IgG and IgA antibodies to HPV-16 VLPs annually for 3 years. Both IgG and IgA antibodies to HPV-16 VLPs were predominantly type specific. Women with cervicovaginal HPV-16 infection were 8–10 times more likely to seroconvert than those with infection of HPV-16-related types. Among subjects who had an incident infection with HPV-16, a maximum of 56.7% became seropositive for IgG within 8.3 months and 37.0% had IgA within 14 months. Detectable seroconversion was a slow process that required sufficient antigenic exposure associated with either a high viral load (relative risk = 5.7 for IgG) or persistent infection of HPV-16 (relative risk = 3.4 for IgA). The median duration for both types of antibodies was ~36 months. Antibodies could persist for a long period of time if the initial antibody levels were high or if there was continued antigenic exposure.

Introduction

Immunization with a papillomavirus virus-like particle (VLP) vaccine can induce high titers of antibodies and seroconversion in all vaccinated subjects (1, 2). However, development of detectable antibodies against VLPs does not always occur after natural human papillomavirus (HPV) infection. For example, the seroprevalence of antibodies to HPV-16 VLPs among women with normal cytology who had HPV-16 DNA detected in cervicovaginal cells ranged from 46 to 59% in various studies (3–6). Host and viral factors associated with development of a humoral response against HPV and persistency of antibodies are not well known because most epidemiological studies on VLP serology have been cross-sectional and could not address the issues of seroconversion and duration of antibodies. This prospective study examined the natural history of antibodies against HPV-16 VLPs.

Materials and Methods

Study Population. Description of the study population was published previously (7, 8). From a state university in New Brunswick, New Jersey, 608 female students were recruited through campus-wide advertisements. The mean age of subjects was 20 years (± SD = 3), and the ethnic distribution was 57% white, 13% Hispanic, 12% black, 10% Asian, and 8% others. At baseline, the cervical HPV DNA prevalence was 26% (9). Subjects were followed at 6-month intervals for a maximum of 3 years, with a median of five visits/subject. At each visit, a self-administered questionnaire, which obtained demographic, behavioral, and lifestyle information since the last visit, was completed, and cervicovaginal lavage was obtained. Serum samples were collected at annual visits initially and at the 6-month visits in the last year of implementation of the study; 70% of consecutive serum samples were obtained within 9–15 months. The study protocol was approved by the Institutional Review Board, and informed consent was obtained from all subjects.

Detection of HPV DNA. Exfoliated cervicovaginal cells were obtained by lavage for HPV determination and typing by the PCR and Southern blot hybridization as described previously (9, 10). HPV PCR products that did not hybridize to type-specific probes were considered to represent uncharacterized HPV types. A sample was considered HPV positive if either PCR or Southern blot was positive, and negative if both assays were negative. HPV types determined by PCR and Southern blot were combined for analyses. An infection with a low viral load was defined as HPV DNA detectable by PCR only, whereas a high level infection was detectable by both Southern blot and PCR.

Detection of Antibodies against HPV-16 VLPs. Serum samples diluted 1:100 were tested for IgG and IgA antibodies to HPV-16 VLPs by a modified polymer ELISA as described previously (8, 11). The absorbance cut points for seropositivity were determined by Receiver Operating Characteristic analyses in which the log-transformed absorbance values of subjects who were positive for HPV-16 DNA were compared with those of subjects who denied a history of vaginal sex and were also tested HPV-16 DNA negative. Because women with HPV-16 infection may not necessarily have detectable antibodies, the seropositivity cut points were chosen to maximize the speci-

Received 7/1/03; revised 9/9/03; accepted 9/15/03.

Grant support: NIH Grant AI-31055 (to R. D. B.). DNA oligonucleotides synthesized in Oligo Synthesis facility of Cancer Research Center at Albert Einstein College of Medicine (Grant CA-13330).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Gloria Y. F. Ho, Department of Epidemiology and Population Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Belfer Building, Room 1312, Bronx, NY 10461. Phone: (718) 430-3558; Fax: (718) 430-3076; E-mail: ho@aecom.yu.edu.

ficity among the women who were HPV-16 DNA negative. For the HPV-16 IgG assay, the cut point of log absorbance ≥ -0.79 gave a sensitivity of 47% and specificity of 100%. For the HPV-16 IgA assay, the cut point of log absorbance ≥ -1.00 gave a sensitivity of 24% and specificity of 100%. Serum samples were assayed in duplicate, and the serostatus was determined by applying the cut point to the mean log absorbance. The interassay reproducibility of serostatus of 575 baseline serum samples tested twice for IgG to HPV-16 VLPs was measured by Kappa statistics with $\kappa = 0.92$;95% confidence interval (CI), 0.88–0.97). For 31 samples tested twice for IgA, $\kappa = 0.86$ (95% CI, 0.67–1.00).

Statistical Analysis. The cumulative probabilities of acquiring and losing antibodies to HPV-16 VLPs were estimated by the Kaplan-Meier method. Baseline was used as the entry time when time to incident seroconversion of antibodies was analyzed. Visit date when HPV-16 DNA was first detected was used as the entry time when the goal was to estimate the time to seroconversion after HPV16 infection. For the analysis of antibody duration, the entry time was at first detection of antibodies to HPV-16 VLPs. The exit time for all analyses was at the time of event (seroconversion or loss of antibodies) or at the last visit (censored).

Five (4.7%) of 106 subjects and 1 (2.0%) of 50 subjects in whom IgG and IgA to HPV-16 VLPs was ever detected, respectively, had fluctuations in serostatus, *i.e.*, seronegativity flanked by seropositivity. In the data analyses, these subjects were considered to have lost their antibodies.

Viral, demographic, and behavioral risk factors were examined for their associations with the time to event (seroconversion or loss of antibodies) by time-dependent Cox proportional-hazards regression. Because the number of events for IgA was small, only descriptive data are presented, whereas more complete analyses were done for IgG. For behavioral variables (*e.g.*, sexual activities and uses of cigarettes, alcohol, and oral contraceptive pills, and so on), the time-dependent exposure in the past 6 months was analyzed. For some time-dependent variables, namely HPV DNA types and number of times HPV16 DNA was detected (0, 1, or $\geq$2 visits), the cumulative exposure status between the entry time and time t (*i.e.*, $\leq$ time t) was analyzed. For example, to estimate the association between HPV DNA types and risk for event by time t, subjects were classified into one of the following hierarchical categories based on the HPV types detected $\leq$ time t: HPV-16 (highest priority), HPV-16- related types (namely HPVs 31, 33, 35, 52, or 58), other types, and HPV negative (lowest priority). If a subject was infected with multiple HPV types at one time, classification into a higher priority category took precedence. The exposure status would change at a later time point only if an HPV type in a higher priority category was detected. Similarly, whether a subject had persistent HPV-16 infection for $\geq$2 visits between the entry time and time t was indicated in a time-varying variable. Because most serum samples for antibodies to HPV-16 VLPs were collected annually, whereas lavage samples for HPV DNA were obtained every 6 months, the outcome measurement (seroconversion or loss of antibodies) was not available for each 6-month visit. By analyzing cumulative HPV DNA status as described above, HPV DNA data of all visits, regardless of whether antibody status was known, were analyzed.

The statistical software packages SAS and Stata were used for data analyses (12, 13). All *P*s presented are two-sided.

Results

Seroconversion of IgG to HPV-16 VLPs. Among the 403 women who were seronegative at baseline and had at least one serum sample during follow-up, 25 women became seropositive, and the cumulative 36-month incidence of seroconversion was 7.2% (95% CI, 4.4–10.1). Table 1 shows the effects of demographic and viral factors on the development of IgG to HPV-16 VLPs. There was no correlation between age and seroconversion [relative risk (RR)/year increase in age = 0.93, $P = 0.474$]. African Americans tended to have a higher risk for seropositivity than other ethnic groups combined (RR = 2.03, $P = 0.158$). Subjects infected with HPV-16 or HPV-16-related types (*i.e.*, HPVs 31, 33, 35, 52, and 58) were more likely to seroconvert than those who were HPV negative or infected with other HPV types. Women with HPV-16 had an 8-fold increased chance of becoming seropositive than women with HPV-16-related types (RR = 8.32, $P = 0.001$). The subjects who had HPV-16 or HPV-16-related types were additionally partitioned by viral load as well as by persistency. Subjects who had a high viral load infection with HPV-16 or HPV-16-related types were more likely to seroconvert than those who only had a low viral load infection with HPV-16 or HPV-16-related types (RR = 2.87, $P = 0.059$). Individuals who had persistent infection with HPV-16 or related types for $\geq$2 visits did not seem to have an increased risk as compared with those without persistent infection (RR = 0.87, $P = 0.756$). Of the 25 individuals with seroconversion of IgG, none had preceding IgA. Rather, IgG and IgA tended to occur together: subjects who had IgA at a given visit were 15 times more likely to have IgG detected at the same visit ($P < 0.001$), and seropositivity of IgG increased with the IgA antibody levels.

Among 28 subjects who had an incident infection of HPV-16, 13 women seroconverted. The median time from detection of HPV-16 DNA to detection of IgG was 8.3 months, and the 12-month cumulative incidence of seroconversion was 56.7% (95% CI, 34.0–79.4). Women who had persistent infection of HPV-16 for $\geq$2 visits did not show an increased risk of seroconversion (Table 1). In fact, 7 (53.8%) of the 13 seroconverters had their first IgG antibodies as well as first HPV-16 DNA detected in the same visit. Seroconversion, however, was significantly associated with viral load of HPV-16 when it was first detected.

The relationships between seroconversion of IgG to HPV-16 VLPs and various behavioral variables were examined univariately by time-dependent Cox regression analysis. Age at first coitus, number of years of vaginal sex experience, frequencies of vaginal and nonvaginal sex, numbers of regular and casual sex partners, and usages of condoms, cigarettes, alcohol, and drugs were not significant risk factors for seroconversion (data not shown). The numbers of men for vaginal sex in lifetime and in the past 6 months were positively associated with the likelihood of seroconversion in univariate analysis but not in multivariate analysis after controlling for HPV infection.

The final multivariate model in Table 2 shows that women who were using oral contraceptive pills at the time of blood draw had almost a 3-fold increased chance for IgG seropositivity, and this relationship was attenuated if age was excluded from the model. Other variables that were significantly associated with seroconversion of IgG included a high viral load of infection with HPV-16 or related types as well as a coexistence of IgA antibodies.

Duration of IgG to HPV-16 VLPs. Of 106 subjects who were seropositive for IgG at baseline or during follow-up, 87 had at least one serum sample after the first seropositive sample, and

Table 1 Association between seroconversion of anti-human papillomavirus (HPV)-16 IgG and viral and demographic factors: univariate Cox proportional hazards regression analysis

	No. seroconverted/ no. person-years	Relative risk (95% confidence interval) for seroconversion	*P*
All subjects[a]			
Age (yrs)			
<20	17/516.5	1	
≥20	8/300	0.79 (0.34–1.83)	0.578
Race			
White, Asian, and others	18/620.5	1	
Hispanic	2/105.4	0.64 (0.15–2.74)	0.543
African American	5/90.6	1.92 (0.71–5.17)	0.199
HPV types detected ≤ time t[b]			
HPV negative	3/493.4	1	
Other types	2/221.5	1.57 (0.26–9.40)	0.622
HPV-16-related: 31, 33, 35, 52, or 58	3/60.3	8.88 (1.79–44.13)	0.008
HPV-16	17/39.4	73.84 (21.57–252.77)	<0.001
HPV types detected ≤ time t[b]			
HPV negative or other types	5/714.9	1	
HPV-16 or HPV-16-related (31, 33, 35, 52, or 58)			
Viral load			
Low	4/40.6	14.44 (3.87–53.84)	<0.001
High	16/59.1	41.47 (15.15–113.53)	<0.001
Type-specific persistence for ≥2 visits			
No	12/54.6	32.43 (11.40–92.24)	<0.001
Yes	8/44.0	28.13 (9.17–86.27)	<0.001
Anti-HPV-16 IgA at time t[b]			
No	20/802.7	1	
Yes	5/13.8	15.14 (5.62–40.83)	<0.001
Anti-HPV-16 IgA absorbance level at time t[b,c]			
No IgA	20/802.7	1	<0.001[d]
<0.2319	2/9.5	8.99 (2.09–38.71)	
≥0.2319	3/4.3	28.33 (8.21–97.75)	
Subjects with HPV-16 incident infection[e]			
Number of visits in which HPV-16 DNA was detected ≤ time t[b]			
1	7/5.9	1	
≥2	6/7.6	1.31 (0.4–4.13)	0.641
HPV-16 viral load when the incident infection was first detected			
Low load	2/7.3	1	
High load	11/6.2	5.67 (1.23–26.18)	0.026

[a] The entry time was at baseline.
[b] The independent variable was analyzed as a time-varying covariate. The sample size presented has the number of seroconverters in the numerator. The person-years contributed by each individual at risk were partitioned by the exposure categories. The denominator for a particular exposure category is the sum of person-years from individuals who were at some point in time being exposed to that category. Because the variable was time dependent, an individual could contribute person-years in more than one exposure category. It is erroneous to directly calculate and compare relative risks based on these person-years. These numbers are shown merely to reflect the sample size.
[c] Median absorbance among samples positive for IgA to HPV-16 virus-like particles.
[d] *P* for linear trend.
[e] The entry time was at the follow-up visit when HPV-16 DNA was first detected.

IgG to HPV-16 VLPs were not detected in 37 women subsequently. The cumulative probabilities of losing IgG by 12, 24, and 36 months were 38.5, 40.0, and 48.2%, respectively. Table 3 presents the factors associated with loss of IgG antibodies. Duration of antibody was not related to age, ethnicity, or whether seropositivity was detected at baseline *versus* during follow-up, but it was associated with the antibody level of the first seropositive sample. Individuals with a low initial absorbance had an increased risk of losing the IgG antibodies (RR = 2.0 for 0.1 unit decrease in log absorbance, $P < 0.0001$). The 24-month probabilities of losing the antibodies were 74.8 and 4.7% for the subjects with initial absorbance ≤0.3055 and >0.3055, respectively. Once an individual became seropositive, those who had no HPV infection thereafter had the highest chance of losing the antibodies as compared with individuals who had infection with any HPV type in at least one visit. However, the specific HPV type with which an individual was infected subsequent to seropositivity did not appear to influence the duration of antibody. That is, subjects infected with HPV-16 were not more likely to retain antibody than those infected with HPV-16-related types or other HPV types. Individuals who had persistent HPV-16 infection for ≥2 visits also did not demonstrate a significantly altered risk to loose antibody. Rather, HPV infection with any types (RR = 0.31, $P < 0.001$) and, particularly, infection in multiple visits (RR = 0.67 for each additional visit with HPV infection, $P = 0.004$), diminished the chance of losing the IgG antibodies

The relationships between behavioral factors and loss of IgG antibodies to HPV-16 VLPs were examined univariately by time-dependent Cox regression analysis (data not shown). Several indices for high sexual activity such as increased numbers of regular male partners for vaginal sex in the past 6 months and lifetime as well as years of vaginal sex experience were associated with a longer duration of antibodies in univariate anal-

Table 2 Risk factors associated with seroconversion of anti-human papillomavirus (HPV)-16 IgG: multivariate time-dependent proportional hazards regression analysis

	Adjusted relative risk (95% confidence interval) for seroconversion	*P*
Age at baseline (per year increase)	0.82 (0.64–1.06)	0.122
User of oral contraceptive pills at time t		
No	1	
Yes	2.88 (1.28–6.47)	0.010
HPV-16 IgA antibodies at time t		
No	1	
Yes	7.89 (2.54–24.50)	<0.001
HPV types detected ≤ time t		
HPV negative or other types	1	
HPV-16 or related (31, 33, 35, 52, or 58) viral load		
Low	14.27 (3.78–53.91)	<0.001
High	37.80 (13.49–105.93)	<0.001

yses but not in multivariate analysis when the number of visits with HPV infection was controlled for. The final multivariate model showed that decreased initial absorbance value as well as decreased number of visits with HPV infection increased the chance of losing IgG antibodies to HPV-16 VLPs (Table 4).

Seroconversion and Duration of IgA to HPV-16 VLPs. Of the 442 women who were seronegative for IgA antibodies to HPV-16 VLPs at baseline and had at least one follow-up serum sample, 14 seroconverted, and the cumulative 36-month incidence of seroconversion was 4.3% (95% CI, 1.9–6.6%). Similar to IgG, seroconversion of IgA was type specific. As compared with women who were HPV negative or had other HPV types, the relative risks for IgA seroconversion were 3.28 ($P = 0.304$) and 33.50 ($P < 0.001$) for women who had HPV-16-related types and HPV-16 ≤ time t, respectively; subjects with HPV-16 infection had a 10-fold increased risk for developing IgA than those with HPV-16-related types ($P = 0.027$). Among the subjects who had an incident infection of HPV-16, 9 women or 37.0% seroconverted within 14 months. Six of the 9 IgA seroconverters had HPV-16 for ≥2 visits. Hence, unlike IgG, seroconversion of IgA tended to be associated with persistent infection with HPV-16 (RR = 3.35, $P = 0.091$).

Among the 40 women who were seropositive for IgA to HPV-16 VLPs and had follow-up after the first seropositive

Table 3 Association between loss of anti-human papillomavirus (HPV)-16 IgG and viral and demographic factors: univariate Cox proportional hazards regression analysis

	No. lost IgG/ no. person-years	Relative risk (95% confidence interval) for loss of IgG	*P*
Age (yrs)			
≥20	13/56.0	1	
<20	24/65.1	1.43 (0.73–2.82)	0.297
Race			
White, Asian, and others	25/84.5	1	
Hispanic	7/13.3	1.80 (0.78–4.17)	0.171
African American	5/23.3	0.71 (0.27–1.87)	0.492
When anti-HPV-16 IgG was first detected			
During follow-up	5/18.5	1	
At baseline	32/102.6	1.39 (0.54–3.60)	0.493
Initial absorbance value when anti-HPV-16 IgG was first detected[a]			
>0.3055	4/82.7	1	
≤0.3055	33/38.4	16.54 (5.78–47.34)	<0.0001
HPV types detected ≤ time t[b]			
HPV-16	5/35.2	1	
HPV-16 related: 31, 33, 35, 52, or 58	5/16.7	2.07 (0.60–7.17)	0.249
Other types	6/36.5	1.10 (0.34–3.63)	0.870
HPV negative	21/32.7	4.05 (1.53–10.77)	0.005
No. visits in which HPV DNA of any types was detected ≤ time t[b]			
≥3	6/45.1	1	
2	7/22.1	1.69 (0.57–5.07)	0.346
1	3/21.2	0.91 (0.23–3.62)	0.888
0	21/32.7	3.88 (1.55–9.68)	0.004
No. visits in which HPV-16 DNA was detected ≤ time t[b]			
≥2	5/21.2	1	
0–1	32/99.9	1.35 (0.53–3.47)	0.530

[a] Median initial absorbance among samples positive for IgG to HPV-16 virus-like particles.
[b] The independent variable was analyzed as a time-varying covariate. The sample sizes presented are number of subjects lost IgG/number of person-years at risk in the particular exposure category.

Table 4 Risk factors associated with loss of anti-HPV-16 IgG: multivariate time-dependent proportional hazards regression analysis

	Adjusted relative risk (95% confidence interval) for loss of IgG	*P*
Initial log absorbance value when anti-HPV-16 IgG was first detected (per 0.1 unit decrease)	1.96 (1.51–2.55)	<0.0001
No. visits in which HPV DNA of any types was detected ≤ time t (per visit decrease)	1.34 (1.05–1.71)	0.018

sample, 16 lost their IgA antibodies. The cumulative probabilities of losing IgA antibodies by 12 and 24 months were 28.1 and 42.5%, respectively, for a median duration of 34 months. The likelihood of losing the IgA antibodies increased with decreasing initial absorbance (RR = 2.26 for 0.1 unit decrease in log absorbance, $P = 0.001$): the 24-month probabilities of losing the IgA antibodies were 78.8 and 7.1% for the subjects with initial absorbance ≤ 0.1660 (median) and > 0.1660, respectively. Unlike IgG, duration of IgA antibodies was not associated with infection with any HPV types for multiple visits.

Discussion

It has been shown cross-sectionally in this and other study populations that antibodies to HPV-16 VLPs were predominantly type specific, and there appeared to be some cross-reactivity with HPV-16-related types (3, 14). At baseline, the seroprevalence of IgG to HPV-16 VLPs was 55% among women with HPV-16 DNA, 33% among those with HPV-16-related types, and 19% among those with other HPV types (15). In the current study, we observed a similar pattern longitudinally that individuals with HPV-16 DNA were 8–10 times more likely to have IgG or IgA antibodies to HPV-16 subsequent to the infection than those with HPV-16-related types. The latter group, in turn, had a higher seroconversion rate than women who had infection with other HPV types (RRs = 5.66 for IgG and 3.28 for IgA). Seropositivity detected in some women infected with HPV-16-related types could be because of nontype-specific antibodies cross-reactive to HPV-16 VLPs.

In our study, the maximum seroconversion rate of IgG after incident HPV-16 infection was 56.7% by 8 months. Carter *et al.* (16) reported that 59.5% of their subjects seroconverted within 18 months. The median seroconversion time was somewhat similar between the two studies (8.3 *versus* 11.8 months). These consistent data suggest that development of antibodies at a detectable level after a natural infection can be a slow process, and it does not necessarily occur in every women. Several factors may explain this phenomenon. (*a*) Antibodies to HPV-16 circulate at a low titer. In our study, the geometric mean absorbances among the seropositive samples were 0.534 for IgG and 0.267 for IgA, which approximated titers of 1:300 and 1:200, respectively. In some women, antibody levels might never have reached the seropositive cut point of the ELISA and were thus not detected as positive. (*b*) Low levels of antibodies need to be boosted over time, so a detectable seroconversion may take time. (*c*) Not every women maintains a detectable level over time. Seroconversion could be missed due to waning of antibodies, especially when blood samples were mostly obtained annually in this study. (*d*) Genetic background of the host such as the class II HLA alleles may determine an individual's humoral response to HPV infection (17, 18). (*e*) Although it is possible that HPV-16 variants may have different immunogenicity (19), most studies suggest that HPV-16 variants belong to a single serotype (20–22).

For the development of IgG antibodies to HPV-16 VLPs, we found that a high viral load of HPV-16 when the incident infection was first detected, rather than persistent infection, was a significant predictor. Other cross-sectional studies had also reported a positive correlation between viral load and the absorbance levels of IgG and hence an association between a high viral load and IgG seroprevalence (3, 6). Some studies, however, identified persistent HPV-16 infection to be a significant factor associated with seroconversion of IgG (4, 5, 14, 16, 23). These different results should not be viewed as contradictory. A high viral load and persistent infection with HPV-16 can be markers indicative of a sufficient antigenic exposure required for development of a detectable level of IgG. A high viral load may provide an antigenic dose that is acute and rapid, whereas a persistent infection maintains a slow gradual boosting effect.

The association between oral contraceptive pill (OCP) use at the time of blood draw and seroconversion of IgG to HPV-16 VLPs was intriguing. It was not due to confounding by condom use because the association remained significant even when usage of condoms was controlled for in the regression model. The fact that current use of OCPs was significantly associated with seroconversion, but having ever used OCPs in the last 6 months was not, argues for some biological effects of exogenous hormones on the detection of IgG antibodies. In this study, we did observe a cross-sectional relationship between current OCP use and high absorbance levels of IgG (data not shown). As glucocorticoid/progesterone response elements have been found in the HPV genome (24, 25), OCPs may induce transcription of the viral capsid gene and increase seroreactivity of the host (5).

In addition to exogenous hormones, another factor that was associated with high absorbance levels of IgG, and hence IgG seropositivity, was the presence of systemic IgA antibodies (8). The level of serum IgA to HPV-16 VLPs could be indicative of a strong antigenic challenge as well as a marker for the host ability to mount a strong humoral response.

Previous studies found IgG antibodies to HPV-16 VLPs to be stable over time (16, 26, 27). In our study, however, ~50% of the seropositive subjects had undetectable IgG levels within 36 months. The discrepancy may likely be due to the differences in the sensitivity and specificity of the ELISA among laboratories as well as the intensity of IgG response to HPV-16 among various populations. Despite these, various prospective studies have yielded some consistent observations: (*a*) The IgG response to HPV-16 after natural infection is generally weak. In another college population, Carter *et al.* (4) reported the average peak geometric mean titer from women who seroconverted was 1:104, which was not far from our average seropositive titer as mentioned above. (*b*) Low levels of IgG antibodies may fluctuate and wane over time, whereas high levels of antibodies are stable. Our study showed that over a maximum of 3-year follow-up since seroconversion, 18.9% of the individuals with initial IgG absorbance ≤ 0.3055 remained seropositive as compared with 84.4% of those with an absorbance of >0.3055. In the study of Geijersstam *et al.* (26), they also reported that

almost all of the samples in the quartile group with the highest absorbance levels remained seropositive.

In addition to a strong initial IgG response, having exposure to HPV of any types in multiple visits subsequent to seroconversion was another factor that was associated with a sustained detectable IgG antibody level. Carter *et al.* (16) also reported that individuals who were repeatedly HPV DNA negative after seroconversion tended not to have persistent IgG antibodies, and this phenomenon was true for antibody responses against HPV-16, HPV-18, and HPV-6. In our study, individuals who had infection with HPV-16, HPV-16-related types, or other HPV types were equally likely to have persistent IgG antibodies, suggesting that the sustained antibodies in women who had any HPV infection were not attributed to nontype-specific antibodies that cross-reacted with HPV-16 VLPs. However, T-helper cell responses to HPV L1 peptides have been shown to have marked cross-reactivity (28). It is possible that infection with different HPV types could induce activation of these cross-reactive T-helper cells, which enhance proliferation and survival of B cells primed by the HPV-16 antigens. An alternative explanation is that detection of HPV DNA is a marker for high-risk sexual behavior and exposure (7, 8). Hence, individuals with frequent HPV infection were likely to have reexposure to HPV-16. Individuals who had antibodies to HPV-16 could be protected from reinfection with HPV-16 (8), but such antigenic reexposure would boost the antibodies to a sustained detectable level.

There were a few differences in the natural history of IgA and IgG antibodies against HPV-16 VLPs. The seroconversion rate of IgA was lower than that of IgG; also, whereas seroconversion of IgA was associated with persistent infection with HPV-16, seroconversion of IgG was related to viral load. Because serum IgA to HPV-16 VLPs had a much lower concentration than IgG, it is not surprising that seroconversion of IgA was more difficult to detect than IgG. A continuous antigenic exposure from persistent HPV-16 infection as well as an intense but short exposure from a high viral load could both augment IgA. However, because IgA antibodies are short lived (29, 30), a persistent HPV-16 infection would be more effective than a high viral load in maintaining the IgA at a detectable level, particularly in this study with annual serum samples.

Our data showed that there were long-term IgA antibodies to HPV-16, and they lasted just as long as the IgG: the median duration for both types of antibodies was ~3 years. However, unlike IgG, the likelihood of having persistent IgA antibodies was not associated with HPV infection in multiple visits, a presumable marker for antigenic exposure. The reason for such a discrepancy is not known.

In summary, antibodies to HPV-16 VLPs are predominantly type-specific, although there is also reactivity with HPV-16-related types. However, the antibody concentration tends to be low. A detectable seroconversion requires boosting of the antibody levels by a strong or sustained antigenic exposure; the markers for such antigenic exposure include high viral load or persistent infection with HPV-16 and use of OCP that may increase expression of the viral capsid gene. Therefore, seroconversion after a natural infection may take several months to become detectable. Antibodies to HPV-16 can persist for years if the initial antibody levels are high. Continued antigenic stimulation through subsequent exposure could serve as a periodic natural booster for sustained antibodies.

Acknowledgments

We thank Dr. Glenn Fennelly for his valuable advice in immunology.

References

1. Harro, C. D., Pang, Y-Y. S., Roden, R. B. S., Hildesheim, A., Wang, Z., Reynolds, M. J., Mast, T. C., Robinson, R., Murphy, B. R., Karron, R. A., Dillner, K. J., Schiller, J. T., and Lowy, D. R. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine. J. Natl. Cancer Inst. (Bethesda), *93:* 284–292, 2001.

2. Schiller, J., and Lowy, D. R. Papillomavirus-like particle vaccines. J. Natl. Cancer Inst. Monogr., *28:* 50–54, 2001.

3. Kirnbauer, R., Hubbert, N. L., Wheeler, C. M., Becker, T. M., Lowy, D. R., and Schiller, J. T. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J. Natl. Cancer Inst. (Bethesda), *86:* 494–499, 1994.

4. Carter, J. J., Koutsky, L. A., Wipf, G. C., Christensen, N. D., Lee, S-K., Kuypers, J., Kiviat, N., and Galloway, D. A. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J. Infect. Dis., *174:* 927–936, 1996.

5. Wideroff, L., Schiffman, M. H., Hoover, R., Tarone, R. E., Nonnenmacher, B., Hubbert, N., Kirnbauer, R., Greer, C. E., Lorincz, A. T., Manos, M. M., Glass, A. G., Scott, D. R., Sherman, M. E., Buckland, J., Lowy, D., and Schiller, J. Epidemiologic determinants of seroreactivity to human papillomavirus (HPV) type 16 virus-like particles in cervical HPV-16 DNA-positive and -negative women. J. Infect. Dis., *174:* 937–943, 1996.

6. Viscidi, R. P., Kotloff, K. L., Clayman, B., Russ, K., Shapiro, S., and Shah, K. V. Prevalence of antibodies to human papillomavirus (HPV) type 16 virus-like particles in relation to cervical HPV infection among college women. Clin. Diag. Lab. Immunol., *4:* 122–126, 1997.

7. Ho, G. Y. F., Bierman, R., Beardsley, L., Chang, C. J., and Burk, R. D. Natural history of cervicovaginal papillomavirus infection in young women. N. Engl. J. Med., *338:* 423–428, 1998.

8. Ho, G. Y. F., Studentsov, Y., Hall, C. B. R., B., Beardsley, L., Lempa, M., and Burk, R. D. Risk factors for subsequent cervicovaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. J. Infect. Dis., *186:* 737–742, 2002.

9. Burk, R. D., Ho, G. Y. F., Beardsley, L., Lempa, M., Peters, M., and Bierman, R. Sexual behavior and partner characteristics are the predominant risk factors for genital HPV infection in young women. J. Infect. Dis., *174:* 679–689, 1996.

10. Burk, R. D., Kadish, A. S., Calderin, S., and Romney, S. L. Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: correlation with biopsy results and Papanicolaou smear. Am. J. Obstet. Gynecol., *154:* 982–989, 1986.

11. Studentsov, Y. Y., Schiffman, M., Strickler, H. D., Ho, G. Y. F., Pang, Y-Y. S., Schiller, J., Herrero, R., and Burk, R. D. Enhanced enzyme-linked immunosorbent assay for detection of antibodies to virus-like particles of human papillomavirus. J. Clin. Microbiol., *40:* 1755–1760, 2002.

12. SAS Institute, Inc. SAS/STAT User's Guide, version 6. Cary, NC: SAS Institute, Inc., 1989.

13. Stata Statistical Software: Release 6.0. College Station, TX: Stata Press, 1999.

14. Sasagawa, T., Yamazaki, H., Dong, Y.-Z., Satake, S.-I., Tateno, M., and Inoue, M. 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, *75:* 529–535, 1998.

15. Studentsov, Y. Y., Ho, G. Y. F., Marks, M. A., R., B., and Burk, R. D. Polymer-based enzyme-linked immunosorbent assay using human papillomavirus type 16 (HPV16) virus-like particles detects HPV16 clade-specific serologic responses. J. Clin. Microbiol., *41:* 2827–2834, 2003.

16. Carter, J. J., Koutsky, L. A., Hughes, J. P., Lee, S. K., Kuypers, J., Kiviat, N., and Galloway, D. A. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J. Infect. Dis., *181:* 1911–1919, 2000.

17. de Gruijl, T. D., Bontkes, H. J., Walboomers, J. M. M., Coursaget, P., Stukart, M. J., Dupuy, C., Kueter, E., Verheijen, R. H. M., Helmerhorst, T. J. M., Duggan-Keen, M. F., Stern, P. L., Meijer, C. J. L. M., and Scheper, R. J. Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. I. Differential T-helper and IgG responses in relation to HPV infection and disease outcome. J. Gen. Virol., *80:* 399–408, 1999.

18. Sanjeevi, C. B., Hjelmstrom, P., Hallmans, G., Wiklund, F., Lenner, P., Angstrom, T., Dillner, J., and Lernmark, A. Different HLA-DR-DQ haplotypes are associated with cervical intraepithelial neoplasia among human papillomavirus type-6 seropositive and seronegative Swedish women. Int. J. Cancer, *68:* 409–414, 1996.

19. Ellis, J. R., Etherington, I., Galloway, D., Luesley, D., and Young, L. S. Antibody responses to HPV16 virus-like particles in women with cervical intraepithelial neoplasia infected with a variant HPV16. Lancet, *349:* 1069–1070, 1997.

20. Cheng, G., Icenogle, J. P., Kirnbauer, R., Hubbert, N. L., St. Louis, M. E., Han, C., Svare, E. I., Kjaer, S. K., Lowy, D. R., and Schiller, J. T. Divergent human papillomavirus type 16 variants are serologically cross-reactive. J. Infect. Dis., *172:* 1584–1587, 1995.

21. Pastrana, D. V., Vass, W. C., Lowy, D. R., and Schiller, J. T. NHPV16 VLP vaccine induces human antibodies that neutralize divergent variants of HPV16. Virology, *279:* 361–369, 2001.

22. Xi, L. F., Carter, J. J., Galloway, D. A., Kuypers, J., Hughes, J. P., Lee, S. K., Adam, D. E., Kiviat, N. B., and Koutsky, L. A. Acquisition and natural history of human papillomavirus type 16 variant infection among a cohort of female university students. Cancer Epidemiol. Biomark. Prev., *11:* 343–351, 2002.

23. de Gruijl, T. D., Bontkes, H. J., Walboomers, J. M. M., Schiller, J. T., Stukart, M. J., Groot, B. S., Chabaud, M. M. R., Remmink, A. J., Verheijen, R. H. M., Helmerhorst, T. J. M., Meijer, C. J. L. M., and Scheper, R. J. Immunoglobulin G responses against human papillomavirus type 16 virus-like particles in a prospective nonintervention cohort study of women with cervical intraepithelial neoplasia. J. Natl. Cancer Inst. (Bethesda), *89:* 630–638, 1997.

24. Chan, W-K., Klock, G., and Bernard, H-U. Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. J. Virol., *63:* 3261–3269, 1989.

25. Pater, A., Bayatpour, M., and Pater, M. M. Oncogenic transformation by human papillomavirus type 16 deoxyribonucleic acid in the presence of progesterone or progestins from oral contraceptives. Am. J. Obstet. Gynecol., *162:* 1099–1103, 1990.

26. af Geijersstam, V., Kibur, M., Wang, Z., Koskela, P., Pukkala, E., Schiller, J., Lehtinen, M., and Dillner, J. Stability over time of serum antibody levels to human papillomavirus type 16. J. Infect. Dis., *177:* 1710–1714, 1998.

27. Shah, K. V., Viscidi, R. P., Alberg, A. J., Helzlsouer, K. J., and Comstock, G. W. Antibodies to human papillomavirus 16 and subsequent *in situ* or invasive cancer of the cervix. Cancer Epidemiol. Biomark. Prev., *6:* 233–237, 1997.

28. Williams, O. M., Hart, K. W., Wang, E. C. Y., and Gelder, C. M. Analysis of $CD4^+$ T-cell responses to human papillomavirus (HPV) type 11 L1 in healthy adults reveals a high degree of responsiveness and cross-reactivity with other HPV types. J. Virol., *76:* 7418–7429, 2002.

29. Rudin, A., Johansson, E-L., Bergquist, C., and Holmgren, J. Differential kinetics and distribution of antibodies in serum and nasal and vaginal secretions after nasal and oral vaccination of humans. Infect. Immun., *66:* 3390–3396, 1998.

30. Schroeder, H. W., Jr., and Torres, R. M. B-cell antigen receptor genes, gene products and coreceptors. *In:* R. R. Rish, T. A. Fleisher, W. T. Shearer, B. L. Kotzin, and H. W. Schroeder, Jr. (eds.), Clinical Immunology. Principles and Practice 1, pp. 4.1. London: Mosby, 2001.

Natural History of Human Papillomavirus Type 16 Virus-Like Particle Antibodies in Young Women

Gloria Y. F. Ho, Yevgeniy Y. Studentsov, Robert Bierman, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:110-116.