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Human Papillomavirus (HPV) Type Distribution and Serological Response to HPV Type 6 Virus-Like Particles in Patients with Genital Warts

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Thirty-nine patients with condylomas (12 women and 27 men) attending a dermatology clinic were tested for genital human papillomavirus (HPV) DNA and for seroprevalence to HPV type 6 (HPV6) L1 virus-like particles. The L1 consensus PCR system (with primers MY09 and MY11) was used to determine the presence and types of HPV in sample specimens. All 37 (100%) patients with sufficient DNA specimens were positive for HPV DNA, and 35 (94%) had HPV6 DNA detected at the wart site. Three patients (8%) had HPV11 detected at the wart site, and one patient had both HPV6 and -11 detected at the wart site. Thirteen additional HPV types were detected among the patients; the most frequent were HPV54 (8%) and HPV58 (8%). Baculovirus-expressed HPV6 L1 virus-like particles were used in enzyme-linked immunosorbent assays to determine seroprevalence among the patients with warts. Seronegativity was defined by a control group of 21 women who were consistently PCR negative for HPV DNA. Seroprevalence was also determined for reference groups that included cytologically normal women who had detectable DNA from either HPV6 or HPV16 and women with HPV16-associated cervical intraepithelial neoplasia. Among the asymptomatic women with HPV6, only 2 of 9 (22%) were seropositive, compared with 12 of 12 (100%) female patients with warts. A similar trend in increased HPV6 seropositivity with increased grade of disease was found with the HPV16 DNA-positive women, whose seroprevalence increased from 1 in 11 (9%) in cytologically normal women to 6 in 15 (40%) among women with cervical intraepithelial neoplasia 1 or 3. However, only 4 of 25 (16%) male patients were seropositive. No factors examined, such as age, sexual behavior, or a history of warts, were found to definitively account for the gender difference in seroresponse.

There are more than 70 types (13) of human papillomaviruses (HPV) which can be categorized by their predilection for mucosal (oral or genital) or keratinized (dermal) epithelium. The viruses are further grouped by their associated clinical manifestations. For example, HPV types 6 (HPV6), -11, -40, and -42 are most commonly associated with condylomata acuminata, while HPV16, -18, -31, -33, and -45 are most commonly associated with cervical intraepithelial neoplasia (CIN), carcinoma in situ, and invasive cervical cancer. Recent analyses suggest that phylogenetic relationships can be used to predict the clinical manifestations of genital HPV types (5, 46).

Although much attention has focused on the HPV types associated with CIN and cancer, fewer studies have characterized specific HPV types found in genital warts. Detection of genital wart-associated viruses has most often been reported as groupings of HPV types (e.g., HPV6 with HPV11). Even with the availability of PCR methods for the detection of specific virus types, few researchers have sought to distinguish wart-associated types in prevalence studies. However, limited studies of patients with genital warts (26, 44) suggest that HPV6 is associated with the majority of condyloma acuminatum cases.

While PCR and other DNA detection systems have been increasingly applied to determine HPV type distribution and the association of HPV with neoplasia, the study of host immune response has been less well characterized. This neglect is largely due to the lack of an in vitro culture system that can generate sufficient quantities of viral antigen for large-scale testing and to the scarcity of virions from HPV-induced lesions. Serological studies utilizing purified virions have included HPV1 isolated from plantar warts (34, 42, 47) and $HPV_{Hershey}11$ virions generated in the nude mouse xenograft system (6–8, 11, 12, 25). These studies have revealed associations between seropositivity and clinical symptoms of infection.

Many of the initial studies of serologic response to HPV employed recombinant DNA methods to clone and express late region 1 (L1) or 2 (L2) proteins with bacterial fusion proteins (16, 23, 49, 50). Recently, the predominant humoral response to HPV virions has been shown to be directed against conformationally dependent structures that were not duplicated in bacterial fusion systems (12, 17). With the advent of insect and vaccinia virus expression systems, researchers have demonstrated the self-assembly of L1 and L1/L2 proteins into virus-like particles (VLPs) for HPV1, -11, and -16 and have evaluated patient humoral response in a limited number of clinical studies (9, 10, 24, 37). To date, there are no studies reported that utilize HPV6 VLPs, the most common HPV in genital warts.

In this study, we determined the genital HPV type distribution in patients with condylomas attending a private practice dermatology clinic. In addition, we sought to develop an en-

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zyme-linked immunosorbent assay (ELISA) to define seropositivity to HPV6 baculovirus-derived VLPs and to determine seroprevalence in patients with condylomas.

MATERIALS AND METHODS

Patient populations and specimen collection. Specimens from two categories of patients were used in this study. The first was a cohort of patients presenting at a private dermatology clinic with vulvar or penile condylomata acuminata, none of whom were identified as sexual partners. Participants completed a brief medical history questionnaire regarding gender, age, marital status, previous history of genital warts and/or abnormal Pap smears, and sexual behavior. Immunocompromised patients were excluded from the study, as were patients with anal warts. In addition, of the female patients with condylomas, only those with normal Pap smears had been referred to this clinic. During a physical examination, swabs for HPV DNA testing were taken from both the clinically apparent wart sites and additional genital sites in order to obtain more complete information concerning HPV genital infection. Clinical personnel were instructed in appropriate methods for collection of samples to be tested by PCR, and measures such as sampling the nonwart site prior to the wart site were taken to prevent sample-to-sample contamination. For females, the wart site specimen included a swab of the wart(s), the posterior fourchette, and the inner aspect of the right and left labia minora. A cervical swab was obtained to represent the nonwart site. From male patients, a swab of the wart(s) was obtained and a nonwart site swab was obtained from the circumference of the coronal sulcus and the distal penile shaft. The Dacron swabs were then placed in 1.0 ml of sterile saline (0.9% NaCl) and stored at −20°C until processing. Samples were processed for subsequent PCR amplification as previously reported (2).

The patients of the second category were used to compose four reference groups. Samples from epidemiologic studies of cervical HPV infection at the University of New Mexico (4, 48) were selected on the bases of both cervical cytology and HPV status by the PCR-based method applied. The HPV-negative reference group included virgins and sexually experienced women who had repeatedly tested negative (12 consecutive weekly visits) for genital HPV DNA and had normal Pap smears. The second group included women that were positive for only HPV6 DNA and were cytologically normal on the basis of colposcopy and a negative cytologic diagnosis taken at the time the HPV DNA specimen was obtained. In addition, none of the women had a past history of cytological abnormalities as determined by self-reporting and a medical history review. A third subset of patients had cervical samples positive for only HPV16 DNA and were also cytologically normal. The fourth and final group included patients who were only positive for HPV16 DNA and had CIN 1 to 3.

PCR. HPV status was determined by PCR with the L1 consensus primers MY09 and MY11 as previously described (2, 21). Amplification products from the University of New Mexico studies and the patients with warts were applied to replicate membranes and tested with type-specific probes for HPV6, -11, -16, -18, -31, -33, -35, -39, -45, -51, -52, -53, -54, -59, -66, and -73 and novel HPV (28) W13B(MM4). Additional probes were used to type the amplification products from the samples from patients with warts that included HPV26, -40, -42, -55, -56, -57, -58, and -68 and novel isolates PAP155(MM8) and PAP291(MM7). Sample sufficiency was determined by coamplification and oligonucleotide-specific probing of a 268-bp β-globin fragment (2). Positive controls and negative controls were included to monitor for contamination as described previously (18) and were included in amplifications and probe hybridizations.

VLP cloning and purification. To construct the baculovirus shuttle vector, pAcCL1-6, the HPV-6b L1 (40) sequence was amplified from the prototype plasmid (a gift of H. zur Hausen) by PCR with an upstream primer encoding the first 20 bp of the L1 open reading frame and a downstream primer encoding the last 22 bp of the coding sequence. To facilitate the subcloning, the primers also introduced an *Asp*718 endonuclease restriction site upstream of the initiating ATG and an *Eco*RI site downstream of the TAA termination codon. The amplified sequence was subcloned into the polylinker region of the shuttle vector pAcC13 (31), behind the polyhedron promoter. The insert was sequenced to confirm that no errors were introduced during PCR. Transfection of *Spodoptera frugiperda* (Sf9) cells and isolation of recombinant virus expressing HPV6b L1 were performed according to standard techniques (32).

Suspension cultures of Sf9 cells were grown in serum-free medium (29) and infected with the L1 recombinant baculovirus at a multiplicity of infection of 0.1. Seventy-two hours postinfection, the cells were collected by centrifugation and lysed in a buffer containing 20 mM Tris (pH 8.2), 1 mM ethylene-bis(oxyethylenenitrilo-tetraacetic acid (EGTA), 1 mM $MgCl_2$, 10 μM leupeptin, and 100 μM phenylmethylsulfonyl fluoride. The nuclei were isolated by centrifugation and lysed by sonication in calcium and magnesium-free phosphate-buffered saline (PBS) with protease inhibitors as noted above. After removal of the nuclear debris by centrifugation, the soluble protein was chromatographed on Sephacryl S-1000 (Pharmacia) as previously described (22). The fractions containing L1 protein, as determined by Western blot (immunoblot) analysis with antibody 5441-4 (16) (a gift of D. A. Galloway), were pooled and further purified by centrifugation for 1 h at 28,000 × *g* in an SW50.1 rotor. The pellet was suspended in PBS, and the presence of VLPs was confirmed by electron microscopy (Fig. 1).

HPV6 VLP ELISA. Purified VLPs (1 μg/50 μl per well in PBS) were added to wells of Immulon II microtiter plates (Dynatech Corp., Chantilly, Va.) and incubated at room temperature for 2 h. Following this incubation, plates were rinsed five times with PBS and then incubated for 2 h at room temperature with 200 μl of blocking agent (5% goat serum and 0.5% instant nonfat dry milk in PBS). Plates were again rinsed five times with PBS, and then 50 μl of diluted serum (diluted 1:25 and 1:50 in blocking buffer) was added to duplicate wells. To determine the nonspecific background for each serum sample, replicate aliquots were applied to plates prepared without antigen. All plates were covered and incubated at room temperature for 2 h. The plates were then rinsed five times with PBS, and 50 μl of a horseradish peroxidase-conjugated goat anti-human immunoglobulin (Ig; including reactivity to human IgA, IgG, and IgM and light chains [Tago Inc., Burlingame, Calif.]) was added to each well and incubated for 1 h at 37°C. The plates were rinsed 10 times with PBS, and 50 μl of peroxidase substrate ortho-phenylenediamine (OPD) buffer (0.1 M Na_2HPO_4, titrated to pH 5.0 with 0.1 M citric acid, 10 mg of OPD [Sigma Chemical Company, St. Louis, Mo.], and 1.25% hydrogen peroxide) was added and incubated for 5 min at room temperature. The reaction was terminated by the addition of 50 μl of 4 N H_2SO_4. The optical densities (OD) were measured in a Titertek Multiskan MCC/340 (ICN Biomedical, Huntsville, Ala.) microplate reader at 492 and 690 nm. Duplicate samples with a coefficient of variation greater than 20% were repeated. On all ELISA plates, two quality control samples were included.

To determine the conformational nature of the patient antibody epitopes, a replicate set of plates with VLPs denatured in 0.2 M Na_2CO_3 (pH 10.6)–10 mM dithiothreitol and dried at 37°C overnight were assayed (11, 15). ELISAs were performed as described above.

Statistical methods. Statistical analyses were performed with InStat for MacIntosh 2.0 (GraphPad Software, San Diego, Calif.). The significance of difference between means was calculated by using Mann-Whitney two-sample tests. Differences in proportions were evaluated by using Fisher's exact *t* test.

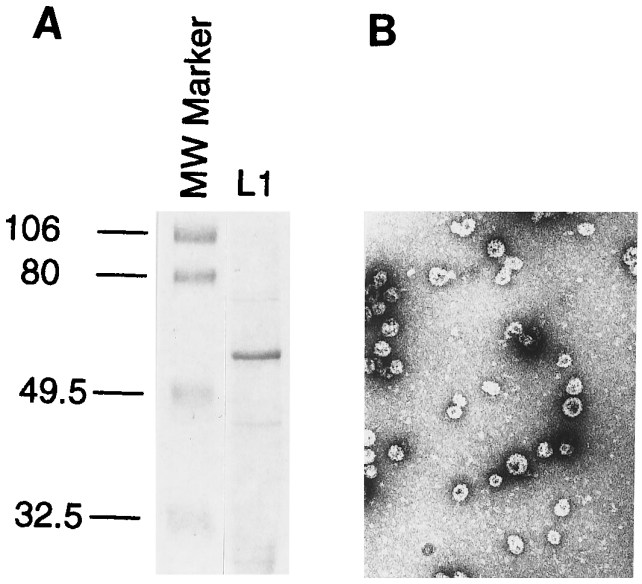

FIG. 1. (A) Sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis analysis of purified HPV6 L1 VLPs. Molecular weight (MW) standards are indicated (in thousands) on the left. (B) Electron micrograph of purified recombinant HPV6 L1 VLPs that were adsorbed onto Formvar-carbon coated grids, stained with 4% uranyl acetate, and photographed with a Zeiss EM10C microscope at a magnification of ×50,000.

RESULTS

HPV type distribution. A total of 39 patients with condylomas were enrolled over a 7-month period. Exfoliated cells from the wart and nonwart site(s) were collected separately to provide a measure of genital HPV infection. HPV typing results were obtained for 37 of the 39 patients (Table 1). Samples from two patients yielded insufficient quantities of DNA and were excluded from further analyses. The HPV types detected at either site included HPV6, -11, -16, -18, -26, -40, -42, -54, -56, -57, -58, and -59 and novel HPV PAP291(MM7). Overall, the types most frequently found were HPV6 (94%), -11 (8%), -54 (8%), and -58 (8%).

TABLE 1. HPV type distribution in patients with condylomas

Specimen site	*n*	No. (%) of HPV-positive patients			
		Total	HPV6	HPV11	Other types
Wart	37[a]	37 (100)	35 (94)	3 (8)	11 (30)
Nonwart	37[a]	19 (51)	11 (30)	1 (3)	6 (16)

[a] Two patients (male) were excluded from the analysis because of insufficient amounts of DNA.

Specimens from the condyloma site(s) in all 37 patients contained HPV DNA. The large majority (94% [95% confidence interval, 0.82 to 0.99]) of wart specimens harbored HPV6 DNA, while 8% (95% confidence interval, 0.02 to 0.22) contained HPV11 (Table 2). Both types were detected in one patient. Additionally, eight patients (all males) had more than one HPV type detected at the condyloma site.

HPV DNA was detected at the nonwart sites in about half of the patients (Table 2). Of these 19 patients, 16 had a concurrence of HPV type in the wart site and the nonwart site and 4 patients had different HPV types at the two sites. More than one HPV type was detected in specimens from 11 patients, 4 of whom were infected with four or more HPV types.

While all 12 female patients had detectable HPV at the wart site (vulva), only 4 (33%) of them had detectable HPV at the cervix. Two of these women had the same HPV type in the cervix as that detected in the wart, while two had HPV types at the cervix that were distinct from that in the wart.

Serology. Serologic responses to HPV6 VLPs were tested in an ELISA by incubation of duplicate, serial dilutions of patient serum samples with 1 μg of HPV6 VLPs. Antigen-bound antibodies were detected by a goat anti-human Ig–horseradish peroxidase conjugate. Replicate plates not coated with VLP antigen were also included to evaluate the nonspecific reactivities generated by each patient's serum.

For each specimen, the mean OD was determined for each set of duplicate serum dilutions (1:25 and 1:50). The ELISA value for a patient's serum was determined by subtracting the mean OD of the non-antigen-coated plate from the mean OD of the antigen-coated plate. Background values of serum specimens ranged from OD 0.03 to 0.2. The coefficient of variation for quality control samples was 12.5%.

The results from the 1:25 dilution are shown in Fig. 2. The ELISA values from the HPV-negative reference group were used to determine the OD cutoff for seropositivity. This negative control group was composed of serum samples from women who were consistently negative for cervical and vulvar HPV DNA during weekly testing for 12 weeks. The cutoff value was defined as the control group mean of the OD at 492 nm plus 2.5 times the standard deviation, and was determined to be 0.57 (mean, 0.298; standard deviation, 0.110). By this criterion, none of the 21 women in the negative control group were seropositive.

The ELISA results indicated that all 12 of the female patients with warts were seropositive for HPV6 VLPs, while only 2 of 9 (22%) asymptomatic, HPV6 DNA-positive women were seropositive ($P = <0.0001$). The female patients with warts were also compared with two additional reference groups of women, one asymptomatic and the other with CIN 1 to 3, in whose genital swabs only HPV16 was detected. Only 1 of the 11 (9%) HPV16 DNA-positive, cytologically normal women was seropositive ($P = <0.00001$). In contrast, 6 of 15 (40%) patients with CIN 1 to 3 were seropositive ($P = 0.0027$).

Since the results from the female patients with condylomas suggested that the serologic response to HPV6 VLPs was strongly associated with current genital warts, we investigated the response in the male patients with condylomas. While all of the female patients with vulvar condyloma were seropositive, only 16% (4 of 25) of the male patients were seropositive ($P = <0.00001$). This apparent difference was also found to be significant by comparing the OD of the serum samples from the male patients with those from the female patients. The mean OD obtained with samples from female patients was 0.837 (95% confidence interval, 0.709 to 0.964) versus 0.367 (95% confidence interval, 0.274 to 0.461) for the male patients.

TABLE 2. HPV type distribution at wart and nonwart sites in patients with condylomas

HPV type(s)[a] at site(s) wart/nonwart	No. of HPV-positive genital specimens for:	
	Female patients	Male patients
6/none	8	6
6/6	2	8
6/other	1	1
11/other	1	
6/6 + other		1
6 + other/none		3
6 + other/6		2
6 + other/other		2
6 + 11/none		1
11/11		1
Total	12	25

[a] Other HPV types include 16, 18, 26, 40, 42, 54, 56, 57, 58, and 59 and PAP291(MM7).

We then defined variables that differed significantly between male and female patients with condylomas and that might explain the apparent gender difference in humoral response. Self-reported variables tested included age, the numbers of sexual partners in the last 6 months and lifetime, age at first intercourse, and previous history of warts.

The ages of the female (mean, 26.75 years) and male (mean, 29.08 years) patients with condylomas did not differ significantly ($P = 0.340$). No significant difference was found between the male and female groups when the number of sexual partners in the last 6 months ($P = 0.559$) was considered.

There was a significant difference in the lifetime numbers of sexual partners ($P = 0.0023$). The females reported an average of 8 partners (range, 4 to 15), while the male patients reported an average of 17 partners (range, 6 to 99).

The reported age at first intercourse for female patients ranged from 16 to 19 years, with an average of 17.4 years. For male patients, the reported age at first intercourse ranged from 9 to 19 years, with an average age of 15.8 years. This difference was significant ($P = <0.0482$).

Only 2 (16.6%) of the female patients reported previous genital warts, compared with 11 (46%) of the male patients. Although suggestive of a difference between gender groups, the difference was not significant ($P = 0.18$).

Analyses to explore whether the age at first intercourse, the lifetime number of partners, or a history of genital warts could explain the gender seroprevalence difference were limited by both the sample size and the extremes in seroprevalence (i.e., the seroprevalence in female patients was 100%). However, neither of the sexual behavior variables of the seropositive male patients differed from those of the seronegative male patients, suggesting that these factors did not explain the gender difference. No history of genital warts appeared to be related to seropositivity in the male patients, although not

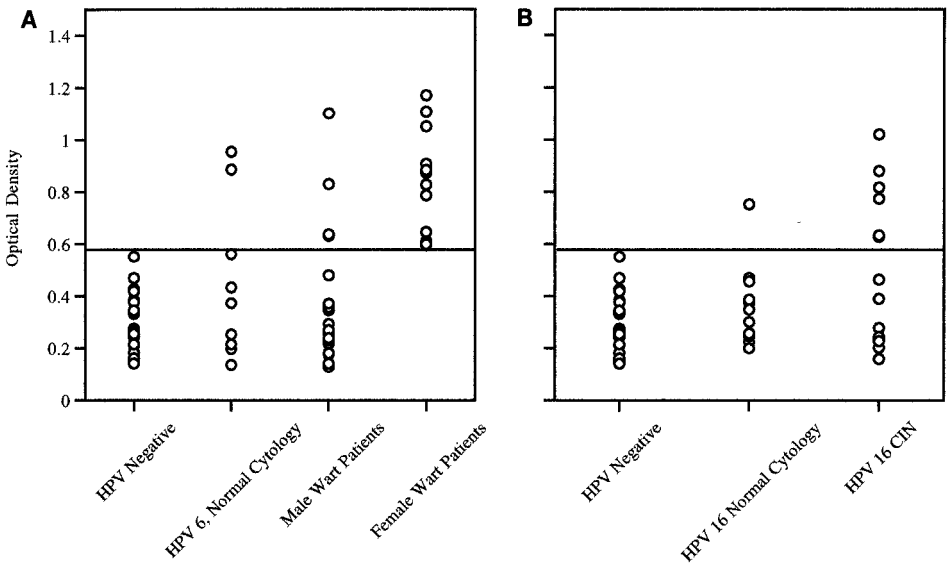

FIG. 2. HPV6 VLP ELISA serology results for reference groups and patients with warts. The OD cutoff for seropositivity was 0.57. Each circle represents one subject. (A) Comparison of HPV-negative female controls, HPV6 DNA-positive and cytologically normal women, and male and female patients with warts. (B) Comparison of HPV-negative female controls, HPV16 DNA-positive and cytologically normal women, and women with HPV16-associated CIN.

significantly ($P = 0.143$). None of the four male patients who were seropositive reported a history of genital warts, while 9 of 20 (45%) seronegative male subjects reported no history.

Results from the 1:50 serum dilution (data not shown) indicated that while they were similar to those found with the 1:25 serum dilution, there was a decrease in the percent seropositive. While the percentage of seropositive female patients with warts dropped to 75%, this was still significantly higher than the percentage of male patients (12% seropositive; $P = 0.00028$), the HPV6 DNA-positive, cytologically normal women (22% seropositivity; $P = 0.03$), the HPV16 DNA-positive, cytologically normal women (9% seropositivity; $P = 0.0028$), and the women with HPV16-associated CIN (33% seropositivity; $P = 0.054$).

Finally, in an effort to partially characterize the nature of the protein determinants recognized by the detected antibodies, the VLPs were denatured at a high pH and in the presence of a reducing agent and used as coating antigens in an ELISA as previously detailed. As analyzed by the same methods and criteria used with the nondenatured capsids, serum samples from only two patients with warts and two reference women (one with HPV6 and one with HPV16, both of whom were cytologically normal) reacted with the denatured VLPs. This result suggests that the majority of antibody responses detected with the HPV6 VLPs were directed against conformational determinants.

DISCUSSION

The purpose of this study of patients with condylomas was to determine the genital HPV type distribution and to characterize the humoral response to HPV6 VLPs. We found an overwhelming majority (94%) of patients with condylomata acuminata had HPV6 DNA at the wart site(s). HPV11 was detected in 8% of patients with condylomas. Limited comparable data are available, since only a few researchers have reported the prevalence of HPV6 and HPV11 independently. In one such recent study (26) HPV6 DNA was detected in 62% of external genital warts and HPV11 was detected in 12%. In another recent study of 26 women (44), HPV typing of exophytic condylomas of the cervix, vulva, and vagina found that 70% contained HPV6 DNA, while 30% contained HPV11 DNA. Interestingly, a similar ratio (90% HPV6 and 10% HPV11) was found in a cross-sectional HPV prevalence study of female students at the nearby University of California—Berkeley (3, 19).

In addition to HPV6 and -11, we detected other genital HPVs in approximately half of the patients with condylomas. The most prevalent types, following HPV6 and -11, were HPV54 and -58 (8% each). Although we were unable to test for additional HPV types, such as HPV43 and -44, these are believed to be very rare and do not represent more than 3% of condyloma-associated HPV infections (27a). While the distribution of HPV types in condylomata acuminata may vary between different populations, it appears that HPV6 predominates significantly in some groups. The biological basis for differences in the distributions of such extremely similar viruses as HPV6 and -11 (92% amino acid similarity in L1 and 82% similarity in L2) is not clear. Additional phylogenetic and epidemiological studies of HPV variants and subtypes may help to elucidate the basis for HPV6 predominance.

Of the women whose wart sites contained HPV6 DNA, only 18% had HPV6 DNA detected at the cervix and only 33% had any cervical HPV DNA detected. This finding is in contrast to data from the University of California—Berkeley study (3, 19). Of the 16 patients undergoing routine gynecologic examination who had HPV6 or -11 DNA detected in that study, the majority (65%) were positive at both the cervix and vulva, while only 35% had HPV6 or -11 DNA detected only at the vulva. It remains to be determined whether this difference in anatomical site infection can be explained by the fact that female patients with condylomas are usually referred to a dermatologist in the absence of accompanying cervical abnormalities or whether it is due to a conveyance of cervical HPV immunity arising in female patients with an external genital condyloma(s). It would be beneficial to carefully investigate the possible conveyance of cervical immunity for an HPV type found in a vulvar condyloma.

In addition to determining the HPV type prevalence in a cohort of patients with condylomas, we developed an ELISA for HPV6 VLPs that was used to define seropositivity and subsequently determined seroprevalence in this population. Our results suggested that in women there is a direct relationship between the level of HPV-associated disease and seropositivity. We found that the seroprevalence among women with normal cytology and detectable HPV6 DNA was 22%, compared with 100% seroprevalence in women with HPV6-associated warts. Similarly, HPV6 VLP seropositivity increased from 9% among women who had cytologically normal Pap smears and were positive for HPV16 DNA to 40% among women who had HPV16-associated CIN. While it is not known whether these data represent cross-reactivity of HPV antibodies against different HPV types, it may be that the women with HPV16-associated CIN had previous or currently undetected HPV6 infections. This possibility is supported by the data of Schiffman et al., who determined that women with multiple HPV types were more likely to have CIN than women with single HPV types (38). In addition, fewer probes were used in typing samples from the HPV-positive reference groups than those from the cohort with warts.

Using VLP and virion-based serologic assays, our data and those of others (6–8, 10–12) suggest that a high-titer humoral response is not generated by the HPV infections tested. In contrast, other sexually transmitted viral infections (such as infections of herpes simplex virus [43], human immunodeficiency virus, and hepatitis B virus) generate significantly greater antibody levels, and therefore seroconversion is a clearly defined marker of infection. Additionally, relatively high antibody titers are detectable for long periods (years) in these infections. Because antibody levels are low in HPV infections, the need to develop and define sensitive and specific serologic assays is of paramount importance for the accurate definition of seropositivity.

While developing our ELISA, we identified the importance of factors such as serum concentration that can affect the sensitivity of the assay. For example, while we have reported the results for serum dilutions of 1:25 and 1:50, we conducted pilot tests with other serum dilutions, such as 1:4 and 1:100. Preliminary experiments indicated that at a 1:4 dilution, there was a significant nonspecific background that we considered unacceptable. At higher-level dilutions (1:50 and 1:100) our seroprevalence among the female patients with warts decreased. This effect of dilution may account in part for the higher seroprevalence in female patients with warts in our study than was observed in previous studies that used higher-level dilutions of sera from patients with warts (6–8, 12, 37). It may be important to consider true serological titers rather than ELISA values for a single serum dilution, particularly in longitudinal natural history studies of sexual debut, and to correlate these findings with HPV status.

A factor not evaluated was the possibility of the detection of antibodies that were generated by other HPV types and that cross-react with HPV6 VLPs. Specifically, in this study we found an increase in seroprevalence in women who had (only) HPV16-associated CIN. Whether this seropositivity reflects previous HPV exposure, current infection levels lower that the sensitivity of the assay, or cross-reactivity of the HPV16 antibodies with HPV6 L1 was not determined. However, data from previous studies using different HPV L1s presented in VLP conformation (10, 17, 36) suggest that little cross-reactivity between distantly related HPV types exists. Because of the small number of patients with HPV11-associated warts, we were unable to critically address the cross-reactivity of a seroresponse to HPV11 infection with HPV6 VLPs. However, the sole female HPV11 patient with a condyloma was seropositive and the two male patients were not.

Perhaps the most important component of this study was the selection of the negative control sera. Our controls were considered to be HPV negative on the basis of repeated PCR testing of multiple genital sites. Clearly, this does not guarantee against misclassification of HPV status, either by previous infection, by infection at anatomical sites not tested, or by levels of infection that were below the limit of the assay, but this was the most sensitive method available. Studies that do not use genital HPV status as a classification criterion may be including data for seropositive individuals that would artificially raise the limits for seronegativity and thereby reduce the seroprevalence in the population under examination.

Our results suggest that gender plays a major role in the level of humoral response. We found a significant difference between the seroprevalence rates of male and female patients with condylomas but were unable to identify the determinants of this difference. A similar difference between male and female humoral levels was recently noted by Tachezy et al. (45) in an HPV6 and HPV11 L2 peptide serology study of laryngeal papillomas. The authors noted that the average ELISA OD from female participant samples were approximately twofold higher than that of the male participants.

Our results suggest that seropositivity was associated with a lack of reported history of genital warts. This may have affected the gender difference, since a majority of males and a minority of females reported a history of warts. Whether the lack of antibody response during an initial episode is related to recurrence of genital warts will need to be addressed in further studies.

While the data indicating gender differences in HPV seroresponsiveness are preliminary, there are other well-documented examples of gender differences in immunologic response (for reviews, see references 1, 20, and 39). In general, females mount a greater natural humoral response than males to microorganisms such as *Escherichia coli* (30) and the causative agents of measles (33), rubella (41), brucellosis (35), and hepatitis B (27) and produce higherlevels of Ig after immunization (14). This increased level in immune response has been correlated with estrogen levels.

Conclusions from our data are limited by the availability of only female HPV-negative controls for comparison with male patients with condylomas and for the establishment of ELISA cutoff values. While the prediction that there is a lower seroprevalence among male patients may be correct, further studies are required to investigate the need for separate negative controls for males and females.

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