Persistent Antibodies to HPV Virus-Like Particles Following Natural Infection Are Protective Against Subsequent Cervicovaginal Infection with Related and Unrelated HPV

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Abstract

Whether persistent human papillomavirus (HPV) IgG antibodies following natural infection are protective against subsequent infection is unknown. In a cohort of 508 college women followed for 3 y, persistent seropositivity was defined as the presence of type-specific HPV virus-like particle (VLP) antibodies at ≥ 2 consecutive visits 1 y apart. Protection from incident infection with any HPV was conferred by persistent antibodies to HPV16 ($p=0.02$), HPV31 ($p<0.001$), HPV33 ($p=0.03$), HPV35 ($p=0.002$), HPV52 ($p=0.007$), HPV45 ($p=0.003$), and HPV53 ($p=0.01$). The risk of incident infection with species-specific HPV types was also decreased in women with persistent antibodies to any HPV type in that group, suggesting that exposure to HPV with persistent development of antibody response can be protective, and may explain the decreased efficacy of HPV vaccine in women with prior exposure.

Introduction

GENITAL INFECTION WITH HUMAN PAPILLOMAVIRUS (HPV) is the most common sexually transmitted disease, and it poses a significant public health burden throughout the world. Although most cervicovaginal HPV infections are self-limited, persistent infection with high-risk HPV types can cause cervical cancer, which is the second most common cancer in women worldwide, accounting for 250,000 deaths annually. The HPV virus-like particle (VLP) vaccine is a significant step towards the prevention of cervical cancer, and is highly efficacious in preventing high-grade cervical, vulval, and vaginal neoplastic lesions caused by HPV types 16 and 18, in women without prior evidence of type-specific infection (1,13).

It has been reported that antibodies present following natural infection with HPV16 are protective against subsequent infection with type-specific and related HPV types (11). Whether naturally-induced antibodies to other types of HPV confer similar protection is not known. The objective of this study was to investigate whether persistent immunoglobulin G (IgG) antibodies to HPV types 16, 18, 31, 33, 35, 45, 52, and 53 following natural infection are protective against subsequent incident infection with phylogenetically related and unrelated HPV types.

Materials and Methods

Study population

Female students at a state university in New Jersey were invited to participate in a longitudinal study investigating the natural history of cervicovaginal HPV infection as previously reported (2). Informed consent was obtained from the 608 women recruited through campus-wide advertisements between September 1992 and March 1994. Participants were followed at 6-mo intervals for 3 y. The study was approved by the Institutional Review Boards of the Albert Einstein College of Medicine and Rutgers University, and human experimentation guidelines of the U.S. Department of Health and Human Services were followed during the conduct of this study.

Data collection

At the baseline visit and every 6 mo, a Pap smear was obtained and participants filled out a self-administered questionnaire that included questions on sociodemographic and sexual history. Serum was collected from 10 mL of blood at the baseline visit and annually; 508 women had at least one follow-up visit after baseline, and constitute the study population for this analysis.

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HPV DNA detection

Exfoliated cervicovaginal cells were collected by lavage for HPV determination and typing for 39 types of HPV by Southern blot hybridization (SB), and MY09/MY11 polymerase chain reaction (PCR), as described previously (2,3). A sample was considered HPV DNA-positive if either assay was positive, and negative if both PCR and SB were negative. The HPV types detected included isolates from the α papillomavirus species groups HPV α -6 (HPV types 30, 53, 56, and 66), HPV α -7 (HPV types 18, 39, 45, 59, 68, 70, and 85), and HPV α -9 (HPV types 16, 31, 33, 35, 52, 58, and 67).

HPV antibody detection

HPV16, 18, 31, 33, 35, 45, 52, and 53 VLPs were produced in insect cells using a baculovirus system that expresses high levels of the HPV L1 protein that self-assembles into VLPs. VLPs were isolated as described previously (11), and used as antigen in a polymer enzyme-linked immunosorbent assay (ELISA) to measure type-specific IgG antibodies to HPV capsid proteins in subjects' serum.

The optical density (OD) cut-off points for seropositivity were determined by receiver operating characteristic (ROC) analyses to maximize the specificity among women who were DNA-negative for each of the different HPV types, as previously described (18). Serum samples were assayed in duplicate, and the median log absorbance was used to determine the subject's serostatus (11).

Statistical analyses

Analyses are based on 508 subjects as described above. Since the majority of serum samples were obtained annually, persistent seropositivity was defined as detectable type-specific HPV VLP IgG antibodies at ≥ 2 consecutive visits. Subjects without persistent antibodies to HPV VLPs (i.e., those with HPV type-specific antibodies detected at a single visit, or no antibody) were grouped together in the analysis. A subject had an incident HPV infection if DNA of a different HPV type, not detected in any previous sample, was detected in a cervicovaginal lavage sample. Subjects' follow-up time was expressed in person-years, and the relative risk (RR) of incident infection with various HPV types was calculated. Statistical analyses were performed using Stata software, version 9.2 (StataCorp LP, College Station, TX). *p*-Values are two-tailed, with *p* < 0.05 considered statistically significant for all analyses.

Results

Enrolled subjects had a mean age ($\pm$ SD) of 20 ± 3 y, and came from diverse ethnic backgrounds: 57% white, 13% Hispanic, 12% African-American, 10% Asian, and 8% other ethnicities. Most women, 88%, were sexually active and reported having one sexual partner in the preceding 6 months (range 0–8). At enrollment, 156 (31%) women were seropositive and remained so to one or more HPV type VLPs; HPV DNA was detected in cervicovaginal lavage specimens in 26% (*n* = 133) of the women. Data on baseline and persistent seropositivity to individual HPV VLP IgG for the 508 women are presented in Table 1. The prevalence of individual HPV DNA types isolated from cervicovaginal specimens at enrollment included: HPV16

TABLE 1. SEROPREVALENCE OF HPV ANTIBODIES

HPV type	Baseline ^a (<i>n</i> = 508)	Persistent ^a (<i>n</i> = 508)
α -PV9		
HPV16	72 (15.0)	44 (9.0)
HPV31	100 (20.0)	94 (18.5)
HPV33	27 (5.5)	27 (5.0)
HPV35	66 (13.5)	56 (11.0)
HPV52	25 (5.0)	28 (5.5)
α -PV7		
HPV18	65 (13.0)	48 (9.5)
HPV45	27 (5.5)	31 (6.0)
α -PV6		
HPV53	29 (6.0)	26 (5.0)
Any HPV	204 (42.0)	156 (31.0)

^aPresented as seropositivity, *n* (%).

α -PV9 group includes HPV types 16, 31, 33, 35, 52, 58, and 67.

α -PV7 group includes HPV types 18, 39, 45, 59, 68, 70, and 85.

α -PV6 group includes HPV types 30, 53, 56, and 66.

in 5%, HPV18 in 2%, HPV31 in 1%, HPV33 in 0.2%, HPV35 in 0.2%, HPV45 in 1%, HPV52 in 1%, and HPV53 in 3%, as previously reported (2). Demographic characteristics of the persistently seropositive and the comparison group are presented in Table 2. Persistent seropositivity to any type of HPV was significantly associated with an annual income of <\$40,000 (*p* = 0.012), and women reporting three or more lifetime male sexual partners (*p* < 0.05).

The association between persistent seropositivity and incident infections with related and unrelated types of HPV is presented in Table 3. The presence of persistent VLP IgG antibodies to most HPV types was protective against incident infection with related types of HPV, and also conferred some cross-protection against non-related HPV. Persistent antibodies to HPV 16, 31, 33, 35, 52, 45, and 53 were also associated with a statistically significantly reduced risk of incident infection with any HPV type (Table 3).

Discussion

In this study we used type-specific HPV VLPs to detect the presence of IgG antibodies to the HPV L1 capsid protein in serum from subjects participating in a natural history study of HPV. We found that the presence of persistent HPV anti-L1 VLP IgG antibodies was associated with a reduced risk of incident infection with type-specific, related and unrelated types of HPV. Similarly to the findings of Viscidi *et al.* (20), we failed to observe immune protection when we defined HPV seropositivity as the presence of VLP IgG at a single visit rather than at ≥ 2 sequential visits, suggesting that sustained, rather than transient, humoral responses following natural infection are required for protection against incident HPV infection (11).

Earlier studies have reported that >40% of young women acquire HPV infection within the first 3 y after sexual debut (10,21). Their risk of incident infection with a new HPV type is highest in the 6 mo following the first infection, and is primarily associated with an increased number of male sexual partners in the preceding year (11). Thus many young

TABLE 2. DEMOGRAPHICS OF PERSISTENT SEROPOSITIVE AND COMPARISON GROUPS

	<i>Not persistent</i> <i>n</i> = 352 (%)	<i>Persistent seropositive</i> <i>n</i> = 156 (%)	<i>Relative risk</i> (95% CI)
Age (y)			
≤19	225 (64)	88 (56)	1
>19	127 (36)	68 (44)	1.24 (0.95, 1.61)
Ethnicity			
White	208 (59)	84 (54)	1
Black/Hispanic/other	144 (41)	72 (46)	1.15 (0.89, 1.50)
Annual income			
<\$40,000	112 (32)	68 (44)	1
≥\$40,000	170 (48)	60 (39)	0.69 (0.52, 0.92)
Age at first vaginal sex			
≤16 y	177 (50)	92 (59)	1
>16 y	175 (50)	64 (41)	0.78 (0.59, 1.02)
Lifetime male partners			
0–2	205 (58)	59 (38)	1
≥3	147 (42)	97 (62)	1.77 (1.35, 2.33)
History of non-HPV STD			
No	305 (87)	131 (84)	1
Yes	47 (13)	25 (16)	1.15 (0.81, 1.63)

women are exposed to multiple types of HPV within the first few years of initiating sexual activity. In multivariate logistic regression models, older women have a lower risk of incident cervicovaginal HPV infection compared to younger women, despite adjusting for markers of sexual activity like the number of sexual partners in the preceding year, suggesting that acquired immunity from prior exposure may play a protective role (4,10).

The HPV major capsid protein, L1, contains multiple epitopes that are highly immunogenic and type-specific, but that also share significant homology with related types of HPV (5,7). The L1 protein is capable of self-assembling into VLPs, which are similar to HPV virions, but lack the viral genome (14). It has been shown that neutralizing antibodies against L1 demonstrate cross-reactivity to related types of HPV (6,12). This may explain why persistent HPV VLP IgG was associated with a reduction in incident infection with type and group-specific HPV, as observed in our study. We noted a trend towards protection against incident infection with the homologous HPV type for most categories, which reached statistical significance only in outcomes with larger numbers. This suggests that natural infection and potentially the HPV vaccine may confer protection that goes beyond the HPV types in the vaccine, to other members of HPV α -6, α -7, and α -9 species types.

What is the mechanism behind the broader cross-protection observed in our study? Humoral and cellular immune responses have been hypothesized to play a role in the persistence and clearance of genital HPV infection. It has been shown that CD4⁺- and CD8⁺-cell responses to genital HPV infection can be directed against the HPV L1 protein (15,17). These T-cell responses accompany humoral responses to genital HPV infection, and may have impacted future infection by other HPV types, as found in our study. In addition, antibodies to highly conserved residues on the L2 protein have been shown to be cross-neutralizing to diverse types of HPV *in vitro* (8). Our subjects may have had L2

antibodies from natural HPV infection (not measured), which may have played a role in conferring broad cross-protection from incident infection with unrelated HPV types. Another hypothesis is the development of an anamnestic response to HPV infection, as has been described for many viral pathogens. It has been suggested that persistence of naturally-occurring HPV antibodies requires ongoing antigenic exposure (12). It is possible that HPV antibody levels in some of our subjects had dropped below the threshold of seropositivity in the absence of persistent antigenic exposure, and incident infection with related HPV resulted in an anamnestic response leading to a boosting of antibody levels, prevention of subsequent viral spread, and rapid clearance of infection. Such an anamnestic response has also been described following vaccination with the quadrivalent HPV vaccine (16). Since our subjects were followed at 6-mo intervals, such transient infections may have been missed.

Vaccine trials have demonstrated a high efficacy of the quadrivalent HPV vaccine in the prevention of subsequent infection and genital lesions related to HPV6, 11, 16, and 18 in women naïve for HPV vaccine types (1,9,19). However, intention-to-treat analyses demonstrate a significantly lower vaccine efficacy in women with evidence of prior HPV16 or 18 infection at the time of first HPV vaccination (1). This suggests that the high efficacy of HPV vaccination is driven primarily by a reduction in disease outcomes in women without prior exposure to vaccine HPV types; rather than by a comparable reduction in both groups.

Our findings demonstrate that the development of persistent antibodies after natural cervicovaginal HPV infection confer protection against diverse HPV types; which suggests that the immune protection conferred by the currently-licensed quadrivalent and bivalent HPV vaccines may extend beyond the types of HPV included in these vaccines. Nevertheless, differences in immunity following natural infection compared to immunization with VLPs may limit the extent of cross-protection.

TABLE 3. ASSOCIATION OF PERSISTENT HPV SEROPOSITIVITY WITH SUBSEQUENT HPV INFECTION

Persistent seropositivity to													
HPV DNA acquisition	Persistent seropositivity	HPV16				HPV31				HPV33			
		Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p
α -PV9 group	No	464	11.4	4.6 (0.8, 185.5)	0.07	414	12.8	5.2 (1.3, 44.4)	0.004	481	11.8	a	0.04
	Yes	44	2.5			94	2.5			27	0.0		
α -PV7 group	No	464	10.7	1.4 (0.4, 7.0)	0.63	414	11.4	2.2 (0.8, 8.7)	0.10	481	11.3	2.7 (0.4, 108.3)	0.33
	Yes	44	7.7			94	5.0			27	4.2		
α -PV6 group	No	464	8.2	3.3 (0.5, 133.2)	0.21	414	8.5	3.3 (0.8, 28.8)	0.06	481	9.2	a	0.10
	Yes	44	2.5			94	2.6			27	0.0		
Any HPV	No	464	26.7	2.8 (1.1, 10.4)	0.02	414	27.5	22.6 (3.9, 901.8)	<0.001	481	26.7	3.5 (1.0, 29.7)	0.03
	Yes	44	9.6			94	1.2			27	7.5		
HPV18													
HPV DNA acquisition	Persistent seropositivity	HPV35				HPV52				HPV18			
		Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p
α -PV9 group	No	452	11.3	2.6 (0.7, 22.4)	0.15	480	11.5	3.2 (0.5, 127.2)	0.2	460	11.4	1.5 (0.5, 7.8)	0.5
	Yes	56	4.3			28	3.7			48	7.4		
α -PV7 group	No	452	11.4	1.7 (0.5, 8.4)	0.41	480	11.6	1.5 (0.4, 13.1)	0.6	460	11.0	1.1 (0.4, 4.3)	0.8
	Yes	56	6.8			28	7.6			48	9.9		
α -PV6 group	No	452	8.6	3.9 (0.6, 159.7)	0.13	480	9.2	a	0.09	460	8.5	3.3 (0.5, 135.6)	0.2
	Yes	56	2.2			28	0.0			48	2.5		
Any HPV	No	452	27.4	4.3 (1.4, 21.3)	0.002	480	26.7	7.3 (1.3, 291.1)	0.007	460	24.8	2.0 (0.8, 6.5)	0.09
	Yes	56	6.3			28	3.7			48	12.0		
HPV53													
HPV DNA acquisition	Persistent seropositivity	HPV45				HPV53				HPV53			
		Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p	Total no.	Events/ 100 py	RR (95% CI)	p
α -PV9 group	No	477	10.9	1.1 (0.4, 5.7)	0.89	482	11.7	a		11.7	a		0.06
	Yes	31	9.6			26	0.0			0.0			
α -PV7 group	No	477	11.0	1.2 (0.4, 5.9)	0.86	482	11.0	0.8 (0.3, 4.1)		11.0	0.8 (0.3, 4.1)		0.69
	Yes	31	9.5			26	13.5			13.5	a		
α -PV6 group	No	477	9.1	3.0 (0.5, 120.4)	0.27	482	9.4			9.4			0.11
	Yes	31	3.1			26	0.0			0.0			
Any HPV	No	477	26.1	a	0.003	482	27.0	6.5 (1.1, 260.8)		27.0			0.01
	Yes	31	0.0			26	4.1			4.1			

^aRelative risk could not be calculated due to zero events in the seropositive group. α -PV9 group includes HPV types 16, 31, 33, 35, 52, 58, and 67. α -PV7 group includes HPV types 18, 39, 45, 59, 68, 70, and 85. α -PV6 group includes HPV types 30, 53, 56, and 66.

py, person-years.

Acknowledgments

This work was supported by a grant from the NIH (no. AI-31055 to R.D.B.). Core support was given by the Cancer Research Center at Albert Einstein College of Medicine (grant CA-13330), and the Einstein/MMC Center for AIDS Research (grant AI51519).

Author Disclosure Statement

No conflicting financial interests exist.

References

1. Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) II Study Group: Quadrivalent vaccine against human papillomavirus to prevent high-grade cervical lesions. *N Engl J Med* 2007;356:1915–1927.
2. Burk RD, Ho GY, Beardsley L, Lempa M, Peters M, and Bierman R: Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. *J Infect Dis* 1996;174:679–689.
3. Burk RD, Kadish AS, Calderin S, and Romney SL: Human papillomavirus infection of the cervix detected by cervicovaginal lavage and molecular hybridization: correlation with biopsy results and Papanicolaou smear. *Am J Obstet Gynecol* 1986;154:982–989.
4. Burk RD, Kelly P, Feldman J, Bromberg J, Vermund SH, DeHovitz JA, and Landesman SH: Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. *Sex Transm Dis* 1996;23:333–341.
5. Chan SY, Delius H, Halpern AL, and Bernard HU: Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J Virol* 1995;69:3074–3083.
6. Combita AL, Bravo MM, Touze A, Orozco O, and Coursaget P: Serologic response to human oncogenic papillomavirus types 16, 18, 31, 33, 39, 58 and 59 virus-like particles in Colombian women with invasive cervical cancer. *Int J Cancer* 2002;97:796–803.
7. Combita AL, Touze A, Bousarghin L, Christensen ND, and Coursaget P: Identification of two cross-neutralizing linear epitopes within the L1 major capsid protein of human papillomaviruses. *J Virol* 2002;76:6480–6486.
8. Gambhira R, Karanam B, Jagu S, *et al.*: A protective and broadly cross-neutralizing epitope of human papillomavirus L2. *J Virol* 2007;81:13927–13931.
9. Garland SM, Hernandez-Avila M, Wheeler CM, *et al.*, and the Females United to Unilaterally Reduce Endo/Ectocervical Disease (FUTURE) I Investigators: Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases. *N Engl J Med* 2007;356:1928–1943.
10. Ho GY, Bierman R, Beardsley L, Chang CJ, and Burk RD: Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998;338:423–428.
11. Ho GY, Studentsov Y, Hall CB, Bierman R, Beardsley L, Lempa M, and Burk RD: Risk factors for subsequent cervicovaginal human papillomavirus (HPV) infection and the protective role of antibodies to HPV-16 virus-like particles. *J Infect Dis* 2002;186:737–742.
12. Ho GY, Studentsov YY, Bierman R, and Burk RD: Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prev* 2004;13:110–116.
13. Joura EA, Leodolter S, Hernandez-Avila M, *et al.*: Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomised clinical trials. *Lancet* 2007;369:1693–1702.
14. Kirnbauer R, Booy F, Cheng N, Lowy DR, and Schiller JT: Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. *Proc Natl Acad Sci USA* 1992;89:12180–12184.
15. Kobayashi A, Greenblatt RM, Anastos K, *et al.*: Functional attributes of mucosal immunity in cervical intraepithelial neoplasia and effects of HIV infection. *Cancer Res* 2004;64:6766–6774.
16. Olsson SE, Villa LL, Costa RL, *et al.*: Induction of immune memory following administration of a prophylactic quadrivalent human papillomavirus (HPV) types 6/11/16/18 L1 virus-like particle (VLP) vaccine. *Vaccine* 2007;25:4931–4939.
17. Passmore JA, Milner M, Denny L, *et al.*: Comparison of cervical and blood T-cell responses to human papillomavirus-16 in women with human papillomavirus-associated cervical intraepithelial neoplasia. *Immunology* 2006;119:507–514.
18. Studentsov YY, Schiffman M, Strickler HD, *et al.*: Enhanced enzyme-linked immunosorbent assay for detection of antibodies to virus-like particles of human papillomavirus. *J Clin Microbiol* 2002;40:1755–1760.
19. Villa LL, Costa RL, Petta CA, *et al.*: Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005;6:271–278.
20. Viscidi RP, Schiffman M, Hildesheim A, *et al.*: Seroreactivity to human papillomavirus (HPV) types 16, 18, or 31 and risk of subsequent HPV infection: results from a population-based study in Costa Rica. *Cancer Epidemiol Biomarkers Prev* 2004;13:324–327.
21. Woodman CB, Collins S, Winter H, *et al.*: Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *Lancet* 2001;357:1831–1836.

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Received June 23, 2009; accepted August 7, 2009.