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Cervical Mucus Antibodies against Human Papillomavirus Type 16, 18, and 33 Capsids in Relation to Presence of Viral DNA

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To investigate whether cervical mucus antibodies against human papillomavirus (HPV) capsids are associated with the detection of HPV DNA or HPV-related cytological diagnoses, 611 samples of cervical secretions from 359 women referred to a colposcopy clinic were tested by an enzyme-linked immunosorbent assay for the presence of immunoglobulin A (IgA) antibodies against HPV capsids of HPV type 16, 18, or 33 and for the presence of cervical HPV DNA by PCR. Among subjects with at least one cervical sample positive for HPV type 16 (HPV-16) DNA, 28.1% also had at least one HPV-16 IgA-positive cervical sample (odds ratio [OR] = 2.9; $P = 0.0003$). IgA to HPV-18 was also more common among HPV-18 DNA-positive subjects (OR = 3.1; $P = 0.0325$) and IgA to HPV-33 was more common among HPV-33 DNA-positive subjects (OR = 4.2; $P = 0.0023$). Cervical IgA antibodies to HPV-16 were also more common among patients with cervical intraepithelial neoplasia, particularly among patients with cervical intraepithelial neoplasia grade I ($P < 0.0005$). The data indicate that an HPV type-restricted IgA antibody response against HPV capsids is detectable in cervical mucus and is associated with a concomitant cervical HPV infection.

There is now strong epidemiological and experimental evidence that the oncogenic types of human papillomavirus (HPV), particularly types 16 and 18, play a crucial role in the development of cervical neoplasia (17, 24). HPV infection is usually diagnosed by the detection of viral DNA. PCR, which is an extremely sensitive technique for the detection of specific DNA sequences, has in recent years become highly reliable (12, 18, 21). Serological diagnosis of HPV infection has only become possible in recent years. The serum antibody response against baculovirus-expressed HPV type 16 (HPV-16) capsids has been validated as a remarkably type-restricted, albeit not very sensitive, marker of sexually transmitted HPV infections (1, 4, 6, 7, 10, 16, 22, 23). However, whether antibodies to HPV capsids can be detected in cervical mucus samples and whether they are related to the presence of cervical HPV DNA have not been investigated. Previously, we have determined that immunoglobulin A (IgA) antibodies against several papillomavirus-derived antigens are detectable in cervical mucus and are more commonly found among women with HPV-associated lesions than among controls (8, 9). However,

TABLE 1. Detection of HPV DNA by PCR in 611 cervical mucus samples from subjects referred for colposcopy because of a previous abnormal cervical smear

Diagnosis	Total no. of samples	No. (%) of samples positive for DNA of[a]:						
		HPV-6	HPV-11	HPV-16	HPV-18	HPV-31	HPV-33	HPV-35
Benign	414	10 (2.4)	0 (0)	45 (10.9)	19 (4.6)	19 (4.6)	20 (4.8)	10 (2.4)
Condyloma	26	8 (30.8)	4 (15.4)	5 (19.2)	4 (15.4)	3 (11.5)	2 (7.7)	0 (0)
CIN grade I	43	0 (0)	1 (2.3)	17 (39.5)	5 (11.6)	4 (9.3)	3 (7.0)	3 (7.0)
CIN grade II	40	1 (2.5)	0 (0)	15 (37.5)	6 (15.0)	4 (10.0)	6 (15.0)	1 (2.5)
CIN grade III	85	1 (1.2)	0 (0)	44 (52.3)	6 (7.1)	8 (9.4)	9 (10.6)	7 (8.2)
Invasive cancer	3	0 (0)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	1 (33.3)	0 (0)
Total	611	20 (3.3)	5 (0.8)	127 (20.8)	41 (6.7)	38 (6.2)	41 (6.7)	21 (3.4)

[a] Because of double ($n = 46$) or even triple ($n = 5$) infections, data for some samples are included in more than one column.

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TABLE 2. Detection of HPV DNA by PCR from the 359 individual subjects referred to colposcopy at either the enrollment visits or subsequent follow-up visits[a]

Diagnosis[b]	Total no. of samples	No. (%) of subjects positive for DNA of:						
		HPV-6	HPV-11	HPV-16	HPV-18	HPV-31	HPV-33	HPV-35
Benign	235	2 (0.1)	0 (0)	30 (12.8)	10 (4.3)	13 (5.5)	10 (4.3)	6 (2.6)
Condyloma	15	4 (26.7)	3 (20.0)	5 (33.3)	2 (13.3)	2 (13.3)	2 (13.3)	0 (0)
CIN grade I	21	0 (0)	1 (4.8)	9 (42.9)	2 (9.5)	3 (14.3)	3 (14.3)	1 (4.8)
CIN grade II	22	1 (4.5)	0 (0)	11 (50)	3 (13.6)	3 (13.6)	3 (13.6)	1 (4.5)
CIN grade III	63	1 (1.6)	0 (0)	36 (57.1)	6 (9.5)	4 (6.3)	8 (12.7)	5 (7.9)
Invasive cancer	3	0 (0)	0 (0)	1 (33.3)	1 (33.3)	0 (0)	1 (33.3)	0 (0)
Total	359	8 (2.2)	4 (1.1)	92 (25.6)	24 (6.7)	25 (7.0)	27 (7.5)	13 (3.6)

[a] Samples from 359 subjects were obtained at the enrollment visit; 169 women attended two or more visits, 69 women attended three or more visits, and 7 women attended four visits.
[b] For women with different histopathological diagnoses at different visits, the most serious diagnosis is entered.

comparisons with the infecting HPV DNA type indicated a low type specificity of the response (9). In this study, we tested whether an HPV serological assay based on HPV capsids of type 16, 18, or 33, which had previously been found to be able to detect an HPV type-restricted antibody response in serum, was also able to detect an HPV type-restricted response in cervical secretions from women with cervical HPV infection.

MATERIALS AND METHODS

Subjects and samples. A total of 359 women (mean age, 38.4 years; age range, 18 to 74 years) participated in the study, which was approved by the Committee on Ethics of the University of Lund. During May 1990 to May 1992, 611 cervical samples were collected consecutively from these women who were referred to a colposcopy clinic because of cytological changes, varying from slight atypia to suspicion of invasive cancer, in cervical smears. For each referral, a final diagnosis was made by the gynecologist on the basis of colposcopy, histopathology, and new cytological smears (but without the knowledge of the result of the virological tests).

Samples were obtained by gentle swabbing of the ectocervix with a brush (Cytobrush; Medscand, Malmö, Sweden), with attempts made to avoid contamination with blood. The brushes were then swirled in test tubes containing 1 ml of saline to release the mucus and cells, and the tubes were transported to the laboratory within 12 h. At arrival, the specimens were centrifuged at 4,400 × *g* for 5 min. The cell pellets and the supernatants were kept at −20°C until analysis of HPV DNA or antibodies, respectively.

Detection of HPV DNA. The PCR technique was performed as described previously (13, 20). Briefly, all the samples were tested for HPV type 6, 11, 16, 18, 31, 33, and 35 DNA by using type-specific primers and probes in the PCR and hybridization tests. As a control for the PCRs, all samples were tested with primers specific for the human β-globin gene. Bovine serum albumin at a final concentration of 0.2% was added to the PCR buffer in order to increase the performance of the PCR test. The HPV DNA results for the initial 359 samples have also been reported elsewhere (14).

HPV serology. An enzyme-linked immunosorbent assay (ELISA) was performed by using a modification of a previously described protocol (15). In short, purified HPV capsids of types 16, 18, and 33 were coated onto microtiter plates (Costar, Cambridge, Mass.) in phosphate-buffered saline (PBS) at a concentration of 1 μg/ml overnight at 4°C. Following blocking with 10% goat serum (GS) in PBS (GS-PBS) for 1 h at room temperature (RT), the secretion samples were diluted 1:2 in GS-PBS and were incubated on the plates for 2 h at RT. Following thorough washing of the plates with PBS–0.5% Tween, mouse monoclonal antibodies to IgA (alpha chain), IgG (gamma chain), or IgM (mu chain) (Eurodiagnostica, Apeldoorn, The Netherlands) or to the secretory component (Sigma, St. Louis, Mo.) were diluted 1:800 in GS-PBS, and the dilutions were incubated on the plates for 90 min at RT. A goat anti-mouse IgG (gamma chain specific)–horseradish peroxidase conjugate (Southern Biotechnology, Birmingham, Ala.) was diluted 1:2,000 in GS-PBS, and the dilution was subsequently incubated on the plates for 1 h at RT. Following washing of the plates, the peroxidase substrate

TABLE 3. Proportion of cervical mucus samples containing IgA antibodies against HPV capsids of type 16, 18, or 33 in relation to concomitant detection by PCR of HPV DNA of the same type in the same sample[a]

HPV DNA type	Cutoff, 0.050				Cutoff, 0.100			
	IgA positivity (%)	OR	95% CI	*P* value	IgA positivity (%)	OR	95% CI	*P* value
HPV-16 positive (n = 127)	24.4[b]	3.2	1.9–5.5	0.0000	13.4	3.4	1.6–7.0	0.0010
HPV-16 negative (n = 484)	9.1[b]				4.3			
HPV-18 positive (n = 41)	14.6[c]	2.7	0.9–7.1	0.086	12.2	3.6	1.0–11	0.049
HPV-18 negative (n = 570)	6.0[c]				3.7			
HPV-33 positive (n = 41)	19.5[d]	3.1	1.2–7.5	0.024	12.2	4.2	1.2–13	0.029
HPV-33 negative (n = 570)	7.1[d]				3.2			

[a] The total number of samples was 611. The OR and CI were estimated by exact logistic regression.
[b] To HPV-16 capsids.
[c] To HPV-18 capsids.
[d] To HPV-33 capsids.

TABLE 4. Proportion of cervical mucus samples containing IgA antibodies against HPV capsids of type 16, 18, or 33 in relation to concomitant detection by PCR of HPV DNA of the same type in the same sample

HPV DNA type	Cutoff, 0.050				Cutoff, 0.100			
	IgA positivity (%)	OR	95% CI	*P* value	IgA positivity (%)	OR	95% CI	*P* value
HPV-16 positive (n = 80)	25.0[b]	3.2	1.6–6.5	0.0009	16.3	5.2	2.0–14	0.0005
HPV-16 negative (n = 279)	9.3[b]				3.6			
HPV-18 positive (n = 22)	18.2[c]	2.8	0.6–9.3	0.18	13.6	3.0	0.5–12	0.23
HPV-18 negative (n = 337)	7.3[c]				5.0			
HPV-33 positive (n = 22)	13.6[d]	1.9	0.3–7.1	0.52	13.6	4.6	0.8–20	0.094
HPV-33 negative (n = 337)	7.7[d]				3.3			

[a] The analysis was restricted to the first donated cervical sample from each individual in the study. The total number of samples was 359. The OR and CI were estimated by exact logistic regression.
[b] To HPV-16 capsids.
[c] To HPV-18 capsids.
[d] To HPV-33 capsids.

TABLE 5. Proportion of individuals in study with at least one of the donated cervical samples testing positive for IgA antibodies against HPV capsids of type 16, 18, or 33 in relation to HPV DNA result by PCR for any one of the donated cervical samples from the same individual[a]

HPV DNA type	Cutoff, 0.050				Cutoff, 0.100			
	IgA positivity (%)	OR	95% CI	*P* value	IgA positivity (%)	OR	95% CI	*P* value
HPV-16 positive ($n = 92$)	28.2[b]	2.9	1.5–5.4	0.0008	15.2	3.2	1.4–7.7	0.0068
HPV-16 negative ($n = 267$)	12.0[b]				5.2			
HPV-18 positive ($n = 24$)	20.1[c]	3.0	8.8–9.2	0.10	16.7	3.5	0.8–12	0.10
HPV-18 negative ($n = 335$)	8.1[c]				5.4			
HPV-33 positive ($n = 27$)	29.6[d]	4.4	1.5–12	0.006	14.8	4.2	0.8–15	0.062
HPV-33 negative ($n = 332$)	8.7[d]				3.9			

[a] The total number of individuals was 359. The OR and CI were estimated by exact logistic regression.
[b] To HPV-16 capsids.
[c] To HPV-18 capsids.
[d] To HPV-33 capsids.

2,2′-azino-di(3 ethylbenzthiazolinesulfonate) diammonium salt was added to the plates and was allowed to develop for 3 h, after which the A_{405}s were recorded. The absorbances of the same samples that reacted with identically treated control antigen-coated wells were subtracted. As a control antigen, we used bovine papillomavirus capsids disrupted with 0.1 M carbonate buffer (pH 9.6) in the initial testing of all samples. In the retesting of 116 IgA-positive samples, similarly disrupted HPV-16 capsids were used as control antigens, with virtually identical results.

Statistical analysis. Data were analyzed by using exact logistic regression (19) with the program Log Xact (Cytel, Boston, Mass.). A P value of <0.05 (two sided) was considered significant.

RESULTS

HPV DNA detection. Among the 414 samples with concomitant cytologically benign smears, 123 (29.7%) contained HPV DNA of one or more specified HPV types (Table 1). No sample was positive for condyloma acuminata-associated HPV-11. All of the malignancy-associated HPV types, types 16, 18, 31, 33, and 35, were represented, with type 16 being the most prevalent one (10.9%) (Table 1). The prevalences of the HPV types were rather similar, regardless of whether all tested samples (Table 1) or the results for individual women (Table 2) were considered.

Interestingly, all of the 26 samples from women with condylomata were positive for HPV DNA of a specified type. HPV-6 was the most prevalent type; this was followed by type 16. Among the samples with a diagnosis of cervical intraepithelial neoplasia (CIN) grade I, II, or III, 76.7, 82.5, and 88.2%, respectively, contained HPV DNA of a specified type. HPV-16 was the most common type (37.5 to 52.3% of CIN cases). Three patients were diagnosed with invasive cervical cancer, and their cervical samples were positive for HPV type 16, 18, or 33 DNA, respectively (Table 1).

HPV capsid IgA in relation to HPV DNA detectability. The proportion of IgA-seropositive samples was compared with the concomitant presence of HPV DNA (Table 3). The IgA antibody levels in cervical secretions were very low. Most samples

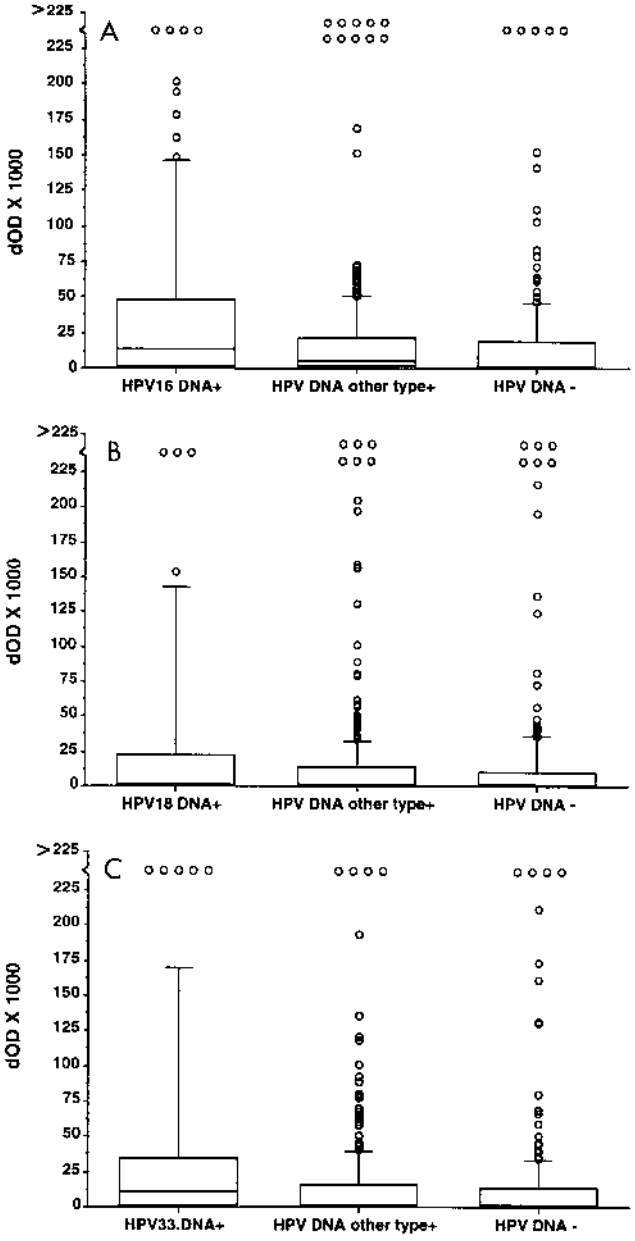

FIG. 1. (A) Box plots of HPV-16 capsid IgA ELISA absorbances in relation to concomitant detection of HPV-16 DNA or other HPV types in the same sample. (B) Box plots of HPV-18 capsid IgA ELISA absorbances in relation to concomitant detection of HPV-16 DNA or other HPV types in the same sample. (C) Box plots of HPV-33 capsid IgA ELISA absorbances in relation to concomitant detection of HPV-33 DNA or other HPV types in the same sample. The box plots show the distributions of antibody levels. The boundaries of the boxes represent 25 and 75% of the values, the line in the box represents the median value, and the lines connected to the boxes by vertical bars represent 10 and 90% of the samples, respectively. In the boxes in which the median line is not visible, it coincides with the lower hinge at the box. dOD, change in optical density.

TABLE 6. Proportion of cervical mucus samples containing IgA antibodies against HPV capsids of type 16, 18, or 33 in relation to concomitant detection of HPV DNA by the same or other HPV type by PCR in the same sample[a]

HPV DNA type	Cutoff, 0.050				Cutoff, 0.100			
	IgA positivity (%)	OR	95% CI	*P* value	IgA positivity (%)	OR	95% CI	*P* value
Any type negative (n = 318)	8.2[b]	Reference			4.1	Reference		
Type 16 positive (n = 127)	24.4[b]	3.6	2.0–6.7	0.0000	13.4	3.6	1.6–8.4	0.0015
Other type positive (n = 166)	10.2[b]	1.3	0.6–2.5	0.55	4.8	1.2	0.4–3.2	0.87
Any type negative (n = 359)	6.0[c]	Reference			4.5	Reference		
Type 18 positive (n = 41)	14.6[c]	2.6	0.8–7.3	0.11	12.2	3.0	0.8–9.1	0.10
Other type positive (n = 211)	5.7[c]	0.9	0.4–2.0	0.99	3.3	0.7	0.3–1.9	0.67
Any type negative (n = 359)	5.9[d]	Reference			3.3	Reference		
Type 33 positive (n = 41)	19.5[d]	3.9	1.4–10	0.010	12.2	4.0	1.0–13	0.043
Other type positive (n = 211)	7.1[d]	1.2	0.6–2.6	0.67	2.4	0.7	0.2–2.2	0.70

[a] The total number of samples was 611. The OR and CI were estimated by exact logistic regression.
[b] To HPV-16 capsids.
[c] To HPV-18 capsids.
[d] To HPV-33 capsids.

gave a zero ELISA absorbance, and only a small fraction of the samples were positive at the preassigned cutoff level (0.100 absorbance unit) (Table 3). However, IgA positivity against HPV capsids of a specific HPV type was three- to fivefold more common in samples testing HPV DNA positive for the corresponding HPV type (Table 3). Analysis by using an alternative, arbitrarily assigned cutoff level (0.050) gave essentially similar point estimates, suggesting that the IgA reactivity of between 0.050 and 0.100 absorbance unit had a specificity comparable to those above 0.100. This was also suggested by plotting the distribution of ELISA absorbances among HPV DNA-positive and -negative samples (Fig. 1). Data analysis restricted to samples taken from different individuals resulted in similar odds ratios (ORs) (Table 4). To investigate whether IgA positivity for samples negative for the DNA of the corresponding HPV type reflects a previous cervical HPV infection, we also determined whether individuals with any one sample testing HPV DNA positive were more likely to have any cervical sample that tested IgA positive. Whereas the proportion of HPV-16-positive samples was similar to that when concomitant infection was considered, some increase in the HPV-18 and the low-level HPV-33 reactivities was seen, suggesting that some of these responses may reflect previous infection (Table 5).

To investigate the type specificity of the response, the IgA reactivities of samples positive for other HPV DNA types were analyzed. The antibody levels were similar to those in samples testing HPV DNA negative, suggesting that most of the IgA antibody response to HPV capsids was type specific (Table 6; Fig. 1).

Persistence of cervical IgA antibodies or of HPV DNA in serial cervical samples from the same women. As expected, a substantial proportion of women who tested HPV DNA positive at the enrollment visit were also HPV DNA positive at follow-up (Table 7). As has also been found in other studies (11), clearance of HPV DNA was associated with a benign cytology after treatment, whereas patients with persistent CIN were persistently HPV DNA positive. For a substantial proportion of IgA antibody-positive women, the follow-up samples were also persistently positive (Tables 8 and 9). Fluctuation between IgA positivity and negativity was mostly found for the very weak reactivities (optical densities, 0.050 to 0.100) (Tables 8 and 9).

HPV capsid IgA and cytological diagnosis. Table 10 shows the relationship between the proportion of the cervical mucus samples that contained IgA antibodies against HPV type 16, 18, or 33 capsids and the cytological diagnosis from the Pap smear taken with the same cervical brush. The presence of HPV type 18 or 33 antibodies showed no association with the presence of HPV-associated disease (Table 10), whereas HPV-16 IgA was more common among CIN patients (Table 10; Fig. 2).

HPV antibodies of different isotypes. To investigate whether the IgA antibodies detected were secretory or were due to the leakage of serum IgA into the mucus, 116 samples that were HPV-16 IgA positive were reanalyzed for IgA, IgG, IgM, or secretory IgA reactivity with the HPV-16 capsids in parallel ELISAs. No IgM reactivity was detectable. Only seven samples contained IgG at levels above 0.100. The IgG reactivity was not related to the IgA reactivity of same sample (R^2 = 0.051; P = not significant) and was not related to the presence of HPV-16 DNA (OR, 1.2; 95% confidence interval [CI], 0.2 to 6.8), although the low number of IgG-positive samples makes this negative result inconclusive. For both IgG and IgM ELISAs, a pool of sera containing IgA, IgG, and IgM to HPV-16 capsids was used as a positive control and reacted strongly at about equal levels for the three isotypes (difference in optical density, 0.42 to 0.56).

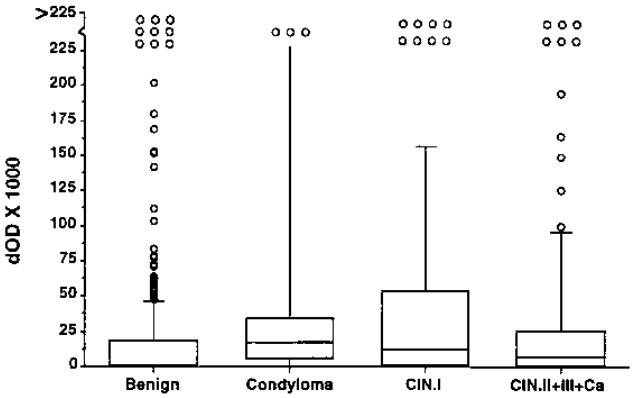

FIG. 2. Box plots of HPV type 16 capsid IgA ELISA absorbances in relation to the cytological diagnosis from the Pap smear taken with the same cervical brush sample. See the legend to Fig. 1 for a description of the symbols and abbreviations. Ca, cancer.

TABLE 7. Detection of cervical HPV DNA by PCR at the enrollment visit in comparison with detection of cervical HPV DNA at subsequent visits

Positive at first visit (no.[a])	% Positive at second visit	OR	CI	*P* value	Positive at first visit (no.[a])	% Positive at third visit	OR	CI	*P* value
HPV-16 DNA positive ($n = 48$)	50.0	16	5.8–49	0.0000	HPV-16 DNA positive ($n = 45$)	37.5	12	2.2–130	0.0015
HPV-16 DNA negative ($n = 121$)	5.7				HPV-16 DNA negative ($n = 24$)	4.4			
HPV-18 DNA positive ($n = 15$)	40.0	24	4.7–137	0.0001	HPV-18 DNA positive ($n = 7$)	42.9	40	2.6–2400	0.0051
HPV-18 DNA negative ($n = 154$)	2.6				HPV-18 DNA negative ($n = 62$)	1.6			
HPV-33 DNA positive ($n = 12$)	41.7	21	3.8–116	0.0003	HPV-33 DNA positive ($n = 10$)	30.0	23	1.6–1300	0.017
HPV-33 DNA negative ($n = 157$)	3.2				HPV-33 DNA negative ($n = 59$)	1.7			

[a] A total of 169 women attended two or more visits, and 69 women attended three or more visits. The OR and CI were estimated by exact logistic regression.

TABLE 8. IgA antibodies against HPV type 16, 18, or 33 capsids in cervical mucus at the enrollment visit in comparison with detection of cervical IgA at subsequent visits, with a cutoff level for positivity of 0.050

Positive at first visit (no.[a])	% Positive at second visit	OR	CI	*P* value	Positive at first visit (no.[a])	% Positive at third visit	OR	CI	*P* value
HPV-16 IgA positive ($n = 23$)	34.7	5.8	4.9–66	0.0000	HPV-16 IgA positive ($n = 13$)	46.2	5.8	1.2–28	0.024
HPV-16 IgA negative ($n = 146$)	12.5				HPV-16 IgA negative ($n = 56$)	12.5			
HPV-18 IgA positive ($n = 14$)	28.6	7.3	3.7–360	0.0008	HPV-18 IgA positive ($n = 7$)	28.6	7.3	0.5–84	0.15
HPV-18 IgA negative ($n = 155$)	4.8				HPV-18 IgA negative ($n = 62$)	4.8			
HPV-33 IgA positive ($n = 14$)	44.4	10	3.6–120	0.0004	HPV-33 IgA positive ($n = 9$)	44.4	10	1.5–78	0.016
HPV-33 IgA negative ($n = 155$)	6.7				HPV-33 IgA negative ($n = 60$)	6.7			

[a] A total of 169 women attended two or more visits, and 69 women attended three or more visits. The OR and CI were estimated by exact logistic regression.

TABLE 9. IgA antibodies against HPV type 16, 18, or 33 capsids in cervical mucus at the enrollment visit in comparison with detection of cervical IgA at subsequent visits, with a cutoff level for positivity of 0.100

Positive at first visit (no.[a])	% Positive at second visit	OR	CI	*P* value	Positive at first visit (no.[a])	% Positive at third visit	OR	CI	*P* value
HPV-16 IgA positive ($n = 11$)	45.5	40	6.2–320	0.0000	HPV-16 IgA positive ($n = 8$)	62.5	42	4.8–620	0.0002
HPV-16 IgA negative ($n = 158$)	1.9				HPV-16 IgA negative ($n = 61$)	3.3			
HPV-18 IgA positive ($n = 6$)	33.3	36	2.2–630	0.0123	HPV-18 IgA positive ($n = 3$)	33.3	27	0.3–2600	0.17
HPV-18 IgA negative ($n = 163$)	1.23				HPV-18 IgA negative ($n = 66$)	1.5			
HPV-33 IgA positive ($n = 8$)	25.0	25	1.6–400	0.023	HPV-33 IgA positive ($n = 6$)	50.0	51	3.2–3300	0.0029
HPV-33 IgA negative ($n = 161$)	1.2				HPV-33 IgA negative ($n = 63$)	1.6			

[a] A total of 169 women attended two or more visits, and 69 women attended three or more visits. The OR and CI were estimated by exact logistic regression.

TABLE 10. Proportion of cervical mucus samples containing IgA antibodies against HPV type 16, 18, or 33 capsids in relation to cytological diagnosis from the Pap smear taken with the same cervical brush

Cytological diagnosis	No. of samples positive/total no. of samples tested (%)[a]		
	HPV-16	HPV-18	HPV-33
Benign	39/414 (9.4)	24/414 (5.8)	31/414 (7.5)
Condyloma	3/26 (11.5)	2/26 (7.7)	3/26 (11.5)
CIN grade I	12/43 (27.9)[b]	4/43 (9.3)	5/43 (11.6)
CIN grades II and III and cancer	21/128 (16.4)[c]	10/128 (7.8)	10/128 (7.8)

[a] IgA to HPV type 16, 18, or 33 capsids was assayed for all 611 samples tested. Absorbances of >0.050 were considered positive. An analysis by using an absorbance of 0.100 as the positivity criterion gave similar point estimates (data not shown).

[b] $P < 0.0005$ relative to the HPV-16 DNA-positive samples from women with a benign diagnosis.

[c] $P < 0.03$ relative to the HPV-16 DNA-positive samples from women with a benign diagnosis.

The ELISAs with the monoclonal antibody to the secretory component for the detection of secretory IgA detected specific reactivities in only six samples. This reactivity correlated well with the presence of a high IgA reactivity. Among the eight samples that had the strongest IgA reactivities to HPV-16 capsids, five samples were also positive in the secretory component HPV-16 ELISA. Since a positive control was not available for the secretory component HPV-16 ELISA, the lower reactivity might have been due to a lower sensitivity of the assay compared with that of the IgA HPV-16 ELISA.

DISCUSSION

Our main result was the finding of an association between cervical HPV DNA positivity for a specific HPV type and cervical IgA reactivity against capsids of the corresponding HPV types. The association was particularly strong for HPV-16 IgA reactivity and HPV-16 DNA positivity.

Although several previous reports have described local antibodies associated with HPV-associated diseases, this is the first report of a local antibody response specific for the type of HPV DNA found. This is of particular interest, since an immunodominant papillomavirus type-specific neutralizing antibody response against intact papillomavirus capsids has been shown to be protective against papillomavirus infection (3, 5). The antibodies detected in this study share the type-specific and conformation-dependent properties with the papillomavirus-neutralizing antibodies, and further study of these antibodies may provide information on whether a local, protective antibody response exists. In animal model systems, the levels of neutralizing antibodies in serum have correlated very well with the degree of protection against infection (3). The very low level of reactivity found does not favor the possibility that the local antibody response protects against reinfection. However, the minimum level of neutralizing antibodies required for protection of humans is not known.

Only the presence of IgA to HPV-16 was associated with CIN, but this is most likely explained by the fact that HPV-16 was the dominant HPV DNA type among women with CIN. The tendency for the highest level of capsid antibodies among CIN grade I patients is in accordance with the biology of HPV capsid antigen expression, since it decreases with an increase in the severity of the CIN lesion (2).

In conclusion, local IgA antibodies against intact HPV capsids are detectable in cervical mucus and are preferentially found in samples testing HPV DNA positive for the same viral type, suggesting that this antibody response is mostly type specific.

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