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Specificity of L1 Peptides versus Virus-Like Particles for Detection of Human Papillomavirus-Positive Cervical Lesions in Females Attending Engativa Hospital, Bogota, Colombia[▽]

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A serological test for the detection of human papillomavirus (HPV) infection in females at risk of developing cervical cancer could be based on conserved L1 peptides with low levels of antigenicity specifically recognized by antibodies from patients with cervical lesions infected with high-risk HPV (HR-HPV) types. The aim was to assess the ability of L1 peptides 18283, 18294, and 18301 compared with the ability of virus-like particles (VLPs) to identify these infections in females. A total of 391 HPV-infected female volunteers were interviewed, and peripheral blood and cervical cells were obtained for detection of anti-HPV antibodies and HPV DNA; all of the patients had a Pap smear test; 287 patients were referred for colposcopy or biopsy, according to gynecological criteria. The level of agreement, as determined by the use of the Lin coefficient (rho value), showed that 75 to 83% of females with HR-HPV DNA-positive cervical lesions had antibodies that recognized VLPs and peptide 18283, 18294, or 18301, while 15 to 23% of the HPV DNA-negative females with a normal cytology had antibodies that recognized these three peptides and 45% had antibodies that recognized VLPs. The rate of agreement between peptides and VLPs for antibody detection was higher for patients with HPV DNA-positive cervical lesions. Peptides 18283, 18294, and 18301 showed similar sensitivities for the detection of HR-HPV DNA-positive cervical lesions and were more specific than VLPs. Peptide 18301 might be detecting protective antibodies in HPV DNA-negative females with atypical squamous cells of undetermined significance. These peptides could be useful for the design of a serology test for the detection of HR-HPV infection in females with cervical lesions and at risk of cervical cancer.

More than 100 human papillomavirus (HPV) types infect human tissues; 40 of these types of viruses (named high-risk HPV [HR-HPV] types) are able to induce carcinomas such as cervical cancer (3, 23). About 70% of females become infected with HPV within the 2 years following the start of sexual activity (22, 37), and 90% of these females have transient HPV infections (2, 13). Cervical lesions caused by persistent HPV infection are detected in about 5% of females; some of these can progress to cervical cancer. Seventy to 90% of HPV-infected females seroconvert 6 to 18 months after HPV DNA is detected, but this rarely occurs in females with transient HPV infection (1, 4, 25).

There is a relationship between a high viral load and the progression of cervical lesions and cervical cancer (13, 27). The anti-HPV antibody response is correlated with the viral load (11, 36). Most potentially oncogenic, persistent, long-term HPV infections elicit an antibody response which can be detected by using virus-like particles (VLPs) (5, 20). In fact, the anti-VLP antibody response is stable; is related to persistent infection, the viral load, and neoplastic lesion development; and is rarely found in patients with transient HPV infections (10, 16, 25, 29).

Seropositivity for VLPs seems to be a good indicator of the risk that a patient will have cervical cancer, despite the controversial results regarding its specificity; in fact, an optimized VLP-based enzyme-linked immunosorbent assay (ELISA) that has 93% sensitivity and 98.5% specificity for discriminating between positive and negative control sera has recently been reported (35).

Some exposed linear epitopes of the L1 protein that interact with antibodies (21) have been identified by using peptides; i.e., the ^{473}GLAKPKFTLGKKATPTTS^{491} peptide is specifically recognized by serum antibodies from 91% of HPV type 16 (HPV-16)-infected patients, 24% of children, and 66% of HPV-16-negative patients (8). The nonapeptide IHSMNSTIL discriminates between HR-HPV types and low-risk HPV types in HPV-infected females (29); peptides 18283 (^{55}PNNNKILV PKVSGLQYRVFR^{74}), 18294 (^{275}LYIKGSGSTANLASSNYF PT^{294}), and 18301 (^{414}EDTYRFVTSQAIACQKHTPPA^{434}) are specifically recognized by antibodies in serum from patients with cervical lesions and show 92 to 97% sensitivity and 89 to 95% specificity for the recognition of patients with precancerous lesions and cervical cancer (35). When the variability in amino acid sequences among the different HPV types is taken into account, it is probable that peptides 18283, 18294, and 18301 and VLPs of HPV-16 have different seroreactivities, sensitivities, and specificities. The aim of this study was to evaluate the agreement in anti-HPV antibody reactivities by pairing peptides 18294, 18283, and 18301 and these peptides with VLPs of HPV-16 and to determine their abilities to discriminate HR-HPV-infected females with cervical lesions from noninfected females presenting with normal cytologies among

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females in a population with a high frequency of HPV infection.

MATERIALS AND METHODS

Peptide synthesis. Twenty-mer peptides corresponding to the L1 peptide of HPV-16 (32) were synthesized with a solid-phase multiple peptide system (14). 4-Methylbenzylhydrylamine resin (0.7 meq/g), *tert*-butoxycarbonyl-protected amino acids, and low-high cleavage techniques were used. The peptides were analyzed by matrix-assisted laser desorption ionization–time of flight mass spectrometry and reverse-phase high-performance liquid chromatography.

HPV-16 VLP production. VLPs of HPV-16 were produced in Sf21 cells by a previously described procedure (34). Recombinant baculoviruses encoding the HPV-16 L1 protein were used to infect Sf21 cells at a multiplicity of infection of 20. The cells were harvested 4 days postinfection; cytoplasmic and nuclear fractions were separated by 0.5% Nonidet P-40 treatment, followed by centrifugation (10,000 × *g*, 15 min). CsCl gradient fractions were collected, and the densities were determined by refractometry. Fractions with a density of about 1,272 g/cm^3 were pooled in 1× phosphate-buffered saline (PBS) and ultracentrifuged (4°C, 1 h, 130,400 × *g*). The assembly of VLPs was verified by electron microscopy.

Population. This study, approved by the institutional review boards and the scientific and ethical committees of the Engativa Hospital and the Fundacion Instituto de Inmunologia de Colombia, was conducted according to the guidelines of the Declaration of Helsinki. A total of 391 females who were from urban areas and who were attending Engativa Hospital in Bogota, Colombia, between 2005 and 2006 voluntarily agreed to participate; signed an informed-consent form; and answered a questionnaire that inquired about their socioeconomic status, gynecological and obstetric histories, current and past sexual behavior, and genital tract infections. These females were between the ages of 15 and 68 years (mean age, 33.5 ± 12 years); most of them were housewives from families with incomes of between $200 and $400 per month. The median number of lifetime male sexual partners was 1.96, and most females reported that they had had two sexual partners or less. A total of 73% of these females were between the ages of 16 and 20 years when they had their first sexual intercourse, 43% reported that they did not use any contraception method, 88% had had children, and 86% reported that they had never smoked. A Pap smear was performed for each female; all 264 females who had abnormal cytologies and 23 of the females who had normal cytologies but who had other cervical diseases were referred for colposcopy and/or punch biopsy, according to the appropriate gynecological criteria. Blood was obtained by venipuncture, and cervical samples were collected with a cytobrush and kept in 95% ethanol for DNA analysis (9, 18, 26).

ELISA. Ninety-six-well ELISA plates were coated with 5 μg/ml L1 peptide 18301, 18294, or 18283 or with 1.25 μg/ml VLPs. The peptides and VLPs had been diluted in PBS at 37°C for 1.5 h. The plates were washed three times with PBS containing 0.05% Tween 20 (PBS-T), blocked with 200 μl PBS-T with 0.01% bovine serum albumin for 20 min at 37°C, and washed; 100 μl serum diluted 1:200 in blocking buffer was added, and the mixture was incubated for 1 h at 37°C. After the mixture was washed, 100 μl peroxidase-conjugated rabbit anti-human immunoglobulin G F(ab′)$_2$ fragment (Vector) diluted 1:5,000 was added. The plates were incubated for 1 h at 37°C, and then 100 μl peroxidase substrate (tetramethylbenzidine; Kirkegaard & Perry Laboratories) was added to the plates. The reaction was stopped by adding 50 μl 1 N sulfuric acid, and the absorbance was read at 450 nm. The assay was performed in triplicate, and the variation was less than 10%. Seroreactivity was calculated as follows: (S − N)/(P − N), where S is the optical density (OD) of the serum sample tested obtained with each antigen, P is the OD of each serum sample obtained with anti-human antibody, and N is the OD obtained with no antigen. The cutoff points for the definition of a seropositive female were 0.15 for the peptides and 0.25 for the VLPs, according to the reactivity of the control serum sample.

HPV DNA detection by PCR. Cervical samples were washed with PBS, centrifuged at 12,000 rpm for 10 min, and incubated in 100 μl lysis buffer (10 mM Tris-HCl [pH 7.9], 0.45% Nonidet P-40, 0.45% Tween 20, 60 μg/ml proteinase K) first at 60°C for 1 h and then at 95°C for 10 min (24). All samples were then amplified by PCR with human β-globin-specific primers GH20 and PC04 (12, 30, 31) and with two different HPV-specific generic primer sets: primers GP5+ and GP6+ and primers MY09 and MY11 (12, 17, 28). The PCR with primers GP5+ and GP6+ (12) was carried out in a final 20-μl volume with 1× amplification buffer (Bioline), 100 μM each deoxynucleoside triphosphate, 3 mM $MgCl_2$, 1 U *Taq* polymerase, and 40 pmol of each primer. The PCR mixture was denatured at 94°C for 10 min, followed by 40 amplification cycles consisting of 94°C for 1 min, 40°C for 2 min, and 72°C for 1.5 min; a final elongation step was carried out at 72°C for 7 min (12). The PCR with primers MY09 and MY11 was carried out in a final 20-μl volume. The PCR mixture was denatured at 94°C for 5 min, followed by 40 amplification cycles consisting of 94°C for 30 s, 45°C for 1 min, and 72°C for 1 min; a final elongation step was carried out at 72°C for 7 min (28). DNA from Sf21 cells transfected with a vector containing the HPV-16 L1 gene was used as a positive control, while ultrapure distilled water (GIBCO) was used as a negative control. All PCR products were run on 2% agarose gels stained with SYBR safe (Invitrogen), and the gels were scanned with a Molecular Imager Fx apparatus (Bio-Rad).

Statistical analysis. Group values are presented as percentages, means, and standard deviations. Agreement between pairs of tests was assessed by using the Lin concordance correlation coefficient (rho value); this coefficient is calculated by combining a measure of accuracy (bias correction factor) and an estimate of precision (Pearson correlation coefficient) (19). STATA (version 9) software was used for statistical analysis.

RESULTS

Two hundred sixty-four females with abnormal cytologies and 127 females with normal cytologies (total, 391 females) were included in this study. The cytologies of the 264 females with abnormal cytologies were classified according to the Bethesda system; and these were classified as atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells, low-grade squamous intraepithelial cervical lesions (cyt-LSILs), or high-grade squamous intraepithelial cervical lesion (cyt-HSILs). All patients with abnormal cytologies and 23 patients with normal cytologies but with other cervical diseases were referred for colposcopy or biopsy; 104 females remained classified as having normal cytologies and no colposcopy (normal cytologies-no col). No cervical lesions (negative colposcopy results) were found in 159 females, LSILs (col-LSILs) were found in 110 females, HSILs (col-HSILs) were found in 14 females, and cervical cancers were found in 4 females.

A total of 307 samples from those 391 female cervical samples that amplified the β-globin housekeeping gene were tested for HPV DNA. HPV DNA was found in 49%, 73%, 73%, 82%, and 100% of the patients with col-normal cytologies, negative colposcopy results, col-LSILs, col-HSILs, and cervical cancer, respectively.

The seroreactivities of the 391 female serum samples to peptides 18283, 18294, and 18301 and VLPs were determined. The median ODs were between 0.22 and 0.25 for the peptides and 0.41 for the VLPs with sera from females normal cytologies-no col; the median ODs for these four antigens increased to between 0.25 and 0.45 for sera from females whose cervical lesions were HPV DNA negative and to between 0.38 and 0.52 for sera from females whose cervical lesions were HPV DNA positive (Table 1). The rates of seropositivity ranged from 18 to 31% for L1 peptides 18283, 18294, and 18301 and 43% for VLPs for normal cytology-no col females. It increased to between 64 and 73% for these three peptides and VLPs for patients negative by colposcopy and patients with col-LSILs and col-HSILs. The four cervical cancer patients were seropositive by the use of these four antigens. The rate of seropositivity was generally higher for HPV DNA-positive females than for HPV DNA-negative females; the exception was females with negative colposcopy results. The rate of seropositivity increased along with lesion severity. The rates of seropositivity were 23% for peptides 18283 and 18301, 15% for peptide 18294, and 45% for VLPs for HPV DNA-negative females with normal cytologies-no col; the rates of seropositivity increased to 83% for peptide 18301 and 75% for peptide

TABLE 1. Rates of seropositivity for patients diagnosed by colposcopy or biopsy

Diagnosis by colposcopy and HR-HPV infection status (no. of patients)	18301			18294			18283			VLPs		
	No. (%) positive	Median OD	IQ[a] range	No. (%) positive	Median OD	IQ range	No. (%) positive	Median OD	IQ range	No. (%) positive	Median OD	IQ range
Normal cytology (104)[b]	23 (22)	0.22	0.17–0.30	19 (18)	0.22	0.18–0.29	32 (31)	0.25	0.20–0.32	45 (43)	0.41	0.30–0.54
Negative (48)[c]	11 (23)	0.21	0.17–0.27	7 (15)	0.22	0.16–0.29	11 (23)	0.24	0.20–0.32	20 (45)	0.43	0.30–0.54
Positive (47)[c]	9 (19)	0.22	0.18–0.32	10 (21)	0.22	0.18–0.29	16 (34)	0.25	0.20–0.35	21 (45)	0.41	0.29–0.55
Negative colposcopy result (159)	109 (69)	0.42	0.30–0.64	102 (64)	0.40	0.30–0.62	111 (70)	0.41	0.29–0.58	105 (66)	0.52	0.39–0.66
Negative (32)[c]	26 (81)	0.50	0.35–0.71	21 (66)	0.42	0.31–0.71	22 (69)	0.40	0.31–0.69	22 (69)	0.56	0.44–0.71
Positive (86)[c]	56 (65)	0.42	0.30–0.59	54 (63)	0.41	0.29–0.59	63 (73)	0.45	0.29–0.63	60 (70)	0.56	0.42–0.69
LSILs (110)	81 (73)	0.49	0.32–0.72	76 (69)	0.41	0.30–0.66	76 (69)	0.38	0.27–0.54	75 (68)	0.51	0.36–0.66
Negative (21)[c]	14 (66)	0.45	0.30–0.69	15 (71)	0.37	0.29–0.64	13 (62)	0.33	0.26–0.49	10 (48)	0.41	0.34–0.58
Positive (58)[c]	47 (81)	0.52	0.35–0.72	42 (72)	0.42	0.30–0.66	41 (71)	0.38	0.29–0.59	41 (71)	0.51	0.38–0.65
HSILs (14)	10 (71)	0.44	0.29–0.64	9 (64)	0.38	0.33–0.66	9 (64)	0.45	0.27–0.56	9 (64)	0.49	0.38–0.73
Negative (2)[c]	1 (50)	0.33	0.18–0.48	1 (50)	0.27	0.21–0.34	0 (0)	0.25	0.24–0.26	0 (0)	0.31	0.24–0.38
Positive (9)[c]	8 (89)	0.44	0.35–0.64	7 (78)	0.41	0.35–0.67	8 (89)	0.49	0.37–0.56	8 (89)	0.51	0.46–0.73
Cervical cancer (4)	4 (100)	0.82	0.55–1.12	4 (100)	0.79	0.63–0.96	4 (100)	0.78	0.62–1.08	4 (100)	0.85	0.63–1.12
Positive (4)	4 (100)	0.82	0.55–1.12	4 (100)	0.79	0.63–0.96	4 (100)	0.78	0.62–1.08	4 (100)	0.85	0.63–1.12
Overall (391)	227 (58)	0.37	0.24–0.63	210 (54)	0.35	0.24–0.57	232 (59)	0.35	0.25–0.51	238 (61)	0.49	0.34–0.64
Negative (103)[c]	52 (51)	0.31	0.21–0.56	44 (43)	0.29	0.21–0.42	46 (45)	0.30	0.23–0.41	52 (51)	0.47	0.32–0.58
Positive (204)[c]	124 (61)	0.40	0.26–0.63	117 (57)	0.37	0.25–0.60	132 (65)	0.38	0.26–0.54	134 (66)	0.50	0.38–0.66

[a] IQ, interquartile.
[b] These 104 patients were not referred for colposcopy because they displayed normal cytologies.
[c] The number of HPV DNA-positive females plus HPV DNA-negative females does not equal the total because only females who were β-globin positive by PCR were analyzed for HPV infection.

18283 and 18294 and VLPs for females with HPV DNA-positive cervical lesions (Table 1). HPV DNA-negative females negative by colposcopy (68% of whom were previously classified as having ASCUS) displayed a higher rate of seropositivity than HPV DNA-positive females.

Analysis of the agreement of the seroreactivities of these four antigens was performed for the overall population and for females classified by cytology report, diagnosis by colposcopy or biopsy, HPV DNA status, and age. There was agreement in the rates of seropositivity for the overall population between the peptide-peptide and the peptide-VLP pairs, with the highest rate being for the 18294-18283 peptide pair (rho value, 0.744) and the lowest being for the peptide 18294-VLP pair (rho value, 0.576) (Table 2).

For all pairs analyzed, the rate of agreement for seroreactivity was higher for HPV DNA-positive females (rho value

TABLE 2. Concordance correlation coefficients for seroreactivities for patients classified by cytology (Bethesda system)

Pap smear result/ HR-HPV infection	18301-VLPs		18294-VLPs		18283-VLPs		18301-18294		18301-18283		18294-18283	
	Rho	95% CI[a]	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI
Normal cytology	0.619	0.52–0.72	0.779	0.72–0.84	0.804	0.74–0.86	0.711	0.63–0.79	0.617	0.51–0.72	0.883	0.85–0.91
Negative	0.150	−0.07–0.37	0.085	−0.02–0.19	0.210	0.04–0.37	0.347	0.21–0.47	0.169	−0.04–0.38	0.583	0.42–0.74
Positive	0.723	0.61–0.83	0.880	0.83–0.92	0.893	0.84–0.94	0.788	0.71–0.86	0.776	0.68–0.87	0.931	0.90–0.95
Abnormal cytology	0.583	0.50–0.65	0.452	0.36–0.54	0.648	0.58–0.71	0.567	0.48–0.64	0.548	0.47–0.62	0.667	0.60–0.72
ASCUS	0.577	0.47–0.68	0.472	0.35–0.59	0.630	0.55–0.71	0.574	0.47–0.68	0.408	0.29–0.53	0.591	0.50–0.68
Negative	0.660	0.49–0.82	0.447	0.22–0.66	0.730	0.58–0.87	0.480	0.23–0.72	0.454	0.22–0.68	0.602	0.44–0.76
Positive	0.516	0.35–0.67	0.477	0.30–0.64	0.661	0.55–0.76	0.578	0.42–0.73	0.406	0.22–0.58	0.547	0.40–0.69
LSILs	0.566	0.45–0.67	0.391	0.25–0.52	0.656	0.55–0.75	0.523	0.37–0.67	0.672	0.56–0.78	0.726	0.63–0.82
Negative	0.087	−0.44–0.62	0.288	−0.24–0.82	0.750	0.53–0.96	0.335	−0.05–0.72	0.101	−0.38–0.58	0.384	−0.19–0.95
Positive	0.616	0.49–0.73	0.379	0.21–0.54	0.655	0.53–0.77	0.485	0.28–0.68	0.732	0.61–0.85	0.739	0.62–0.85
HSILs[b]	0.759	0.61–0.90	0.816	0.65–0.97	0.782	0.60–0.96	0.859	0.73–0.98	0.504	0.26–0.75	0.671	0.44–0.89
Positive	0.802	0.64–0.95	0.843	0.66–1.02	0.833	0.64–1.02	0.937	0.86–1.01	0.572	0.28–0.85	0.640	0.32–0.95
Overall	0.607	0.54–0.66	0.576	0.52–0.64	0.712	0.66–0.76	0.624	0.56–0.98	0.584	0.52–0.64	0.744	0.71–0.78

[a] CI, confidence interval.
[b] The analysis was not performed for HSIL-negative females because of the small number of patients.

TABLE 3. Concordance correlation coefficients for the seroreactivities of patients diagnosed by colposcopy or biopsy

Diagnosis and HR-HPV infection status	18301-VLPs		18294-VLPs		18283-VLPs		18301-18294		18301-18283		18294-18283	
	Rho	95% CI[a]	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI
Negative lesion (normal cytology, negative by colposcopy)	0.602	0.53–0.97	0.481	0.40–0.56	0.648	0.58–0.71	0.634	0.56–0.70	0.633	0.56–0.70	0.715	0.66–0.76
Normal cytology-no col[b]	0.118	−0.03–0.26	0.148	0.05–0.24	0.208	0.10–0.31	0.398	0.27–0.52	0.121	0.03–0.28	0.403	0.28–0.58
Negative	0.134	−0.09–0.60	0.071	−0.02–0.16	0.187	0.02–0.35	0.307	0.18–0.43	0.135	−0.08–0.35	0.557	0.39–0.71
Positive	0.095	−0.11–0.30	0.616	0.47–0.75	0.208	0.05–0.36	0.509	0.30–0.71	0.103	−0.15–0.35	0.358	0.11–0.60
Negative by colposcopy	0.648	0.56–0.72	0.645	0.36–0.56	0.681	0.60–0.76	0.601	0.50–0.69	0.631	0.54–0.72	0.687	0.61–0.75
Negative	0.660	0.48–0.83	0.065	−0.03–0.16	0.690	0.51–0.86	0.443	0.16–0.72	0.430	0.16–0.69	0.599	0.42–0.77
Positive	0.669	0.56–0.77	0.223	0.05–0.38	0.683	0.57–0.78	0.650	0.53–0.76	0.786	0.70–0.86	0.724	0.63–0.81
Positive for lesions (LSILs, HSILs, cancer)[c]	0.587	0.47–0.70	0.693	0.60–0.78	0.782	0.71–0.84	0.577	0.46–0.69	0.490	0.37–0.61	0.783	0.72–0.84
LSILs	0.579	0.45–0.70	0.699	0.61–0.79	0.780	0.71–0.84	0.555	0.43–0.68	0.487	0.35–0.62	0.787	0.72–0.85
Negative	0.404	0.08–0.72	0.558	0.26–0.85	0.843	0.73–0.95	0.579	0.30–0.85	0.299	−0.04–0.64	0.648	0.39–0.90
Positive	0.614	0.45–0.77	0.780	0.68–0.88	0.856	0.79–0.92	0.505	0.32–0.69	0.548	0.37–0.72	0.817	0.74–0.89
HSILs	0.675	0.40–0.95	0.755	0.53–0.98	0.773	0.58–0.96	0.743	0.53–0.96	0.361	−0.02–0.70	0.682	0.39–0.97
Positive	0.628	0.25–1.01	0.775	0.49–1.05	0.683	0.38–0.99	0.680	0.34–1.02	0.267	−0.19–0.73	0.686	0.35–1.02
Overall												
Negative	0.505	0.37–0.63	0.441	0.30–0.57	0.605	0.49–0.71	0.548	0.41–0.68	0.441	0.30–0.57	0.679	0.59–0.76
Positive	0.638	0.55–0.71	0.610	0.53–0.68	0.760	0.71–0.81	0.628	0.54–0.71	0.678	0.61–0.75	0.773	0.72–0.82

[a] CI, confidence interval.
[b] These 104 patients were not referred for colposcopy because they displayed normal cytologies.
[c] The analysis was not performed for HSIL-negative females and cervical cancer patients because of the small numbers of patients.

range, 0.610 to 0.773) than for HPV DNA-negative females (rho value range, 0.441 to 0.679); it was higher for the 18283-VLP pair (rho value, 0.760) and the 18294-18283 pair (rho value, 0.773) for the HPV DNA-positive group and lower for the 18301-18283 and 18294-VLP pairs (rho values for both pairs, 0.441) for the HPV DNA-negative group (Table 3).

The rate of agreement for seroreactivity was higher for HPV DNA-positive females with normal cytologies, with rho values ranging from 0.723 for the 18301-VLP pair to 0.931 for the 18294-18283 pair, than for HPV DNA-negative females with normal cytologies, particularly for the peptide-VLP pairs (rho value range, 0.085 to 0.210) (Table 2).

There were no differences in the rates of agreement between HPV DNA-positive and HPV DNA-negative females with ASCUS for all pairs analyzed or between HPV DNA-positive and HPV DNA-negative females with cyt-LSILs for the 18294-VLP, 18283-VLP, and 18301-18294 pairs. On the contrary, there was a higher rate of agreement for HPV DNA-positive females (rho value range, 0.616 to 0.739) than for HPV DNA-negative females (rho value range, 0.087 to 0.384) with cyt-LSILs for the 18301-18283, 18294-18283, and 18301-VLP pairs. The highest level of agreement for HPV DNA-positive females with cyt-HSILs was found for the 18301-VLP pair (rho value, 0.802), the 18294-VLP pair (rho value, 0.843), the 18283-VLP pair (rho value, 0.833), and the 18301-18294 pair (rho value, 0.937) (Table 2).

The agreement for the normal cytology-no col group was lower than that for the normal cytology group for the peptide-VLP pair (rho value range, 0.071 to 0.616) and the 18301-18283 pair (rho value, 0.121). The agreement for females with negative colposcopy results was higher for HPV DNA-positive females than for HPV DNA-negative females for the 18294-VLP and 18301-18283 pairs. The lowest levels of agreement for HPV DNA-negative and HPV DNA-positive females with negative colposcopy findings were for the 18294-VLP pair (rho value, 0.223) and the 18294-VLP pair (rho value, 0.065), respectively (Table 3).

Among females in whom cervical lesions were detected by colposcopy, the highest seroreactivity agreement was for the VLP-18283 pair (rho value, 0.782) and the 18294-18283 pair (rho value, 0.783), and the lowest was for the 18301-18283 pair (rho value, 0.490) (Table 3). Among the col-LSIL patients, the level of agreement was higher for HPV DNA-positive females than for HPV DNA-negative females, particularly for the 18301-VLP, 18294-VLP, and 18301-18283 pairs. The highest level of agreement for the HPV DNA-positive females with col-LSILs was for the 18283-VLP pair (rho value, 0.856) and the 18294-18283 pair (rho value, 0.817), and that for the HPV DNA-negative females with col-LSILs was for the 18283-VLP pair (rho value, 0.843). The highest level of agreement for HPV DNA-positive females with col-HSILs was for the 18294-VLP pair (rho value, 0.775), and the lowest was for the 18301-18283 pair (rho values, 0.361 and 0.267 for HPV DNA-positive and -negative females, respectively) (Table 3). Agreement was not determined for females with atypical squamous cells but in whom HSILs could not be excluded, atypical glandular cells, noninfected col-HSILs, or cervical cancer because of the small numbers of cases.

The rate of agreement was high for the 18294-18283 pair for most of the age groups analyzed, irrespective of whether they were HPV DNA positive or not, but it was higher for HPV DNA-positive females than for HPV DNA-negative females,

TABLE 4. Concordance correlation coefficients for seroreactivities for the different age groups

Age group (yr) and HR-HPV infection status	18301-VLPs		18294-VLPs		18283-VLPs		18301-18294		18301-18283		18294-18283	
	Rho	95% CI[a]	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI	Rho	95% CI
<25	0.641	0.53–0.75	0.576	0.47–0.67	0.798	0.73–0.86	0.512	0.39–0.63	0.503	0.36–0.64	0.743	0.68–0.80
Negative	0.444	0.11–0.77	0.615	0.34–0.88	0.587	0.35–0.82	0.727	0.52–0.92	0.441	0.07–0.80	0.768	0.61–0.91
Positive	0.681	0.55–0.80	0.576	0.45–0.70	0.848	0.78–0.91	0.516	0.38–0.64	0.628	0.48–0.77	0.753	0.68–0.82
25–34	0.469	0.34–0.59	0.495	0.36–0.62	0.600	0.49–0.70	0.702	0.61–0.78	0.648	0.54–0.75	0.781	0.70–0.85
Negative	0.084	−0.25–0.41	0.184	−0.07–0.44	0.302	0.05–0.55	0.519	0.29–0.74	0.300	0.01–0.59	0.830	0.70–0.95
Positive	0.507	0.34–0.67	0.615	0.45–0.77	0.688	0.57–0.80	0.673	0.54–0.80	0.747	0.62–0.86	0.809	0.72–0.89
35–44	0.715	0.61–0.82	0.661	0.54–0.78	0.716	0.62–0.80	0.759	0.67–0.84	0.601	0.48–0.72	0.737	0.64–0.82
Negative	0.665	0.49–0.83	0.494	0.23–0.75	0.732	0.55–0.90	0.549	0.26–0.83	0.414	0.14–0.68	0.552	0.31–0.78
Positive	0.745	0.61–0.88	0.724	0.59–0.85	0.703	0.57–0.82	0.876	0.81–0.94	0.711	0.58–0.84	0.898	0.84–0.95
>44	0.603	0.48–0.72	0.532	0.40–0.65	0.679	0.57–0.78	0.614	0.48–0.74	0.536	0.40–0.66	0.670	0.58–0.75
Negative	0.496	0.27–0.72	0.271	0.03–0.51	0.689	0.52–0.85	0.323	0.02–0.622	0.502	0.24–0.76	0.734	0.61–0.85
Positive	0.606	0.43–0.77	0.586	0.40–0.76	0.668	0.52–0.81	0.723	0.57–0.86	0.509	0.33–0.68	0.624	0.48–0.76

[a] CI, confidence interval.

particularly for the 25- to 34-year-old and the 35- to 44-year-old age groups, for most pairs analyzed. The highest rate of agreement for HPV DNA-positive females ages 35 to 44 years was for the 18301-18294 pair (rho value, 0.876) and the 18294-18283 pair (rho value, 0.898). Very low levels of agreement were found for HPV DNA-negative females in the 25- to 34-year-old age group for the 18301-VLP pair (rho value, 0.084), the 18294-VLP pair (rho value, 0.184), the 18283-VLP pair (rho value, 0.302), and the 18301-18283 pair (rho value, 0.300) and females in the >44-year-old age group for the 18301-18294 pair (rho value, 0.323) and the 18294-VLP pair (rho value, 0.271) (Table 4).

DISCUSSION

A reliable serological VLP-based ELISA for the detection of HPV DNA-positive females at risk of developing cervical cancer presents several drawbacks. The most relevant of these is the high frequency of antibodies that react with VLPs in females with normal cytologies (low specificity), and most antibody responses detected seem to be HPV type specific. In principle, both of these drawbacks could be resolved by identifying specific HR-HPV type B-cell epitopes with low levels of sequence variability; moreover, the immunogenicities of these B-cell epitopes should be associated with the viral load. In fact, several reports have described the use of peptides for the identification of females persistently positive for HPV DNA (6–8, 29, 33). This work compared the abilities of peptides 18283, 18294, and 18301 and VLPs to identify HPV DNA-positive females with cervical lesions.

The rates of seropositivity obtained with the four antigens were similar (54% to 61%) for the general population; were higher for HPV DNA-positive females than HPV DNA-negative females; and increased with lesion severity, particularly for peptide 18283, which seemed to be more specific for the discrimination of HPV DNA-positive females from HPV DNA-negative females. Peptide 18283 could contain specific strong B-cell epitopes, since antibodies against this peptide were detected before lesions appeared. There was a higher rate of seropositivity for the VLPs than for peptides for HPV DNA-negative females with normal cytologies; this could have been due to the cross-reactivity of antibodies elicited by some low-risk HPV types with VLPs (e.g., HPV-6 and HPV-11) (15, 37, 38).

The rates of seropositivity for peptides 18294 and 18301 were lower than those for the VLPs and 18283 for noninfected females with negative cytologies-no col, suggesting that the B-cell epitopes displayed on peptides 18294 and 18301 could be less immunogenic than the epitopes present on 18283 and VLPs; there is also the possibility that the antibodies detected by these peptides remained present for a shorter time than antibodies detected by VLPs or 18283. Moreover, peptides 18294 and 18301 presented abilities to detect cervical lesions in HPV DNA-positive females similar to and greater than those of VLPs, respectively, suggesting that peptides 18294 and 18301 are more specific than VLPs for the discrimination of HPV DNA-positive females with cervical lesions from HPV DNA-negative females with normal cytologies-no col (Table 1).

A comprehensive analysis of all the data revealed that there was agreement for most of the pairs analyzed for HPV DNA-positive females, suggesting that there are similarities in peptide and VLP antigenicities during natural HPV infection. Good agreement was found between the VLP-18283 and the 18283-18294 pairs; but the level of agreement was lower for the VLP-18294 pair, suggesting that there are at least two epitope types on 18283, one of which has antigenicity similar to that of 18294 and the other of which has antigenicity similar to that of the VLPs. On the contrary, a low level of agreement for some of the antigen pairs analyzed (especially VLP-18301) could have been due to the differences in their antigenicities. VLPs contain epitopes that range from being poorly antigenic to highly antigenic (immunodominant), but the peptide epitope repertoire is restricted; some peptides mainly contain immunodominant epitopes, and others contain poorly antigenic epitopes. This is also supported by the fact that immune pressure on these sequences seems to be different according to differences in amino acid sequence variability; the VLP surface contains a highly variable sequence; on the contrary, peptide 18301 contains a highly conserved sequence. The lowest level

of agreement among the peptide pairs was found between peptides 18301 and 18283, especially for HPV DNA-negative females with normal cytologies, and the highest level of agreement was found between peptides 18294 and 18283. Peptide 18283 (the most variable of these peptides) and peptide 18294 probably contain VLP surface-exposed dominant B-cell epitopes and seemed to be more antigenic for HPV than the most conserved peptide 18301 sequence.

Good agreement was found for the 18283-VLP pair for all females with cervical lesions detected by colposcopy, and the rate of agreement was very low for females with normal cytologies-no col; also, the 18294-VLP pair displayed a low level of agreement for HPV DNA-negative females with normal cytologies-no col. This could have been due to the lower frequency of anti-18283 and anti-18294 antibodies than anti-VLP antibodies. This finding suggests that these peptides are more sensitive for the detection of HPV-associated cervical lesions than cytology in this setting (sensitivity, about 70%). The good agreement for the 18294-18283 pair and the low level of agreement for the 18301-18283 pair for females classified according to their colposcopy results suggests similar antigenicities between 18294 and 18283 and different antigenicities between 18301 and 18283 in patients with natural HPV infections. Females negative by colposcopy (65% of whom were classified as having ASCUS) displayed a higher frequency of HPV DNA positivity and seropositivity than females with normal cytologies-no col; these females who were negative by colposcopy and in whom cell abnormalities were detected by Pap smear but not by colposcopy could have had endocervical or microscopic lesions, or their cervical lesions could have disappeared when they were examined by colposcopy. Interestingly, among the females in this group, the rate of seropositivity was higher for HPV DNA-negative females than for HPV DNA-positive females for peptide 18301, suggesting that this peptide detected antibodies involved in controlling the development of detectable cervical lesions and/or HPV infection.

The antibody immune response during infection was roughly parallel to the age of the female, which is consistent with the fact that most females had their first sexual intercourse at the same age and became HPV DNA positive in the 2 years following their first sexual intercourse. There was good agreement in the rates of seroreactivity to the various antigens among the different age groups (each age group contained between 84 and 108 females), and the rate of agreement was slightly lower for most noninfected females. The antibody repertoire during natural HPV infection depends on different epitope types, ranging from immunodominant epitopes (rapidly inducing antibodies) to epitopes with low levels of antigenicity, which induce antibodies only after long-term exposure to the immune system. Moreover, the concentrations of these antibodies could slowly or rapidly become reduced after the HPV infection disappears. The VLP-based ELISA measures all antibodies induced by the different epitopes. On the contrary, peptides could be used to measure some of these antibodies, since they contain fewer epitopes, thus displaying sensitivities and specificities different from those of VLPs for the detection of HPV DNA in females with cervical lesions.

The rates of agreement for HPV DNA-positive females <25 years old was high for the 18283-VLP pair (rho value, 0.798) and then decreased as the age increased, supporting the idea that some of the VLP immunodominant epitopes that induce antibodies shortly after infection are contained on peptide 18283 and that as females age other B-cell epitopes on HPV became immunodominant. It also seemed that the levels of the antibodies recognized by peptide 18283 decreased soon after the HPV infection disappeared, since the frequency of antibodies to this peptide detected was higher for HPV DNA-positive females than for noninfected females. The rate of agreement was higher for HPV DNA-positive females in the 35- to 44-year-old age group than for females in the other age groups; this is the age range at which females display a high frequency of HPV-associated cervical lesions, HPV infection, and anti-HPV antibodies. The rate of agreement between peptides 18283 and 18294 was high for all age groups analyzed; these peptide sequences build the top of the spikes on the VLP model, and parts of their sequences vary, probably due to immune pressure. On the contrary, the rate of concordance between peptides 18301 and 18283 was low in most age groups, even though they are on the VLP surface.

In conclusion, peptides 18283 and 18294 proved to be more specific and peptide 18301 was more sensitive than VLPs for the detection of HPV DNA-positive cervical lesions and for the discrimination of HPV DNA-positive females and HPV DNA-negative females with normal cytologies. Peptide 18283 is useful for the detection of HPV in females. According to the results presented above, a serological test for the detection of HR-HPV-positive females at risk of developing cervical cancer can probably be designed.

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