Research Article

Cancer Prevention Research

Durable Antibody Responses Following One Dose of the Bivalent Human Papillomavirus L1 Virus-Like Particle Vaccine in the Costa Rica Vaccine Trial

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Abstract

The Costa Rica HPV16/18 Vaccine Trial (CVT) showed that four-year vaccine efficacy against 12-month HPV16/18 persistent infection was similarly high among women who received one, two, or the recommended three doses of the bivalent HPV16/18 L1 virus-like particle (VLP) vaccine. Live-attenuated viral vaccines, but not simple-subunit vaccines, usually induce durable lifelong antibody responses after a single dose. It is unclear whether noninfectious VLP vaccines behave more like live-virus or simple-subunit vaccines in this regard. To explore the likelihood that efficacy will persist longer term, we investigated the magnitude and durability of antibodies to this vaccine by measuring HPV16- and HPV18-specific antibodies by VLP-ELISA using serum from enrollment, vaccination, and annual visits through four years in four vaccinated groups; one-dose ($n = 78$), two-doses separated by one month ($n = 140$), two doses separated by six months ($n = 52$), and three scheduled doses ($n = 120$, randomly selected). We also tested enrollment sera from $n = 113$ HPV16- or HPV18 L1-seropositive women prevaccination, presumably from natural infection. At four years, 100% of women in all groups remained HPV16/18 seropositive; both HPV16/18 geometric mean titers (GMT) among the extended two-dose group were non-inferior to the three-dose group, and ELISA titers were highly correlated with neutralization titers in all groups. Compared with the natural infection group, HPV16/18 GMTs were, respectively, at least 24 and 14 times higher among the two-dose and 9 and 5 times higher among one-dose vaccinees. Antibody levels following one-dose remained stable from month 6 through month 48. Results raise the possibility that even a single dose of HPV VLPs will induce long-term protection. *Cancer Prev Res; 6(11); 1242–50. ©2013 AACR.*

Introduction

In a community-based phase III trial of Cervarix in young Costa Rican women, we recently reported that, unexpectedly, the efficacy of two or even a single dose was similar to that of three doses in preventing persistent HPV16/18 infection over 4 years (1). Vaccination with two or even one vaccine dose could simplify the logistics and reduce the cost of vaccination in the developing world, where more than 85% of cervical cancers occur and it is the most common cause of cancer-related death in women (2, 3).

While we observed high vaccine efficacy among those who received one vaccine dose for 4 years, it is not known whether this protection was despite antibody titers that waned over time or whether antibody levels stabilized during follow-up. Addressing this question helps to determine whether the virus-like particle (VLP) vaccine behaves more like live attenuated vaccines, which often confer long-term protection, or simple protein vaccines, which require periodic boosting.

Most antiviral prophylactic vaccines are thought to function primarily by inducing virus-neutralizing antibodies (4). Antibody responses to vaccination, or to infections that clear, generally have a biphasic decline from their peak (5). Initially, there is a rapid decline, as short-lived antibody-secreting cells expire, followed by a gradual decline or plateauing in levels, with the antibodies predominantly produced by long-lived plasma cells in the bone marrow. The rate of decline during the second phase varies greatly across vaccines. Antibody titers induced by live-attenuated virus vaccines, for example, measles and mumps, tend to be very durable, despite being administered in one, or at most,

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two doses (6). In contrast, the half-lives of the antibody titers induced by simple protein-subunit vaccines, for example, tetanus and diphtheria, are much shorter, despite the routine administration of multiple doses. Consequently, multiple initial doses and periodic boosters are generally recommended for most protein-subunit vaccines, with profound effects on the cost of vaccination programs.

The ordered, repetitive, and dense display of epitopes on a virion surface is believed to induce exceptionally strong antibody responses. The commercial HPV prophylactic vaccines provide an opportunity to evaluate in humans the durability of the antibody response to virus-like displayed foreign antigens in the absence of infection because they are based on noninfectious VLPs with an ordered array of 72 pentamers of the L1 major capsid protein that structurally and antigenically mimics the surface of authentic virions (7). In phase III clinical trials, both Cervarix, a bivalent vaccine which contains HPV16 and HPV18 VLPs, and Gardasil, a quadrivalent one which contains HPV6, HPV11, HPV16, and HPV18 VLPs, induced durable antibody responses and strong protection from infection and anogenital neoplastic diseases by the vaccine-targeted types after administration of the recommended three doses over 6 months (8). After three doses, the geometric mean antibody titers (GMT) for Cervarix were shown to be essentially stable between years 2 and 8.4, the longest reported follow-up (9), supporting the conjecture that antigen structure might be more critical than active infection for inducing a durable antibody response. However, it remains unclear whether two doses, or a single priming dose, of a VLP vaccine is sufficient to induce an antibody response that stabilizes over time.

Here, we investigated the immunogenicity of Cervarix among women from The Costa Rica HPV16/18 Vaccine Trial (CVT) who received one or two vaccine doses to those who received the full three-dose schedule and to antibody levels among unvaccinated women who were seropositive at enrollment (before vaccination) presumably from natural infection. Using a VLP-based ELISA and an *in vitro* neutralization assay, we analyzed the serum antibody responses to the vaccine over 4 years, to evaluate whether the strong protection induced by two or even a single dose of Cervarix is likely to be sustained. The results obtained support the recently proposed "imprinted lifespan" model of plasma cell longevity (5) and an optimistic projection of the long-term efficacy of less than the recommend three doses of Cervarix and potentially of future virus-like display vaccines.

Materials and Methods

Study population

Participants and samples were from the publicly funded community-based randomized phase III CVT, described in detail elsewhere (10). In brief, between 2004 and 2005, a total of 7,466 women were consented and randomized to receive either the HPV or the hepatitis A vaccine and followed for 4 years. At enrollment and all follow-up visits, participants provided a serum sample, and for sexually experienced women, a pelvic exam was performed, and exfoliated cervical cells were collected for cytology and HPV DNA infection determination. The protocol was approved by the Institutional Review Boards (IRB) of the U.S. National Cancer Institute and the Costa Rican INCIENSA, and all participants signed IRB-approved informed consent forms.

Approximately 20% of women in the CVT received less than three doses of Cervarix or the control Hepatitis A vaccine, even though all women were randomized to receive three doses (1). There was balance between arms, and the HPV16/18 infection rate in the control group was similar for women who received one, two, or three Hepatitis A vaccine doses. Reasons for missing vaccine doses in both groups were largely involuntary, with major reasons being pregnancy and colposcopic referral (approximately 35% compared with 13% who missed doses due to refusals; ref. 1). In this study, we evaluated and compared four groups with sera available from all study visits: (i) received one HPV16/18 vaccine dose ($n = 78$); (ii) received two HVP16/18 vaccine doses at enrollment and 1 month later (two-dose$_{0/1}$, n = 140); (iii) received two HVP16/18 vaccine doses at enrollment and 6 months later (two-dose$_{0/6}$, $n = 52$); and (iv) 120 randomly selected participants who had received all three HPV16/18 vaccine doses. To compare the vaccine-induced antibody titers with those that develop after natural infection, we included a fifth group of women ($n = 113$) who were HPV16 or HPV18 L1 seropositive at enrollment. Antibody levels for this last group were evaluated at enrollment only, as it was established previously that antibodies in this group are stable over 4 years (data not shown).

Laboratory methods

HPV serological measurements. *ELISA* Serum was used to determine HPV16 and -18 IgG serostatus using an L1 VLP-based ELISA that measures polyclonal antibodies (11). The laboratory-determined seropositivity cutoffs for HPV16 and HPV18 were 8 EU/mL and 7 EU/mL, respectively. Five percent of the total number of samples ($n = 291$) was included as laboratory-blinded replicates as quality control (QC) samples. On the basis of the QC samples, the inter-plate and intra-plate coefficient of variation (CV) for both HPV16 and HPV18 were less than 20%.

SEAP neutralization assay HPV16 and HPV31 neutralization titers were determined using a previously described pseudovirion-based secreted alkaline phosphatase neutralization (SEAP) assay (12), using specimens collected at the last (48 month) clinic visit. The inter-plate and intra-plate CVs for both HPV16 and HPV31 neutralization assays were less than 20%.

HPV DNA measurements. *HPV DNA- SPF$_{10}$/DEIA/LiPA$_{25}$* HPV DNA detection and genotyping were performed at DDL Diagnostic Laboratory (13–15). Extracted DNA was used for PCR amplification with the SPF$_{10}$ primer sets. The same SPF$_{10}$ amplimers were used on SPF$_{10}$-DEIA–positive samples to identify HPV genotype by reverse hybridization on a line probe assay (LiPA; SPF10-DEIA/HPVLiPA25, version 1; Labo Bio-Medical Products, Rijswijk, the Netherlands), which detects 25 HPV genotypes.

Statistical analysis

Positivity at the laboratory's suggested cutoff was used to dichotomize results at each visit. Contingency tables and χ^2 tests were used to compare the different groups. We report GMTs and 25th and 75th percentiles at each visit by vaccine dose received. To exclude the rare outliers, we also present the 10th and 90th percentile antibody levels.

We compared the two-dose$_{0/1}$, two-dose$_{0/6}$, and one-dose group against the three-dose group and naturally infected group. Confidence intervals (CI) were estimated for the ratios of the type-specific GMTs measured at the last (48 month) visit for each of the two-dose and one-dose group compared with the three-dose group. In keeping with other HPV-immunogenicity studies (16, 17), we defined noninferiority for the two and one-dose compared with the three-dose schedule, if the lower bound of the CI of the GMT ratio was more than 0.5.

While, thus far, no correlates of protection have been identified, we reasoned that because of the near complete vaccine efficacy observed in those who received all three doses over 8.4 years of follow-up (9), the minimum antibody levels at 48 months might serve as a conservative immunogenicity benchmark of long-term protection. Thus, we compared the GMTs of the one- and two-dose groups with the antibody range in levels among women in the three-dose groups at 48 months. Among the three-dose group, the lowest antibody level at 48 months was 105 EU/mL for HPV16 and 28 EU/mL for HPV18. We then calculated the percentage of participants whose antibody levels dropped below these levels at 48 months, by vaccine dose.

To investigate stability of antibody responses with less than three doses, we determined the fraction of participants whose antibody levels decreased, increased, or remained constant between the 24 and 48 month study visits, and between the 36 and 48 month visits, for those receiving one, two$_{(0/1)}$, two$_{(0/6)}$, and three doses. We defined stability as antibody level at 48 months that either remained within two-fold of the level at 24 months or decreased/increased relative to the 24-month levels (same for 36 month comparison).

All statistical analyses were performed in Stata SE11.

Results

Participant characteristics are shown in Supplementary Table S1. There were no differences by age at vaccination, enrollment HPV infection, HPV16/18 seropositivity, smoking, or oral contraceptive use; however, women in the two-dose groups were more likely to report a higher number of lifetime partners.

Figure 1A and B show, respectively, HPV16- and HPV18-specific antibody GMTs over time for all study groups. For both HPV16 and HPV18, almost all participants in all vaccine dose groups were seropositive at approximately 1 month after receiving the first vaccine dose and remained seropositive throughout the 48 months follow-up period.

Detailed HPV16 and HPV18 antibody levels for each group at each study visits are shown in Table 1. The GMTs were comparable one month after the initial vaccine dose for subjects who received one, two, or three vaccine doses (HPV16 $P = 0.8$, HPV18 $P = 0.7$), indicating that all groups had similar intrinsic ability to respond to the vaccine.

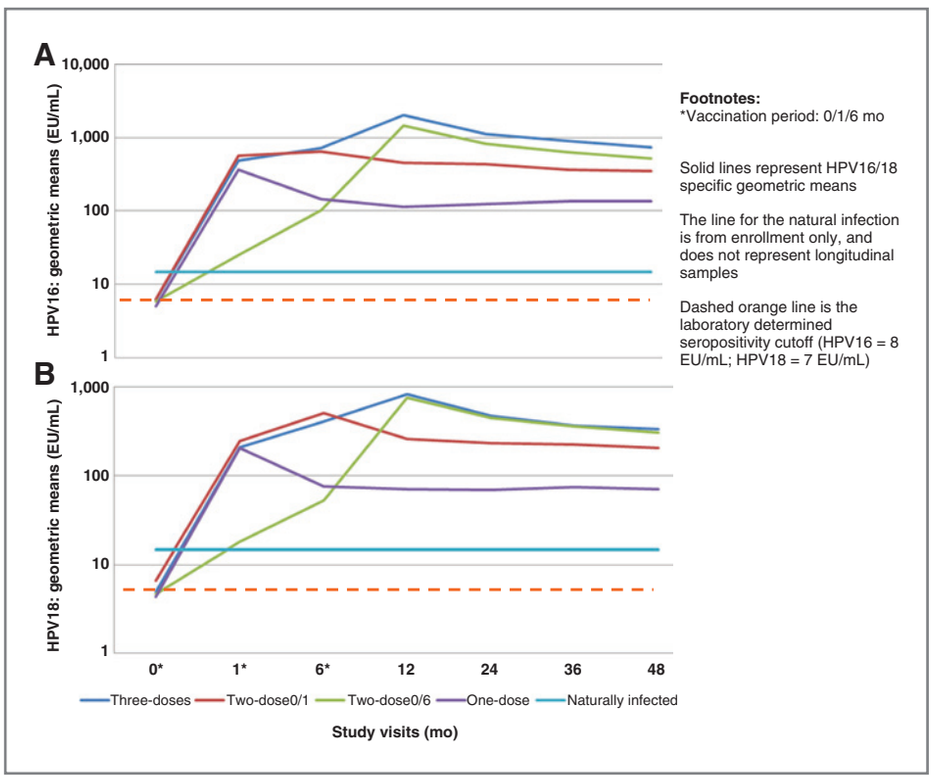

Figure 1. A and B, HPV16 (top) and HPV18 (bottom) specific antibody geometric means: by number of vaccine doses and study visit.

Table 1. HPV16 and HPV18 antibody levels, at all visits, by vaccine-dose

	3-dose	2-dose$_{0/1}$	2-dose$_{0/6}$	1-dose	3-dose	2-dose$_{0/1}$	2-dose$_{0/6}$	1-dose
Visit	**HPV16 antibody levels (EU/mL)**				**HPV18 antibody levels (EU/mL)**			
				Geometric means				
Enrollment	<LOD[a]	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	484.59	567.42	NA[b]	363.78	207.85	244.06	NA	207.01
6	723.78	655.45	102.42	145.27	407.77	510.99	52.72	75.79
12	2,035.95	459.06	1,483.91	114.82	826.83	258.24	763.19	70.60
24	1,115.26	433.82	837.19	124.34	470.77	234.94	446.09	68.55
36	899.16	369.57	641.86	135.78	369.30	224.22	357.61	73.81
48	748.25	352.99	519.99	137.49	334.55	206.75	304.97	70.21
				25th percentile				
Enrollment	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	212.37	173.52	NA	151.74	87.3	82.01	NA	87.65
6	387.21	340.19	32.89	57.6	227.99	224.19	23.54	31.09
12	1,075.52	223.9	849.66	48.94	387.03	114.84	445.72	31.37
24	641.08	231.15	492.79	45.76	261.56	101.26	195.65	30.76
36	493.39	203.92	362.17	50.18	214.17	111.58	181.35	37.64
48	438.17	214.18	283.93	59.61	200.42	108.03	155.02	35.29
				75th percentile				
Enrollment	<LOD	<LOD	<LOD	<LOD	<LOD	8·87	<LOD	<LOD
1	860.98	1,440.16	NA	491.02	362.69	452.26	NA	270.02
6	1,449.64	1,665.54	278.15	237.62	739.9	925	81.88	111.18
12	3,784.49	981.2	2,578.44	242.32	1,659.59	574.61	1,563.59	126.28
24	1,948.35	897.24	1,491.34	277.31	946.73	502.92	992.89	135.85
36	1,674.05	715.92	1,117.75	265.8	736.04	463.97	688.59	112.34
48	1,359.28	670.29	944.39	305	624.36	420.55	551.12	130.66
				10th percentile				
Enrollment	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	94.51	86.97	NA	83.55	49.95	42.33	NA	42.57
6	185.09	172.18	20.13	27.38	136.63	137.781	16.31	13.09
12	648.88	119.265	467.84	22.81	244.26	67.00	176.70	18.23
24	379.73	117.17	276.93	32.76	122.81	62.57	124.93	15.469
36	285.04	103.185	240.14	32.27	100.60	58.61	126.57	18.924
48	274.83	97.14	180.94	36.85	85.99	50.84	96.13	18.05
				90th percentile				
Enrollment	744.62	30.655	25.68	<LOD	12.39	51.83	10.21	10.05
1	41,258.15	12,704.86	NA	22,78.53	714.81	3,254.77	NA	1,184.962
6	8,841.191	2,822.65	1,115.71	1,969.53	1,520.91	2,090.46	177.02	310.551
12	19,493.58	1,922.23	5,567.41	730.16	2,519.34	1,152.09	2,348.99	462.298
24	7,578.942	1,588.88	1,928.73	834.36	1,314.06	967.92	1,304.85	447.95
36	6,898.308	1,400.06	1,723.84	759.6	1,103.11	875.68	1,117.35	454.82
48	5,380.097	1,160.3	1,345.07	703.17	970.32	711.43	1,013.60	358.99

[a]LOD, limit of detection.

[b]NA, not applicable—the two-dose group participants did not come for a 1-month visit, thus did not have blood drawn at 1 month for serological testing.

Antibody GMTs and 25th and 75th percentile levels were fairly stable between months 36 and 48 for the two-dose$_{0/1}$ group and the one-dose group for HPV16 and for the all dosage groups for HPV18. Moreover, GMTs for the one-dose group persisted at approximately 130 for HPV16 and approximately 70 for HPV18 between months 6 and 48. Among participants who were HPV16/18 seronegative at enrollment, GMTs for the one-dose group were approximately 100 for HPV16 and approximately 60 for HPV18 between months 6 and month 48 (data not shown). Individual kinetics by vaccine doses are shown in Supplementary Figs. S1A–S1H.

Table 2. HPV16 and HPV18 antibody levels at visit 48 months

HPV16					
Vaccine dose	*n*	GMTs (95% CI) EU/mL	GMT ratio (95% CI) (Alternate dose/3 dose)	GMT ratio (95% CI) (Alternate dose/NI)	*n* (%) above the lowest 3-dose levels at 48 months[a]
1	78	137.49 (106.16–178.07)	0.18 (0.14–0.24)	9.36 (6.26–13.81)	42 (53.85)
2 (0/1)	140	352.99 (302.03–412.55)	0.47 (0.38–0.58)	24.03 (17.78–32.52)	123 (87.86)
2 (0/6)	52	519.99 (422.02–640.70)	0.69 (0.53–0.89)	35.40 (22.84–54.16)	52 (100)
3	120	748.25 (647.63–864.49)	—	50.94 (37.48–69.15)	120 (100)
Natural infection	113	14.69 (11.12–19.40)	0.02	—	12 (10.62)
HPV18					
Vaccine dose	*n*	GMTs (95% CI) EU/mL	GMT ratio (Alternate dose/3 dose)	GMT ratio (Alternate dose/NI)	*n* (%) above the lowest 3-dose levels at 48 months[a]
1	78	70.21 (54.37–90.67)	0.21 (0.16–0.28)	4.79 (3.37–6.86)	63 (80.77)
2 (0/1)	140	206.75 (174.31–245.24)	0.62 (0.49–0.78)	14.10 (10.70–18.84)	136 (97.14)
2 (0/6)	52	304.97 (237.76–391.18)	0.91 (0.68–1.22)	20.80 (14.15–30.86)	52 (100)
3	120	334.55 (285.31–392.30)	—	22.82 (17.23–30.37)	119 (99.17)
Natural infection	113	14.66 (11.53–18.63)	0.04	—	31 (27.43)

[a]Calculated percentage of the participants that had values above 105 or 28 which are the lowest HPV16 and HPV18 level, respectively, at 48 month in CVT in the 3 doses group for whom vaccine efficacy has been observed.

Table 2 shows 48-month HPV16 and HPV18 GMTs and the GMT-ratios of each alternate dose compared with the three-dose schedule and to natural infection. At 4 years, HPV16 and HPV18 antibody levels of the two-dose$_{0/6}$ group were noninferior to the three-dose group (GMT ratio: HPV16$_{0/6}$ = 0.69, 95% CI: 0.53–0.89; HPV18$_{0/6}$ = 0.91, 95% CI: 0.68–1.22). Although the ratio for the two-dose$_{0/1}$ group did not meet the 0.5 threshold of the lower bound CI for noninferiority, the HPV16 and HPV18 antibody levels were high (GMT HPV16 two-dose$_{0/1}$ = 353 vs. three-dose = 748, GMT ratio = 0.47, 95% CI: 0.38–0.58; HPV18 two-dose$_{0/1}$ = 207 vs. three-dose = 335, GMT ratio = 0.62, 95% CI: 0.49–0.78). Compared with the natural infection group, the HPV16 and HPV18 antibody levels were at least 24 and 14 times higher, respectively, among those who received two-doses (Table 2).

At 4 years, the ratios of HPV16 and HPV18 GMTs of participants in the one-dose group to those in the three-dose group were 0.18 (95% CI: 0.14–0.24) and 0·21 (95% CI: 0.16–0.28), respectively. Nevertheless, this meant the one-dose HPV16 and HPV18 antibody GMTs were still 9 times and 5 times higher, respectively, than the natural infection group (HPV16 one-dose = 137, and natural infection = 15; $P < 0{\cdot}001$; HPV18 one-dose = 70, and natural infection = 15; $P < 0{\cdot}001$; Table 2).

Table 2 also presents the fraction of participants whose HPV16 or HPV18 antibody levels at 48 months was above the lowest antibody levels observed among women who received all three doses. By these criteria, at 48 months, 100% of women who received two doses$_{0/6}$ had HPV16 and HPV18 antibody levels within the range observed among women who received all three vaccine doses. Similarly, among the two-dose at 0/1 recipients, 88% and 97%, respectively, had HPV16 and HPV18 antibody levels within the range observed among women who received all three doses. Finally, among the one-dose group, 54% and 81% had HPV16 and HPV18 antibody levels at 48 months within the range observed among women who received all three doses. To further characterize the one-dose group, we explored whether antibody levels at 12 months (earliest available data after last vaccine dose administered) predicted antibody levels at 48 months, which shows that women whose antibody levels at 48 month fell below the lowest levels observed among women who received all three doses tended to already have reduced levels at 12 months (Supplementary Figs. S2A and S2B).

HPV16 and HPV18 antibody durability between 24 to 48 and 36 to 48 months are shown in Table 3. By our definition of less than a two-fold change, stability between 24 to 48 and 36 to 48 months was high in all dose groups (stability$_{24-48\ \text{months}}$ in the one dose group: HPV16 = 86%; HPV18 = 95%). Neither HPV16 nor HPV18 antibodies decreased between 24 to 48 or 36 and 48 months and increases in levels during these time intervals were few in all dose groups.

At 4 years, we observed strong correlations between HPV16 ELISA and neutralization titers, overall and by number of doses (Fig. 2A–E; all Spearman rank correlation coefficient over 0.7), and participants in all vaccine dose groups remained seropositive for HPV16 neutralization (Table 4), suggesting that the ELISA levels reflect the neutralizing potential. When we evaluated the HPV31 neutralization potential by vaccine dose, we observed that HPV31

Table 3. HPV16 and HPV18 antibody stability between months 24, 36, and 48 by number of doses

		1-dose	2-doses$_{(0/1)}$	2-doses$_{(0/6)}$	3-doses
			(Stable between 24–48 month)		
			n (%)		
HPV16					
	Stable	67 (85.90)	137 (97.86)	52 (100)	119 (99.17)
	Increase	11 (14.10)	3 (2.14)	0	1 (0.83)
HPV18					
	Stable	74 (94.87)	133 (95.00)	52 (100)	120 (100)
	Increase	4 (5.13)	7 (5.00)	0	0
			(Stable between 36–48 month)		
			n (%)		
HPV16					
	Stable	72 (92.31)	136 (97.14)	52 (100)	119 (99.17)
	Increase	6 (7.69)	4 (2.86)	0	1 (0.83)
HPV18					
	Stable	75 (96.15)	137 (97.86)	51 (98.08)	117 (97.50)
	Increase	3 (3.85)	3 (2.14)	1 (1.92)	3 (2.50)

HPV16 Spearman's rank correlation coefficient at 24- and 48-month visits was 0.88 ($P < 0.0001$).
HPV18 Spearman's rank correlation coefficient at 24- and 48-month visits was 0.90 ($P < 0.0001$).

neutralization increased with number of vaccine doses received (Table 4).

Discussion

Our study is the first to show that even a single HPV16/18 vaccine dose induces an antibody response that was readily detected in all vaccinated young women at end of the 4-year follow-up, although the titers were lower than after two or three doses and the number of one and two dose recipients was limited. Critically, the GMTs remained at a stable plateau level from 6 months post vaccination until study end that was higher than the GMTs induced from natural infection. Our

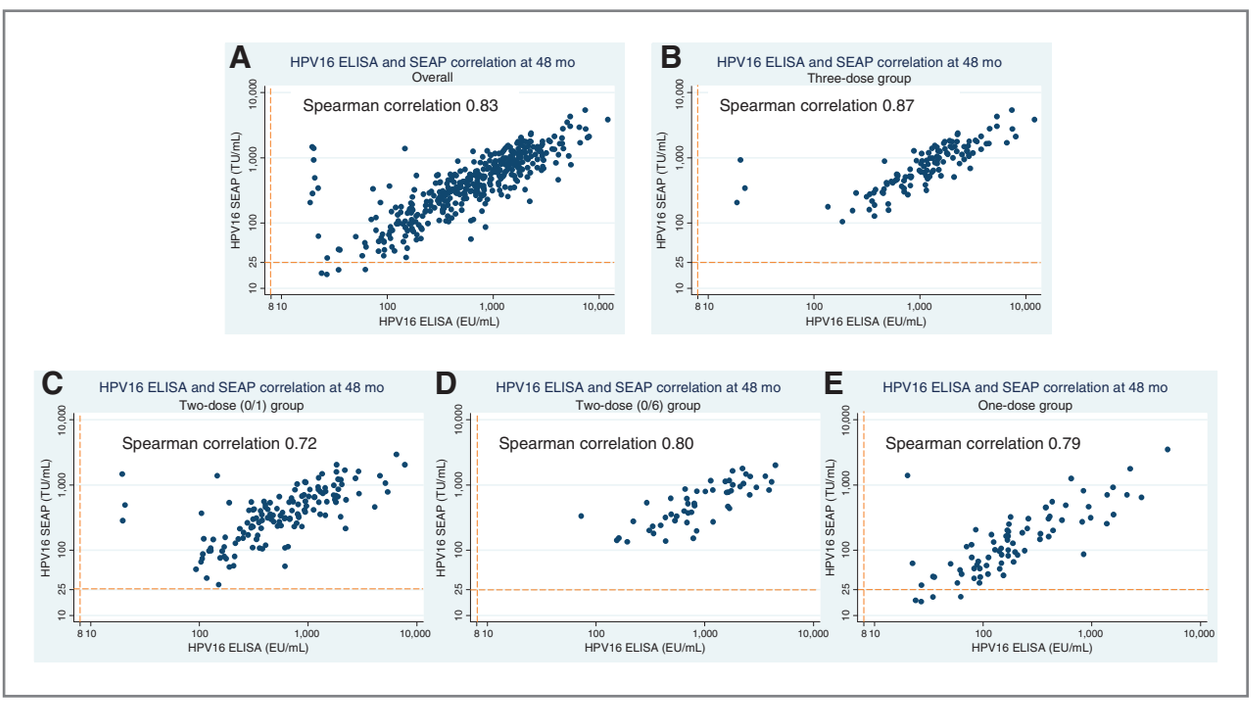

Figure 2. A–E, Correlation between HPV16 ELISA and SEAP at 48 months – overall and by dose-group. For all the graphs (A–E), the orange dashed lines represent the assay seropositivity cutoff (ELISA16 = 8 EU/mL and SEAP16 = 25.1 TU/mL).

Table 4. Seropositivity[a] by HPV16 ELISA and HPV16-neutralization and HPV31-neutralization at 48 months by number of doses

	1-dose ($n = 78$)	2-doses$_{0/1}$ ($n = 140$)	2-doses$_{0/6}$ ($n = 52$)	3-doses ($n = 120$)	
	n (%)	*n* (%)	*n* (%)	*n* (%)	*P*
HPV16 ELISA	78 (100)	140 (100)	52 (100)	120 (100)	0.9
HPV16 neutralization	75 (96.15)	137 (97.86)	52 (100)	117 (97.50)	0.6
HPV31 neutralization	19 (24.36)	54 (38.57)	26 (50.00)	76 (63.33)	<0.001

[a]Positivity defined at the laboratory determined cutoff (HPV16 ELISA = 8 EU/mL, HPV16 neutralization = 15·1 TU/mL; HPV31 neutralization = 10 TU/mL).

findings for the two dose groups are similar to two-dose immunogenicity studies of the GSK (18) and Merck (19, 20) vaccines with shorter follow-up. Coupled with our previous demonstration that a single vaccine dose of Cervarix induced strong protection in the CVT, the findings suggest that antibody titers induced by two or three vaccine doses may be substantially higher than needed for long-term protection.

The durability of observed antibody response to a single dose is similar to that of an attenuated live-virus vaccine, rather than a more typical protein immunogen, whose antibody titers continue to decline. We observed durable antibody responses in women who were seronegative prevaccination, indicating that the longevity of the response after a single vaccine dose was not dependent on prior natural infection. To our knowledge, similar findings have not been reported for other noninfectious vaccines in humans. We speculate that this durable response may be attributable, at least in part, to the HPV vaccines being the first noninfectious licensed vaccines whose virus-like surface characteristics truly mimic the ordered, high density structure of authentic capsids. Vaccines based on inactivated whole viruses, such as injected polio and hepatitis A vaccines, may have attenuated virus-like character with respect to closely spaced epitopes, because of the random formaldehyde-induced cross-linking of surface peptides associated with the inactivation process (21). To ensure long-term protection, such vaccines are administered in multiple doses. Similarly, the S proteins floating in the lipid bilayer of hepatitis B particle vaccines may not present the ordered dense array of antigens that characterize authentic virions, and antibody titers after three doses of these vaccines continue to wane over time. An anamnestic response by memory B cells can prevent disease by preventing breakthrough infections in vaccinees who become seronegative, although a proportion of individuals do not generate an anamnestic response to the booster vaccine (22).

The particular nature of the antigen, which may promote induction of T helper responses, and the ability of the HPV VLPs to induce innate immune activation in a variety of immunocytes probably also contribute to the strong and durable B-cell responses induced by the HPV VLPs (23, 24). While the HPV vaccine has been shown to be immunogenic even in the absence of an adjuvant (25), Cervarix's two adjuvants, a simple aluminum salt and the toll-like receptor (TLR)-4 agonist monophosphoryl-Lipid A, may contribute additional important signals to the B cells (26). Gardasil does not contain a TLR agonist adjuvant and so it is uncertain if it would be as effective after a single dose.

It was somewhat unexpected to find similar quantitative relationship between ELISA and *in vitro* neutralization titers among women receiving one, two, and three doses. Infection inhibition may be more dependent on antibody affinity than simple antigen binding, and we recently found a clear increase in antibody avidity with each of the two boosts in CVT participants who received all three doses (27, 28). Possible explanations for the observed one-dose neutralizing activity include: (i) low avidity antibodies may be able to neutralize HPV virions; and (ii) B-cell receptor affinity-dependent selection for plasma cell survival may be the predominant driving force of antibody avidity long term, regardless of the number of doses.

The finding that even a single dose of the VLP vaccine induces a stable plateau level of antibodies supports a recently proposed model by Amanna and Slifka (5), postulating that survival potential of a plasma cell and its level of antibody production are determined during its initial interaction with antigen, in combination with concurrent costimulatory signals provided by T helper cells and activation molecules such as TLR ligands. Our study clearly establishes that activation of memory B cells by a booster dose is not required for induction of persistent antibody responses by a protein-subunit vaccine. It seems unlikely that exposure to HPV virions contributes substantially to the longevity of the VLP antibody response in most vaccinees. First, it is doubtful that most of the CVT participants would be routinely exposed to the viruses, particularly to HPV18, which is relatively uncommon (10, 29). If natural virus exposure were important, one would expect considerable heterogeneity in the durability of the individual antibody responses, but it was consistent and indistinguishable for the two HPV types in most women. Second, boosting by natural exposure would be expected to induce at least a transient increase in the antibody titer after the initial response to vaccination has waned in individual women. However, such increases in titer were infrequently observed. Third, similarly persistent antibody levels were observed among participants who were HPV16/18 seronegative prevaccination.

The durability and magnitude of the antibody response to a single vaccine dose may have important implications for VLP vaccination strategies, although it would be desirable to verify our findings in independent studies with formal randomization by dose. The high efficacy after single dose suggests that long-term protection may not require the 5-fold higher titers induced by three doses of the vaccine. Fewer doses would be less expensive and logistically easier to deliver, therefore increasing vaccine accessibility worldwide. The findings also suggest that second generation vaccines might be strongly protective even if they do not induce the high levels of antibodies induced by the licensed vaccines as used according to current recommendations (30). However, one potential disadvantage of alternate vaccine schedules is that protection against phylogenetically related HPV types, which is probably attributable to cross-neutralizing antibodies, is likely to be lower than that observed with the standard three-dose schedule. We previously reported that cross-neutralizing titers against HPV31 and HPV45 are much lower, or undetectable, compared with neutralizing titers against vaccine-targeted types in women receiving three doses (27), and we found here that lower numbers of vaccine doses were correlated with a lower percentage of sera that exhibited cross-neutralizing activity against HPV31. This potential limitation should be less important for second generation vaccines that target a larger number of oncogenic HPV types. For the vaccinology field, our results provide strong support for the conclusion that virus-like display of antigens is a remarkably effective and efficient strategy for safely inducing durable antibody responses in humans that should be considered in the design of future vaccines against other targets susceptible to antibody-mediated intervention.

Disclosure of Potential Conflicts of Interest

J. Schiller received royalties on U.S. government-owned patents. D.R. Lowy is a named inventor on U.S. government-owned HPV vaccine patents that are licensed to GlaxoSmithKline and Merck and is entitled to limited royalties as specified by federal law. M. Schiffman has GSK agreement with NCI. L.-J. van Doorn has ownership interest (including patents) in DDL Diagnostic Laboratory. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The Costa Rica HPV Vaccine Trial is a long-standing collaboration between investigators in Costa Rica and the National Cancer Institute (NCI).

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