Original
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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

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Objectives: We performed a study to establish the pattern of serological reactivity for immunoglobulins (Ig), to capsids of human papilloma virus (HPV) after new HPV infection in two groups of subjects.

Methods: The pattern of serological reactivity after acquisition of infection with HPV was investigated by measuring IgA, IgM, and IgG antibodies to capsids containing L1 and L2 proteins of HPV types 6, 11, 16, 18, and 33 in longitudinal studies of groups with different patterns of sexual activity. Individuals who tested negative for HPV DNA by the polymerase chain reaction at enrolment, but who became HPV DNA positive during follow up, were examined for antibodies to HPV capsids by enzyme linked immunosorbent assay. One group consisted of 15 young girls (with eight controls who remained HPV DNA negative) who were becoming sexually active and the other comprised 12 male (with five controls) and 35 female (with seven controls) heterosexual attenders of a sexually transmitted disease clinic who had had multiple sexual partners.

Results: The sexually inexperienced girls showed IgA and IgG responses, but seldom an IgM response to infection with HPV types 6/11, 16, and 18. No consistent pattern of serological reactivity was apparent for the heterosexuals with multiple partners. The lack of association between current HPV DNA positivity and detectable antibodies in these individuals was possibly related to the duration of infection or to prior exposure to HPV. For the latter group serological reactivity to HPV capsids was significantly greater in women than in men ($p = 0.001$, $p = 0.003$, and $p = 0.024$, for IgG to HPV 6, 11, and 16, respectively).

Conclusion: The sex difference in antibody response detected in previous studies with assays based on peptide antigens was thus corroborated in the present study with capsid based serological assays. This sex difference might reflect a difference in sexual activity and prior exposure to HPV between men and women in this particular group.

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Introduction

The laboratory identification of human papillomavirus (HPV) infections has relied mainly on the detection of HPV DNA in clinical specimens. However, because of the localised nature of HPV infections, detection of HPV DNA is subject to sampling errors and it may be difficult to obtain representative specimens for epidemiological studies. Furthermore, in the case of transient infections, prior exposure to virus is not necessarily reflected in the current presence of HPV DNA.^{1,2} Thus, the test approach to assess previous HPV infection is the detection of an HPV immune response in serum.

Although the use of several early HPV serological assays revealed associations between antibody response and HPV related diseases or the detection of HPV DNA in longitudinal studies, the sensitivity and specificity of these assays were insufficient for routine diagnostic purposes.^{3–5} Serological assays based on HPV capsids, which present conformational virion epitopes, have been evaluated and appear to present type restricted markers of past or present HPV 16 infection among various cohorts.^{6–14} The HPV serological approach based on HPV capsids has already established associations between HPV 16 infection and

human anogenital neoplasia or other extragenital cancers both in case-control studies based on incident cases and in prospective studies.^{8,15–19}

Previous longitudinal studies have described production of immunoglobulin G (IgG) to HPV 6, 16, or 33 capsids after infection, whereas other studies described the generation of IgA and IgM antibodies.^{6,7,14,20–22} We have performed a study to establish the pattern of serological reactivity for IgA, IgG, and IgM to capsids of HPV 6, 11, 16, 18, or 33 after new HPV infection in two groups of subjects. The subjects in both groups were followed in a prospective cohort study. The main difference between both groups was the measure of sexual exposure and age. A new infection was defined to be present when a participant being negative for HPV by polymerase chain reaction (PCR) at entry of the study became positive for HPV DNA afterwards during the follow up visits.

Materials and methods

STUDY POPULATIONS

Teenage girls in Karlstad

From September 1989 to the end of September 1990, all girls starting their education in the healthcare programme of an upper secondary school in Karlstad, Sweden, were invited to

participate in a longitudinal study on sexuality and sexually transmitted diseases (STDs). The study design has been described in detail elsewhere.⁶⁻²⁰ The girls visited the youth clinic every 6 months for a total of five visits, at which an interview was performed and a blood sample was taken. A gynaecological examination was performed at the first or second visit and at the end of the study period, as well as whenever the participants requested one. Ectocervical and endocervical specimens were collected as follows: a Papanicolaou smear with a cytobrush, a swab specimen for detection of *Chlamydia trachomatis* by an enzyme immunoassay, and then four additional samples on glass slides. The cytobrush was subsequently placed in a vial containing 96% (v/v) ethanol for subsequent PCR analysis for HPV DNA which was performed at the Stockholm Central Microbiology Laboratory as described by Skyldberg *et al.*²³⁻²⁴ with both the consensus primers MY 09 and MY 11 and primers specific for HPV 6/11, 16, 18, 31, or 33.²⁴⁻²⁵ A total of 168 girls were invited to participate, 98 of whom entered the study. Nine girls did not attend the last visit, and data on HPV DNA detection were incomplete for seven. The mean age of the 82 girls with complete data was 16.1 (range 15-17) years at entry into the study. For the present investigation we focused on the 15 girls who tested negative for HPV DNA at entry into the study (first visit) and subsequently tested positive (second to fifth visit).

Fourteen of the 15 girls reported having had penetrating sex before the first visit. The distribution of HPV types was as follows: HPV 6/11 (n=0), HPV 16 (n=4), HPV 18 (n=2), HPV 31 (n=2), HPV 33 (n=5), and HPV x (n=3). Fourteen girls were positive for one type and one was positive for two types (HPV 16 and 31). Eight girls who remained HPV DNA negative during follow up and who reported never having had penetrating sex at the end of the study were randomly chosen as negative controls.

STD clinic visitors in Amsterdam

From May 1988 to the end of December 1990, participants were recruited among visitors attending the STD clinic of the municipal health service of Amsterdam. The design of the study has been described previously.²⁶ Criteria for enrolment in the study were an age of >18 years and sexual contact with five or more heterosexual partners within the previous 6 months. At each visit a physical examination, including colposcopy, was performed and participants were asked to complete a standardised questionnaire on medical history, risk factors for human immunodeficiency virus (HIV) and HPV infection, sexual behaviour, and social psychological variables. A total of 162 women and 85 men agreed to enter into the HPV study, of whom 110 women and 48 men were asked for follow up visits approximately every 4 months until January 1992. The mean age of the 48 men was 37 (range 20-65) years and of the 110 women 31 (range 19-58) years. For the women the mean (SD) duration of the follow up was 513 (256) days, with a mean interval

between visits of 188 (96) days. For the 48 men, the mean duration of the follow up was 498 (269) days with a mean interval between visits of 193 (110) days. For women, the mean number of commercial sex partners (that is, paid sexual contacts with male clients) during the month before entry into the study was 41, whereas men had four commercial sex encounters over the previous 4 months. Specimens were collected from standard sites in three different anatomical regions: the genital region (urethra and coronal sulcus in men; cervix and labia minora in women), the anorectal region (anus and rectum in both women and men), and the oral cavity (tongue and buccal epithelium together in both women and men). In women, specimens were also obtained from the cervical transformation zone for routine cytological examination.

HPV DNA was detected by PCR with four different primer sets.²⁷ The amplified products were subjected to dot blot analysis; the filters were hybridised with a mixture of 5'-end labelled (³²P) HPV type specific oligonucleotide probes under conditions of high stringency.

For the present investigation, we selected all 47 participants (35 women and 12 men) who were HPV DNA negative at entry into the study and who became HPV DNA positive during follow up. The serum specimen collected at the first visit, the sample taken at the same visit as that at which HPV DNA was first detected, and, if available, a specimen collected at the last visit were analysed for HPV serology. The distribution of HPV types among these 47 individuals was as follows: HPV 6/11 (n=12), HPV 16 (n=18), HPV 18 (n=16), and HPV 33 (n=6) with five subjects positive for two types. Twelve subjects (seven women and five men) were randomly chosen as controls from those who remained HPV DNA negative throughout the study period.

HPV SEROLOGY

Serum specimens were tested by enzyme linked immunosorbent assay (ELISA) with baculovirus expressed L1 and L2 capsids of HPV 6, 11, 16 (clone 114/K), 18, and 33. (HPV 6, 11, 16, and 18 capsids were kindly provided by J T Schiller, and HPV 33 capsids by Dr M Sapp.) The capsids were purified by CsCl centrifugation from insect cells infected with baculoviruses encoding L1 plus L2.^{10 12 28 29} Capsids (1.0 µg in 50 µl of phosphate buffered saline (PBS)) were added to each well of a 96 well microtitre plate (Costar, Cambridge, MA, USA), which was then covered and incubated overnight at room temperature. The wells were washed three times with Ca²⁺ and Mg²⁺ free PBS containing 0.1% Tween 20, and then exposed for 1 hour at room temperature to PBS containing 10% horse serum. Serum specimens were tested at a dilution of 1:30; after the addition of 50 µl to each well, the plate was covered and incubated at room temperature for 2 hours. The wells were washed five times and, after the addition of 50 µl of mouse monoclonal antibodies to human IgG (1:800 dilution), to IgM (1:800 dilution), or to IgA

Table 1 HPV ELISA absorbance values ($\times 1000$) for the first serum specimens (visit 1) obtained from 23 young girls in Karlstad and from 59 heterosexuals in Amsterdam with multiple sexual partners

HPV type	Ig type	Cut off	Karlstad (n=23)				Amsterdam (n=59)			
			Median	Range	N pos*	(%)	Median	Range	N pos*	(%)
6	A	468	163	0–452	0	(0)	119	0–1195	7	(12)
6	M	399	83	0–367	0	(0)	20	0–165	0	(0)
6	G	124	39	0–458	1	(4)	172	0–735	34	(58)
11	A	420	80	0–444	1	(4)	65	0–813	5	(9)
11	M	200	31	0–174	0	(0)	10	0–91	0	(0)
11	G	100	20	0–276	4	(17)	90	0–1080	28	(48)
16	A	100	13	0–143	2	(8)	50	4–1173	14	(24)
16	M	NT†	NT	NT	NT		158‡	22–807	36	(74)
16	G	134	7	0–855	1	(4)	41	0–395	10	(20)
18	A	127	42	0–294	1	(4)	25	0–1398	4	(7)
18	M	195	31	0–205	1	(4)	36	0–223	1	(2)
18	G	100	7	0–376	1	(4)	27	0–677	15	(25)
33	A	171	0§	0–883	2	(9)	26	0–1097	4	(7)
33	M	205	41	0–178	0	(0)	17	0–98	0	(0)
33	G	100	0	0–140	2	(9)	0	0–220	4	(7)

*ELISA absorbance > cut off value; †not tested, because of insufficient volume; ‡10 specimens missing, all from subjects who tested HPV DNA negative during follow up; §one specimen missing.

(1:400 dilution) (Eurodiagnostics, Apeldoorn, Netherlands), incubated at room temperature for 90 minutes. After five washes, 50 μ l of horseradish peroxidase conjugated goat antibodies to mouse IgG (1:2000 dilution), were added to each well, and the plate was incubated for 1 hour at room temperature. After another five washes, 50 μ l substrate were added to the well and the plate was incubated at room temperature for 45 minutes. Absorbance at 405 nm was measured with a Dynatech MR 4000 spectrophotometer. The assays were performed in duplicate, and samples with discrepant results were tested two additional times. Each plate included a panel of positive and negative control sera that had been validated in previous studies.^{6,9} For each serum, the difference in absorbance between plates coated with intact capsids and plates coated with control antigens (disrupted bovine papilloma virus capsids) was calculated. The cut off level was designated as the mean plus 3 SD of the absorbance obtained with sera from virginal women analysed at the same time or as an absorbance of at least 0.100, which was the value of a preassigned cut off in a previous study of IgG antibodies to HPV 16 and 33 capsids.⁶ The intra-assay coefficients of variation for HPV 16 assays varied between 9.4% and 10.7% in different assay runs and the interassay coefficients of variation varied between 9.9% and 23.9%.

DATA ANALYSIS

Data are expressed as the proportion having an absorbance above the cut off level and as medians with ranges. Serological results were compared between HPV DNA positive and negative participants with the Mann–Whitney U test. The Wilcoxon paired signed rank test was used for paired analysis of the serological results of the first visit versus those of subsequent visits. A p value of <0.05 was considered statistically significant. All analyses were performed with the Statistical Package for Social Sciences (SPSS/PC+).³⁰

Results

TEENAGE GIRLS IN KARLSTAD

The results of the sera obtained at entry into the study are presented in table 1. The calculated cut off values varied for the different assays and the prevalence of antibodies to the various capsids based on these cut off values was low with the exception of IgG to HPV 11. With the exception of the IgA and IgM reactivity to HPV 6 and IgA reactivity to HPV 11, the median reactivity of antibodies to HPV capsids was also relatively low. The serological results obtained after PCR detectable HPV infection are shown in table 2. The ELISA absorbance values of the last serum specimens obtained from the girls who became positive for a specific HPV type as determined by PCR, were

Table 2 HPV ELISA absorbance values ($\times 1000$) for the last serum specimens (visit 4 or 5) obtained from 23 young girls from Karlstad

HPV DNA status	N	IgA					p Value*†	IgM				IgG					p Value*†
		Median	Range	N pos	(%)			Median	Range	N pos	(%)		Median	Range	N pos	(%)	
Anti-HPV 16																	
HPV 16	4	231	92–381	3	(75)	0.01		Anti-HPV 16				Anti-HPV 16					
Other types	11	41	0–196	1	(9)			NT‡				500	338–588	4	(100)	<0.01	
All types neg	8	9	0–99	0	(0)			NT				46	0–498	2	(20)		
Anti-HPV 18																	
HPV 18	2	289	212–336	2	(100)	0.04		NT				0	0–120	0	(0)		
Other types	13	21	0–120	0	(0)			NT				0	0–120	0	(0)		
All types neg	8	36	8–90	0	(0)			NT				0	0–120	0	(0)		
Anti-HPV 33																	
HPV 33	5	8	0–50	0	(0)			NT				0	0–52	0	(0)		
Other types	10	0	0–113	1	(10)			NT				0	0–52	0	(0)		
All types neg	8	0	0–92	0	(0)			NT				0	0–52	0	(0)		

*p Value (Mann–Whitney U test), for comparison with all types negative; †all p values not shown are >0.10; ‡not tested, because of insufficient volume.

Table 3 HPV ELISA absorbance values ($\times 1000$) of the last serum (visit 2 to 5) obtained from 59 heterosexuals in Amsterdam with multiple sexual partners

HPV DNA status	N	IgA				IgM				IgG			
		Median*	Range	N pos	(%)	Median*	Range	N pos	(%)	Median*	Range	N pos	(%)
Anti-HPV 6													
HPV 6/11	12	138	0–817	3	(25)	21	0–164	0	(0)	171†#	14–908	8	(67)
Other types	35	156†#	0–1187	5	(14)	25	0–243	0	(0)	206	0–710	23	(66)
All types neg	12	35	0–514	1	(8)	42	0–119	0	(0)	114	0–743	5	(42)
Anti-HPV 11													
HPV 6/11	12	83	22–1258	1	(8)	6	0–129	0	(0)	181†#	0–858	7	(58)
Other types	35	91	0–884	2	(6)	12	0–65	0	(0)	111†*	0–1219	19	(54)
All types neg	12	112	0–443	1	(8)	24	0–49	0	(0)	45	6–1222	4	(33)
Anti-HPV 16													
HPV 16	18	79†#	0–114	6	(33)	253†#	70–617	16	(89)	85†#	6–681	5	(28)
Other types	29	54	11–1987	9	(31)	174	42–1249	24	(83)	36	0–560	7	(24)
All types neg	11	47	19–448	2	(18)	222	16–283‡	2	(100)	223	44–401‡	1	(50)
Anti-HPV 18													
HPV 18	16	5	0–1232	2	(12)	40†\$	7–129	0	(0)	41	0–691	5	(31)
Other types	31	47	0–1056	6	(19)	59	0–253	1	(3)	34†#	0–979	10	(32)
All types neg	12	48	31–872	2	(17)	72	31–113	0	(0)	19	0–761	5	(42)
Anti-HPV 33													
HPV 33	6	45	11–1406	1	(17)	1†*	0–37	0	(0)	0	0–70	0	(0)
Other types	41	47	0–746	5	(12)	18	0–67	0	(0)	0	0–179	4	(10)
All types neg	12	66	0–136	0	(0)	5	0–70	0	(0)	0	0–147	1	(8)

†p Value (Wilcoxon matched paired signed rank test), for comparison with first serum specimens; *0.05 < p < 0.10, #0.01 < p < 0.05, \$p ≤ 0.01; all p values not shown are > 0.10; ‡n = 2 because of missing data.

compared with those of the participants who remained HPV DNA negative. Because the numbers of subjects in the HPV DNA positive subgroups were too small, a Wilcoxon analysis comparing the paired serum samples collected at the first and the last (fourth or fifth) visits was not performed. Participants who became positive for HPV 16 or 18 DNA showed higher IgA and IgG reactivities to the corresponding HPV capsids than did the HPV DNA negative girls; these differences were statistically significant with the exception of IgG reactivity to HPV 18. Antibody reactivity to HPV 33 capsids was low in the sera of the five girls who became positive for HPV 33 DNA.

When we analysed the data starting from the HPV serological results in relation to HPV DNA positivity, we observed 11 girls whose last serum specimen tested did not show reactivity to any HPV capsids. Seven of them remained HPV DNA negative, two became positive for HPV DNA 33, and two became HPV DNA type x positive. Of the seven girls who showed in the last serum specimen reactivity to HPV 6 or 11 capsids, none became HPV DNA type 6 or 11 positive, one became HPV 16 DNA positive, three became HPV DNA 18 positive, one became HPV 31 DNA positive, one became positive for HPV 33 DNA, and one became HPV type x DNA positive.

STD CLINIC VISITORS WITH MULTIPLE SEXUAL PARTNERS

The antibody reactivity at enrolment into the study for the group of male and female heterosexuals with multiple sexual partners is shown in table 1. The median IgG reactivities to HPV 6, 11, 16, and 18 capsids in this group were greater than those for the girls in Karlstad. The prevalence of IgA and IgG reactivity to HPV 6 in the Amsterdam heterosexuals was 12% and 58%, respectively; 9% and 48% for HPV 11, 24% and 20% for HPV 16, and 7% and 25% for HPV 18. The serological reactivity to HPV 33 capsids was low.

The serological reactivity to the various capsids for serum specimens obtained after PCR detectable HPV infection is shown in table 3. In common with the analysis of the Karlstad group, using the Mann-Whitney test we firstly compared the reactivity of specific HPV DNA positive participants against the corresponding capsid and also the levels found in the subjects positive for another HPV type, with the participants who remained HPV DNA negative. The IgA reactivity to HPV 6 capsids was greater in both subjects positive for HPV 6/11 DNA and those positive for DNA of other HPV types ($p = 0.06$ and $p = 0.01$, respectively) than for participants who remained HPV DNA negative. Neither IgG reactivity to HPV 11 capsids in subjects positive for HPV 6/11 DNA nor IgA and IgG reactivity to HPV 16 capsids in subjects positive for HPV 16 DNA was significantly greater than that in HPV DNA negative participants. The values of IgA and IgM reactivity to HPV 18 were greater in subjects negative for HPV 18 DNA than in the HPV 18 DNA positive individuals ($p = 0.07$ and $p = 0.03$, respectively).

The antibody reactivity of serum specimens collected at the last visit from the participants who became positive for HPV DNA was also compared with that of sera collected at the time of enrolment by Wilcoxon analysis (table 3). IgG reactivity to HPV 6 capsids increased significantly over time in HPV 6/11 DNA positive subjects, whereas IgA reactivity to HPV 6 increased in subjects positive for DNA of HPV types other than HPV 6 (that is HPV 16, 18, or 33). The extent of IgG reactivity to HPV 11 capsids also increased significantly in HPV 6/11 DNA positive subjects. The participants who became positive for HPV 16 DNA showed a significant increase over time in IgA, IgM, and IgG reactivity to HPV 16 capsids. Finally, the reactivity of IgM and IgG to HPV 18 capsids increased significantly in HPV 18 DNA positive subjects and individuals positive for DNA of other HPV types, respectively.

Figure 1 IgA, IgM, and IgG reactivity to HPV 16 capsids over time in 10 individuals for whom at least three serum specimens were tested. These subjects were HPV DNA negative at the first visit, HPV DNA positive for type 16 in between, and HPV DNA negative at their last visit.

The development of antibodies over time was examined in more detail in 34 individuals for whom at least three serum specimens were tested. Twenty six subjects were HPV DNA negative at the first visit, HPV DNA positive in between, and HPV DNA negative at their last visit. Eight participants tested HPV DNA negative at all three visits. Subjects positive for DNA of specific HPV type showed a greater serum reactivity to the corresponding antigen than those who remained HPV DNA negative or became positive for DNA of a different type of HPV in the case of HPV 6 (IgA), HPV 11 (IgA; only the serum specimen collected in between), and HPV 16 (IgA, IgM, and IgG). To illustrate this, serum reactivity over time for individuals who became positive for HPV 16 is displayed in figure 1. For participants who became positive for HPV 18 or HPV 33 DNA, the reactivity of antibodies to HPV 18 and 33, respectively, was similar to that for participants who remained HPV DNA negative.

SEX DIFFERENCE

We previously detected a difference in antibody reactivity to HPV derived synthetic peptides between men and women.⁵ We therefore analysed separately the data for male (n=17)

and female (n=42) participants from the Amsterdam cohort separately (table 4). Serological reactivity to HPV 6, 11, 16, and 18 capsids was greater in women than in men at the first visit. The difference was statistically significant for IgG to HPV 6, IgA and IgG to HPV 11, IgM and IgG to HPV 16, and IgM to HPV 18.

Because of the observed sex difference, we compared the results at entry of the study of the Karlstad girls with the Amsterdam women, who differed in age and number of sexual encounters. Reactivities of IgG antibodies to HPV 6 and 11 at entry to the study were significantly greater in the Amsterdam women who had a much longer history of sexual experience than for the Karlstad girls ($p < 0.01$ for both). The reactivities of IgA and IgG to HPV 16 capsids were significantly greater in the Amsterdam women than in the Karlstad girls ($p < 0.01$ for both). Similarly, the Amsterdam women showed a significantly higher reactivity of IgG to HPV 18 ($p < 0.01$). Also, IgA reactivity to HPV 33 differed significantly between the two groups ($p < 0.01$).

Discussion

The aim of the present study was to determine the serological reactivity to HPV capsids after acquisition of a new HPV infection in two groups with a different level of sexual exposure. We hypothesised that the different patterns of sexual behaviour would be reflected in the prevalence of antibodies to HPV capsids in the sera obtained at entry into the study as well as at the end of the study period. Acquisition of a new HPV infection was defined when a participant who was HPV DNA negative at entry of the study became HPV DNA positive afterwards. In the Karlstad group the number of observations for HPV DNA examination was limited to the first and the last visit. Therefore, only one visit with an HPV negative result by PCR was taken as criterion of entry into the study, although two or more consecutive negative tests should have been more convincing. For the Amsterdam group, we reported in a previous study that at least 20% of the infections in women and 6% in men were

Table 4 HPV ELISA absorbance values ($\times 1000$) for first serum specimens obtained from 42 women and 17 men in Amsterdam with multiple sexual partners

HPV type	Ig type	Women (n=42)				Men (n=17)				Differences p† value
		Median	Range	N pos*	(%)	Median	Range	N pos*	(%)	
6	A	149	0–1195	5	(12)	86	0–1097	2	(12)	
6	M	20	0–165	0	(0)	22	0–99	0	(0)	
6	G	218	0–735	29	(69)	68	0–276	5	(29)	<0.01
11	A	98	0–813	4	(10)	27	0–714	1	(6)	0.02
11	M	4	0–53	0	(0)	22	0–91	0	(0)	0.08
11	G	170	0–1080	25	(60)	36	0–267	3	(18)	<0.01
16	A	51	15–1173	11	(26)	37	4–144	3	(18)	0.09
16	M‡	214	32–807	NA§		97	22–484	NA§		0.01
16	G‡	52	0–395	12	(34)	20	5–127	1	(7)	0.02
18	A	34	0–1398	4	(10)	21	0–102	0	(0)	0.06
18	M	46	0–223	1	(2)	18	0–93	0	(0)	<0.01
18	G	44	0–677	13	(32)	10	0–112	2	(12)	0.07
33	A	31	0–1097	4	(10)	24	0–118	0	(0)	
33	M	23	0–98	0	(0)	6	0–83	0	(0)	
33	G	0	0–220	4	(10)	0	0–54	0	(0)	

*ELISA absorbance > cut off value; †p value (Mann–Whitney U test) for comparison between men and women; all p values not shown are >0.10; ‡specimens missing from 7 women and 3 men, all of whom were HPV DNA negative during follow up; §not applicable because of lack of cut off value.

persistent.²⁶ An intermittent pattern of infection was not observed in case the duration of the infection could be established.

The low prevalence at entry of antibodies to HPV 6 or 11 capsids in the teenage girls was consistent with the low prevalence of clinically overt condylomata and the fact that HPV 6 or 11 DNA was not detected in this group. Only one girl (HPV x positive at the last visit) had suspect condylomata at the second visit and she showed obvious condylomata in the vulva at the last visit; for this girl high IgG reactivity to HPV 6 and 11 at the first and last visit was found. The relatively high absorbance values obtained with HPV 6 and HPV 11 capsids in the teenage girls might be explained by cross reacting antibodies against non-sexually transmitted HPV types, although the origin of the weak antibody reactivities below the cut off could not be determined in the present study. After infection with HPV 16 or 18 as proved by PCR, the Karlstad girls showed a specific IgA and IgG response to the corresponding capsids. For the Karlstad girls, infection with HPV 33 was not significantly associated with the presence of serological reactivity to HPV 33 capsids.

The serological pattern for the STD clinic group was less clear, possibly because of frequent prior exposure of the participants to HPV as demonstrated by greater IgG values to HPV 6, 11, 16, and 18 than in the Karlstad girls. After acquisition of an HPV 6 or 11 infection as detected by PCR, a significant IgG response to HPV 6 and 11 capsids was observed.^{5 14 22} Likewise, HPV 16 specific IgA and IgG responses were detected in subjects who became infected with HPV 16. For HPV 18 the results of the Amsterdam cohort differed from those for the Karlstad girls. The higher IgM reactivity to HPV 18 capsids in HPV 18 DNA negative subjects than in HPV 18 DNA positive subjects might be attributed to a short lasting shedding of HPV 18 during infection precluding detection by PCR or to infection at a site that was not sampled. The value of both the HPV 16 and HPV 18 IgM assays has to be established in further studies. As in the Karlstad girls, serological reactivity to HPV 33 capsids was low in the Amsterdam cohort. For the Amsterdam subjects who became PCR positive for HPV 33, a specific serological response to HPV 33 was not apparent, although in some individuals a specific IgA response was detected. Such an apparent lack of response might be attributable to rapid clearance of HPV 33 from cervical epithelium or slow development of weak reactivity to HPV 33, as postulated previously.⁶ Another explanation might be that the HPV 33 assays performed less well.

The high prevalence of IgG and IgA antibodies to HPV 6, 11, 16, and 18 at entry in the group of heterosexuals with multiple partners might be caused by the high exposure to HPV in the past as indicated by the great number of sexual partners before entry into the study. The relatively poor specific serological responses apparent after HPV infection in comparison with the participants who re-

mained HPV DNA negative, might have several explanations. The greater reactivity of some antibodies in HPV DNA negative controls than in subjects who became HPV DNA positive might be ascribed to already existing antibodies, indicating that serology reflects past rather than present exposure.³¹⁻³³

Olsen *et al*³² showed that serum reactivity of antibodies to HPV 16 was above the cutoff in three out of 17 (17.6%) HPV 16 DNA positive individuals compared with 33 of 205 (16.1%) subjects who were either HPV DNA negative or positive for other HPV types. Wideroff *et al*¹³ also showed that 17% of HPV 16 DNA negative women were seropositive. Thus, studies must take into account the fact that the duration of genital HPV infections varies and that antibodies to HPV capsids may persist after the viral DNA has been cleared.

The observed patterns of the antibody responses for the various HPV types can be summarised as follows. For HPV 6 and HPV 11 infection, an IgA and IgG antibody response was apparent without a concomitant IgM response. IgG antibodies remained detectable over a long period of time. An IgA and IgG antibody response to HPV 16 was also detected; again specific IgG reactivity may remain high over a long period of time. In the Karlstad group, an IgA and IgG antibody response to HPV 18 was observed in the presence of a weak IgM response. However, the presence of HPV 18 DNA was not significantly associated with a detectable specific antibody response in the Amsterdam group. A low level antibody response was demonstrated for IgA, IgM and IgG after infection with HPV 33; IgA reactivity to HPV 33 capsids in some HPV 33 DNA negative individuals was even greater than that in those positive for HPV 33 DNA. In general, the reliability of the IgM assays using HPV capsid as antigen to demonstrate recent mucosal infection was low. In general, IgA has a much shorter half life than IgG. The multiple exposure to HPV might provide a biological explanation as to why the IgA levels were in some instances at about the same level as those for IgG.

The difference between men and women in the Amsterdam group at entry into the study was marked. We previously detected the sex difference in a serological study with HPV peptide antigens.⁵ A sex difference was later described by Carter *et al*⁷ and Greer *et al*³⁴ for antibodies to HPV 6 capsids. The ages of men (median 39.9 years) and women (median 30.9 years) in the present study differed significantly. However, in our study, age was not associated with antibody reactivity to any antigen (data not shown). The estimated number of lifetime sexual partners was markedly greater for women than for men.^{5 26} Thus, the sex difference might be explained by the difference in the number of estimated lifetime sexual encounters. This explanation is also consistent with the observations of Carter *et al*²¹ for HPV 6b capsids. The researchers showed that the average ELISA value for women with warts was significantly greater than that for men, and that the number of sex partners was associated with

ELISA reactivity for women. Eisemann *et al.*³⁵ also detected a sex difference for antibodies to HPV 11 capsids in control groups consisting of hospitalised individuals without known HPV infections and blood donors without information on HPV infection, but not in patients with condylomata acuminata. Recently, lower seroreactivity to HPV 16 virus-like particles in men attending STD clinics in Denmark and Greenland compared with seroreactivity in women attending the same clinics was reported by Svare *et al.*³⁶ As possible explanations biological differences—for example, the area of affected mucosal surface, and variables related to sexual life were given.

In conclusion, capsid based ELISAs revealed a specific IgA and IgG response after infection with HPV 6/11, 16, or 18. Only weak immune response was detected after infection by HPV 33. A sex difference in the antibody response detected in previous studies with assays based on HPV peptide antigens was confirmed by the present study with HPV capsid serology. Furthermore, serological methods may be more sensitive than PCR based assays to measure cumulative exposure to genital HPVs.

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