Published in final edited form as:

Vaccine. 2011 March 3; 29(11): 2011–2014. doi:10.1016/j.vaccine.2011.01.001.

HPV16/18 L1 VLP Vaccine Induces Cross-Neutralizing Antibodies that May Mediate Cross-Protection

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Abstract

Human papillomavirus (HPV) L1 VLP-based vaccines are protective against HPV vaccine-related types; however, the correlates of protection have not been defined. We observed that vaccination with CervarixTM induced cross-neutralizing antibodies for HPV types for which evidence of vaccine efficacy has been demonstrated (HPV31/45) but not for other types (HPV52/58). In addition, HPV31/45 cross-neutralizing titers showed a significant increase with number of doses (HPV31, $p < 0.001$; HPV45, $p < 0.001$) and correlated with HPV16/18 neutralizing titers, respectively. These findings raise the possibility that cross-neutralizing antibodies are effectors of cross-protection observed for the HPV16/18 vaccine.

Keywords

Human papillomavirus; antibody; vaccine

1. Introduction

Human papillomavirus (HPV) L1 VLP-based vaccines are efficacious at preventing infections and pre-cancerous lesions caused by HPV vaccine-related types [1-2]. These vaccines have shown significant protection against infection with a limited number of phylogenetically-related HPV types but not against more distantly related types [1,3].

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Potential conflicts of interest: Troy J. Kemp, No conflict; Allan Hildesheim, No conflict; Mahboobeh Safaeian, No conflict; Joseph G. Dauner, No conflict; Yuanji Pan, No conflict; Carolina Porras, No conflict; John T. Schiller, listed as inventor on US government-owned patents covering the papillomavirus virus-like-particle-based vaccine technology. These patents have been licensed coexclusively to Merck and GlaxoSmithKline; Douglas R. Lowy, listed as inventor on US government-owned patents covering the papillomavirus virus-like-particle-based vaccine technology. These patents have been licensed coexclusively to Merck and GlaxoSmithKline; Rolando Herrero, No conflict; and Ligia A. Pinto, No conflict.

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Specifically, significant vaccine efficacy against 6-month persistent HPV type-specific infection has been demonstrated for HPV31 after vaccination with the alum-adsorbed HPV-6/11/16/18 Gardasil[®] vaccine and for HPV types 31, 33, and 45 after vaccination with the AS04-adsorbed HPV16/18 Cervarix[™] vaccine.

Based on pre-clinical papillomavirus animal models that show passive transfer of immunized sera is protective in naïve animals[4-5] and clinical trials showing that vaccinated individuals develop robust antibody titers[6-7], neutralizing antibodies are thought to be the primary immune mechanism of protection by HPV vaccination. However, efficacy studies have not fully elucidated the mechanism of protection against infection with vaccine and non-vaccine HPV types, and only a few studies have suggested cross-neutralization potential within sera collected from participants in HPV vaccine clinical trials[8-9].

We hypothesized that the observed cross-protection afforded by HPV vaccination would be due to vaccine-induced antibodies capable of cross-neutralizing HPV types for which evidence of cross-protection has been demonstrated but not HPV types for which such evidence is lacking. As additional corollaries, we hypothesized that the ability to neutralize cross-protective types would increase with number of vaccine doses received and that it would be higher for vaccinated individuals who developed higher neutralizing titers against the phylogenetically-related HPV vaccine types (i.e., HPV16/18).

2. Materials and Methods

We evaluated neutralizing antibody responses to HPV16 and HPV18 and to phylogenetically-related types in sera from a subset of women vaccinated with the HPV16/18 Cervarix[™] vaccine as part of the NCI-sponsored Costa Rica HPV Vaccine Trial (CVT), which has been described in detail elsewhere [10-11]. For the present study, we randomly selected 50 women from those who had at least 12 months of follow-up and serum available from the three time points of interest (month 0 (pre-vaccination), month 1 (approximately 1 month after first dose of vaccine), and month 12 (approximately 6 months after 3 doses of vaccine). Forty-six of these women received all three vaccine doses, three received two doses, and one received a single dose, and only the women who received the three vaccine doses were analyzed and presented. All participants provided informed consent, and the trial was approved by human subjects review committees at the National Cancer Institute (NCI) and Costa Rica.

For the 46 women that were selected based on receiving all three vaccine doses, we examined the levels of neutralizing antibodies to the vaccine-related types, two phylogenetically-related HPV types for which pseudovirions were available and validated and for which evidence of cross-protection was previously reported (HPV31, which is closely related to HPV16; HPV45, which is closely related to HPV18), and two phylogenetically-related HPV types for which such evidence is lacking (HPV52 and HPV58, both related to HPV16). Although, cross-protection was reported for another HPV16 closely-related type, HPV33, there are not any pseudovirions currently available for testing. Bovine papillomavirus type 1 (BPV) served as a negative control.

Neutralizing antibody titers were determined using a Pseudovirion (PsV)-based neutralization assay (SeAP-NA) as previously described[12]. The reported neutralization titers reflect the mean value of triplicate testing for each sample. Neutralizing antibody titers below our lowest dilution (1/10) were arbitrarily given a value of 5. SeAP-NA titers greater than or equal to 1/10 dilutions were defined as positive for the respective HPV types and are presented. The Kruskal Wallis test was used to determine statistical significance between

median titers ($p < 0.05$), and the Spearman's rank correlation coefficients were calculated to determine correlations between HPV16/18 and phylogenetically-related types.

3. Results

As previously reported[12], vaccination induced high neutralizing titers against HPV16 and HPV18, and titers increased with number of doses administered (Table 1). Prior to vaccination, only a minority of the participant's sera neutralized phylogenetically-related HPV types (11% for HPV31, 13% for HPV45, 26% for HPV52, and 11% for HPV58 – Table 1). Significant increases in median titers were observed after the three doses of vaccine were administered for HPV31 ($p < 0.001$) and HPV45 ($p < 0.001$) but not for HPV52 ($p = 0.14$) or HPV58 ($p = 0.09$). To account for intra-individual correlation, we also examined the difference in titers between visits 0 and 1 month, and 1 and 12 months following the start of the vaccination series. Similar patterns were observed with significant increases in median titers for HPV16, 18 and 31 (data not shown). The highest levels of response and proportion of responders were observed after all vaccine doses were administered (month 12 visit) for both HPV31 (median titer = 45.9; percent responders = 73.9%) and HPV45 (median titer = 19.8; percent responders = 60.9%) (Table 1). Neutralizing antibody titers observed for phylogenetically-related HPV types at the month 12 visit were typically 100-fold lower than those observed for HPV16 or HPV18 (Table 1). Anti-BPV neutralization titers, examined as controls, were, with one exception, negative for each time point tested (Table 1).

When we assessed the correlations between neutralizing titers against HPV16/18 and related HPV types from the same phylogenetic species (i.e., HPV16 correlation with HPV31, HPV52, and HPV58; HPV18 correlation with HPV45) following completion of the vaccination series (month 12 visit), we observed significant correlations between HPV16 and HPV31 neutralizing titers ($\rho = 0.54$, $p = 0.0001$) and between HPV18 and HPV45 neutralizing titers ($\rho = 0.31$, $p = 0.04$) (Table 2). This is consistent with the 6-month persistent HPV infection vaccine efficacy data reported for HPV31 (79%) and HPV45 (76%) within a HPV16/18 Cervarix™ vaccine study [1]. No significant correlations were seen between HPV16 and either HPV52 ($\rho = 0.05$, $p = 0.76$) or HPV58 ($\rho = -0.24$, $p = 0.11$), also consistent with the lack of 6-month persistent HPV infection vaccine efficacy observed for these two HPV types (Table 2)[1]. Consistent with these overall correlations, when data was evaluated within tertiles of HPV16 or HPV18 neutralizing titers, we observed that the overall median cross-neutralization titer was highest among individuals with the highest titer for the corresponding vaccine-containing HPV type (Table 3). Furthermore, the proportion of individuals with a titer ≥ 10 for anti-HPV31 and HPV45 SeAP-NA increased with increasing tertiles of HPV16 and HPV18 neutralization titers, respectively (Percent responders for HPV31 at month 12 visit = 53.3%, 68.8%, and 100% for individuals in the low, middle, and high tertiles of HPV16 responses, respectively; Percent responders for HPV45 at month 12 visit = 33.3%, 75.0%, and 73.3% for individuals in the low, middle, and high tertiles of HPV18 responses, respectively) (Table 3).

4. Discussion

Understanding the important factors associated with vaccine efficacy are essential in developing and improving the next generation of vaccines. Vaccine efficacy may be the result of a strong humoral response, cell-mediated response, or both. The effector mechanism of the HPV16/18 L1 VLP vaccine has been primarily associated with a humoral response due to the lack of a therapeutic benefit[11] as well as the high induction of anti-HPV16/18 antibodies produced following vaccination[6-7]. For this study, we focused on the antibody responses induced via the HPV16/18 Cervarix™ vaccine.

It is noteworthy that neutralization titers increased with vaccination schedule and correlated with titers observed for the vaccine types and the two HPV types (HPV31 and HPV45) for which 6-month persistent HPV infection vaccine efficacy has been demonstrated[1]. In contrast, we observed no significant increase in HPV52 and HPV58 neutralization titers following vaccination and no correlation with HPV16 titers, a finding consistent with the lack of 6-month persistent HPV infection vaccine efficacy observed for these two HPV types[1].

Our observation that levels of HPV31 and HPV45 neutralization titers following vaccination are modest and about 100-fold lower than those observed for HPV16 and HPV18 have two important implications. First, it suggests the need to track efficacy against HPV31 and HPV45 over time, since vaccine efficacy might wane with time as neutralization titers against HPV31 and HPV45 decrease. Second, it suggests that protection against HPV16 and HPV18 afforded by vaccination might be long-lasting, since titers higher than those observed for HPV31 and HPV45 are typically seen even years after vaccination[13].

To our knowledge, this is the first demonstration that sera from individuals vaccinated with the HPV16/18 Cervarix™ vaccine can cross-neutralize two phylogenetically-related HPV types for which 6-month persistent HPV infection vaccine efficacy has been demonstrated (HPV31 and HPV45) but not other types for which Cervarix™ vaccination does not appear to confer protection.

Acknowledgments

We would like to thank Brian Befano for all of his help with data management and statistical analysis for this project. We would also like to thank Tadahito Kanda for the plasmids used to generate the HPV52 and 58 pseudovirions, Susana Pang and Cynthia Thompson for pseudovirion production as well as the Costa Rica Vaccine Trial (CVT) group members.

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Financial support. The Costa Rica HPV Vaccine Trial is a long-standing collaboration between investigators in Costa Rica and the NCI. The trial is sponsored by the NCI, and conducted with support from the Ministry of Health of Costa Rica. This project has been funded in whole or in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. Other funding sources for this project were from the NCI Intramural Research Program and the National Institutes of Health Office for Research on Women's Health (ORWH). Vaccine was provided for our trial by GlaxoSmithKline Biologicals (GSK Bio, Rixensart, Belgium), under a Clinical Trials Agreement with the NCI. GSK Bio also provided support for aspects of the trial associated with regulatory submission needs of the company under grant FDA BB-IND 7920. Laboratory testing was performed at the NCI-sponsored SAIC-Frederick, Inc. HPV

Immunology Laboratory in Frederick, MD. The NCI and Costa Rica investigators are responsible for the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation of the manuscript. The NCI and Costa Rica investigators make final editorial decisions on this and subsequent publications; GSK Bio has the right to review and comment.

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Table 1

HPV Neutralizing Antibody Titers by Visit.

HPV Type	Visit	Overall Median (IQR)	p-value ^a	Titers ≥10, N (%) ^b	Median (IQR) among titers ≥10
16	Month 0	5 (5.0-11.2)	<0.001	13 (28.3)	18.6 (15.6-27.2)
	Month 1	215.2 (102.7-828.5)		46 (100)	215.2 (102.7-828.0)
	Month 12	4028.9 (2114.8-6923.1)		46 (100)	4028.9 (2114.8-6923.1)
18	Month 0	5 (5.0-5.0)	<0.001	3 (6.5)	25.4 (11.8-77.0)
	Month 1	218.8 (83.0-582.1)		46 (100)	218.8 (83.0-582.1)
	Month 12	3804.6 (1701.6-7389.2)		46 (100)	3804.6 (1701.6-7389.2)
31	Month 0	5 (5.0-5.0)	<0.001	5 (10.9)	119.0 (78.0-224.2)
	Month 1	5 (5.0-9.0)		11 (23.9)	81.9 (31.7-640)
	Month 12	45.9 (9.0-143.2)		34 (73.9)	100.5 (31.4-302.2)
45	Month 0	5 (5.0-5.0)	<0.001	6 (13.0)	25.6 (16.5-57.9)
	Month 1	5 (5.0-22.0)		19 (41.3)	34.4 (17.5-268.8)
	Month 12	19.8 (5.0-55.3)		28 (60.9)	51.5 (27.2-136.4)
52	Month 0	5 (5.0-10.2)	0.14	12 (26.1)	18.4 (15.1-77.8)
	Month 1	5 (5.0-17.0)		17 (37.0)	21.4 (16.2-70.4)
	Month 12	7.3 (5.0-40.1)		20 (43.5)	70.1 (15.1-174)
58	Month 0	5 (5.0-7.0)	0.09	5 (10.9)	45.1 (38.5-55.4)
	Month 1	5 (5.0-9.0)		11 (23.9)	47.9 (17.6-77.0)
	Month 12	6.1 (5.0-19.0)		15 (32.6)	30.5 (19.0-57.6)
BPV	Month 0	5 (5.0-5.0)	0.8	0	N/A
	Month 1	5 (5.0-5.0)		0	N/A
	Month 12	5 (5.0-5.0)		1 (2.2)	15.1 (15.1-15.1)

NOTE. SeAP-NA titers were evaluated from 46 participants at each time point, except BPV Month 1 (N=48). N/A refers to not applicable and IQR refers to interquartile range.

^aKruskal Wallis p-value which tests for the difference in median titers between visits.

^bN (%) are the number (and %) of women with titers ≥10.

SEAP-NA antibody titer correlations, sequence identity, and vaccine efficacy between HPV vaccine-related and cross types (Month 12).

Table 2

Vaccine-related Type	Vaccine-cross Type	Vaccine Efficacy (96.1% CI) ^a	Vaccine-cross Type Identity to HPV16 (%) ^c	Vaccine-cross Type Identity to HPV18 (%) ^c	N	Spearman (ρ)	(p-value)
HPV16	HPV31	79% (70.2-85.2) ^b	83	66	46	0.54	0.0001
	HPV52	7.8% (-8.7-21.8)	80	66	46	0.05	0.76
	HPV58	1.8% (-26.0-23.4)	80	66	46	-0.24	0.11
HPV18	HPV45	76% (60.4-85.7) ^b	67	88	46	0.31	0.04

^a - Vaccine efficacy (6-month persistent infection) results from Paavonen et. al, Lancet 2009; 374: 301–14.

^b - Vaccine efficacy was only significant (p<0.0001) for these vaccine cross-types.

^c - HPV vaccine-cross type amino acid identity results were obtained from Brown et. al, JID 2009; 199:926–35.

Table 3

Evaluation of HPV Cross-Related Neutralization Titers by HPV16/18 Neutralization Titers (Month 12).

	SEAP-31			
SEAP-16	N	Overall Median (IQR)	Titer ≥ 10 (%) ^c	Median (IQR) Among SEAP-31 (≥ 10)
Low Tertile (436.7-2439.8) ^a	15	18.3 (5.0-30.6)	53.3	29.2 (21.6-69.5)
Middle Tertile (2555.6-5808.0) ^a	16	62.9 (8.1-144.8)	68.8	104.7 (47.5-302.2)
High Tertile (6203.9-50716.6) ^a	15	140.6 (52.1-368.4)	100.0	140.6 (52.1-368.4)
<i>p-value</i> ^b		0.002		

	SEAP-45			
SEAP-18	N	Overall Median (IQR)	Titer ≥ 10 (%) ^c	Median (IQR) Among SEAP-45 (≥ 10)
Low Tertile (283.5-2165.4) ^a	15	5.0 (5.0-19.2)	33.3	29.0 (19.2-55.3)
Middle Tertile (2748.0-4996.6) ^a	16	32.0 (10.4-56.7)	75.0	45.8 (28.4-67.3)
High Tertile (6131.6-80647.9) ^a	15	35.1 (7.0-141.2)	73.3	59.2 (33.6-150.0)
<i>p-value</i> ^b		0.03		

SEAP refers to HPV type-specific antibody titers detected in SEAP-NA.

IQR-Interquartile range

^a-The values indicate the range (low-high) within each tertile.^b-Kruskal Wallis test for the difference in medians.^c-Titer ≥ 10 (%) are the percentage of women with titers ≥ 10 .