RESEARCH PAPER

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Comparison of adaptive and innate immune responses induced by licensed vaccines for Human Papillomavirus

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Two HPV virus-like particle (VLP) vaccines, HPV-16/18 (GlaxoSmithKline, Cervarix®) and HPV-6/11/16/18 (Merck, Gardasil®), are currently licensed in the United States. Given the similar antigenic content but different adjuvant formulations in the 2 vaccines, they provide an efficient method for evaluating adjuvants and comparing the kinetics of the innate and adaptive immune responses. We randomized women to receive either Cervarix® or Gardasil®, followed 6 month vaccination delivery schedules per manufacturer's recommendations, and analyzed the humoral immune response, T cell response, and circulating plasma cytokine levels in response to vaccination. Cervarix® recipients had higher anti-HPV-16 antibody and neutralization titers at month 7, and elevated anti-HPV-18 antibody and neutralization titers at months 7 and 12. Antibody avidity was similar for the 2 vaccines. HPV-31 was the only phylogenetically related non-vaccine HPV type, for which there is evidence of cross-protection, to be cross-neutralized and only in response to Cervarix®. Comparing $CD4^+$ T cell cytokine responses at month 12, there was a trend of increased levels of IL-2 and TNF-α in the Cervarix® groups versus the Gardasil® groups that was consistent across all 4 tested HPV types (16/18/33/45). Elevated levels of circulating plasma cytokine/chemokines were observed post first vaccination in Gardasil® recipients and proinflammatory cytokines were elevated following 1st and 3rd Cervarix® vaccinations. Cervarix® and Gardasil® are both highly immunogenic vaccines. Higher antibody levels and CD4 T cell responses were achieved with Cervarix® after 3 doses, although similar affinity maturation was measured for the 2 vaccines. The clinical implications of the differences in immune responses are unknown.

Introduction

HPV is one of the most common sexually transmitted infections in the United States. There are more than 130 subtypes of HPV, of which 15 are classified as oncogenic and are important causes of anal, cervical, and oropharyngeal cancers in men and women.[1] HPV-16/-18 account for 70% of cervical cancer cases,[2] and 25% of cervical cancer cases are associated with the closely related non-vaccine types within the Alpha-papillomavirus species group A9 (HPV 16-like: 31/33/35/52/58) and A7 (HPV 18-like: 39, 45, 59, 68).[1,3] Globally, there are an estimated 530,000 cases diagnosed with more than 275,000 cervical cancer deaths each year.[4]

There are 2 licensed HPV vaccines (HPV-16/18/6/11, Gardasil® and HPV-16/18, Cervarix®) with excellent efficacy against vaccine types and some degree of protection against HPV-16 and HPV-18 phylogenetically related non-vaccine types.[5–8] Both HPV vaccines are virus-like particles (VLPs) based on the major HPV capsid protein L1. Antigenically the vaccines are very similar but are produced in different systems and contain different adjuvants. The Gardasil® vaccine is adjuvanted with aluminum hydroxyphosphate sulfate. The Cervarix® vaccine is formulated with AS04, which contains aluminum hydroxide salts and the TLR4 agonist MPL (3-O-desacyl-4'-monophosphoryl lipid A). The AS04 adjuvant is likely to play a role in the higher immunogenicity observed in Cervarix® recipients but because of other differences in production, it is difficult to attribute these results solely to the adjuvant.[9–11]

Several studies have compared immunogenicity responses between Cervarix® and Gardasil®. The majority of work has

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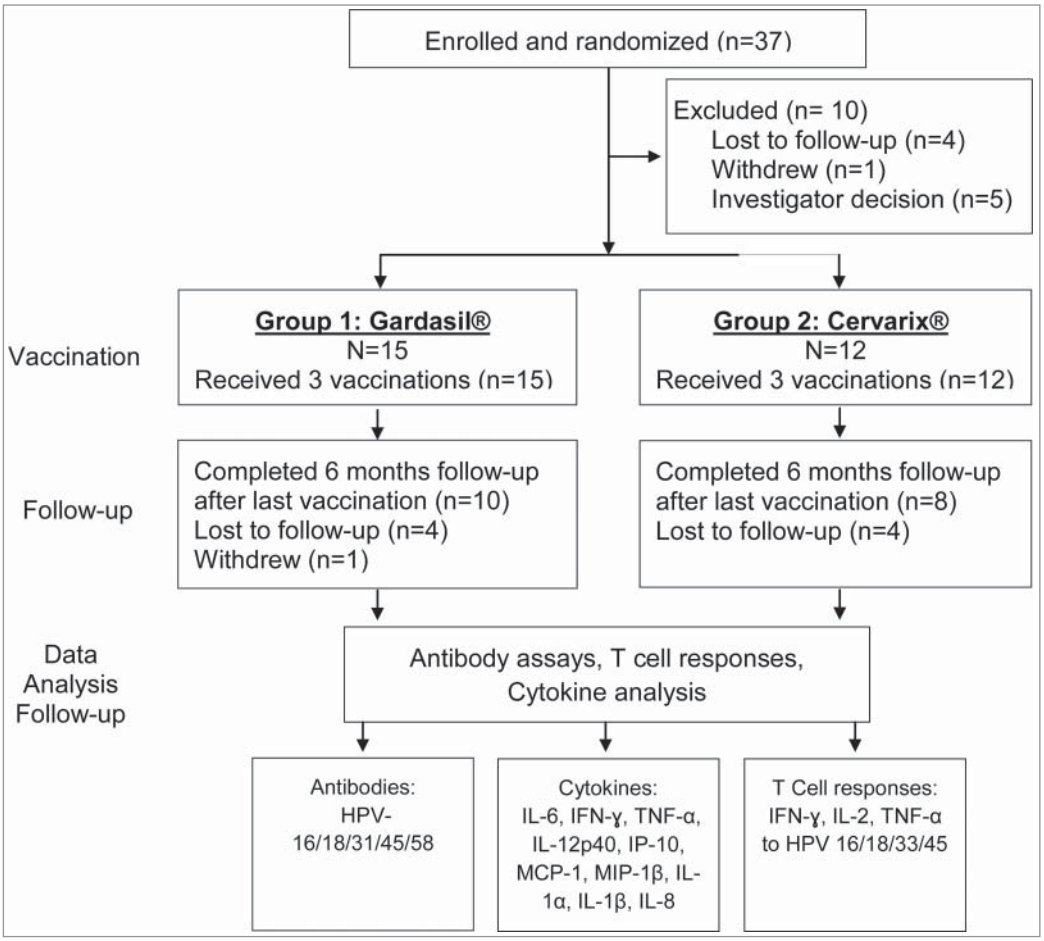

Figure 1. VRC 902 CONSORT Diagram. Subject randomization is shown.

focused on the magnitude of antibody responses as these are likely the main mediators of protection.[9,10] While both vaccines result in robust neutralizing antibody production against vaccine types, Cervarix® induces significantly higher serum HPV-16 and HPV-18-specific antibody titers as well as specific memory B-cell frequencies.[11,12] Comparisons of antibody avidity and early cytokine responses induced by the 2 vaccines have not previously been evaluated. Differences in T-cell responses to the vaccine types (HPV-16 and HPV-18) and non-vaccine types (HPV-31 and HPV-45) between the vaccines have been observed, but it is unclear exactly how these differences impact underlying mechanisms of protection.[12,13] It is known that HPV VLP vaccination elicits a broad spectrum of ex vivo cytokine responses in whole blood samples, and analysis of cytokine production in L1 VLP-stimulated peripheral blood mononuclear cells (PBMCs) following HPV-16 L1 vaccination showed that cytokine responses followed similar patters as neutralizing antibody responses.[14] However, the role of circulating cytokines in immunogenicity and long-term protection remains unknown. 'The Vaccine Research Center (VRC) trial described here (CONSORT diagram shown in **Fig. 1**) was conducted to comprehensively evaluate and compare humoral and cellular immune responses induced by Cervarix® and Gardasil® recipients.

Results

Antibody titers

HPV-16 and -18 ELISA. Antibody responses to vaccination with either Cervarix® or Gardasil® are shown in **Figure 2**. There is a trend of elevated antibody titers in Cervarix® recipients at the later time points. The geometric mean titer (GMT) was 2.8-fold greater ($p = 0.03$) for anti-HPV-16 at month 7 in Cervarix® than in Gardasil® recipients, and this trend continues at months 12 and 24. Anti-HPV-18 antibody GMTs were statistically higher at months 7 and 12 in Cervarix® recipients (fold differences of 3.6 and 4.7, $p = 0.015$ and $p = 0.02$, respectively) than Gardasil® recipients. Peak levels of anti-HPV-16 and -18 antibodies were achieved after the third dose of Cervarix®, in contrasts to Gardasil® where maximum response is seen after the second dose.

HPV SeAP Neutralization. The kinetic patterns of HPV pseudovirion neutralization for Cervarix® and Gardasil® are shown in **Figure 3**. HPV-16 and -18 neutralization results are consistent with the ELISA titers (**Fig. 2**), as previously observed.[15] Both anti- HPV-16 and -18 antibody neutralization levels are significantly (at least 3 times) greater in Cervarix® recipients vs. Gardasil® recipients at month 7. For all time points the HPV-18 neutralization levels are higher for Cervarix® with statistically significant difference at months 1, 7, and 12. Cervarix® induces neutralization titers against the phylogenetically related type HPV-31 (**Fig. 3C**) at month 7 after 3 doses of vaccine while Gardasil® does not induce significant levels of cross neutralization antibody titers against HPV-31. Neither vaccine induced neutralizing antibody against HPV-45 (**Fig. 3D**). Gardasil® induces significantly higher titers of neutralizing antibodies to HPV-58 (**Fig. 3E**) at months 1, 3, and 7 compared to Cervarix® with titers near the limit of detection of the assay. However, the titers to HPV phylogenetically related types were 2–4 logs lower than the titers to the HPV vaccine types (HPV-16 and HPV-18).

HPV-16 and -18 Avidity. The kinetic patterns of HPV-16 and -18 avidity for Cervarix® and Gardasil® are shown in **Figure 4**. In all cases highest avidity indices were achieved after 3 doses of vaccine. At month 3, avidity indices induced by

Gardasil® were statistically higher ($P < 0.03$) than those induced by Cervarix® for both HPV-16 and HPV-18. Conversely, at month 7 HPV-16 avidity was higher in Cervarix® recipients (1.18-fold, p = 0 .04). At months 12 and 24 avidity indices are similar for both vaccines for HPV-16 and −18.

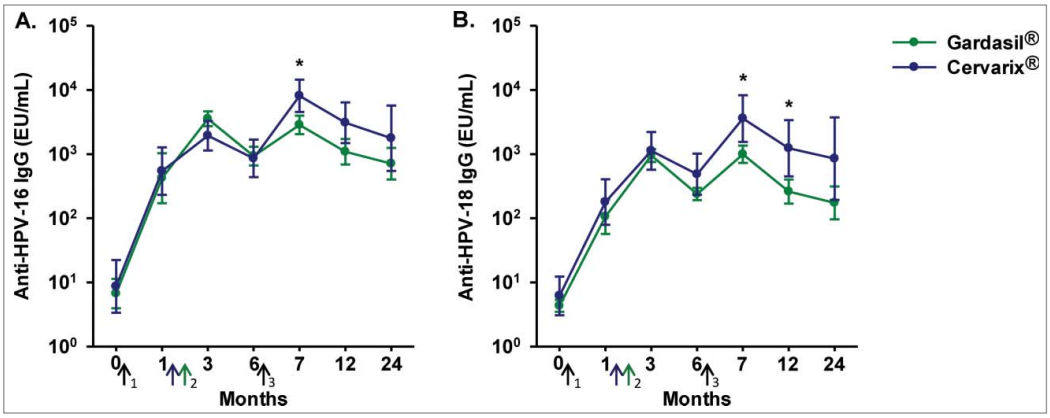

Figure 2. ELISA antibody levels (IgG) for anti-HPV-16 (**A**) and anti-HPV-18 (**B**). *$P < 0.05$ (Mann-Whitney). Arrows indicate time of first (month 0), second (Cervarix® month 1, Gardasil® month 2), and third (month 6) vaccinations with respect to time points. Data presented as Geometric mean Titer (GMT) (95% CI).

HPV L1-specific T cell responses

IFN-γ ELISpot. An ELISpot assay was performed to measure the IFN-γ response PBMCs stimulated with L1 peptide pools. Vaccination with either Cervarix® or Gardasil® increases the frequency of IFN-γ producing cells within PBMCs from pre vaccination levels to post vaccination levels in response to vaccine (HPV-16 and -18) and non-vaccine HPV types (HPV-33 and -45) (Figure S1).

T Cell Responses by Intracellular Cytokine Staining (ICS). To further characterize the HPV L1-specific T cell-mediated immune responses, we analyzed HPV L1-specific $CD4^+$ and $CD8^+$ T cell cytokine responses to Cervarix® and Gardasil® at baseline and post vaccination by flow cytometry. No subjects had detectable HPV L1-specific $CD4^+$ T cell responses prior to vaccination. As a result of limited responses, $CD8^+$ T cell cytokine responses were not analyzed further (data not shown).

Comparing $CD4^+$ cytokine responses at month 12, there is a trend of increased production of IL-2 and TNF-α in the Cervarix® group versus the Gardasil® group that is consistent across all 4 HPV types (**Fig.** 5). This trend is statistically significant in the HPV-18 IL-2 response, where the Cervarix® response

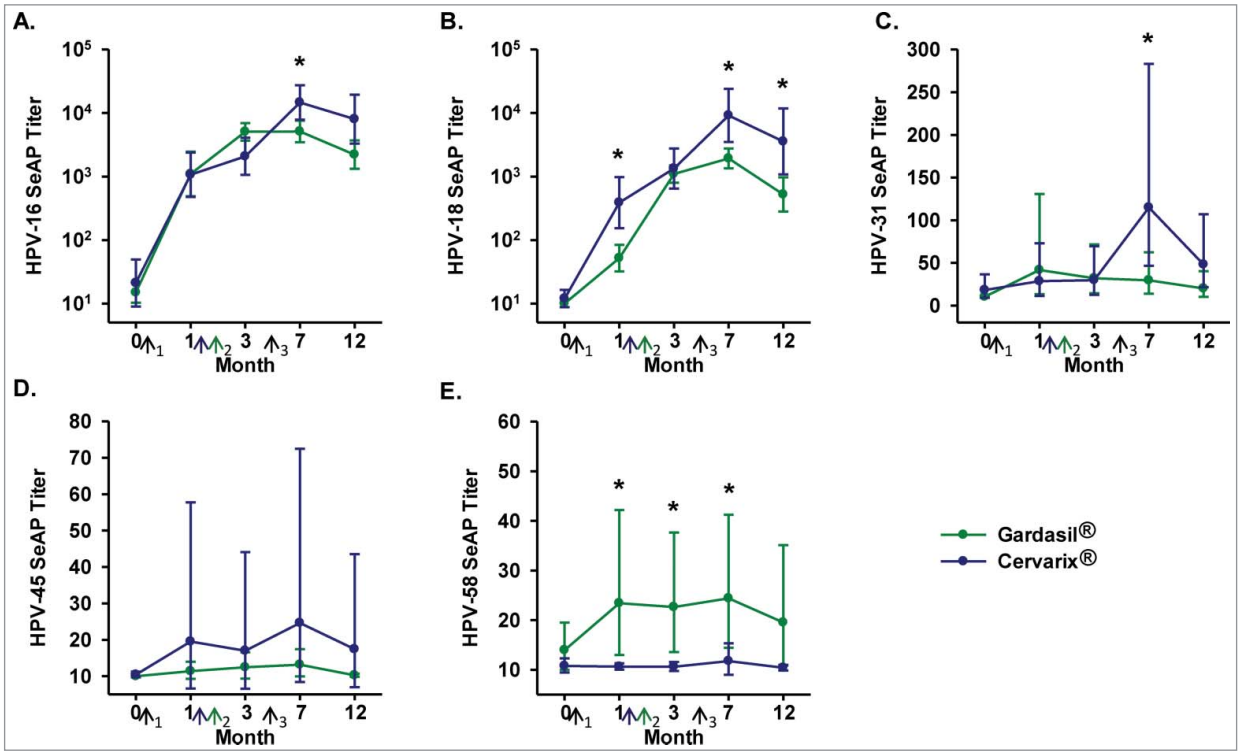

Figure 3. SeAP antibody titers for HPV-16 (**A**), -18(**B**), -31(**C**), -45(**D**), and -58(**E**). *$P < 0.05$ (Mann-Whitney). Arrows indicate time of first (month 0), second (Cervarix® month 1, Gardasil® month 2), and third (month 6) vaccinations with respect to time points. Data expressed as GMT (95% CI).

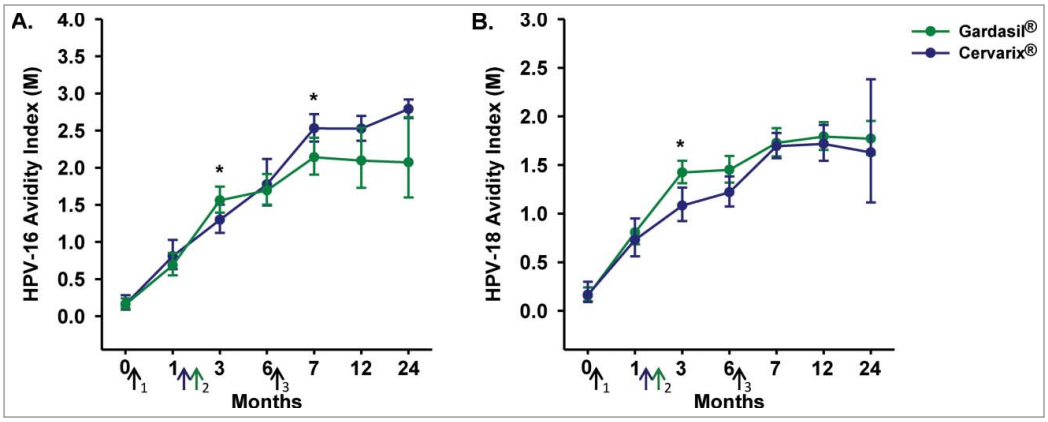

Figure 4. Modified HPV L1 VLP ELISA avidity assay using chaotropic elution for HPV-16 (**C**) and HPV-18 (**D**). Arrows indicate time of first (month 0), second (Cervarix® month 1, Gardasil® month 2), and third (month 6) vaccinations with respect to time points. *$P < 0.05$ (Mann-Whitney). Data expressed as Geometric Mean of Avidity Indices (95% CI).

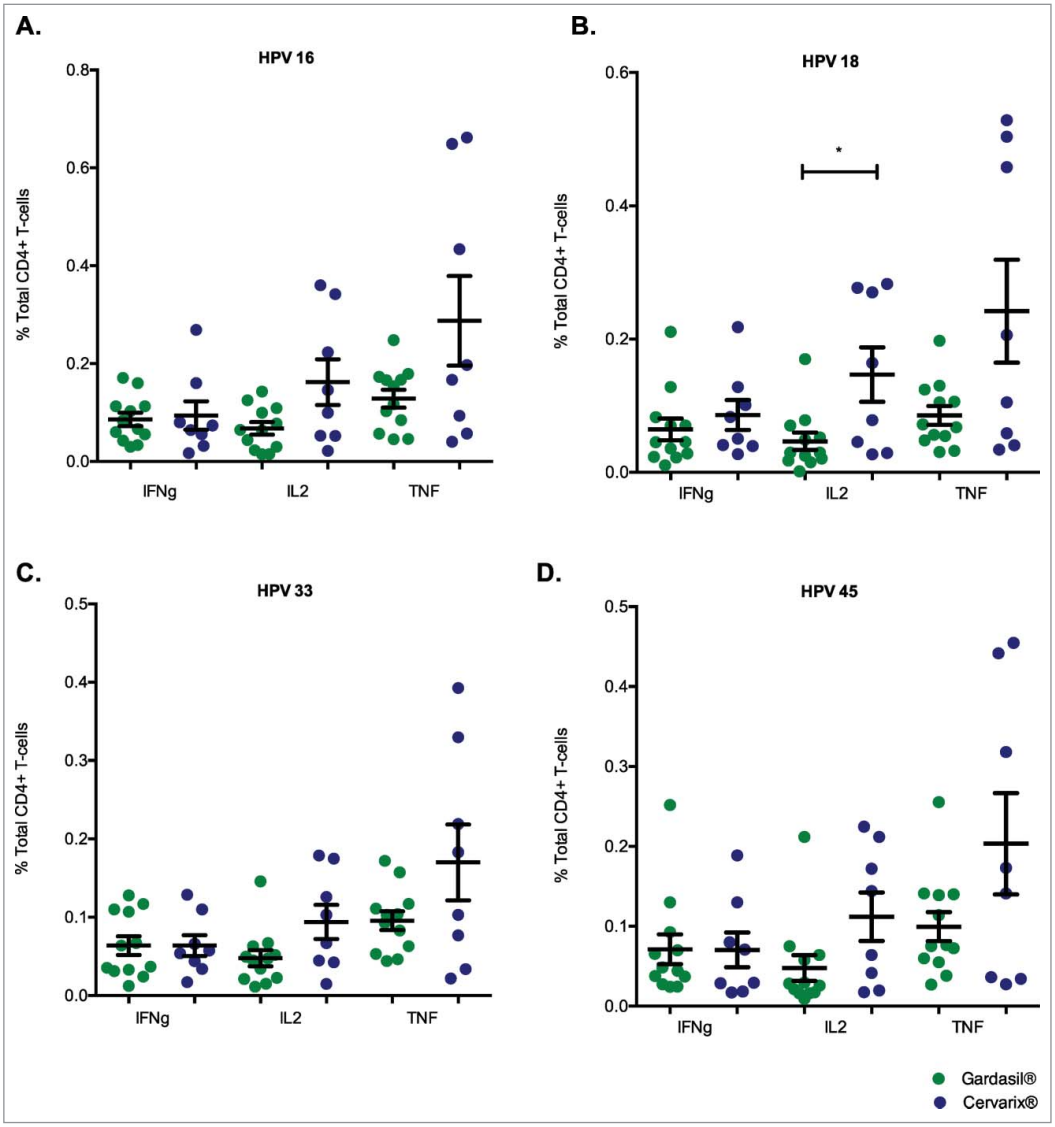

Figure 5. CD4$^+$ T cell HPV-specific responses in Cervarix® or Gardasil® recipients post-vaccination in response to stimulation in vitro with HPV-16 (**A**), HPV-18 (**B**), HPV-33 (**C**), or HPV-45 (**D**) L1 peptides. The horizontal bars represent the mean frequency and the error bars represent the SEM. *$P < 0.05$ (Mann-Whitney) Note: pre-vaccination levels for all responses (data not shown, Figure S2A) were significantly lower than post-vaccination for all responses ($P < 0.001$, Wilcoxon signed-rank test).

is 3-fold greater ($p = 0.045$) than the Gardasil® response. The ICS responses among all vaccine recipients combined reveal that the T cell responses to the phylogenetically related pairs [(HPV-16 & HPV-33) and (HPV-18 & HPV 45)] were similar, despite the fact that only HPV-16 and -18 are vaccine encoded, where as their genetically related pair is not included in the vaccine (Figure S1B). This may indicate detection of conserved T cell epitopes by this assay.

Plasma cytokine and chemokine responses

Plasma was analyzed by vaccine group for 10 circulating cytokines and chemokines, IL-12p40, TNF-α, IFN-γ, IL-6, IL-8, IL-1α, IL-1β, IP-10, MIP-1β, and MCP-1, at pre-vaccination and post first and third vaccination hours of 1, 3, 7, and days 1, 2, 5, 14 and 28 and at month 7. No statistically significant trends were observed for IFN-γ or TNF-α and data is not presented. The two vaccines induced significantly different responses at

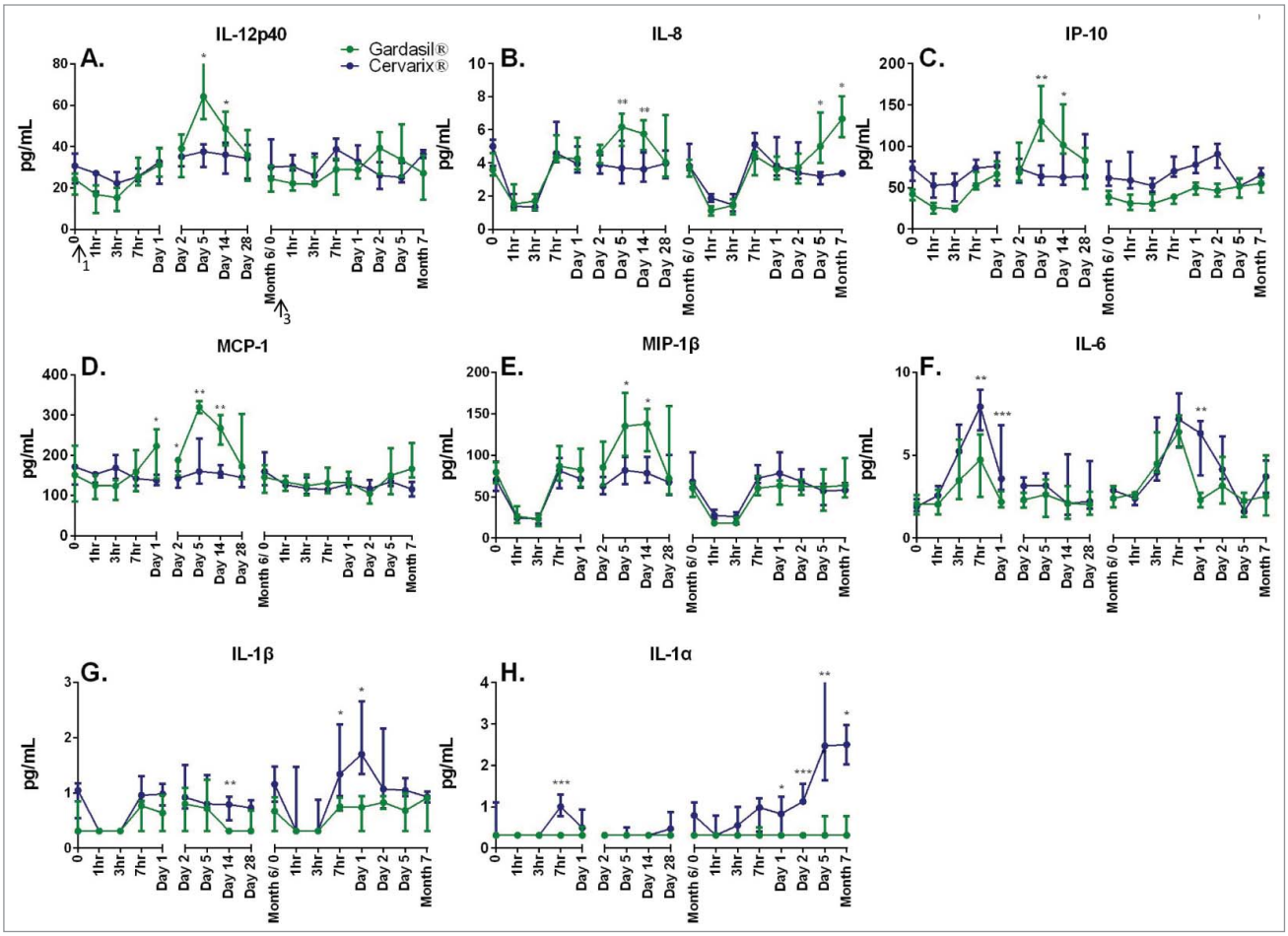

Figure 6. Circulating cytokine levels pre and post first and third vaccination. Arrows indicate first and third vaccinations. Note: second vaccination time points are not shown. IL-12p40 (**A**), IL-8 (**B**), IP-10 (**C**) MCP-1 (**D**), MIP-1 β (**E**), IL-6 (**F**), IL-1β (**G**), IL-1α (**H**). Data expressed as median values with interquartile range. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann-Whitney).

varying time points for the remaining cytokines, which are shown in **Figure 6**. Gardasil® induced significantly higher early cytokine/chemokine responses than Cervarix® (**Figs. 6A-E**) which peaked at day 5 for the following cytokines; IL-12p40: 1.70 fold greater (p = 0 .046), IL-8: 1.98 fold greater (p = 0 .045), IP-10: 2.03 fold greater (p = 0 .001), MCP-1: 2.00 fold greater (p = .006), and MIP-1β: 1.65 fold greater (p = .035). All of these cytokines/chemokines remained significantly elevated in Gardasil® recipients compared to Cervarix® recipients at the day 14 time point. Conversely, median levels of inflammatory markers, IL-6, IL-1α, and IL-1β, were significantly higher in Cervarix® recipients following the first, and in some cases third, dose of vaccine (**Figs. 6F-H**).

Discussion

The VRC independently evaluated innate and adaptive immune responses in healthy young women following vaccination with the 2 FDA licensed HPV vaccines. This study reports a comprehensive immune comparison between Cervarix® and Gardasil®, with novel data on T cell responses, avidity, and an extensive time course of circulating plasma cytokine profiles following vaccination. These aspects of immunogenicity have not previously been broadly evaluated in the context of these HPV vaccines.

Both vaccines are efficacious and are known to produce a robust antibody response against oncogenic HPV vaccine types (HPV-16 and HPV-18). Although correlates of immune protection have not yet been determined,[16,17] partially due to the lack of unambiguous vaccine failures, preclinical data has demonstrated that neutralizing antibodies can mediate type-specific protection against infection in the absence of other immune effectors.[18,19] The thresholds for B and T cell responses associated with protective immunity are not currently known. Understanding the kinetics, breadth, and magnitude of the innate and adaptive immune response can contribute to the identification of candidates for correlates of protection against infection and aid in building predictive models of vaccine immunogenicity and efficacy.

In this study we observed that both vaccines induced strong antibody responses with differences in magnitude of titers achieved. Levels of HPV-16 and HPV-18 antibodies after 3 doses of vaccine were significantly higher for Cervarix® than levels induced in Gardasil® recipients, and peak antibody titers were achieved after only 2 doses of Gardasil®, whereas peak titers were observed after 3 doses of Cervarix® - indicating a more prominent booster effect from the third dose of Cervarix®. These results are compatible with previous reports of head-to-head comparison of immunogenicity between the 2 vaccines.[11,13] It should be noted that dose 2 is delivered at month 1 for Cervarix® vs. month 2 for Gardasil®, allowing an additional month for Cervarix® antibody titers to potentially wane prior to the month 3 time point. In terms of clinical dose recommendations there is increasing evidence that the antibody response induced after 2 vaccinations, or even one, may be sufficient for protection.[20] As such the World Health Organization (WHO) recently changed dose recommendations to 2 vaccine doses, administered at month 1 and 6, in girls when vaccination is initiated prior to 15 y of age.[21] Furthermore, the minimum level of antibodies needed for protection are likely much lower than those induced by the HPV vaccines, [20,22] so observed differences in antibody titers may be related to the mechanisms associated with the different adjuvants but may not translate into clinical differences.

We evaluated levels of phylogenetically related, non-vaccine neutralizing antibodies and observed neutralizing HPV-31 antibodies were induced at statistically higher levels in Cervarix® recipients than Gardasil® recipients following 3 doses of vaccine, a trend consistent with observation in Cervarix® recipients within the Costa Rica Vaccine Trial.[7,15] For example, Cervarix® has shown approximately 77.1% cross-protection against HPV-31,[23] while Gardasil® has only shown significant efficacy against HPV-31 persistent infection.[24] The induction of low level HPV-58 neutralizing titers following Gardasil® administration is also in agreement with a previous report [25] but remains difficult to interpret, as this vaccine does not seem to show cross-protection against HPV-58.[5]

Patterns of antibody avidity were overall similar between the 2 vaccines, with durable maintenance of plateauing levels up to 24 months after last dose of vaccine. Antibody binding avidity is a marker of antibody quality that reflects the degree of affinity maturation in the B cells in the germinal centers.[26] For both vaccines the avidity index increases with each vaccine dose, indicating that each vaccine dose may improve antibody quality and that both vaccines induce similar quality antibody responses.

Although protection is believed to be primarily antibody-mediated induction of antigen-specific $CD4^+$ T cell responses by the HPV vaccines have been previously demonstrated.[14,27] Head-to-head comparison studies of the 2 vaccines have shown that HPV specific $CD4^+$ T cell frequencies are higher in Cervarix® than Gardasil®.[12,13] Consistent with these findings, in our study $CD4^+$ T cells produce significantly higher amounts of IL-2 in response to HPV-18 L1 in Cervarix® recipients compared to Gardasil® recipients, and there is a general trend toward a higher frequency of $CD4^+$ T cells producing IL-2 and TNF-α for HPV-16/18/33, and -45 in Cervarix® recipients. T cells are known to play an important role in the induction, duration and quality of antibody responses[26,28] and thus, are likely to have some involvement in the differences in the antibody responses observed between the 2 vaccines.

This is the first study that evaluated and compared circulating cytokine responses within hours and days after vaccination with the HPV vaccines. The most notable difference observed is an increased chemokine and cytokine (IL-12p40, IL-8, IP-10, MCP-1, and MIP-1β) response in Gardasil® recipients following first vaccination which peaks at day 5 and extends through day 14. With the exception of IL-12p40, a T cell stimulating factor, Gardasil® induced mostly increases in chemokines. Conversely, Cervarix® recipients showed elevated circulating pro-inflammatory markers (IL-6, IL-1α, IL-1β) following first (3–7 hrs) and third vaccination (7 hrs through month 7) when compared to Gardasil®. These data suggest that the TLR4 ligand adjuvant may have a stimulatory effect on pro-inflammatory cytokines and this is supported by previous studies in animal models showing that MPL, in contrast to aluminum hydroxide, induces high amounts of IL-1α and IL-6 [29] and distinct local inflammatory signals. As both vaccines induce antibody responses well above the level anticipated needed for protection against infection,[22] the observed differences represent mechanistic differences likely due to the adjuvant formulation and/or VLP particle composition that may not necessarily translate into distinct clinical efficacy effects. The field is just beginning to understand the potential implications of innate response signatures and adjuvant effects on the generation of effective adaptive immune responses and protection against infection. These patterns should be evaluated in larger, comprehensive studies to further assess the impact of innate patterns associated with specific adjuvants and antigen immunogenicity.

Until the 2009 Food and Drug Administration (FDA) approval of a lipid-based adjuvant with a TLR4 ligand, the only adjuvant approved for use in the United States was aluminum salts (alum). Aluminum salt is the main adjuvant in the Gardasil® vaccine, while Cervarix® contains the AS04 adjuvant. Alum promotes a Th2 response and extends the time for antigen exposure[30] through reorganization of antigen presenting cell (APC) lipid membranes.[31] The AS04 adjuvant includes an aluminum salt and a TLR4 agonist MPL (3-O-desacyl-4'-monophosphoryl lipid A) that activates the MyD88/Trif pathways.[32,33] AS04, delivered in the Cervarix® vaccine formulation, has been shown to induce a transient local cytokine response which leads to the activation of antigen-loaded dendritic cells and monocytes resulting in an enhanced activation of antigen-specific T cells as compared to alum alone. The AS04-induced innate responses are primarily due to MPL.[34] These results are consistent with our findings of transient elevated pro-inflammatory circulating cytokines and increased antigen-stimulated T cell cytokine production in Cervarix® recipients. Early induction of circulating IL-6 6 hours following vaccination has also been shown in rhesus monkeys immunized with HIV-1 envelope gp140 (B.63521) adjuvanted with a TRL4 agonist,[35] and these results are consistent with the increases we observed in Cervarix®

in IL-6 at 7 hours following first vaccination. Given the potential differences in mechanisms of action of the adjuvants included in these 2 vaccines, the observed cytokine profiles may suggest an influx and activation of different cell types that may translate into differences in the circulating cytokines. Innate cellular responses at the time points studied, including microarray analyses, would aid in interpretation of the differences and the cell components underlying the cytokine profiles observed. Such future work may aid in development of a systems biology approach to vaccine evaluation and have implications in the design of future efficacious vaccines.

In addition to adjuvant, the different systems of production utilized for the 2 vaccines may cause differences in particle composition and epitope presentation. For Gardasil® the L1 protein is produced from L1 expressing yeast while for Cervarix® the protein is produced in insect cell lines infected with L1 recombinant baculovirus.[36] Another difference between the vaccines is the number of antigens, Gardasil® contains 4 HPV antigens (HPV-16/18/6/11) and Cervarix® contains only 2 (HPV-16/18). The immunological differences observed may be related to immune interference events leading to lower immunogenicity of the quadrivalent vaccine, a trend seen in other studies.[37]

In conclusion, our study demonstrated induction of higher levels of HPV-16 and HPV-18 specific antibodies and $CD4^+$ T cell cytokine responses following Cervarix® vaccination compared to Gardasil®. The quality of HPV-16 and HPV-18 specific antibodies as measured by avidity was similar for both vaccines at months 12 and 24. Our data show that for the HPV vaccine types maximum antibody titers are achieved in Gardasil® after the second dose of vaccine, whereas with Cervarix® the titers are further boosted after the third dose of vaccine. The two vaccines additionally showed differences in circulating cytokine profiles, which may be attributed to the differences in adjuvant formulation. These findings indicate that future larger studies of early cytokine profiling, both at protein and transcript level are warranted and may contribute to identification of novel, early correlates of immunogenicity and efficacy against vaccine and phylogenetically related HPV types.

Subjects and Methods

Study design and participants

Women were enrolled into a National Institute of Allergy and Infectious Diseases (NIAID), Vaccine Research Center (VRC) clinical trial at the National Institutes of Health (NIH) Clinical Center, Bethesda, Maryland, USA (ClinicalTrials.gov #NCT01132859). The study was reviewed and approved by the NIAID Institutional Review Board. The study team followed human experimental guidelines for conducting clinical research from the US Department of Health and Human Services.

Twenty-seven women ages 18 y to 25 were enrolled and randomized in a 1:1 ratio to receive either the Cervarix® or Gardasil® 3 injection regimen (months 0, 1, 6 or months 0, 2, 6, respectively) as per package insert (open label) recommendations for vaccination (**Fig. 1**). First vaccinations were delivered immediately following the month zero collection time point (baseline), Cervarix® and Gardasil® second vaccinations were delivered immediately following the month 1 and 2 time points respectively, and third vaccinations were delivered immediately following the 6 month collection time point. Whole blood was collected prior to and at different time points following vaccination and was processed for serum, EDTA plasma, and isolation of peripheral blood mononuclear cells (PBMC), as previously reported.[38]

Antibody assays

HPV-16 and HPV-18-specific antibody levels by ELISA, neutralization titers for HPV-16/18/31/45/58 by pseudovirus neutralization assays, and antibody avidity for HPV-16/18 by modified ELISA were determined in serum obtained at pre-vaccination (month 0) and post-vaccination at months 1, 3, 6, 7, 12, and 24. All of the VLPs and pseudovirions used in this study were produced at the HPV Immunology Laboratory using mammalian cells as previously reported. [39] For antibody assay details see supplemental methods.

HPV L1-specific T cell Assays

Blood sampling was conducted prior to the first vaccination and at month 12 to analyze T cell responses. PBMC were used to measure responses to L1 peptides by IFN-γ enzyme-linked immunosorbent spot (ELISpot). Intracellular cytokine staining (ICS) and flow cytometry were used to measure pre and post vaccination CD4 and CD8 T cell cytokine response to L1 peptides. A total of 20 individuals had sufficient PBMCs available both prior to and following completion of the vaccination series. Two individuals were excluded from ELISpot analysis due to high background. All 20 individuals were included in the flow cytometric analysis. For T cell assay details see supplemental methods.

Plasma cytokine and chemokine analysis

Cytokine and chemokine profiles of subjects were characterized with ultra-sensitive MULTI-ARRAY® Technology from Meso Scale Discovery (MSD). We custom designed plates to assess 10 cytokines and chemokines: IL-12p40, IL-6, IL-8, IL-1α, IL-1β, TNF-α, IFN-γ, IP-10, MIP-1β, and MCP-1. These cytokines and chemokines were measured in plasma from vaccine recipients of Cervarix® (n = 12) and Gardasil® (n = 13) in duplicate at pre-vaccination and first and third post-vaccination hours of 1, 3, 7, and days 1, 2, 5, 14 and 28. An additional time point was taken at month 7 (1 month after final vaccination).

Statistics.

Antibody levels are expressed as geometric mean titer (GMT) (95% CI), T cell frequencies are expressed as means and standard error of the mean (SEM), and circulating cytokine levels are expressed as median levels. Statistical differences were determined using the nonparametric Mann-Whitney U test or the Wilcoxon matched-pairs signed rank test, and $P < 0.05$ was considered significant.

Notes

Cervarix® is a registered trade mark of the GlaxoSmithKline group of companies. Gardasil® is a registered trade mark of Merck and Co., Inc..

Disclosure of Potential Conflicts of Interest

John Schiller is an inventor of US government-owned patents that have been licensed to Merck and GlaxoSmithKline and so is entitled to limited royalties as specified by US law. The authors declare that we have no additional conflicts of interest.

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Supplementary Material

Supplemental data for this article can be accessed on the publisher's website.

The VRC 902 Team

Includes Mary E. Enama, Ingelise Gordon, Sarah Plummer, Cynthia Starr Hendel, Laura Novik, Kathy Zephir, Floreliz Mendoza, Jamie Saunders, Nina Berkowitz, Brandon Wilson, Sandra Sitar, Brenda Larkin, Joseph Casazza, Leejah Chang, Olga Vasilenko, Iris Pittman, Hope Decederfelt, and Robert T. Bailer.

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