Comparison of Human Papillomavirus Types 16, 18, and 6 Capsid Antibody Responses Following Incident Infection

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The relationship between human papillomavirus (HPV) DNA in the genital mucosa and serum IgG to HPV-16, -18, and -6 was studied in a cohort of 588 college women. Among women with incident HPV infections, 59.5%, 54.1%, and 68.8% seroconverted for HPV-16, -18, or -6, respectively, within 18 months of detecting the corresponding HPV DNA. Transient HPV DNA was associated with a failure to seroconvert following incident HPV infection; however, some women with persistent HPV DNA never seroconverted. Antibody responses to each type were heterogeneous, but several type-specific differences were found: seroconversion for HPV-16 occurred most frequently between 6 and 12 months of DNA detection, but seroconversion for HPV-6 coincided with DNA detection. Additionally, antibody responses to HPV-16 and -18 were significantly more likely to persist during follow-up than were antibodies to HPV-6.

Genital human papillomavirus (HPV) types are the etiologic agents of genital warts and squamous intraepithelial lesions, and a subset of types are causally linked with the development of anogenital cancers [1, 2]. Most HPV infections resolve without medical intervention; however, a small proportion do not [3]. Persistent detection of viral DNA may be a prerequisite for neoplastic progression. Presently, there is little understanding of the host, viral, environmental, or behavioral factors that influence viral persistence. It is likely that failure to develop an appropriate antiviral immune response influences subsequent recurrence or persistence [4]. The immune functions important for viral clearance and acquired immunity have not been defined.

Serum antibodies to HPV capsids are not thought to clear infections, but they may provide protection against reinfection by the same type or types, and they can serve as markers for ongoing or previous infections [5–7]. In an animal model, immunity to infection was passively transferred by serum [8], suggesting that antibodies protect against reinfection with the same type. Serologic studies have demonstrated that ~20%–50% of women with HPV DNA– or HPV-associated lesions do not have detectable type-specific anti-HPV antibodies [9–14]. This may be due to antibody titers declining over time among individuals with cancer; however, HPV antibody responses have been shown to persist for many years [15, 16]. With a few exceptions [17, 18], studies measuring HPV antibody responses have been cross-sectional or have enrolled subjects already known to be infected with HPV. Although these studies have been valuable, it was not possible for them to address the kinetics of antibody responses in relationship to HPV DNA detection and clinical outcome.

To provide insight into the natural history of antibody responses to HPVs, we conducted a longitudinal study of young women. At enrollment, the majority of women in the cohort had few or no sex partners; therefore, the HPVs detected were likely to represent their first exposure to the virus, rather than reactivation of latent infections. Previously, we examined HPV-16 antibody responses among 25 women who had acquired HPV-16 DNA during the course of follow-up [9]. Here, the antibody responses to HPV-6, -16, and -18 were examined in a larger cohort to determine if the earlier analyses of the HPV-16 antibody responses were representative of anti-HPV antibody responses in general. Because one of the limitations of the previous study was the small number of women who acquired HPV-16 infections during the study, serum samples from an additional 263 women were tested for HPV-16 antibodies. The time to seroconversion and antibody persistence were examined for HPV-6, -16, and -18. Antibody responses to these 3 types were evaluated and compared in terms of detection and persistence of HPV DNA and detection of clinical lesions.

Subjects and Methods

Study subjects. Recruitment methods have been reported elsewhere [9]. Briefly, between September 1990 and September 1998,
female university students 18–20 years of age were invited to participate in a longitudinal cohort study of genital HPV infection. Women were eligible for participation if they were residents of the state of Washington with plans to stay in the area for at least 3 years and if they were able to provide informed consent. The majority of the 603 women (80.8%) reported 2 or fewer sex partners at enrollment (171 reported 0, 199 reported 1, and 117 reported 2 previous partners). This study concerns the 588 subjects enrolled for whom there were HPV DNA and serologic data. Among these women, the average time (± SD) between visits was 4.7 ± 0.9 months, and the average length of follow-up was 31.3 ± 18.8 months.

**Interview, physical examination, and collection of specimens for testing.** After completing informed-consent procedures, subjects underwent standardized interviews concerning socioeconomic status, gynecological and obstetrical history, current and past sexual behavior, and history of genital-tract infections. Standardized examination of the external genitalia, perianal region, vagina, and cervix included colposcopic evaluation of the cervix and collection of separate endo-ectocervical and vulvovaginal Dacron-tipped swab specimens for HPV DNA analysis by polymerase chain reaction (PCR)-based methods and Pap smears of both the cervical transformation zone and lateral vaginal walls. In addition, 20 mL of blood was collected for serologic testing. An experienced health care specialist enrolled and examined all subjects.

**PCR.** To test for HPV DNA by PCR, each sample was amplified in duplicate with consensus primers MY09 and MY11, as described elsewhere [9]. The PCR products were probed with a biotin-labeled generic HPV probe and with biotin-labeled type-specific oligonucleotide probes, including probes for HPV-6 and -11, -16, and -18, as well as other type-specific and group-specific probes. “Other type” HPV DNA included all samples that tested positive with the consensus primers, whether typed or untyped, but excluded samples that tested positive for HPV-16, -18, or -6.

**HPV antibody test.** To test for serum IgG antibodies to HPV-6, -16, and -18, a capture antibody test similar to the HPV-16 assay described elsewhere [9] was used. In brief, capture monoclonal antibodies were coated on carbonate buffer and used to coat microtiter plates (Immulon II; Dynex Technologies, Chantilly, VA). The monoclonal antibodies used were H11.B2 (used at a dilution of 1 : 5000), H6.B10 (1 : 10,000), or H6.M48 (1 : 7000), H16.V5 (1 : 5000), and H18.J4 (1 : 7500), all generously provided by Dr. N. Christensen (Pennsylvania State University, Hershey Medical Center, Hershey, PA). One test was conducted with only H16.V5, a second with H6.C6, H11.B2, and H18.J4, and a third with H16.V5, H6.M48, and H18.J5. There was no significant difference between tests in the proportion of seropositive women (data not shown). Nonspecific binding was blocked by the addition of PBS with 5% goat serum (Sigma, St. Louis) and 0.05% Tween-20 (Fisher Scientific, Fair Lawn, NJ). Capsids were produced with recombinant vaccinia viruses and partially purified on cesium chloride gradients, as described elsewhere [19]. Capsids were added at an optimum dilution, determined with a positive control serum. Human serum samples that had been frozen at −70°C were thawed, diluted 1 : 100 in blocking buffer, and tested in triplicate in wells containing HPV-6, -16, or -18 capsids. Nonspecific binding for each serum sample was determined, also in triplicate; we used wells containing capture antibodies without added capsids. Bound human IgG was detected as described elsewhere [9], and the plates were read at 405 nm. The ELISA values were calculated as follows: the ELISA value equaled the natural logarithm of the median optical density (OD) of wells with capsids minus the natural logarithm of the median OD of the wells without capsids. A total of 3959 sera from 588 women was tested for antibodies to HPV-16. There were not sufficient sera for all samples to be tested for HPV-6 and HPV-18 antibodies. Of 588 women tested for HPV-16 antibodies, 3625 sera from 577 of these women were tested for antibodies to HPV-6 and -18.

The cut point for an initial screen was computed as 2 SDs above the mean of a set of 76 serum samples from women in whom no HPV DNA was detected and who reported no previous male sex partners. Subsequent tests were performed on sera collected after the initial screen. For these tests, cut points were calculated daily by linear regression by use of 12 sera that had been tested in the original screen, some of which were positive for each HPV type. To make the values of tests directly comparable, the cut points were subtracted from all of the values; thus, a positive adjusted value was always seropositive.

**Statistical analysis.** Statistical analyses were performed using Egret (Statistics and Epidemiology Research, Seattle) and S-plus (Mathsoft, Cambridge, MA). Plots were prepared by Prism (GraphPad Software, San Diego).

All analyses were specific for individual HPV types. Time to event data (e.g., time from first DNA positive to seroconversion; time from seroconversion to seronegativity) was analyzed with survival analysis methods. Time to seroconversion analysis was limited to women who acquired HPV DNA during follow-up, who were seronegative at all visits prior to HPV DNA detection, who had serology results available after the first HPV DNA–positive visit, and who were not missing serology data from the visit that corresponded to the first detection of HPV DNA, unless the subsequent visit was seronegative. Product limit estimates [20] were used to estimate the distribution of time to an event. Log-rank tests were used to compare survival curves between subgroups. For women with incident HPV infections, stratified product limit estimates were used to depict the time to seroconversion distribution for subjects who remained HPV DNA positive vs. those who became HPV DNA negative. In addition, smoothed hazard function estimates [21], stratified by current HPV DNA status, were used to depict the risk of seroconversion for subjects who remained HPV DNA positive vs. those who became HPV DNA negative. In this analysis, all subjects start in the DNA-positive stratum but switch between strata during follow-up if their HPV status changes from positive to negative.

To determine if some women were predisposed to respond or not respond to HPV infections, we counted the number of incident HPV infections that were followed by at least 1 year of additional study participation, and we also counted the number of incident infections that led to type-specific seroconversion within 1 year. Dividing the latter by the former gave the probability of seroconverting within 1 year. Under the null hypothesis that women who seroconvert for 1 HPV type are not more or less likely to seroconvert for other types, one may compute the expected number of women who would seroconvert to 1, 2, or 3 HPV infections within 1 year of DNA positivity. Comparing the actual number of woman seroconverting to 1, 2, or 3 HPV infections within 1 year with the
expected number gives a statistical test (a \( \chi^2 \) statistic) of the hypothesis that some woman are more or less likely to respond to HPV infections. The data we present include HPV-16 data previously published [9].

Results

**Serum antibody responses in relation to detection of prevalent HPV-16, -18, or -6 DNA at enrollment.** The association of serum IgG antibody responses with the type-specific detection of HPV DNA among women who entered the study HPV DNA positive is shown in table 1. At enrollment, there were 24 women who were HPV-16 DNA positive, 11 women who were HPV-18 positive, and 13 women who were HPV-6 DNA positive. The highest percentage of seropositive results at enrollment was observed among women with HPV-16 infections (54.2%) and the lowest percentage among women with HPV-18 infections (36.4%). At 18 months of follow-up, the highest rate of seroconversion was observed among women with HPV-6 infections (91.7%), and, again, women with HPV-18 infection had the lowest rate of seroconversion (54.5%).

**Time to seroconversion following incident HPV-16, -18, or -6 DNA detection.** The great advantage of this cohort study was the ability to detect incident infections among women who had recently initiated sexual activity and to examine changes in the levels of HPV antibodies over time. Women who had entered the study as HPV DNA negative for a particular type but had become HPV DNA positive for that type at a subsequent visit were considered to be incident cases. Among the 584 women enrolled in this study for whom there were HPV DNA and serology data available, incident infection with HPV-16 was detected among 56 women; 35 women had incident HPV-18 infection; and 36 women had incident HPV-6 infection. To accurately estimate time to seroconversion after DNA detection, the analyses were restricted to women with the most complete serologic data (see Subjects and Methods). Forty-two women were used to determine the time required to seroconvert following incident infection for HPV-16; 30 women were used for HPV-18; and 31 women were used for the HPV-6 analysis. For women with incident HPV-16 infections, the median time to seroconversion from DNA detection was 11.8 months (figure 1). The median time to HPV-6 seroconversion among women with incident HPV-6 infections was 11.7 months, and the median time to HPV-18 seroconversion was 12.6 months among women with incident HPV-18 DNA. Seroconversion rates were similar for these 3 types (log-rank test; \( P = .63 \)). Serologic responses were primarily type specific, because the proportion of women who seroconverted during 1 year of follow-up for HPV-16, -18, or -6 was estimated as 41.6%, 27.1%, and 13.8%, respectively, among women in whom other types of DNA were detected. In the same time interval, <6% of women who were always negative for HPV DNA seroconverted for any HPV type (data not shown).

Although the HPV-6 and HPV-16 survival curves converge after 12 months, most HPV-6 seroconversions occurred sooner after detection of HPV-6 DNA than did HPV-16 seroconversions after detection of HPV-16 DNA (figure 1). Eight (25.8%) of 31 HPV-6 DNA–positive women seroconverted at the initial visit at which HPV-6 DNA was detected, whereas only one (2.4%) of 42 HPV-16 DNA–positive women seroconverted coincident with the detection of HPV-16 DNA (figure 1; \( P = .004 \)). Among women with incident HPV-18 infections, 5 (16.7%) of 30 were seropositive at the first visit at which HPV-18 DNA was detected.

Few seroconversions occurred \( \geq 18 \) months after the detection of HPV DNA (figure 1); therefore, that time was chosen to analyze the percentage of women who developed HPV antibodies. Eighteen months after the detection of incident HPV DNA, 59.5%, 54.1%, and 68.8% of women in whom HPV-16, -18, and -6 DNAs had been detected seroconverted, respectively (the SE ranged from 8.1% for HPV-16 to 9.8% for HPV-18).

Figure 2 shows the individual serologic responses among women in whom incident HPV infections by HPV-16, -18, or -6 were detected. There was considerable heterogeneity of antibody responses to the same type, and there were differences in the patterns of responses between types. Among women with the most vigorous antibody responses, those with HPV-16 produced stronger responses than those with HPV-6 or -18 (note the differences in the scales of the ELISA values in figure 2). It was also evident that, whereas the majority of the HPV-6 serologic responses declined after an initial peak, most of the HPV-16 and -18 responses persisted following seroconversion (see below).

**Analysis of factors that could influence seroconversion.** The data presented in figures 1 and 2 suggest that, after infection with genital-type HPVs, some women may never develop an anticapssid antibody response. We and others have shown [9, 22, 23] that women in whom HPV-16 DNA was detected at only 1 visit were significantly less likely to seroconvert than were women in whom HPV-16 DNA was detected at several visits. This finding was reproduced in the analysis of HPV-16 seroconversion (figure 3A). Women with only 1 HPV-16 DNA–positive visit were less likely to seroconvert than women with >1 HPV-16 DNA–positive visit (\( P = .036 \)). HPV-18 seroconversion (figure 3B) was less likely to occur among women

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<th>Table 1. Type-specific seroconversion among women who tested positive for human papillomavirus (HPV) DNA at enrollment.</th>
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<td>DNA type detected (n)</td>
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<td>HPV-16 (24)</td>
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* The percentage of women who seroconverted for each type of HPV was determined using survival analysis. At 18 months, the SE for HPV-16 seropositivity was 7.6%; for HPV-18, 15.0%; and for HPV-6, 8.0%.
Figure 1. Estimation of time to seroconversion by Kaplan-Meier survival analysis, using seroconversion as the outcome. Solid lines represent the proportion of women who seroconverted for human papillomavirus (HPV) type 16 (△; n = 42), HPV-18 (●; n = 30), or HPV-6 (■; n = 31) who were positive for HPV-16, -18, or -6, respectively. Dotted lines represent the proportion of HPV-16 (Δ; n = 108), HPV-18 (⊙; n = 126), or HPV-6 (□; n = 102) seroconversions among women in whom other types of HPV DNA were detected. SE bars are shown at the last visit on which seroconversion was detected for each line.

who were HPV-18 DNA positive only once, but the difference did not achieve statistical significance (P = 0.08). In contrast, women who were HPV-6 DNA positive at only 1 visit were as likely to seroconvert as were women who were HPV-6 DNA positive at ≥2 visits (P = 0.8; figure 3C).

One weakness of this analysis is that it groups together women with 2–8 HPV DNA–positive visits. Thus, the dotted survival curve represents a mixture of women who had become HPV DNA negative and women who remained HPV DNA positive. An alternative analysis is presented in figures 3D, 3E, and 3F. Seroconversion data are presented as smoothed curves that estimate the risk of seroconversion at various times after initial detection of HPV DNA among women who remained HPV DNA positive (dotted lines) and among women who became HPV DNA negative (solid lines). In these analyses, at any time after the first detection of HPV DNA, women may be represented in either the dotted or solid lines, depending on their most recent HPV DNA result. Data from an individual could be represented in both lines if she changed DNA status during follow-up. For HPV-16 and -18, the rate of seroconversion among DNA-positive women was highest at ∼7–8 months after DNA detection (figure 3D, 3E). The rate of HPV-6 seroconversion was highest at the time of, or soon after, the detection of HPV-6 DNA (figure 3F). What was interesting was that for all types, after reaching a peak, the rate of seroconversion declined even in the continued presence of HPV DNA. It was concluded from these analyses that transient DNA was not responsible for the failure to observe seroconversion in all women with HPV-16 or -18 DNA and few, if any, women with HPV-6 DNA.

Another possible explanation for women failing to seroconvert was that they were genetically or immunologically predisposed to not recognize or otherwise respond to all or most genital HPV infections. To examine this possibility, we analyzed the serologic responses of women with single and multiple HPV-16, -18, and -6 infections. Among women who were infected with one of the 3 types, 33 (41.8%) of 79 failed to seroconvert within the first year. Assuming that there was no subpopulation of women unable to respond to HPVs, then it would be expected that 17.4% (41.8% × 41.8%) of women infected with 2 types would fail to seroconvert for both types. Thus, if a subpopulation of nonresponding women existed, then the percentage of women with 2 HPV infections who failed to respond to both types should be significantly higher than 17.4%. Among women who were infected with 2 of the 3 types (HPV-16, -18, or -6), only 3 (11.5%) of 26 women failed to seroconvert for both types within the first year. An analysis of the overall expected and observed proportions of women who seroconverted among
Figure 2. Changes in ELISA reactivity after detection of human papillomavirus (HPV) DNA. Each line represents the adjusted ELISA values from 1 woman over time. A, HPV-16 ELISA values from 45 women with incident HPV-16 DNA; B, HPV-18 ELISA values from 34 women with incident HPV-18 DNA; and C, HPV-6 ELISA values from 33 women with incident HPV-6 DNA. Not shown are women with no serologic follow-up after the detection of HPV DNA.

women with single and multiple infections confirmed this analysis ($P = .254$), suggesting that there was not a predisposition on the part of some of the women to be unable to seroconvert to genital-type HPVs in general.

Failure to seroconvert was examined relative to the site of HPV DNA detection (cervical, vulvovaginal, or anal), the presence of other HPV DNAs, the detection of squamous intraepithelial lesions (HPV-16 or -18), or the detection and treatment of genital warts (HPV-6). None of these factors showed either a positive or negative association with seroconversion (data not shown).

Persistence of antibody responses. Persistence of serum antibodies was examined among women with incident infections in whom HPV-16 DNA and serum antibodies were detected (figure 4A), and the same analyses were conducted for HPV-18 and -6 (figure 4B, 4C). The majority of women remained seropositive throughout follow-up for HPV-16 and -18. Among women for whom there was $\geq 1$ follow-up visit after the initial seropositive visit, 20 (71.4%) of 28 women with incident HPV-16 infections were HPV-16 seropositive at all visits, and 11 (78.6%) of 14 women with incident HPV-18 infections were HPV-18 seropositive at all visits. In comparison, among women with incident HPV-6 infections, only 8 (34.8%) of 24 were seropositive at all subsequent visits. Persistent HPV-6 responses were detected significantly less frequently than HPV-16 or -18 responses (log-rank test, HPV-16 compared with HPV-6, $P = .01$; HPV-18 compared with HPV-6, $P = .03$; HPV-18 compared with HPV-16, $P = .80$). Loss of antibodies to any HPV type was not related to longer follow-up time; on the contrary, most women who became consistently seronegative did so within the first year of follow-up (figure 4).

In contrast to the general pattern of persistent type-specific antibody responses among women who were HPV-16 or -18 DNA positive, type-specific serum antibody responses among women who were repeatedly HPV DNA negative tended not to persist. Among women who were repeatedly HPV-16 DNA negative but were HPV-16 seropositive at least once and who had a follow-up visit, only 12 (15.6%) of 77 were seropositive at $\geq 2$ consecutive visits.

Discussion

In this study, we examined type-specific serologic responses among women with incident HPV-16, -18, and -6 infection. For all 3 types of HPV, the kinetics and magnitude of antibody responses following the detection of HPV DNA varied considerably from 1 individual to the next. Some women generated vigorous sustained antibody responses, whereas others had no detectable reactivity. The rate of seroconversion and the proportion of women who seroconverted within 18 months following detection of HPV DNA were similar for HPV-16, -18, and -6. However, type-specific differences in antibody responses were noted in the persistence of antibodies and in the timing of seroconversion in relation to initial detection of HPV DNA.

In agreement with a previous study [24], we found that antibodies to HPV-6 were detected on the visit at which HPV-6 DNA was initially detected among 25.8% women. However, HPV-16 seroconversions were rarely detected coincident with the detection of HPV-16 DNA (only 2.4%). Most HPV-16 sero-
Figure 3. Association between persistent detection of human papillomavirus (HPV) DNA and seroconversion. Survival curves (A–C) compare rates of seroconversion between women who were HPV DNA positive only once (solid lines) and women having several DNA-positive visits (dotted lines) for HPV-16 (A), -18 (B), or -6 (C). Smoothed curves (D–F) show the estimated risk of seroconversion among women with incident HPV infection for HPV-16 (D), -18 (E), or -6 (F). Solid lines represent the risk of seroconversion among women with incident infection after they became HPV DNA negative for HPV-16 (D), -18 (E), or -6 (F). Dotted lines represent the risk of seroconversion following incident HPV infection, while the women remained HPV DNA positive for that type (includes all women with incident HPV infections).

Conversions were detected 6–12 months after the detection of HPV-16 DNA (figures 1 and 3). The pattern of HPV-18 seroconversion was more similar to the HPV-16 pattern than it was to the HPV-6 pattern (figure 3). Twelve months after initial HPV DNA detection, the proportion of women who had seroconverted was similar for all 3 types. The median time to seroconversion ranged from 11.7 months for HPV-6 to 12.6 months for HPV-18. HPV-16 was found to have a median time to seroconversion of 11.8 months. We had reported earlier that the median time to HPV-16 seroconversion was 8.3 months [9]; the current estimate is more precise because of the larger sample size.

It is possible that apparent differences in the timing between HPV-6 and -16 seroconversion occurred because of differential detection of HPV-6 vs. -16 DNA. This may have resulted from methodological differences in the sensitivity of PCR amplification or detection or from higher viral DNA loads present in HPV-16 infections relative to HPV-6 infections. Higher levels of HPV-16 DNA relative to HPV-6 DNA would make HPV-16 detectable sooner after exposure than HPV-6. This hypothesis was supported by the observation that, among women who became infected, HPV-16 DNA was detected earlier after exposure to a new sex partner than was HPV-6 DNA (data not shown).

We and others have shown that detection of HPV-16 antibodies is associated with HPV-16 DNA persistence [9, 22, 23]. Accordingly, this study showed that failure to seroconvert was associated with having had only 1 HPV DNA–positive visit for...
HPV-16. Seroconversion to HPV-18 was also detected less frequently among women who had had only 1 HPV-18 DNA–positive visit than among women with multiple HPV-18 DNA–positive visits; however, the result did not achieve statistical significance. In contrast, seroconversion for HPV-6 was not dependent on the number of HPV DNA–positive visits. This finding could also be explained by a reduced ability to detect HPV-6 DNA relative to HPV-16 or -18 DNA. Alternatively, it could be argued that detection of HPV-16 DNA at only 1 visit was due to a false-positive PCR test. This is unlikely, because all HPV-16–positive results were verified by repeated testing and most HPV DNA specimens were sequenced (data not shown). Additional analysis of the relationship between seroconversion and DNA persistence indicated that even in the presence of persistent HPV DNA, the likelihood of seroconversion declined over time after an initial peak. Thus, failure to seroconvert was not due solely to transient detection of HPV DNA, even for HPV-16 or -18.

De Grujil et al. [22] found that persistent HPV-16 DNA and HPV-16 serum antibodies were present in all 11 women who developed severe dysplasia in their study. This was not the case in our study. Of the 9 women who developed high grade intraepithelial lesion (HSIL) following incident HPV-16 or -18 infections, 5 failed to seroconvert for HPV-16, -18, or both. Of these 5 women, 4 were HPV-16 or -18 DNA positive at every visit for >1 year but never seroconverted. Overall, HPV-16 seroconversion was observed in 41 (73%) of 56 HPV-16 DNA–positive women who did not develop HSIL, compared with 4 (44%) of 9 HPV-16 DNA–positive women who developed HSIL ($P = .13$).

Factors other than DNA persistence that may have contributed to a failure to seroconvert were examined (e.g., anogenital sites positive for HPV DNA, presence of other HPV DNAs, and detection and treatment of squamous intraepithelial lesions or genital warts). None of these factors appeared to influence seroconversion. An alternative explanation is that infection with certain HPV variants resulted in serologic responses that were not detected by these assays. Cheng et al. [25] reported that the 2 most divergent variants of HPV-16 are serologically cross-reactive; however, Ellis et al. [26] showed that women infected with a particular variant were less likely to be HPV antibody positive than were women with other variants. In this study, the proportion of women who seroconverted to HPV-16 was similar for women infected by different HPV-16 variants (L. F. Xi, personal communication). Differences in the host genetic background or immune status could also explain why some individuals failed to seroconvert. We attempted to determine if a subset of women appeared to be predisposed to not respond to the HPV types tested for (and perhaps all genital HPVs). No evidence of such a subpopulation was found here. One limitation of this analysis was the relatively small number of women with combinations of HPV-16, -18, and -6 infections.

A previous study examining HPV-16 variants in a subset of this same cohort of women found no evidence of women becoming reinfected with a different HPV-16 variant [27]. This implied that women developed effective protective immunity after their first infection. However, analysis of the data presented here and in previous studies demonstrates that a significant proportion of women with HPV infections do not develop detectable serum antibodies [13, 22], and others have shown that not all women develop mucosal antibodies [28]. It must be concluded either that serum or local antibodies below the level of detection are capable of providing protection or that cellular responses are sufficient to induce HPV immunity. Possibly the current generation of HPV serologic tests lacks the
sensitivity required to detect low levels of antibody produced after some infections.

This is the first report showing type-specific differences in antibody persistence. Whereas approximately three-quarters of HPV-16 and HPV-18 responses persisted, only about a third of HPV-6 antibody responses persisted throughout follow-up (figure 4). Our findings are consistent both with studies of HPV-16 antibody persistence that have concluded that serum antibody responses persist for years [15, 16] (T. A. Grubert, personal communication) and with studies indicating that HPV-6 antibodies are often lost after treatment [24, 29]. It will be important to determine if women who lose detectable antibodies can be reinfected with the same HPV type.

One caveat to the interpretation of the persistence data is that the sensitivity of detecting serum antibodies to HPV-16 and -18 capsids may be different from the sensitivity of detecting antibodies to HPV-6 capsids. It is difficult to compare assay sensitivity, because reactivity in these assays depends not only on the amount of capsid antigen present but also on having intact conformational epitopes present in equivalent numbers for the different types. We do not believe that there were major differences in assay sensitivity for these types, because the proportion of women who seroconverted was similar for the 3 types. In fact, from the information presented in table 1 and figure 1, it appears that of the 3 assays, the HPV-18 ELISA may have been less sensitive than the HPV-6 ELISA, because seroconversion was less likely to be observed among women with HPV-18 infections than among women with HPV-6 infections.

Unlike the HPV-16 and -18 antibody tests, the HPV-6 assay may have detected some cross-reactive responses. All of the serologic responses were generally type specific, being more frequently detected among women in whom the HPV DNA type corresponded to the antibody test than among women in whom other types of HPV DNA were detected. However, among women in whom other types of HPV DNA were detected, HPV-6–seropositive women were detected at a higher frequency than were HPV-16– or HPV-18–seropositive women (figure 1). It is possible that the HPV-6 capsids are less stable, resulting in more denatured 6L1 in the ELISA. An alternative explanation is that, because HPV-6 infections are not as persistent as HPV-16 or -18 infections [30] (L.A.K., unpublished data), either for biological or methodologic reasons, the detection of other HPV DNA could have been a surrogate marker for transient, undetected HPV-6 infections.

In summary, we have shown herein that the proportion of women who seroconverted was ~60% within 18 months of DNA detection for all 3 types. However, detection of HPV-6 antibodies differed from detection of HPV-16 and -18 antibodies in 2 important aspects. First, HPV-6 seroconversions were detected coincident with the detection of HPV-6 DNA more frequently than were HPV-16 seroconversions among women with HPV-16 DNA. Second, HPV-16 and -18 antibody responses were more likely than HPV-6 antibody responses to persist throughout follow-up.

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References

18. Andersson-Ellstrom A, Dillner J, Hagmar B, et al. Comparison of development of serum antibodies to HPV16 and HPV33 and acquisition of cer-