JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2001, p. 4344–4348 Vol. 39, No. 12
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.12.4344–4348.2001

Prevalence of Anti-Human Papillomavirus Type 16, 18, 31, and 58 Virus-Like Particles in Women in the General Population and in Prostitutes

ANTOINE TOUZÉ,[1] SILVIA DE SANJOSÉ,[2] PIERRE COURSAGET,[1]* M. ROSA ALMIRALL,[3] VIRGILIO PALACIO,[4]† CHRIS J. L. M. MEIJER,[5] JANET KORNEGAY,[6] AND F. XAVIER BOSCH[2]

Laboratoire de Virologie Moléculaire, INSERM EMI-U 00-10 Protéases et Vectorisation, Faculté de Pharmacie, 37200 Tours, France[1]; Servei d'Epidemiologia i Registre del Cancer, Institut Catala d'Oncologia, Barcelona,[2] Centre d'Atenció a la Dona, Cornella,[3] and Unidad de Dermatologia, Hospital Monte Naranco, Oviedo,[4] Spain; Department of Pathology, University Hospital Vrije Universiteit, Amsterdam, The Netherlands[5]; and Roche Molecular Systems, Alameda, California[6]

Received 23 April 2001/Returned for modification 1 July 2001/Accepted 8 October 2001

Genital human papillomavirus (HPV) infection is sexually transmitted. The aim of the study was to characterize serological responses to HPV types 16, 18, 31, and 58 by exploring type-specific virus-like particles (VLPs) in two groups of women with very distinct sexual behaviors. Anti-VLP antibodies for types 16, 18, 31, and 58 and HPV DNA in cervical cells were investigated with 177 prostitutes and 283 age-matched controls from the female general population in Spain. Anti-VLP positivity increased with number of lifetime sexual partners in women from the general population, and no seroresponse was found in virgins. However, in prostitutes HPV infection was characterized by higher multireactivity to three or four VLPs (25%) than the general population (3%) and by a more frequent antibody response to HPV-58 than in the general population. About 75% of the women seropositive for type 58 had been born in a Latin American country. Seroprevalence of HPV and cervical HPV DNA in prostitutes were 14 and 10 times higher than observed in women in the general population (prevalence odds ratio [POR] of HPV seropositivity, 14.04 [95%; CI = 8.4 to 23.6] and POR for HPV DNA, 10.4 [95% CI = 3.9 to 27.6). Our results indicate that prostitutes are at an increased risk of oncogenic HPV infections, and they confirm the validity of anti-VLPs as markers of present or past HPV infection, that the number of sexual partners is the major determinant in acquisition of oncogenic HPV, and that anti-VLPs could be used as a marker of repeated infection in prostitutes.

Genital human papillomavirus (HPV) infection is the most common viral sexually transmitted disease, and it has been estimated that at least 50% of sexually active adults have had a genital HPV infection (20). Cohort studies indicate that genital HPV infection with oncogenic types is mostly transient and that only a small proportion of those infected become carriers and then develop cervical intraepithelial neoplasia (14, 17–19).

More than 100 HPV genotypes have been fully cloned and sequenced (34), and the etiologic role of papillomavirus in cervical cancer has been recognized for a limited number of them (i.e., HPV-16, -18, -31, -33, -35, -45, -52, -58, and -59) (27). The most common HPV types associated with cervical cancers worldwide are HPV-16 followed by HPV-18. Other types have an uneven geographical distribution. For example, HPV-33, -39, -58, and -59 are more common in Latin America than in other regions (5, 16).

Numerous serologic studies mainly using HPV-16 virus-like particles (VLPs) have demonstrated that infection with genital HPV is followed by a serologic immune response to viral capsid proteins. However, the titer of detectable serum antibodies to HPV VLPs is low. This immune response is largely HPV type-specific and directed against conformational epitopes (8, 9, 9a, 32, 39, 40). Moreover, not all HPV-infected subjects have detectable levels of antibodies, since 20 to 50% of women with HPV DNA do not have detectable type-specific anti-HPV antibodies (6, 22, 26). This may be due to the decline in antibody titers over time in infected individuals (2, 7). Follow-up studies have demonstrated that seroconversion most frequently occurs between 6 and 18 months after DNA detection (6, 7, 10, 11, 14). Anti-VLP antibodies are rarely observed in patients with transient HPV DNA (6) but are associated with persistence of HPV DNA detection. Anti-VLP antibodies persist for many years (1, 33) and may be an indicator of past as well as current infection.

Acquisition of HPV infection is strongly related to sexual behavior. HPV prevalence increases with number of sexual partners and with earlier age at first sexual intercourse (3, 13, 23, 24, 25, 32, 38). Women working as prostitutes are consequently at high risk of HPV infection. The aim of the study was to characterize the serological response to HPV type 16, 18, 31, and 58 VLPs in two groups of women with very distinct patterns of sexual behavior.

* Corresponding author. Mailing address: Laboratoire de Virologie Moléculaire, Faculté des Sciences Pharmaceutiques, 31 Ave. Monge, 37200 Tours, France. Phone: 33 2 47 36 72 56. Fax: 33 2 47 36 71 88. E-mail: coursaget@univ-tours.fr.
† Deceased.

MATERIALS AND METHODS

Study subjects. The subjects were recruited in Oviedo and Barcelona, Spain, and included 177 practicing prostitutes and 283 women randomly selected from the general population. Prostitutes were invited to participate during their regular visits to a specialized sexually transmitted disease clinic. Women from the general population were extracted from a larger follow-up study that included a random sample of the general population stratified in 11 age groups. Women were invited to participate via a personal letter. Of the women invited, 50% agreed to participate (n = 1,127), and 283 of these women matched by age to the group of prostitutes were selected for this study. All participants had a gynecological examination that included collection of cervical cells by means of an Ayre's spatula and a cervix brush to scrape the endocervix. Cervical scrapes were used to prepare a Pap smear, and the remaining cells were immediately sent to the laboratory to be processed for HPV DNA detection. Reading of the Pap smears and HPV detection were carried out without knowledge of the other test results. Specially trained nurses interviewed all women. Structured questionnaires explored sexual and reproductive histories and use of intravenous drugs in detail. All participants gave written informed consent, and the institution's Ethics Committees cleared all protocols.

HPV VLPs and detection of anti-VLP antibodies. Anti-VLP antibodies were measured by enzyme-linked immunosorbent assay using recombinant VLPs. HPV VLPs were produced in Sf21 insect cells using recombinant baculoviruses encoding the L1 gene of HPV-16, -18, -31, and -58. VLPs were purified by CsCl density gradient centrifugation, as described previously (25, 36). VLPs were further pelleted by ultracentrifugation and resuspended in phosphate-buffered saline (PBS) (pH 7.4).

Microtiter plates (Maxisorp; Nunc) were coated with either 100 ng of each purified HPV VLP per well (test wells) or newborn bovine serum (NBS) (control well). The plates were incubated at 4°C overnight. After four washes with PBS–0.1% Tween 20, nonspecific binding sites were blocked by incubation for 30 min at 37°C with PBS–1% NBS. The blocking solution was replaced by 100 μl of human serum diluted 1/20 in 5× PBS containing 10% NBS and 2% Tween 20. Following incubation at 45°C for 90 min and four washes, bound antibodies were detected with mouse anti-human immunoglobulin G (IgG) antibodies covalently linked to horseradish peroxidase, and 100 μl of a substrate solution containing *o*-phenylene diamine and H_2O_2 was added after incubation at 45°C for 90 min and four washes. The reaction was stopped after 30 min by addition of 100 μl of 4 N H_2SO_4, and optical densities (OD) (492 nm) were read with an automated plate reader. The OD of the control well was subtracted from the OD of test wells. The cutoff values were set at 0.2.

Detection of HPV DNA. HPV testing in women from the general population was performed by PCR enzyme immunoassay (EIA) using GP5+/bio 6+ primer-mediated PCR, as described earlier (12, 21). Fifteen high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73) and six low-risk HPV types (6, 11, 40, 42, 43, and 44) could be demonstrated in one test at the subpicogram level. Moreover, amplification products were analyzed for the individual HPV types. GP5+/bio 6GP+ PCR products were also analyzed by conventional Southern blot hybridization with a radioactive labeled general probe (12). Positive PCR EIA samples were also positive after Southern blot analysis.

HPV DNA testing in prostitutes was performed using the PCR-reverse line hybridization (PCR-RLH) assay: PCR using biotinylated L1 consensus primers PGMYB09/PGMYB11 and biotinylated primers for beta-globin (PC04/GH20). The PCR mix included 5 μl of target DNA, dNTPs (dATP, dCTP, and dGTP, 10 mM; dUTP, 30 mM) and Ampli*Taq* Gold polymerase. The first cycle was 9 min at 95°C followed by 40 cycles of 1 min at 95°C, 1 min at 55°C, and 1 min at 72°C. The PCR product was subsequently denatured (0.13 N NaOH) and hybridized to a strip with 27 HPV probes and 2 β-globin probes at different concentrations. The high-risk probes included were 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, MM4, MM7, and MM9. Washing was performed under high-stringency conditions (53°C with agitation for 15 min). Detection was carried out with peroxidase-labeled streptavidin.

HPV-DNA tests using MY09/11 and GP5+/6+ primers identified nearly equivalent prevalence of HPV infection, but tests using GP5+/6+ primers have been shown to be more sensitive in the detection of specific types like HPV 35, 53, and 61 (31). However, the PGMY09/11-line blot assay has been shown to have higher sensitivity than MY09/11 line blot assay (15). Thus, it has been assumed that PGMY09/11-line blot and GP5+/6+ assays are of similar sensitivity in detecting HPV types.

Statistical analysis. The statistical differences in HPV prevalence between the two groups according to different characteristics was analyzed using the χ^2 test. The χ^2 test for linear trend was used when exploring the association with accumulative exposure for different characteristics. Logistic regression techniques were used to assess the relationship between HPV VLP seropositivity and demographic variables, behavior characteristics, and HPV DNA status. The association between anti-HPV VLPs and potential risk factors is presented by the prevalence odd ratio (POR) for anti-HPV VLPs and 95% confidence intervals.

TABLE 1. Prevalence odds ratios of anti-VLP antibodies and of cervical HPV DNA in women in the general population and in prostitutes[a]

Antibody or DNA	General population		Prostitutes		Prevalence odds ratio (95% CI)
	No. +ve (%)	% Among +ve	No. +ve (%)	% Among +ve	
Anti-VLPs:					
HPV-16	17 (6.0)	50.0	81 (45.8)	88.0	13.2 (7.4–23.4)
HPV-18	5 (1.8)	14.7	41 (23.2)	44.0	16.9 (6.5–43.7)
HPV-31	8 (2.8)	23.5	34 (19.2)	36.9	8.1 (3.7–18.2)
HPV-58	5 (1.8)	14.5	38 (21.5)	41.3	15.3 (5.9–39.8)
Any HPV	29 (10.2)	100	109 (61.6)	100	14.0 (8.4–23.6)
HPV DNA[b]	5 (1.8)		28 (15.8)		10.4 (3.9–27.6)

[a] Reference group: HPV DNA-negative women in the general population. +ve, positive.
[b] Considered as positive only for HPV-16, -18, -31, and 58.

RESULTS

The mean age of the women enrolled in this study was 30.3 years (range, 19 to 49). Women from the general population were largely of Spanish nationality and monogamous (61%). Prostitutes were largely immigrants (76%), mostly from South America, and three women were from Africa, four from Europe, and one from Singapore.

The distribution of HPV DNA and anti-VLP antibodies both for women in the general population and for prostitutes is summarized in Table 1. To allow comparison between HPV seroresponse and HPV DNA, HPV DNA prevalence is calculated only based on the presence of HPV-16, -18, -31, and -58 DNA.

Prevalence rates and POR were significantly higher for HPV-16, -18, -31, and -58 VLPs and for HPV DNA among prostitutes than among women in the general population. Prostitutes were at 14 times greater risk of having antibodies to one or more of the HPV VLPs investigated than were women in the general population. Anti-VLP antibodies were mainly directed against HPV-16 VLPs in both study groups.

Women in the general population were five times more likely to have a seroresponse to HPV than to have detectable HPV DNA in cervical cells. In prostitutes, the ratio of the prevalence rate of both markers was 3.3. The overall prevalence of anti-HPV antibodies was analyzed in both groups according to age, country of origin, educational level, number of pregnancies, age at first sexual intercourse, number of lifetime sexual partners, and alcohol consumption (Table 2). Only number of lifetime sexual partners was related to the prevalence of anti-VLPs with statistical significance (P = 0.04). In both groups women with higher educational levels had a higher prevalence of anti-VLPs. Anti-VLPs were more prevalent among women from the general population who began sexual activity before the age of 18. Anti-VLPs were not detected in women reporting no sexual intercourse (n = 13). Anti-VLP antibodies were present in 8% of women reporting one sexual partner and in 18% of those with two sexual partners. In agreement with the high number of sexual partners, anti-VLPs were detected in 62% of the prostitutes.

TABLE 2. Distribution of anti-VLP antibodies by different characteristics of women in the general population and in prostitutes[a]

Characteristic[b]	General population		Prostitutes	
	No. anti-VLP +ve[c]	(%)	No. anti-VLP +ve	(%)
Age				
<25	9/79	11.3	27/42	64.3
25–29	3/35	8.6	31/54	57.4
30–34	10/65	15.4	22/33	66.7
>34	7/104	6.7	29/48	60.4
P value		0.31		0.82
Country of origin				
Europe	28/277	10.1	30/49	61.2
Latin America	1/4	25	74/121	61.1
Africa/Asia	0/1	0	5/5	83
P value		0.6		0.66
Education				
Up to primary	9/125	7.2	75/121	62.0
Secondary	14/112	12.5	26/46	56.5
Higher education	6/46	13.0	8/9	88.9
P value test for trend		0.32		0.5
Pregnancies				
0–1	18/165	10.9	43/71	60.6
>1	11/118	9.3	66/106	62.3
P value		0.66		0.8
Age at first sexual intercourse (*)				
≤18	17/130	13.1	89/147	60.6
>18	12/140	8.6	20/30	66.7
P value		0.23		0.52
No. of lifetime sexual partners (*)				
1	14/176	7.9	0	0
2	7/51	13.7	0	0
3	2/11	18.2	0	0
4 or more	6/32	18.8	109/177	61.6
P value test for trend		0.04		n.a.
Alcohol				
No	15/150	10	22/36	61.1
Yes	14/133	10.5	87/141	61.7
P value		0.88		0.9

[a] Anti-VLP positivity represents cumulative results from individual VLP assays for each genotype. +ve, positive.
[b] *P* values for hetereogeneity test or for linear trend when indicated (*) excluding 13 virgins.
[c] No. positive/no. tested.

Study of the concordance between the detection of type-specific HPV DNA in cervical cells and the serologic response to VLPs indicates that the agreement was high for the negative samples (range, 66.9 to 90.4) but very low for the detection of positive samples (range, 0 to 4.13). Overall kappa values were very low, which indicates poor concordance between the two tests (data not shown).

TABLE 3. Prevalence of single and multiple anti-VLP-antibodies in women from the general population and in prostitutes[a]

Seropositivity to no. of HPV anti-VLPs	General population [no. (%)]	Prostitutes [no. (%)]
1	25 (86.2)	59 (54.1)
2	3 (10.3)	23 (21.1)
≥3	1 (3.4)	27 (24.8)
Total	29	109

[a] Test for linear trend, *P* value = 0.002

Seropositivity for the different HPV VLPs was studied in both groups. As shown in Table 3, prostitutes were statistically more likely to have antibodies to multiple HPV types, since a quarter of them had antibodies to three or four HPV types, compared to only 3.4% of women in the general population.

In this study, prostitutes were mainly immigrants, and we therefore explored anti-HPV seropositivity according to country of birth (Table 4). The most prevalent anti-HPV antibody in European women was anti-HPV-16 (65.5%), followed by anti-HPV-18 and -31 (24.1 and 29.3%, respectively) and finally anti-HPV-58 (19%). In women from Latin America, the most prevalent antibody was anti-HPV-16 (76%) followed by anti-HPV-58 (42,7%). Anti-HPV-16 and -18 (60%) were the types mainly found in African and Asian women, and none of them was anti-HPV-58 positive.

DISCUSSION

The very high rate of antibodies to four oncogenic HPV VLPs in 61.6% of prostitutes compared to 10.4% of women from the general population suggests that high lifetime cumulative exposure to HPV is related to the number of lifetime sexual partners. Accordingly, we did not observe anti-VLP antibodies in women reporting no sexual intercourse. These results are corroborated by the fact that virgins starting sexual activity seroconvert after the acquisition of HPV DNA and that cervical lesions had been described only in HPV-16 DNA-positive women who had seroconverted (27). In our study, only one woman of the prostitute group was HIV infected, and she had a seroresponse to HPV-18. Interestingly, HIV infection has been reported to be followed by an increased prevalence of anti-HPV VLP, probably due to increased expression of capsid proteins due to cellular immunodeficiency (30).

In contrast to other studies (35, 38), we did not observe an increase in HPV seropositivity with age. The association with young age at first sexual intercourse in the general population did not reach statistical significance. No association was de-

TABLE 4. Distribution of anti-VLP antibodies to type-specific HPVs according to country of birth

Country	No. (%) positive for anti-VLP HPV type:				
	16	18	31	58	Any[a]
Europe	38 (65.5)	14 (24.1)	17 (29.3)	11 (19.0)	58 (100)
Latin America	57 (76.0)	29 (38.7)	24 (32.0)	32 (42.7)	75 (100)
Africa/Asia	3 (60.0)	3 (60.0)	1 (20.0)	0 (0.0)	5 (100)
Total	98 (71.0)	46 (33.3)	42 (30.4)	43 (31.2)	138 (100)

[a] Women with multiple infections were counted once.

tected among prostitutes. This could be due to the limited age range of the women explored and to the very high rate of HPV infection in prostitutes in all age groups.

Twenty-five percent of the prostitutes had antibodies to three or four HPV genotypes, compared to only 3% of women in the general population. The low prevalence rate observed in the latter is consistent with the HPV DNA detection and anti-VLP 33 detection reported in previous studies (28) and the low incidence rates of cervical cancer in Spain (29). The different patterns observed between the two groups suggests that multiple reactivity is more likely to be a marker of lifetime cumulative exposure to HPV than cross-reactivity between genotypes. The only women from the general population with seroreactivity to three or four VLPs reported five sexual partners and age at first sexual relationship of 16. The high rate of seroreactivity to multiple HPV types observed in prostitutes is believed to be the consequence of multiple, sequential or concomitant infections with different HPVs.

Prevalences of overall DNA and antibodies were very distinct in the two groups. HPV DNA (types 16, 18, 31, and 58) was found in 1.8 and 15.8% of the general population and prostitutes, respectively, whereas anti-VLP antibodies were detected in 10 and 62%, respectively. This emphasizes the need to investigate anti-HPV antibodies in addition to HPV DNA to evaluate the overall HPV infection in a population more effectively.

Women in the general population group were more likely to have a serological response to HPV-16 and HPV-31, whereas the predominant serological types in prostitutes were HPV-16, followed by HPV-18 and HPV-58. The majority of prostitutes in Spain were born overseas, particularly in Latin American countries. The higher seroprevalence of anti-HPV-58 VLPs observed among prostitutes could be due to a higher level of exposure to HPV-58 in the country of origin compared to Spain. The presence of many prostitutes from Latin America, where HPV-58 is recognized as the second-most-frequent type (4; M. Molano, A. J. C. Van den Brule, H. Posso, C. J. L. M. Meijer, M. Ronderos, O. Orozco, et al., 18th Int. Papillomavirus Conf. Program Abstr., p. 181, 2000), could be responsible for dissemination in the Spanish population of an HPV genotype which is rarely detected in the general population. HPV DNA typing in the general population in Spain suggests that unless future HPV vaccines induce some cross-protection, three genotypes, 16, 18, and 31, should be included in a vaccine to prevent the majority of cases of cervical neoplasia in this population. In a situation comparable to the one observed in Spain, the dissemination of an infrequently detected genotype introduced by prostitutes from foreign countries could be expected. It is thus essential either to produce a multivalent vaccine directed against the main oncogenic genotypes observed worldwide or ideally to develop a vaccine which would induce cross-protection.

In conclusion, our study strongly supports the importance of sexual intercourse in the transmission of oncogenic HPV infection and that anti-VLPs could be used as a marker of repeated infection in prostitutes. The data obtained confirms the validity of anti-VLPs as a marker of present or past HPV infection.

ACKNOWLEDGMENTS

Financial support was provided by the Spanish Fondo de Investigación Sanitaria (FIS 98-0646), French Association pour la Recherche sur le Cancer (grant no. 9970), and Ligue Contre le Cancer (Comité d'Indre et Loire).

We thank Rebeca Font and Judit Sola for data handling and analysis.

REFERENCES

1. **Af Geijersstam, V., M. Kibur, Z. Wang, P. Koskela, E. Pukkala, J. Schiller, M. Lehtinen, and J. Dillner.** 1998. Stability over time of serum antibody levels to human papillomavirus type 16. J. Infect. Dis. **177:**1710–1714.
2. **Andersson-Ellstrom, A., J. Dillner, B. Hagmar, J. Schiller, M. Sapp, L. Forssman, and I. Milsom.** 1996. Comparison of development of serum antibodies to HPV16 and HPV33 and acquisition of cervical HPV DNA among sexually experienced and virginal young girls. A longitudinal cohort study. Sex. Transm. Dis. **23:**234–238.
3. **Andersson-Ellstrom, A., J. Dillner, B. Hagmar, J. Schiller, and L. Forssman.** 1994. No serological evidence for non-sexual spread of HPV 16. Lancet **345:**197–198.
4. **Bosch, F. X., N. Muñoz, S. de Sanjosé, C. Navarro, P. Moreo, N. Ascunce, L. C. Gonzalez, L. Tafur, M. Gili, I. Larranaga, et al.** 1993. Human papillomavirus and cervical intraepithelial neoplasia grade III/carcinoma in situ: a case-control study in Spain and Colombia. Cancer Epidemiol. Biomark. Prev. **2:**415–422.
5. **Bosch, F. X., M. Manos, N. Muñoz, M. Sherman, A. Jansen, J. Peto, M. Schiffman, V. Moreno, K. V. Shah, and the IBSCC study group.** 1995. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J. Natl. Cancer Inst. **87:**796–802.
6. **Carter, J. L., L. A. Koutsky, G. C. Wipf, N. D. Christensen, S. Lee, J. Kuypers, N. Kiviat, and D. A. Galloway.** 1996. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. J. Infect. Dis. **174:**927–936.
7. **Carter, J. J., L. A. Koutsky, J. P. Hughes, S. K. Lee, J. Kuypers, N. Kiviat, and D. A. Galloway.** 2000. Comparison of human papillomavirus type 16, 18, and 6 capsid antibody responses following incident infection. J. Infect. Dis. **181:**1911–1919.
8. **Christensen, N. D., R. Kirnbauer, J. T. Schiller, S. J. Ghim, R. Schlegel, A. B. Jenson, and J. W. Kreider.** 1994. Human papillomavirus types 6 and 11 have antigenically distinct strongly immunogenic conformationally dependent neutralizing epitopes. Virology **205:**329–335.
9. **Christensen, N. D., J. Dillner, C. Eklund, J. J. Carter, G. C. Wipf, C. A. Reed, N. M. Cladel, and D. A. Galloway.** 1996. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. Virology **223:**174–184.

9a. **Combita, A. L., A. Touzé, L. Bousarghin, S. El Mehdaoui, P. Y. Sizaret, N. Muñoz, and P. Coursaget.** Gene transfer using human papillomavirus pseudovirions varies according to virus genotype and required cell surface heparan sulfate. FEMS Microbiol. Lett., in press.

10. **de Gruijl, T. D., H. J. Bontkes, J. M. M. Walboomers, J. T. Schiller, M. J. Stukart, B. S. Groot, M. M. R. Chabaud, A. J. Remmink, R. H. M. Verheijen, T. J. M. Helmerrhorst, C. J. L. M. Meijer, and R. J. Scheper.** 1997. Immunoglobulin G responses against human papillomavirus type 16 virus-like particles in a prospective nonintervention cohort study of women with cervical intraepitheliel neoplasia. J. Natl. Cancer. Inst. **89:**630–638.
11. **de Gruijl, T. D., H. J. Bontkes, J. M. M. Walboomers, P. Coursaget, M. J. Stukart, C. Dupuy, E. Kueter, R. H. M. Verheijen, T. J. M. Helmerrhorst, M. F. Duggankeen, P. L. Stern, C. J. L. M. Meijer, and R. J. Scheper.** 1999. 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.
12. **de Roda Husman, A. M., J. M. M. Walboomers, A. J. C. Van den Brule, C. J. L. M. Meijer, and P. J. F. Snijders.** 1995. The use of general primers GP5 and GP6 elongated at their 3′ ends with conserved sequences improves human papillomavirus detection by polymerase chain reaction. J. Gen. Virol. **76:**1057–1062.
13. **Dillner, J., I. Kallings, C. Brihmer, B. Sikstrom, P. Koskela, M. Lehtinen, J. T. Schiller, M. Sapp, and P. A. Mardh.** 1996. Seropositivities to human papillomavirus type 16, 18, or 33 capsids and to Chlamydia trachomatis are markers of sexual behavior. J. Infect. Dis. **173:**1394–1398.
14. **Franco, E. L., L. L. Villa, J. B. Sobrinho, J. M. Prado, C. Rousseau, M. Désy, and T. E. Rohan.** 1999. Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high risk area for cervical cancer. J. Infect. Dis. **180:**1415–1423.
15. **Gravitt, P. E., C. I. Peyton, T. Q. Alessi, C. M. Wheeler, F. Coutlée, A. Hildesheim, M. H. Schiffman, D. R. Scott, and R. J. Apple.** 2000. Improved amplification of genital human papillomavirus. J. Clin. Microbiol. **38:**357–361.
16. **Herrero, R., A. Hildesheim, C. Bratti, M. E. Sherman, M. Hutchinson, J.**

Morales, I. Balmaceda, M. D. Greenberg, M. Alfaro, R. D. Burk, S. Wacholder, M. Plummer, and M. Schiffman. 2000. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J. Natl. Cancer. Inst. **92:**464–474.

17. **Hildesheim, A., M. H. Schiffman, P. E. Gravitt, A. G. Glass, C. E. Greer, T. Zhang, D. R. Scott, B. B. Rush, P. Lawler, M. E. Sherman, et al.** 1994. Persistence of type specific human papillomavirus infection among cytologically normal women. J. Infect. Dis. **169:**235–240.
18. **Hinchliffe, S. A., D. Van Velzen, H. Korporaal, P. L. Kok, and M. E. Boon.** 1995. Transience of cervical HPV infection in sexually active young women with normal cervicovaginal cytology. Br. J. Cancer **72:**943–945.
19. **Ho, G. Y. F., R. Bierman, L. Beardsley, C. J. Chang, and R. D. Burk.** 1998. Natural history of cervicovaginal papillomavirus infection in young women. N. Engl. J. Med. **338:**423–428.
20. **International Agency for Research on Cancer.** 1995. Monographs on the evaluation of the carcinogenic risks to humans, p. 1–378. Human papillomaviruses, vol. 64. IARC, Lyon, France.
21. **Jacobs, M. V., P. J. F. Snijders, A. J. C. Van den Brule, T. J. M. Helmerhorst, C. J. L. M. Meijer, and J. M. M. Walboomers.** 1997. A general primer GP5+/GP6+-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. J. Clin. Microbiol. **35:**791–795.
22. **Kirnbauer, R., N. L. Hubbert, C. M. Wheeler, T. M. Becker, D. R. Lowy, and J. T. Schiller.** 1994. A virus-like particle ELISA detects serum antibodies in a majority of women infected with human papillomavirus type 16. J. Natl. Cancer Inst. **86:**498–498.
23. **Kjaer, S., B. Chackerian, A. Van Den Brule, E. I. Svare, G. Paull, J. M. M. Walboomers, J. T. Schiller, J. E. Bock, M. E. Sherman, D. R. Lowy, and C. L. M. Meijer.** 2001. High-risk human papillomavirus is sexually transmitted: evidence from a follow up study of virgins starting sexual activity (intercourse). Cancer Epidemiol. Biomark. Prev. **10:**101–106.
24. **Kjellberg, L., Z. Wang, F. Wiklund, K. Edlund, T. Angstrom, P. Lenner, I. Sjoberg, G. Hallmans, K. L. Wallin, M. Sapp, J. Schiller, K. Wadell, C. G. Mahlck, and J. Dillner.** 1999. Sexual behaviour and papillomavirus exposure in cervical intraepithelial neoplasia: a population-based case-control study. J. Gen. Virol. **80:**391–398
25. **Le Cann, P., P. Coursaget, S. Iochmann, and A. Touzé.** 1994. Self-assembly of human papillomavirus type 16 capsids by expression of the L1 protein in insect cells. FEMS Microbiol. Lett. **117:**269–274.
26. **Le Cann, P., A. Touzé, N. Enogat, D. Leboulleux, C. Mougin, M. C. Legrand, C. Calvet, J. M. Afoutou, and P. Coursaget.** 1995. Detection of antibodies against the human papillomavirus (HPV) type 16 virions by ELISA using recombinant HPV 16 L1 capsids produced by recombinant baculovirus. J. Clin. Microbiol. **33:**1380–1382.
27. **Muñoz, N.** 2000. Human papillomavirus and cancer: the epidemiological evidence. J. Clin. Virol. **19:**1–5.
28. **Nonnenmacher, B., N. L. Hubbert, R. Kirnbauer, K. V. Shah, N. Munoz, F. X. Bosch, S. de Sanjose, R. Viscidi, D. R. Lowy, and J. T. Schiller.** 1995. Serologic response to human papillomavirus type 16 (HPV-16) virus-like particles in HPV-16 DNA-positive invasive cervical cancer and cervical intraepithelial neoplasia grade III patients and controls from Colombia and Spain. J. Infect. Dis. **172:**19–24.
29. **Parkin, D. M., P. Pisani, and J. Ferlay.** 1999. Estimates of the worldwide incidence of 25 major cancers in 1990. Int. J. Cancer **80:**827–841.
30. **Petter, A., K. Heim, M. Guger, A. Cisera-König, N. Christensen, M. Sarcletti, U. Wieland, H. Pfister, R. Zangerle, and R. Höpfl.** 2000. Specific serum IgG, IgM and IgA antibodies to human papillomavirus types 6, 11, 16, 18 and 31 virus-like particles in human immunodeficiency virus-seropositive women. J. Gen. Virol. **81:**701–708.
31. **Qu, W., G. Jiang, Y. Cruz, C. J. Chang, G. Y. F. Ho, R. S. Klien, and R. D. Burk.** 1997. PCR detection of human papillomavirus: comparison between MY09/MY11 and GP5+/GP6+ primers systems. J. Clin. Microbiol. **35:**1304–1310.
32. **Roden, R. B., H. L. Greenstone, R. Kirnbauer, F. P. Booy, J. Jessie, D. R. Lowy, and J. T. Schiller.** 1996. In vitro generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype. J. Virol. **70:**5875–5883.
33. **Shah, K. V., R. P. Viscidi, A. J. Alberg, K. J. Helzlsouer, and G. W. Comstock.** 1997. Antibodies to human papillomavirus 16 and subsequent in situ or invasive cancer of the cervix. Cancer Epidemiol. Biomark. Prev. **6:**233–237.
34. **Stoler, M. H.** 2000. Human papillomaviruses and cervical neoplasia: a model for carcinogenesis. Int. J. Gynecol. Pathol. **19:**16–28.
35. **Strickler, H. D., G. D. Kirk, J. P. Figueroa, E. Ward, A. R. Braithwaite, C. Escoffery, J. Drummond, B. Goebel, D. Waters, R. McClimens, and A. Manns.** 1999. HPV 16 antibody prevalence in Jamaica and the United States reflects differences in cervical cancer rates. Int. J. Cancer **80:**339–344.
36. **Touzé, A., S. El Mehdaoui, P. Y. Sizaret, C. Mougin, N. Muñoz, and P. Coursaget.** 1998. The L1 major capsid protein of human papillomavirus type 16 variants affects the yield of virus-like particle produced in an insect cells expression system. J. Clin. Microbiol. **36:**2046–2051.
37. **Van Doornum, G., M. Prins, A. Andersson-Ellstrom, and J. Dillner.** 1998. Immunoglobulin A, G, and M responses to L1 and L2 capsids of human papillomavirus types 6, 11, 16, 18, and 33 L1 after newly acquired infection. Sex. Transm. Infect. **74:**354–360.
38. **Viscidi, R. P., K. L. Kotloff, B. Clayman, K. Russ, S. Shapiro, and K. Shah.** 1997. Prevalence of antibodies to human papillomavirus (HPV) type 16 virus-like particles in relation to cervical HPV infection among college women. Clin. Diagn. Lab. Immunol. **4:**122–126.
39. **White, W. I., S. D. Wilson, W. Bonnez, R. C. Rose, S. Koenig, and J. A. Suzich.** 1998. In vitro infection and type-restricted antibody-mediated neutralization of authentic human papillomavirus type 16. J. Virol. **72:**959–964.
40. **White, W. I., S. D. Wilson, F. J. Palmer-Hill, R. M. Woods, S. J. Ghim, L. A. Hewitt, D. M. Goldman, S. J. Burke, A. B. Jenson, S. Koenig, and J. A. Suzich.** 1999. Characterization of a major neutralizing epitope on human papillomavirus type 16 L1. J. Virol. **73:**4882–4889.