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ORAL AND SYSTEMIC HPV ANTIBODY KINETICS POST-VACCINATION AMONG HIV-POSITIVE AND HIV-NEGATIVE MEN

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Abstract

Duration and functional aspects of the oral and systemic antibody responses following HPV vaccination in HIV-negative (HIV^-) and HIV-positive (HIV^+) men are not well characterized. Oral and systemic HPV-16 and HPV-18-specific antibody levels were evaluated over 18-months of follow-up, in HIV^+ and HIV^- men. Sera and oral gargles from 147 HIV^- men, ages 27–45 and 75 HIV^+ men, ages 22–61, who received 3-doses of quadrivalent HPV vaccine were tested for HPV-16 and HPV-18 antibodies at Day 1, Month 7 (1 month post-dose 3), and Month 18 (12 months post-dose 3) and HPV avidity (Day 1, and Month 7) using L1-VLP ELISA.

All individuals seroconverted, regardless of HIV-status, following 3-doses of vaccine for HPV-16 and HPV-18. Serum HPV-16 and HPV-18 antibody geometric mean levels were >2-fold lower in HIV^+ compared to HIV^- men at Month 7 (HPV-16: 808.5 versus 2119.8 EU/mL, and HPV-18: 285.8 *versus* 611.6 EU/mL, $p<0.001$) but not significantly different at Month18 (HPV-16: 281.8 *versus* 359.7 EU/mL, $p=0.145$, and HPV-18: 120.2 *versus* 93.4 EU/mL, $p=0.372$). Post-vaccination, only oral HPV-16 antibody levels at Month7 were significantly different between HIV^+ and HIV^- men (127.7 *versus* 177.1 EU/mg of IgG, $p=0.008$). Among baseline HPV-seronegative men, circulating levels of HPV-16 and HPV-18 antibodies were up to >3 fold lower in HIV^+ men, at Months 7 and 18. In contrast, levels of HPV-16 and HPV-18 antibodies after vaccination were not inferior in baseline HPV-seropositive, HIV^+ men. HPV-16 and HPV-18 avidity was lower among HIV^+ compared to HIV^- men at Month7 (HPV-16: 1.95 M *versus* 2.12 M, $p=0.027$; HPV-18: 1.50 M *versus* 1.72 M, $p<0.001$).

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Although differences in peak antibody levels were observed between HIV^+ and HIV^- men following 3 doses of vaccine, plateau antibody levels were overall comparable, and avidity was relatively high for both groups. These data indicate that vaccine induced antibody affinity maturation in both HIV^+ and HIV^- men and likely will result in long-term protective immune responses.

Introduction

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses [1]. In the United States (U.S.), HPV infections are responsible for over 70% of oropharyngeal cancers (OPCs), with the majority due to HPV-16 [2, 3]. U.S. incidence rates of HPV-positive OPCs are increasing, with a concomitant increase in the proportion of OPCs attributable to HPV [4]. OPC incidence is 4–5-fold higher in males compared to females and 2–3-fold higher among individuals with HIV [5, 6]. In the U.S., OPC incidence among males ages ≥50 years exceeds 23/100,000, approximately twice the incidence of cervical cancer among women of a similar age, with no routinely available method for early detection. Approximately 18,000 U.S. adults are diagnosed annually with HPV-related OPC with an estimated 5-year survival of 64% [7]. As current HPV vaccines have not been evaluated for efficacy against OPC, there is also no established method to prevent OPC.

The quadrivalent HPV (qHPV) vaccine is highly efficacious in reducing HPV-6, HPV-11, HPV-16, and HPV-18 anogenital infection and subsequent development of HPV-related external genital lesions (EGL) and anal disease in young males (ages 16–26 years) [8]. This led to the licensure of qHPV vaccine in males, ages 9–26 years, for the prevention of HPV-6 and HPV-11 related genital warts and HPV-16 and HPV-18 related anal cancers. However, HPV measurements at the oral epithelium were not included in this trial, and there are no prospective data on the efficacy of HPV vaccination to prevent OPC, to support an indication for vaccine prevention of OPC in men. Data from our group indicate that at one-month post dose three of vaccine, men ages 27–45 years mount a strong serum anti-HPV vaccine type specific antibody response [9, 10], achieving antibody levels in serum that are comparable to those seen in young adult males, the age group in which clinical efficacy against genital and anal HPV and related diseases was demonstrated. Importantly, HPV antibodies were also detectable at the oral cavity following vaccination in this same group of men.

The qHPV vaccine is also highly immunogenic in HIV-infected adults [11–13], resulting in seroconversion to each of the 4 vaccine HPV type components among HIV^+ mid-adult aged men [13], although lower antibody levels have been reported in HIV^+ individuals [11, 12, 14]. Secondary endpoint analyses of a Phase III Trial of the qHPV vaccine among mid-adult aged HIV^+ men indicate trends toward efficacy against persistent oral HPV infections in this population [15]. Although no correlates of protection have been formally identified, neutralizing antibodies are believed to be the main mechanism of protection afforded by virus-like particle (VLP) HPV vaccines [16]. However, successful prevention of HPV-related cancers depends upon sustaining antibody titers long term, as individuals remain at risk for cancer for many years after immunization. No studies have yet addressed duration or functional aspects of the immune response to vaccination in mid-adult aged men,

systemically or locally at the oral cavity. This is important information to understand the local mucosal and systemic HPV-specific antibody responses to the qHPV vaccine as well as to inform and assess the potential for the HPV vaccine to prevent OPC in HIV^- and HIV^+ men.

In this study we evaluated and compared the kinetics and duration of the oral and systemic HPV-16 and HPV-18 specific antibody response to three doses of the qHPV vaccine in mid-adult aged HIV^+ men from the AIDS Malignancy Cohort (AMC) 052 trial and mid-adult aged HIV^- men from the Mid-Adult Male [MAM] trial. Additionally, antibody avidity was determined. This is the first study evaluating longevity of the mucosal antibody responses, and serum HPV antibody avidity following HPV vaccination in HIV^+ males when compared to healthy subjects. With this work we aim to develop better understanding of the local and systemic HPV- specific antibody responses to the qHPV vaccine in an HIV-infected male mid-adult vaccinated cohort.

Materials and Methods

Study Population

The study population consisted of a subset of mid-adult aged males from two different clinical trials, one among a cohort of HIV^- men (n=147) and one among a cohort of HIV^+ men (n=75). The *Mid-Adult Men Trial Study* (The MAM Trial; www.clinicaltrials.gov,) is a single-arm intervention trial (n=150) that enrolled, vaccinated, and assessed the circulating antibody response to the qHPV vaccine in men ages 27–45 [10]. Briefly, subjects were vaccinated intramuscularly with qHPV vaccine at Day 1, Months 2, and 6. A total of 150 men from Tampa, FL, USA, and Cuernavaca, Mexico who met eligibility criteria (male, 27–45 years, completed four years of follow-up in the *HPV Infection in Men (HIM) Study*) were enrolled and received at least one dose of vaccine. A subset of vaccine recipients had oral gargle (n= 147, 147, and 103) and serum (n= 126, 126, and 104) available for HPV antibody testing at Day 1, Month 7, and Month 18, respectively. The institutional review boards at each participating center (University of South Florida in the U.S. and Instituto Nacional de Salud Publica in Mexico) approved the protocol, and informed consent was obtained from all participants. The study was conducted in conformance with applicable country or local requirements regarding ethical committee review, informed consent, and other statutes or regulations regarding the protection of the rights and welfare of human subjects participating in biomedical research.

The AMC052 study (www.clinicaltrials.gov) is a single-arm Phase II trial of the qHPV vaccine in HIV-infected men (n=112) that enrolled, vaccinated and assessed the serum antibody responses in men ages 22–61 years [13]. The institutional review boards at each participating center approved the protocol, and informed consent was obtained from all participants. Participants were excluded from the trial if they had anal infection with both HPV16 and HPV18, and if they had high grade squamous intraepithelial lesions on anal histology or cytology. All recipients of three doses of vaccine with oral gargles (n= 62, 50, and 53) and serum (n= 75, 75, and 74) available at Day 1, Month 7 and Month 18, respectively, who provided consent for future unspecified research and storage of residual study specimens at the AIDS Cancer Specimen Repository were included in this analysis.

HIV viral load and CD4 T cell counts were determined as previously reported [13]. This planned combined analysis of samples and data from both protocols was reviewed by the AMC, the AIDS Cancer Specimen Repository, and USF Institutional Review Board and monitored by the Moffitt Cancer Center Protocol Support Office.

Specimens

Oral gargle and serum specimens were obtained and archived from scheduled clinical visits at Day 1, Months 7 (1 month post-dose 3) and 18 (12 months post-dose 3). At each visit, 10 mL of blood were collected in a serum collection tube (BD Cat# 366430). Following centrifugation, sera were aliquoted into cryovials and stored at −80°C until testing. Oral gargle specimens were collected by rinsing/gargling 15 mL mouthwash (e.g., Target UP & UP) for 30 seconds in the MAM trial using a previously described protocol [17] or 10 mL Phosphate Buffer Saline (PBS) for 60 seconds in the AMC052 trial. Specimens were centrifuged and the supernatant as well as the cell pellets were archived at −80°C until analysis.

Direct L1 VLP ELISA

Anti-HPV IgG antibodies were detected by an enzyme-linked immunosorbent assay (ELISA), as previously described [18, 19]. This ELISA measures total levels of HPV-16 and 18-specific IgG antibodies (both neutralizing and non-neutralizing) and is amenable for use in large epidemiologic and clinical studies. The assay is highly reproducible, with a reported CV of 11.4% [18]. Briefly, polystyrene flat-bottom microtiter plates (MaxiSorp, high binding; Nunc, Cat# 439454 Thermo Fisher Scientific, USA) were coated with HPV-16 or HPV-18 L1 VLPs and incubated at 4°C. Prior to use, the plates were washed with a phosphate-buffered saline containing 0.05% Tween 20 (VWR, Cat# EMPX1296–1). After blocking the plates with blocking buffer containing 4% skim milk (BD, Cat# 232100) and 0.2% Tween 20 in phosphate-buffered saline (Gibco, Cat# 14190–136), the plates were washed again. Serum (starting dilution 1:100) and oral fluids (oral gargle, starting dilution 1:2) from participants were serially diluted in the blocking solution in two-fold increments in the assay plate. The plates were incubated for one hour at room temperature. After washing four times, a solution of peroxidase-labeled goat anti-human IgG (KPL, Inc., Gaithersburg, MD) was added for one hour at room temperature. Plates were then developed with a tetramethylbenzidine (TMB) substrate solution (KPL, Inc.) for 25 minutes in the dark at room temperature. Next, the reaction was stopped, and the absorbance measured with a microtiter plate reader (Spectramax M5; Molecular Devices, Sunnyvale, CA). Antibody levels, expressed as ELISA units (EU)/mL, were calculated by interpolation of OD values from the standard curve by averaging the calculated concentrations from all dilutions that fall within the working range of the standard curve. The seropositivity lower cut points for serum were set at 19 EU/mL for anti-HPV-16 and 18 EU/mL for anti-HPV-18 [9]. Cut points for oral gargles were set at 0.042 EU/mL for anti-HPV-16 and 0.032 EU/mL for anti-HPV-18 [9]. The HPV-16 and −18 ELISA has been calibrated with the World Health Organizations International Standards for anti-HPV-16 and anti-HPV-18 and the respective conversion factor for anti-HPV-16 antibodies is 1 IU/mL = 5.29 EU/mL and anti-HPV-18 antibodies is 1 IU/mL = 6.81 EU/mL.

Avidity ELISA

Avidity values were determined using a modified ELISA-based method established and qualified at the Frederick National Laboratory for Cancer Research that approximates the binding strength of IgG antibodies specific for HPV VLPs using guanidine hydrochloride as the chaotropic agent [20, 21]. Briefly, polystyrene flat-bottom microtiter plates (MaxiSorp, high binding; Nunc, Cat# 439454 Thermo Fisher Scientific, USA) were coated with 2.7 μg/mL HPV-16 or HPV-18 L1 VLPs and incubated at 4°C for 3–5 days. Prior to use, the plates were washed with a phosphate-buffered saline containing 0.05% Tween 20 (VWR, Cat# EM-PX1296–1). After blocking the plates with blocking buffer containing 4% skim milk (BD, Cat# 232100) and 0.2% Tween 20 in phosphate-buffered saline (Gibco, Cat# 14190–136), the plates were washed again. Next, serum samples were diluted based on previous testing in the HPV-16 or HPV-18 ELISA to yield an absorbance reading of 1.0 ± 0.5. Of note, if a sample's absorbance reading at 1:100 did not yield 1.0 ± 0.5, then the sample was excluded from testing in the avidity assay. Regardless of a sample's cut point in the HPV-16 or HPV-18 L1 VLP ELISA, if the sample's absorbance reading fulfilled the criteria of 1.0 ± 0.5, then it was included in the avidity testing. Each sample was tested in duplicate. The diluted serum was incubated for 1 hour with low speed shaking at room temperature. Following sample incubation, buffer alone (control) or 0.5 to 3.5 M guanidine hydrochloride (GuHCl; Sigma, St. Louis, MO) was added for 15 minutes at room temperature. After washing the plate, a solution of peroxidase-labeled goat anti-human IgG (KPL, Inc., Gaithersburg, MD) was added for one hour at room temperature. Plates were then developed with a tetramethylbenzidine (TMB) substrate solution (KPL, Inc.) for 25 minutes in the dark at room temperature. The reaction was stopped with 0.36 N H_2SO_4, and the absorbance at 450 nm and 620 nm were measured with a microtiter plate reader (Spectramax M5; Molecular Device, Sunnyvale, CA). Avidity data are reported as Geometric Mean Avidity Levels. Avidity levels are the concentrations of GuHCl, expressed in Molar (M), that reduces the optical density by 50% compared to sample wells without GuHCl treatment (control).

Total IgG ELISA

Total human IgG levels were measured in duplicate per specimen type (serum and oral gargle) using an ELISA according to the manufacturer's protocol (Bethyl Laboratories, Montgomery, TX, USA) [9]. Total IgG levels in each different sample type (serum and oral gargle) were used to normalize levels of HPV specific antibodies across different biological specimens and to compare levels between different collection time points.

Statistical analysis

The p values from the demographic characteristics were calculated using Pearson χ^2 analysis for categorical variables and the Wilcoxon Mann-Whitney test for continuous variables. To determine the percentage seropositive, the proportion of men who had detectable serum or oral HPV-16/18 IgG and the 95% confidence interval (CI) were estimated. Geometric mean levels (GMTs) and 95% CIs for HPV-16 and 18 antibody levels were calculated and reported. GMTs were compared across groups using the Wilcoxon-Mann-Whitney test. Correlations between serum- and oral-specific IgG levels were

determined by Spearman correlation coefficients. Among trial participants with detectable antibodies in both serum and oral gargle, HPV-specific antibody levels were normalized to the total IgG level in oral specimens due to variations in collection volume and reported as ratios of the HPV-specific IgG concentrations/total concentrations of IgG. As serum concentrations of HPV antibodies is stable regardless of collection volume, normalization to total IgG was not assessed in this analysis.

Results

Basic demographic characteristics for both cohorts are shown in Table 1. The men enrolled in AMC052 were older than those in MAM (median of 45 versus 36 years). There were also differences in race and HPV sero-status at Day 1. The median CD4+T-cell count among men in AMC052 was 498 cells/mm^3 (Inter-Quartile Range: 408, 680), and 61/75 (81%) were virally suppressed with a plasma HIV-1 RNA <200 copies/mL. Furthermore, 63/75 (84%) of the men in AMC052 were on antiretroviral therapy.

Serum Antibody Kinetics:

On Day 1, the percent seropositivity for HPV-16 and HPV-18 antibodies, in serum, was approximately two-fold higher in HIV^+ compared with HIV^- men. All seroconverted 1 month following three doses of vaccine for both HPV-16 and HPV-18 (Table 2). More than 96% of vaccinated individuals remained seropositive for HPV-16 at Month 18, but HPV-18 seropositivity declined to 84% and 90% among HIV^+ and HIV^- men, respectively at Month 18.

Prior to HPV vaccination on Day 1, the Geometric Mean Levels (GMT based on non-normalized data) among HIV^+ and HIV^- mid-adult aged males who were HPV-16-seropositive at baseline were comparable for HPV-16 (among those who had detectable HPV-16 antibodies). One month after receiving three doses of qHPV vaccine, HPV-16 antibody levels rose sharply among both populations but achieved levels >2 fold lower in HIV^+ men compared with HIV^- men (808.5 *versus* 2119.8 EU/mL, $p<0.0001$). However, at Month 18 HPV-16 antibody levels dropped in both groups leading to non-significant differences in HPV-16 antibody levels by HIV status (281.8 *versus* 359.7EU/mL, $p=0.1451$).

At Day 1, HIV^+ men seropositive for HPV-18 at baseline had higher serum HPV-18 antibody levels than HIV^- men (49.9 *versus* 26.9 EU/mL, $p<0.0004$ [Table 2]). However, one month post-dose three of qHPV vaccine, HPV-18 GMTs among HIV^+ men were >2 fold lower than among HIV^- men (285.8 *versus* 611.6 EU/mL, $p<0.0001$). As with HPV-16 GMTs, at Month 18, HPV-18 GMTs had decreased in both groups and were no longer significantly different (120.2 *versus* 93.4 EU/mL, HIV^+ and HIV^- respectively, $p=0.3715$).

As HPV sero-status at time of vaccination may affect levels of response to vaccination, we estimated antibody levels in HIV^+ and HIV^- individuals with and without pre-existing antibodies at Day 1 (Table 2). HIV^+ individuals with HPV-16 or HPV-18 antibodies at the time of vaccination developed 2.5- to 3.6-fold higher antibody levels at Month 7 and 18 following vaccination, compared with the HPV-seronegative men. Among Day 1 HPV-seropositive HIV^- men, no significant differences were observed at Month 7 or Month 18

compared with HPV-seronegative HIV$^-$ men. Responses in HPV-seronegative, HIV$^+$ men were significantly lower (1.6- to 3.6-fold) than in HIV$^-$ individuals at both time points after vaccination.

In addition, we observed that HIV$^+$ men with viral suppression (n=61/75) developed significantly higher systemic antibody responses to HPV-16 and HPV-18 (GMT in serum of 923.3 *versus* 453.4 EU/mL; p=0.0133 and 324.4 *versus* 164.5 EU/mL; p=0.0379, respectively) as well as higher oral HPV-16 antibody responses (0.320 *versus* 0.134 EU/mL; p=0.0183) than individuals without viral suppression, at Month 7 but not at Month 18 (Supplemental Tables 1 and 2).

Oral Gargle Antibody Kinetics:

On Day 1, 33.9% of HIV$^+$ men had detectable oral HPV-16 antibodies compared to none in HIV$^-$ men (Table 3). At Month 7, oral HPV-16 antibodies were detected in 94.0% of HIV$^+$ men and 93.2% of HIV$^-$ men. At Month 18, a higher percentage of HIV$^+$ remained oral HPV-16 antibody positive (69.8%) compared with HIV$^-$ men (39.8%). Oral HPV-18 antibody prevalence at Day 1 was higher than HPV-16 antibody prevalence, with oral HPV-18 antibodies observed among 62.9% of HIV$^+$ men and 4.1% of HIV$^-$ men. At Month 7, oral HPV-18 antibodies were detected in 88.0% and 72.1 % of HIV$^+$ and HIV$^-$ men, respectively. At Month 18, most of HIV$^+$ remained oral HPV-18 antibody positive (71.7%), compared with only 10.7% of HIV$^-$ men with detectable oral HPV-18 antibodies.

To account for differences in oral gargle collection volumes, oral HPV-16 and HPV-18 antibody levels were normalized for total IgG in corresponding specimens (Table 3). At Month 7, HIV$^+$ men had lower oral HPV-16 antibody levels than HIV$^-$ men (127.7 versus 177.1 EU/mg of IgG, p=0.0081); however, by Month 18 oral HPV-16 antibody levels were comparable. In contrast, no differences in oral HPV-18 antibody levels were observed by HIV status at Months 7 or 18. IgG normalized HPV-18 antibody levels were only significantly different in HIV$^+$ individuals at Day 1 (41.1 versus 17.4 EU/mg of IgG, p=0.0316), when compared with HIV-negative men. However, a very small number of HIV$^-$ individuals were seropositive for HPV-18 compared with HIV$^+$ men (4.1% versus 62.9%). No significant differences in oral antibody levels were observed by baseline oral HPV serostatus, except for higher oral HPV-18 antibodies at Month 18 in HPV-seropositive HIV$^+$ men when compared with the HPV-seronegative men.

Anti-HPV-16 and anti-HPV-18 Antibody Avidity:

Table 4 presents HPV-16 and HPV-18 antibody avidity levels, expressed as Geometric Means, at Day 1 and Month 7 among HIV$^+$ and HIV$^-$ participants. HPV-16 antibody avidity was slightly, yet significantly, lower among HIV$^+$ men one-month (Month 7) following receipt of three vaccine doses compared with HIV$^-$ men (2.0 *versus* 2.1 M, p=0.0258). Similarly, HPV-18 antibody avidity was lower among HIV$^+$ men one-month following receipt of three vaccine doses compared with HIV$^-$ men (1.5 *versus* 1.7 M, p<0.0001). Despite the significance observed, overall differences in avidity levels were of small magnitude and levels observed in both groups were high suggesting that the vaccine induced affinity maturation in both HIV$^+$ and HIV$^-$ individuals.

Avidity levels following vaccination were not significantly different by baseline HPV-serostatus among HIV^+ or HIV^- men. Avidity levels in HPV-seronegative, but not in HPV-seropositive, HIV^+ men were lower than in HIV^- individuals at both time points after vaccination.

In HIV^+ men, HPV-16 antibody avidity levels were higher overall in HIV^+ participants with viral suppression as compared to those without, at Day 1, and Month 7 (1.4 *versus* 0.7 M, p= 0.0036, at Day 1; 2.1 *versus* 1.6 M, p=0.0159, at Month 7) (Supplemental Table 1).

Correlations between Antibody Levels and Antibody Avidity:

Correlations between avidity and antibody levels following qHPV vaccination were overall low ($\rho<0.36$), indicating that these measures are likely independent (Table 5).

Among HIV^+ men, none or weak correlations were observed between CD4 levels or HIV viral load) measurements at Day 1 and anti-HPV-16 or HPV-18 antibody levels or avidity following vaccination (Supplemental Tables 3 and 4). The highest inverse correlations were observed between HIV viral load and avidity (Day 1 HPV-16 and HPV-18 antibody avidity *versus* HIV viral load: $\rho=-0.45$; $\rho=-0.46$, respectively, Supplemental Table 4

Discussion

This is the first study to directly compare the immune response of HIV^+ and HIV^- mid-adult aged men following receipt of a 3-dose regimen of the qHPV vaccine. A high and comparable percentage of HIV^+ and HIV^- men sero-converted following receipt of three doses of vaccine. While peak serum antibody levels were lower among HIV^+ compared with HIV^- men, geometric mean plateau levels at Month 18 were comparable. As has been demonstrated in other trials, HIV^+ individuals entering HPV-seropositive prior to receiving vaccine achieve considerably higher antibody levels at Month 7 compared to those who are HPV-seronegative at the time of vaccination. Interestingly, the higher antibody response in HPV- seropositive men was sustained among HIV^+ men but not HIV^- men through the 18-month time point.

Overall, HIV-infected individuals have higher rates of HPV infection, they are more likely to have multiple HPV types and persistent infections, and are at increased risk of oropharyngeal cancer and other HPV-associated cancers compared with HIV^- individuals [22–24]. Recently, we published that the quadrivalent HPV vaccine induces HPV-specific antibodies in the oral cavity following HPV vaccination in mid adult, HIV^- males, although at much lower levels than the ones found in serum [9]. However, the longevity and the concentration of antibodies at plateau levels, particularly at the oral cavity in HIV-infected individuals was unknown. Serum HPV-16 and HPV-18 antibody geometric mean levels were >2-fold lower in HIV^+ men at Month 7 but not significantly different at Month 18 compared with HIV^- men. Significantly lower antibody levels were observed in individuals without viral suppression at Month 7, indicating the inverse association between plasma HIV-1 RNA and peak antibody responses. This association has been observed in other studies among HIV-infected individuals [25]. Lack of significance for other time points or for HPV-18 antibody measurements may be related to the small sample size of men with >200 copies/ml

(n=14). Larger sample sizes are required to appropriately evaluate this association. However, results from this study are in agreement with previous findings demonstrating a lower antibody response in HIV^+ individuals, which was reported to be influenced by CD4 T cell depletion and higher HIV viral loads [11, 12, 14]. This is also consistent with immunogenicity findings from other vaccines administered in HIV^+ individuals [26, 27].

Findings from this study indicate that oral anti-HPV-16 and anti-HPV-18 antibody levels at Month 18 are markedly reduced compared with the levels observed with peak responses, at Month 7 (1 month following administration of the three doses of vaccine), particularly for HPV-18 among HIV^- men. HIV^+ individuals showed markedly higher oral HPV antibody prevalence on Day 1 and Month 18, for both HPV-16 and HPV-18, but not at peak time of antibody responses, at Month 7. Only oral HPV-16 antibody levels at Month 7 were significantly lower in HIV^+ compared with HIV^- men, while antibody levels, for both HPV-16 and HPV-18, were comparable with a tendency for higher levels in HIV^+ individuals once plateau levels were achieved.

As the presence of HPV-specific antibodies at time of vaccination is related to antibody response to vaccination [28], we evaluated its influence on the antibody responses among HIV^+ and HIV^- individuals at Months 7 and 18. Despite the small numbers, we observed that HIV^+ men with detectable serum HPV antibodies at the time of vaccination, developed stronger antibody responses than the HPV-seronegative men at both time points after vaccination. HIV^- men who were HPV- seropositive at Day 1 did not mount significantly higher antibody responses when compared with HPV-seronegative individuals. In contrast to the serum, oral antibody levels were not significantly different by baseline HPV serostatus, except for higher oral HPV-18 antibodies at Month 18 in HPV-seropositive HIV^+ men when compared with the HPV-seronegative men.

The observed loss in oral antibody detectability may be related to assay sensitivity issues, as even in serum, only 80% of individuals had detectable responses to vaccination. Loss of detectability of HPV-18 antibody responses has been observed in previous studies [29], despite the lack of breakthrough infections, suggesting that new assays with higher sensitivity are warranted for use at mucosal sites, where levels are much lower. Furthermore, different methods of collection were used in the two cohorts studied. To minimize influence of the method of collection and adjust for differences in oral collection volumes, oral antibody data were normalized by total IgG levels determined in each sample.

The role of antibody avidity has been demonstrated in vaccine protection against other infections [30] and it is likely to play an important role in protecting against HPV infection. HPV-16 and HPV-18 avidity was slightly lower among HIV^+ compared with HIV^- men at Month 7. Of significance, lower HPV-16 avidity was observed among individuals without viral suppression. In contrast to serum antibody levels, avidity levels did not appear to be influenced by Day 1 HPV sero-status. Overall, these findings suggest that vaccine induced affinity maturation in HIV-infected and uninfected mid-adult aged males is similar, with some influence of plasma HIV-1 RNA in HPV-16 avidity levels. Weak correlations were observed between avidity and antibody levels following HPV vaccination suggesting these measures are independent as they relate to different aspects of the B cell response.

Correlations were weak between CD4 levels and viral load and antibody measurements. This could be due to the fact that most of the individuals were virally suppressed with relatively high CD4 T cell counts. To address the role of plasma HIV-1 RNA and CD4 depletion extent in HPV-specific antibody measurements, larger studies in populations with a wider range of clinical parameters are needed.

In conclusion, HIV^- and HIV^+ men developed comparable plateau antibody levels following qHPV vaccination, although lower peak antibody levels were observed among HIV^+ men. HPV seropositivity at baseline played a critical role in antibody levels achieved after vaccination among the HIV^+ men. The comparable plateau antibody responses and high antibody avidity levels among the HIV^+ and HIV^- groups are suggestive that long-term immunogenicity and protection may be observed. Although other factors need to be taken into account, overall, these findings strongly support the consideration of vaccinating both HIV^- and HIV^+ mid-adult aged men for the prevention of infection and cancer at the oral cavity and at other HPV-susceptible anatomic sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1:

Demographic characteristics of the study participants.

Baseline (Day 1) Characteristics	MAM Trial[a]	AMC Trial[a]	P Value[b]
Age at visit			
N, mean (std)	N=150, 34.8 (5.1)	N=75, 44.6 (8.7)	
Median (range)	36 (27– 45)	45 (22–61)	<0.0001
Race			
White	68 (45%)	54 (72%)	<0.0001
Black/African American	12 (8%)	8 (11%)	
Hispanic	63 (42%)	9 (12%)	
Other	7 (5%)	4 (5%)	
HPV-16 sero-status (Day 1)			
Negative	102 (81%)	46 (61%)	0.0032
Positive	24 (19%)	29 (39%)	
HPV-18 sero-status (Day 1)			
Negative	100 (79%)	45 (60%)	0.0036
Positive	26 (21%)	30 (40%)	
Antiretroviral Therapy (ART)			
Yes	N/A	63 (84%)	
No	N/A	12 (16%)	
CD4 count (cells/mm^3)			
Total; N, Median (range)	N/A	N=74, 498 (408–680)	
Viral Load >200; N, Median (range)	N/A	N=14, 545 (434–683)	
Viral Load <200; N, Median (range)	N/A	N=60, 488 (393–669)	
HIV-1 Viral Load >200 copies/mL			
N, Median (range)	N/A	N=14, 8670 (2425–29193)	

Abbreviations: std = Standard deviation;

HIV-1 Viral Load <200 copies/mL is defined as HIV suppression.

[a]Unless otherwise indicated, data are number (percentage) of study participants.

[b]P value represents comparison between trials.

Table 2:

HPV-16 and HPV-18 antibody levels among HIV^+ and HIV^- mid-adult men (Serum).

	Visit	Group	N	Antibody positive (%)[a]	Total GMT (95% CI)	Day 1 Seropositive GMT (95% CI)	Day 1 Seronegative GMT (95% CI)	HIV Status Total p-value[b]	HPV Serostatus p-value[c]	HIV Status Seropositive p-value[d]	HIV Status Seronegative p-value[d]
HPV-16	D1	HIV−	126	24 (19.0)	34.5 (28.3 – 42.0)	34.5 (28.3 – 42.0)	-	0.0602		0.0602	-
		HIV+	75	29 (38.7)	60.6 (40.2 – 91.1)	60.6 (40.2 – 91.1)	-				
	M7	HIV−	126	126 (100)	2119.8 (1858.2 – 2418.1)	2335.6 (1646.2 – 3313.7)	2072.0 (1795.6 – 2390.9)	<0.0001	0.5847	0.0641	<0.0001
		HIV+	75	75 (100)	808.5 (623.7 – 1048.1)	1408.4 (930.0 – 2132.9)	569.8 (421.7 – 769.9)		0.0002		
	M18	HIV−	104	103 (99.0)	359.7 (293.0 – 441.6)	458.5 (221.7 – 948.2)	344.0 (278.9 – 424.4)	0.1451	0.6487	0.4041	0.0002
		HIV+	74	71 (95.9)	281.8 (208.0 – 382.0)	600.6 (388.5 – 928.5)	167.2 (118.2 – 236.4)		<0.0001		
HPV-18	D1	HIV−	126	26 (20.6)	26.9 (23.6 – 30.7)	26.9 (23.6 – 30.7)	-	0.0004		0.0004	-
		HIV+	75	30 (40.0)	49.9 (36.6 – 67.9)	49.9 (36.6 – 67.9)	-				
	M7	HIV−	126	126 (100)	611.6 (526.4 – 710.6)	666.6 (479.8 – 926.1)	598.1 (504.0 – 709.7)	<0.0001	0.5957	0.7383	<0.0001
		HIV+	75	75 (100)	285.8 (216.9 – 376.6)	604.4 (398.0 – 917.9)	173.4 (129.3 – 232.6)		<0.0001		
	M18	HIV−	104	94 (90.4)	93.4 (77.7 – 112.3)	91.5 (47.8 – 175.3)	93.7 (77.2 – 113.7)	0.3715	0.9224	0.0069	0.0095
		HIV+	74	62 (83.8)	120.2 (88.3 – 163.6)	283.4 (183.8 – 436.9)	59.3 (45.6 – 77.2)		<0.0001		

D1= Day 1; M7= Month 7; M18= Month 18

[a] Antibody positive represents the number (%) of subjects with an antibody response above the cutoff.

[b] p-value: Wilcoxon-Mann-Whitney test comparing total GMT for HIV+ and HIV- men. Analysis was conducted on the total population at each visit.

[c] p-value: Wilcoxon-Mann-Whitney test comparing HPV seropositive and seronegative men. HPV serostatus was based on Day1 antibody responses and thereafter these groups were maintained for Month7 and Month18 analysis.

[d] p-value: Wilcoxon-Mann-Whitney test comparing between HIV+ and HIV- men within Day 1 Seropositive group and Day 1 Seronegative group at each visit.

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GMT: Geometric Mean Levels expressed as ELISA Units/mL.

Table 3:

HPV-16 and HPV-18 oral gargle levels (IgG normalized) among HIV^+ and HIV^- mid-adult men.

					IgG normalized						
	Visit	**Group**	**N**	**Antibody positive (%)[a]**	**Total GMT (95% CI)**	**Day 1 Seropositive GMT (95% CI)**	**Day 1 Seronegative GMT (95% CI)**	**HIV Status Total p-value[b]**	**HPV Serostatus p-value[c]**	**HIV Status Seropositive p-value[d]**	**HIV Status Seronegative p-value[d]**
HPV-16	D1	HIV−	147	0 (0.0)	-	-	-	-		-	-
		HIV+	62	21 (33.9)	43.2 (24.8 – 75.4)	43.2 (24.8 – 75.4)	-				
	M7	HIV−	147	137 (93.2)	177.1 (156.3 – 200.7)	-	177.1 (156.3 – 200.7)	0.0081	-	-	0.0421
		HIV+	50	47 (94.0)	127.7 (94.9 – 171.9)	133.7 (81.4 – 219.6)	124.5 (84.0 – 184.4)		0.9913		
	M18	HIV−	103	41 (39.8)	79.9 (59.9 – 106.6)	-	79.9 (59.9 – 106.6)	0.8108	-	-	0.5255
		HIV+	53	37 (69.8)	86.2 (59.7 – 124.4)	117.7 (67.2 – 206.3)	67.9 (41.1 – 112.1)		0.1951		
HPV-18	D1	HIV−	147	6 (4.1)	17.4 (9.8 – 30.9)	17.4 (9.8 – 30.9)	-	0.0316		0.0316	-
		HIV+	62	39 (62.9)	41.1 (30.2 – 55.9)	41.1 (30.2 – 55.9)	-				
	M7	HIV−	147	106 (72.1)	73.7 (64.3 – 84.4)	52.4 (20.4 – 134.5)	74.9 (65.2 – 86.1)	0.0615	0.2794	0.1395	0.8353
		HIV+	50	44 (88.0)	97.8 (71.6 – 133.6)	108.6 (75.6 – 156.1)	68.6 (35.0 – 134.3)		0.2271		
	M18	HIV−	103	11 (10.7)	31.4 (17.2 – 57.2)	-	31.4 (17.2 – 57.2)	0.1024	-	-	0.4623
		HIV+	53	38 (71.7)	57.5 (40.8 – 81.0)	68.6 (47.6 – 99.0)	22.4 (11.4 – 43.9)		0.0072		

D1= Day 1; M7= Month 7; M18= Month 18

[a] Antibody positive represents the number (%) of subjects with an antibody response above the cutoff.

[b] p-value: Wilcoxon-Mann-Whitney test comparing total GMT HIV+ and HIV- men. Analysis was conducted on the total population at each visit.

[c] p-value: Wilcoxon-Mann-Whitney test comparing HPV seropositive and seronegative men. HPV serostatus was based on Day1 antibody responses and thereafter these groups were maintained for Month7 and Month18 analysis.

[d] p-value: Wilcoxon-Mann-Whitney test comparing between HIV+ and HIV- men within Day 1 Seropositive group and Day 1 Seronegative group at each visit.

GMT: Geometric Mean Levels expressed as ELISA Units/mL; Total IgG normalized GMT are expressed as EU/mg total IgG.

Table 4:

HPV-16 and HPV-18 antibody avidity among HIV^{+} and HIV^{-} mid-adult men (Serum).

Avidity	Visit	Group	N	Day 1 Total GMA (95% CI)	Day 1 Seropositive GMA (95% CI)	Day 1 Seronegative GMA (95% CI)	HIV Status p-value[a]	HPV Serostatus p-value[b]	HIV Status Seropositive p-value[c]	HIV Status Seronegative p-value[c]
HPV-16	D1	HIV−	10	1.1 (0.9 – 1.5)	1.2 (0.9 – 1.5)	0.8 (-)	0.4025	-	0.1517	-
		HIV+	42	1.2 (1.1 – 1.4)	1.4 (1.2 – 1.7)	0.9 (0.7 – 1.2)		0.0078		
	M7	HIV−	126	2.1 (2.0 – 2.2)	2.1 (1.9 – 2.4)	2.1 (2.0 – 2.2)	0.0258	0.9791	0.7431	0.0111
		HIV+	75	2.0 (1.8 – 2.1)	2.1 (1.9 – 2.3)	1.9 (1.7 – 2.1)		0.1542		
HPV-18	D1	HIV−	2	0.9 (0.5 – 1.6)	0.9 (0.5 – 1.6)	-	0.5057	-	0.3446	-
		HIV+	28	1.1 (0.9 – 1.3)	1.2 (1.0 – 1.3)	0.7 (0.4 – 1.3)		0.0235		
	M7	HIV−	126	1.7 (1.7 – 1.8)	1.7 (1.6 – 1.9)	1.7 (1.7 – 1.8)	<0.0001	0.8821	0.0661	0.0003
		HIV+	75	1.5 (1.4 – 1.6)	1.5 (1.4 – 1.7)	1.5 (1.4 – 1.6)		0.6039		

GMA= Geometric Mean Avidity Levels expressed as concentration of GuHCl (Molar), which reduced the optical density by 50% compared to sample wells without GuHCl treatment.

D1= Day 1; M7= Month 7.

[a] p-value: Wilcoxon-Mann-Whitney test comparing total GMT HIV+ and HIV- men. Analysis was conducted on the total population at each visit.

[b] p-value: Wilcoxon-Mann-Whitney test comparing HPV seropositive and seronegative men. HPV serostatus was based on Day1 antibody responses and thereafter these groups were maintained for Month7 analysis.

[c] p-value: Wilcoxon-Mann-Whitney test comparing HIV+ and HIV- men within Day 1 Seropositive group and Day 1 Seronegative group at each visit.

Table 5:

Correlations between HPV-16 and HPV-18 antibody avidity and antibody levels among HIV^{+} and HIV^{-} mid-adult men in serum, at Month 7.

Group		Antibody Avidity versus ELISA correlations		
		N	Spearman ρ	p-value
HPV-16	HIV−	126	0.18	0.0465
		126	0.19	0.0380
	HIV+	75	0.24	0.0351
		75	0.29	0.0113
HPV-18	HIV−	126	0.36	<0.0001
		126	0.32	0.0002
	HIV+	75	0.27	0.0171
		75	0.25	0.0315