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Antibody to the gp120 V1/V2 Loops and CD4+ and CD8+ T Cell Responses in Protection from SIV<sub>mac251</sub> Vagal Acquisition and Persistent Viremia

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The human papillomavirus pseudovirions (HPV-PsVs) approach is an effective gene-delivery system that can prime or boost an immune response in the vaginal tract of nonhuman primates and mice. Intravaginal vaccination with HPV-PsVs expressing SIV genes, combined with an i.m. gp120 protein injection, induced humoral and cellular SIV-specific responses in macaques. Priming systemic immune responses with i.m. immunization with ALVAC-SIV vaccines, followed by intravaginal HPV-PsV-SIV/gp120 boosting, expanded and/or recruited T cells in the female genital tract. Using a stringent repeated low-dose intravaginal challenge with the highly pathogenic SIV<sub>mac251</sub>, we show that although these regimens did not demonstrate significant protection from virus acquisition, they provided control of viremia in a number of animals. High-avidity Ab responses to the envelope gp120 V1/V2 region correlated with delayed SIV<sub>mac251</sub> acquisition, whereas virus levels in mucosal tissues were inversely correlated with antienvelope CD4+ T cell responses. CD8+ T cell depletion in animals with controlled viremia caused an increase in tissue virus load in some animals, suggesting a role for CD8+ T cells in virus control. This study highlights the importance of CD8+ cells and antienvelope CD4+ T cells in curtailing virus replication and antienvelope V1/V2 Abs in preventing SIV<sub>mac251</sub> acquisition.  The Journal of Immunology, 2014, 193: 6172–6183.

The development of a vaccine that prevents HIV acquisition remains a formidable challenge. Most currently licensed protective viral vaccines induce neutralizing Abs that mediate long-lasting immunity. However, broadly neutralizing Abs take an average of 2.5 y to develop during natural HIV infection (1) and often have extensive somatic hypermutation (2), a property likely to be difficult to induce via vaccination. In addition, clinical trials using a protein vaccine that primarily induced Ab responses failed to prevent HIV infection (3, 4) and led to increased emphasis on vaccines that induce HIV-specific T cell responses. However, vaccines that induced robust T cell responses failed to prevent HIV infection in clinical efficacy trials (Merck STEP trial–HVTN 502, HVTN 503, and HVTN 505) (5–7). In addition, in some of the trials, a higher number of infections occurred in vaccinated individuals than in the placebo arms. All three trials included systemically administered adenovirus vectors, and although the role of vector-specific responses remains unclear, the results suggest that systemic CD8 T cells alone are not sufficient to prevent HIV acquisition.

The RV144 Thai trial was the first HIV vaccine clinical trial to demonstrate measurable protective efficacy. Vaccination significantly reduced the risk of HIV infection, with an estimated efficacy of 31.2% (8). The vaccine regimen consisted of an i.m. injection of the canarypox vector ALVAC expressing HIV genes, paired with a bivalent envelope protein gp120 boost. This regimen induced mainly nonneutralizing Abs and CD4+ T cell responses (8, 9). Abs directed to the V1/V2 region of gp120 were found to be a primary correlate of a reduced risk of HIV acquisition, whereas Ab-dependent cellular cytotoxicity (ADCC) was a secondary correlate (9). These findings highlighted the potential of vaccine-induced Abs in preventing HIV acquisition. Binding nonneutralizing functional Abs could prevent virus entry and dissemination by impairing virus mobility at the portal of entry, or by destroying newly infected cells by activating the complement pathway, and/or coordinating with macrophages or NK cells (10).
Repeated low-dose mucosal challenges with simian HIV or SIV viruses in macaques are reasonable models of HIV sexual transmission (11). The SIVmac251 challenge used in this study is a pathogenic CCR5 user that is resistant to neutralization, similar to most HIV primary isolates. To date, HIV vaccine candidates tested in this macaque model, using mucosal repeated low doses of SIV, have recapitulated the results of HIV clinical trials in humans (12–14).

Preventing HIV transmission remains the primary goal of HIV vaccines; however, once infection has occurred, the reduction of chronic-phase viremia and the slowing or halting of disease progression are also important objectives. Increasing evidence suggests that although a vaccine-induced humoral response is important for protection from virus acquisition (15–17), CD8+ T cell responses contribute to virus control after lentiviral transmission (16, 18–20). In the RV144 Thai trial, the ALVAC-HIV/gp120 regimen induced negligible CD8+ T cell responses, and vaccinees that became infected had virus levels and CD4+ T cell counts similar to those of the placebo group, requiring the initiation of antiretroviral therapy (21). Multiple lines of evidence implicate CD8+ T cells in the control of HIV/SIV replication; for example, CD8+ T cell depletion of macaques during SIV infection causes a rapid increase in viral burden (22, 23). In addition, during primary HIV infection, the postpeak decline in viremia is temporarily associated with the induction of CD8+ T cell responses (24, 25). The immunologic pressure imposed by CD8+ T cells on HIV is evidenced by the emergence of MHC I–restricted escape mutations (26–28).

Intriguingly, recent studies have demonstrated potent control of SIV infection by broadly distributed T cell responses, induced by rhesus CMV vaccine vectors that generate unusual MHC class II–restricted CD8+ T cells targeting promiscuous SIV epitopes (29, 30).

Our goal was to develop a novel vaccine regimen that induces mucosal CD8+ T cells together with binding functional Abs and to ask whether this vaccine regimen alone could protect, or whether prior priming with systemic immunization could further improve protection. Human papillomaviruses (HPVs) are small non-enveloped DNA viruses that naturally infect epithelial cells within the genital tract; we used HPV-based vectors as a delivery system to specifically target Ag expression to the vaginal epithelium. We created HPV-pseudovirions (PsVs) that express SIV genes and to specifically target Ag expression to the vaginal epithelium. We created HPV-pseudovirions (PsVs) that express SIV genes and delivered them to basal epithelial cells in the female genital tract; we used HPV-based vectors as a delivery system to specifically target Ag expression to the vaginal epithelium. We created HPV-pseudovirions (PsVs) that express SIV genes and delivered them to basal epithelial cells in the female genital tract. Infection in the vaginal tract is facilitated by microtrauma that allows access to the basal epithelial layers (31). HPV-PsVs–mediated gene expression in the female genital tract has been shown to be transient, lasting for ~5 d, during which priming of T cells in the genital draining lymph nodes and Ag recall in the genital mucosa have been reported in murine models (32, 33). Initiation of an adaptive response is likely enhanced by the adjuvant-like potential of the HPV capsid, with its ordered protein arrangement (34). HPV-like particles have been shown to induce maturation of dendritic cells, resulting in the production of IL-6, IL-12, and TNF-α, and may be recognized by TLRs on mucosal cells engaging pathogen-associated molecular patterns on the HPV capsid (35, 35, 36). In previous studies, we demonstrated the feasibility of this vaccine approach using model Ags and the ability of HPV-PsVs to express foreign genes in the vaginal tract and induce HPV capsid–specific Abs in serum to each HPV serotype (37).

In this study, we evaluated whether the local mucosal immune responses, induced by HPV-PsVs vaccines paired with a gp120 protein boost, might prevent SIVmac251 intravaginal transmission. In addition, because ALVAC/gp120 regimens demonstrate limited but significant protection from infection in humans as well as in nonhuman primates (8, 13, 20, 38, 39), we examined whether an ALVAC-SIV systemic prime, paired with an HPV-PsV-SIV/gp120 boost, by inducing also higher systemic response, could increase vaccine efficacy.

Materials and Methods

**Animals, immunization, and SIV challenge**

This study used 36 female rhesus macaques of Indian origin, aged 3.5–7 y. All animals were housed and cared for under the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, and the study was conducted with the approval of the Institutional Animal Care and Use Committee at Advanced Biosciences Laboratories in Rockville, MD. The animals were divided into 3 groups of 12 animals, each based on their MHC alleles. In one group, the 12 animals were vaccinated with 105 IU of ALVAC-SIV encoding SIVmac251 gag-pol, and env (gp160) by i.m. injection in the thigh at weeks 0 and 4. The ALVAC-SIV vector was made as previously described (40). The 12 ALVAC-SIV–vaccinated animals and 12 additional macaques were vaccinated intravaginally with HPV-PsVs expressing SIVmac251 genes (HPV-PsV-SIV) at weeks 0, 10, and 24. HPV-PsVs were produced in 2 doses, delivered into the vaginal vault, using a positive displacement pipette. In addition, all 24 vaccinated macaques were given 2 i.m. injections with 200 μg gp120 protein, as done previously (39). The protein was mixed with the adjuvants alum and monophosphoryl lipid A (MPL) and administered in the thigh muscle at weeks 10 and 24. A total of 12 macaques were used as controls. Control animals were given the ALVAC vector that did not express SIV genes, HPV-PsVs that expressed luciferase, and the adjuvants alum and MPL at similar doses and times as the vaccinated animals.

At week 28, 4 wk after the last HPV-PsV vaccination, all 36 rhesus macaques were challenged intravaginally with 250 tissue culture–infective doses, 50%, of SIVmac251. The virus was kindly provided by Nancy Miller in the Division of AIDS, National Institutes of Health. Blood was collected, and SIV RNA was quantified in plasma 7 d after challenge; animals with virus loads <50 copies per milliliter were retested. Animals with two successive viral determinations >105 were considered persistently SIV infected; repeated SIV challenges were stopped, and virus loads were monitored weekly in the acute phase and monthly in the chronic phase. Animals with virus loads between 50 and 104 copies were retested at day 10. If the virus load increased at day 10 to >105, the animal was considered persistently SIV infected; the challenge phase was stopped, and virus load in plasma was monitored thereafter. If, however, the virus load at day 10 was <50 copies per milliliter, the animal was considered transiently infected, and repeated low-dose challenges were resumed. A maximum of nine repeated low doses of SIVmac251 were administered at 10-d intervals.

**Mucosal Abs**

Vaginal secretions were collected using absorbent cotton sponges. To elute secretions, the sponges were incubated for 10 min in elution buffer, on ice; transferred into a Salivette column (Sarstedt); and then centrifuged at 3000 rpm for 30 min at 4°C. For SIV-specific IgA and IgG, serially diluted vaginal secretions were transferred into 96-well half-area plates (Greiner Bio-One), previously coated with 50 μl (10 μg/ml) SIVmac251 gp120 (Advanced Bioscience Laboratories), and blocked with 1% BSA Blocking Solution (KPL). After overnight incubation at 4°C, plates were washed with PBS-Tween, reacted with peroxidase-conjugated anti-monkey IgA or IgG Ab (Alpha Diagnostic), and incubated for another hour at room temperature. After washing, 50 μl TMB peroxidase substrate solution was added to each well and incubated for 20 min at room temperature. Reactions were stopped by adding 50 μl 2 M H2SO4, and plates were read at 450 nm within 30 min. Titer was defined as the reciprocal of the dilution at which the absorbance of the test sample was twice that of the negative control sample diluted 1:5. Total IgA and IgG concentrations were similarly determined by incubating serially diluted mucosal samples and a dilution series of a standard normal rhesus macaque serum pool with known concentrations of IgA and IgG, and then incubated in microplates coated with rhesus/perfused goat anti-monkey IgA or IgG Ab. The Env-specific IgA or IgG titer was divided by the corresponding total IgA or IgG concentration in each secretion and reported as titer per microgram of total IgG or IgA.
Comparisons between groups were performed using the Mann–Whitney–Wilcoxon test for continuous factors, and paired comparisons between two times were assessed by the Wilcoxon signed rank test. Correlations were performed using the Spearman rank correlation method. The difference between groups in the binding of each of the overlapping gp120 peptides was tested using the exact Mann–Whitney–Wilcoxon test, and the p values were corrected for multiple comparisons by the Hochberg method. Graphical analysis was performed using GraphPad Prism, and error bars on graphs represent the SEMs.

IFN-γ ELISPOT
SIV-specific T cells were assessed using an IFN-γ ELISPot kit from Mabtech, as previously described (39). Cryopreserved PBMCs were thawed, rested, and stimulated with either SIVmac251 Gag or gp120 (Env) overlapping 15-mer peptides, Con A, or were left unstimulated. PBMCs and stimulants were added to IFN-γ-coated plates and incubated for 24 h. The plates were developed, and the frequency of IFN-γ-positive spot-forming cells per 10⁶ PBMCs was determined after background subtraction.

Pentamer staining and intracellular cytokine assays
Ten-color flow cytometric analysis was performed on mononuclear cells from blood, tissue, and vaginal and rectal biopsy specimens. Each biopsy specimens obtained from the cervix, vaginal, rectal, or wound were washed and incubated for 1 h with collagenase D at a concentration of 2 mg/ml in Iscove’s medium with antibiotics and amphotericin. Following incubation, the remaining tissue was mechanically disrupted to obtain a mononuclear cell suspension. Filtered single-cell suspensions of mononuclear cells were used in an intracellular cytokine assay performed as previously described (37). Cells were stimulated with either Env peptides at a concentration of 2 μg/ml or with PMA and ionomycin, or were left unstimulated in the presence of Golgi transport inhibitors, CD107a, clone HA43; anti-CD28ECD, clone CD28.2 (eBiosciences); and CD49D, clone 9F10 (BD Biosciences) for 6 h. Cells were then surface stained with CD3 (cloneSP34-2), CD4 (clone L200), CD8 (clone H120C10), CD95 (clone R11C9), and CD25 (clone B72), TNF-α (clone MAB11), and IL-2 (clone MQ1-17H12). Reagent stocks were obtained from BD Biosciences unless otherwise stated. Cytokine production after background subtraction from memory (CD95*), CD4*, and CD8* cells and the proportion of monofunctional and polyfunctional (simultaneous production of multiple cytokines) responses were determined. For Gag CM9 pentamer detection (obtained from ProImmune), cells were stained for 15 min with the Gag CM9 PE pentamer, washed, and then stained with the amine dye and CD3, CD4, CD8, and CD95, using the same clones as above. Samples were washed, permeabilized with Cytofix/Cytoperm, and stained intracellularly with K67 (clone B56, BD Biosciences). All cells were fixed with 1% paraformaldehyde and acquired on an LSR II (BD Biosciences). Data analysis was performed with FlowJo (TreeStar) and with SPICE (National Institute of Allergy and Infectious Diseases) (42).

CFSE proliferation assay
The lymphoproliferation assay was performed as previously described (39). Cells were briefly incubated with 5 nM CFSE (Invitrogen), washed, enumerated, and stimulated with 5 μg/ml SIV Env or Con A, or were left unstimulated for 5 d. Cells were then harvested and stained with CD3, CD4, CD8, CD28, and the amine dye, as described above. Samples were acquired on an LSR II flow cytometer, and the frequency of CD3⁺CD4⁺ or CD3⁺CD8⁺ cells with diminished expression of CFSE (proliferated) after 5 d of culture was determined and the background subtracted (%CFSE dim in unstimulated cells).

Binding Abs and pepscans
An ELISA was used to detect SIVmac251–gp120 binding Abs in blood, as previously described (40), and to detect binding to overlapping peptides spanning gp120. A serial dilution of plasma was added to microtiter plates coated with native purified gp120 Env protein of SIVmac251 or individual peptides, and the Ab titer determined. The absorbance at OD 450 nm was reported for peptide mapping. For binding Abs to gp120, the endpoint titers were defined as 2× the OD 450 of the negative control serum.

B cell ELISPOT
SIV Env-specific or total IgG or IgA Ab-secreting cells were analyzed by a B cell ELISPOT, as described previously (39). Briefly, MultiScreen 96-well plates (Millipore MAIPS4510) were incubated with 70% ethanol, rinsed, and coated with SIVmac251 gp120 protein or IgG or IgA (KPL). Coated plates were incubated at 4°C overnight, washed, and blocked for 2 h at 37°C. PBMCs were stimulated with Con A (ODN-2006; Operon), CD40L, and IL-21 (Peprotech) for 3 d at 37°C in 24-well plates. Stimulated PBMCs were next harvested and washed, and 3 × 10⁶ cells were plated and incubated overnight at 37°C. Plates were then washed and incubated with biotinylated goat anti-monkey IgG or IgA (Rockland), and HRP–avidin D conjugate (Vector Laboratories) was added. After several washes, plates were developed using 3-amoino-9-ethyl-carbazole (Sigma-Aldrich). Spot quantitation was performed with an ELISPOT reader.

ADCC
ADCC activity mediated by Abs in plasma samples was detected by the GranToxixLux (GTL) procedure, as previously described (20, 43). Briefly, CEM.NKRCCR5 target T cells were coated with recombinant SIVmac251 gp120 and labeled with a fluorescent target-cell marker and a viability marker. Labeled target cells were washed and plated. Cryopreserved human PBMCs from an HIV-seronegative donor served as effectors and were added to the assay wells at an E:T ratio of 5:1. Fluorescent granzyme B substrate (OncoImmunin) was added to each well. After incubation, serially diluted plasma samples were added to the assay wells. The plates were incubated for 15 min at room temperature, centrifuged, and incubated for 1 h at 37°C. The plates were then washed, cells were resuspended in PBS, and ~2500 events representing viable target T cells were acquired for each well, using an LSR II flow cytometer (BD Biosciences). Data analysis was performed using the FlowJo 8.8.4 software (TreeStar). The final results are expressed as ADCC titer and maximum granzyne B activity.

Neutralization assays
Neutralization was measured as a reduction in luciferase reporter gene expression after a single round of infection in TZM-bl cells, as described previously (20, 44). TZM-bl cells were obtained from the NIH AIDS Research and Reference Reagent Program. Virus was incubated with serial 10-fold dilutions of samples in duplicate. Freshly trypsinized cells were added to each well. One set of control wells received cells and virus (virus control), and another set received cells only (background control). After a 48-h incubation, cells were transferred to 96-well black solid plates (Costar) for measurements of luminescence. Neutralization titers are the dilution at which relative luminescence units were reduced by 50% compared with that in virus control wells after subtraction of background relative luminescence units. Assay stocks of molecularly cloned Env-pseudotyped viruses, SIVmac251.6 and SIVmac251.30, were prepared by transfection in 293T cells and titrated in TZM-bl cells. The SIVmac251 challenge stock was obtained from Nancy Miller in the Division of AIDS, National Institutes of Health, expanded on rhesus PBMCs, titered, and used.

Ab Avidity
Three recombinant SIV envelope proteins—full-length gp120, gp120 deleted of the V1V2 region, and the V1V2 mini protein—were made from codon-optimized SIVmac251 gp120. The V1V2 mini protein was fused to the C-terminal tag of HIV-1 gp120. These proteins were used as an Ag for the capture ELISA to detect SIV Abs against conformational epitopes, as previously described (39). Parallel ELISAs were used to determine Ab avidity. Heat-inactivated plasma samples were serially diluted and applied to a 96-well plate capturing SIVmac239 gp120 proteins in parallel duplicate spots. After 1 h of incubation, the plate was washed, and half the samples were treated with TBS, whereas the paired samples were treated with 1.5 M sodium thiocyanate (Sigma-Aldrich) for 10 min at room temperature. The plate was washed, and a goat anti-monkey IgG-detecting Ab ( Fitzgerald) was used. The avidity index (%) was calculated by taking the ratio of the sodium thiocyanate–treated plasma dilution, giving an OD of 0.5 to the TBS-treated plasma dilution giving an OD of 0.5, and multiplying by 100. Plasma of uninfected normal macaques served as negative controls. A high-avidity monkey mAb of 3.11H was included on every plate as the standard.

Viral load and transmitted founder variants

Plasma SIV RNA was quantified by nucleic acid sequence–based amplification, as previously described (45, 46). SIV DNA was quantified in mucosal tissues 3 wk after SIV infection by a real-time quantitative PCR assay with sensitivity ≤10 copies per 10⁶ cells, as previously described (45). Briefly, genomic DNA was extracted from tissue homogenates and subjected to the DNeasy Blood & Tissue Kit (QIAGEN), according to the manufacturer’s protocol, except the DNA elution step. The quantity and quality of the DNA were assessed by OD 260 measurements using an ND-1000
spectrophotometer (NanoDrop). The TaqMan probe and PCR primers for the real-time PCR were designed within the conserved gag gene of SIVmac239, and probe and primer sequences were used for the monkey albumin gene detection. The reaction conditions are as follows: the 25 μl PCR mixture consisted of 500 ng genomic DNA extracted from tissues; 200 nM primers; 100 nM probe; 2× TaqMan Universal PCR Mastermix (Applied Biosystems) consisting of 10 nM Tris–HCl (pH 8.3); 50 nM KCl; 5 mM MgCl2; 300 μM each of 2′-deoxyadenosine triphosphate, deoxyctydylate triphosphate, and deoxyguanosine triphosphate; 600 μM 2′-deoxyuridine 5′-triphosphate; 0.625 U AmpliTaq Gold DNA polymerase; and 0.25 U uracil N-glycosylase. Amplification was performed using one cycle at 50°C for 2 min and one cycle at 95°C for 10 min, followed by a two-step PCR procedure consisting of 50 cycles of 15 s at 95°C and 1 min at 60°C. PCR amplification was performed using the ABI Prism 7500 Sequence Detector System (Applied Biosystems). The normalized value of the SIV proviral DNA load was calculated as SIV DNA copy number/Mac albumin gene copy number × 2 × 10^6, and expressed as the number of SIV proviral DNA copies per 10^6 PBMCs or cells. In addition, an ultrasensitive nested quantitative real-time PCR and RT-PCR approach was also used to identify SIV RNA or DNA in vaginal and rectal tissues before and after CD8+ T cell depletion, as previously described (18).

Transmitted/founder viruses and their progeny were identified by single-genome amplification of SIV RNA from plasma or rectal pinch. SIV RNA or DNA was extracted, and limiting-dilution PCR of newly synthesized cDNA was performed. Reverse transcription of RNA to single-stranded cDNA was performed using SuperScript III reverse transcriptase according to the manufacturer’s recommendations (Invitrogen), using gene specific priming: SIVEnvR1, 5′-TGT AAT AAA TCC CTT CCA GGA CTA GC-3′; SIVEnvR2, 5′-ATG AGA CAT RTC TAT TGC CAA TTT GTA-3′ and antisense primer SIVEnvR1 under the following conditions: 1 cycle of 94°C for 2 min, 35 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 4 min, followed by a final extension of 72°C for 10 min. Nested PCR was performed with the following primers: SIVEnvF2, 5′-TAT CCC CCT CCA GGA CTA GC-3′; and SIVEnvR2, 5′-ATG AGA CAT RTC TAT TGC CAA TTT GTA-3′ under the same conditions used for first-round PCR, but with a total of 45 cycles. Transmitted/founder virus lineages were determined phylogenetically by identifying all distinct, low-diversity lineages, as described previously (20, 39, 47–49). All 388 sequences are deposited in GenBank under accession numbers KF646830–KF647217 (www.ncbi.nlm.nih.gov/genbank).

**CD8+ cell depletion**

CD8+ lymphocyte depletion was performed in 11 macaques. Animals were infused with the αCD8-depleting rehsus recombinant Ab M-T807R1, ob-
tain from the Non-Human Primate Reagent Resource. Animals were given three doses of αCD8 Abs on days 0, 3, and 7. The first dose was administered at a concentration of 10 mg/kg, whereas the other two doses were given at 5 mg/kg. The number or frequency of CD3 (clone SP34-2), CD4 (clone L200), CD8 (clone DK25 Dako), and CD20 (clone B9E9 Beckman Coulter) expressing cells was monitored in the blood and in the vaginal and rectal biopsy specimens. The CD8 Ab used for flow cytometry was chosen, as it has been shown not to compete with or mask the epitope of the CD8-depleting Ab (50).

Results

Induction of T cell responses by intravaginal HPV-PsV vaccination

The 36 rhesus macaques were distributed into three groups (Fig. 1A): HPV, group 1; ALVAC/HPV, group 2; and controls, group 3. Each group contained 3 Mamu-A*01–positive animals. The HPV vaccine group was given three intravaginal vaccinations with HPV-PsVs that expressed the SIV genes gag-pro, gp120, and rev, tat, nef (HPV-PsV-SIV) at weeks 6, 10, and 24. At weeks 10 and 24, animals were also given monomeric gp120 protein adjuvanted in alum and MPL. In the second cohort of animals (ALVAC/HPV, group 2), the systemic immune system was primed with ALVAC expressing SIV genes gag, pol, and gp160 (40) at weeks 0 and 4 and then boosted intravaginally with HPV-PsV-SIV and i.m. with gp120 protein in alum and MPL, similar to the group 1 animals. Twelve control macaques (group 3) were vaccinated with the ALVAC empty vector, HPV-PsVs that expressed luciferase and were given the adjuvants at the same time and dose as the other groups.

Previously, we demonstrated that intravaginal delivery of HPV-PsVs expressing SIV Gag recruited CD4+ and CD8+ T cells to the female genital tract, and resulted in SIV-specific responses in the cervicovaginal lamina propria (37). In this study, we extend and confirm those findings and measured T cell responses in the blood throughout the study; however, cervicovaginal biopsies were limited to baseline (before vaccination) and weeks 11 and 17 (1 and 6 wk after the second HPV vaccination) (Fig. 1A). This was done to allow sufficient time for healing before the intravaginal SIV challenge at week 28. Vaccination induced similar SIV Gag- and Env-specific immune responses in the blood of both vaccine groups measured by IFN-γ ELISPOT at 17 wk after vaccination (Fig. 1B). Proliferative responses were measured 1 wk after the last vaccination (Fig. 1C). ALVAC-primed HPV + gp120–boosted animals had higher levels of CD4+ Gag and Env T cell proliferation compared with the HPV group, although the difference was not significant (Fig. 1C). Env-specific CD8 T cell proliferative responses were higher in the ALVAC/HPV group compared with the HPV group (p = 0.039). Gag CM9 staining in Mamu-A*01–positive animals revealed no Gag-specific CD8+ T cells in the blood of animals from the HPV or control groups (Fig. 1D). As expected, the ALVAC/HPV group developed a systemic Gag-specific CD8+ Gag response that was boosted by intravaginal HPV-PsV-SIV vaccinations. The frequency of GagCM9 CD8+ T cells was also determined in the vaginal tract after the second HPV vaccination at weeks 11 and 17. Similar to the blood, Gag-specific CD8+ T cells were detected in the ALVAC/HPV group at week 11, but not in the HPV group (Fig. 1E). HPV-PsV entry in wounded keratinocytes is a slow process taking many hours, with peak expression on days 2–3 (31). Thus, 7 d after vaccination may not have been sufficient time for Ag presentation and T cell expansion. However, 6 wk later, T cell responses were detected in the HPV group and were expanded in the ALVAC/HPV group. The frequency of Gag-specific T cells was 14–to 16-fold greater in the vaginal tract compared with the blood, and 2– to 3-fold higher in the vaginal tract of the ALVAC/HPV group compared with the

FIGURE 2. Vaccination induced mainly monofunctional responses in the blood and vaginal tract. (A) Cytokine production following Env peptide stimulation of mononuclear cells from vaginal biopsies obtained at week 17. Shown is the sum of IFN-γ, TNF-α, IL-2, and CD107 production after background subtraction in CD95+CD4+ and CD95+CD8+ T cells. (B) The functional capacity of the SIV-specific response is represented by the pie charts; they show the proportion of cells that responded to stimulation by producing either IFN-γ, TNF-α, IL-2, or CD107, or a combination thereof. The fraction of cells that responded to stimulation by producing one cytokine is shown in gray; two cytokines, black; three cytokines, green; or four cytokines, orange. CD4 responses are represented in the top pie panel and CD8 responses are in the lower pie panel. (C) Total Env-specific cytokine production in blood after the last vaccination, week 26, in CD95+CD4+ and CD95+CD8+ T cells. Shown is the sum of IFN-γ, TNF-α, IL-2, and CD107 production. (D) Pies show the fraction of cells that responded to stimulation by producing one cytokine (gray), two cytokines (black), three cytokines (green), or four cytokines (orange). CD4 responses are in the top pie panel and CD8 responses are in the lower pie panel.
HPV group. Gag-specific CD8+ T cells were not detected in the rectum of vaccinated animals at week 17 (data not shown). The increased T cell response, observed in the vaginal tract of the ALVAC/HPV group, and the absence of Gag-specific T cells in the rectum of ALVAC-SIV–primed animals suggest that systemic priming followed by intravaginal boosting recruits and/or expands cell-mediated responses in the female genital tract.

Pinch biopsies of cervicovaginal tissues yielded a limited number of mononuclear cells. Mononuclear cells isolated from the Mamu-A*01–positive animals were used to measure the frequency of GagCM9+ CD8+ T cells, whereas cells from the remaining 27 Mamu-A*01–negative animals were used to measure functional mucosal responses to envelope peptides at week 17 (Fig. 2A). Intracellular cytokine staining for IFN-γ, TNF-α, IL-2, as well as the expression of CD107, was determined following 6-h stimulation with overlapping Env peptides. Vaccination induced mainly monofunctional CD4+ and CD8+ T cell responses that secreted IFN-γ, TNF-α, or CD107 (Fig. 2B). The frequency of Env-specific T cells was similar in the two vaccination regimens. At 2 wk before the first SIV challenge (week 26), a similar analysis of cytokine profile was performed in the blood of all vaccinated animals (Fig. 2C). ALVAC/HPV-vaccinated animals had a greater frequency of blood CD4+ T cell responses compared with the HPV group. Similar to the vaginal tract, primarily monofunctional memory responses were induced in blood (Fig. 2D); however, TNF-α was the dominating cytokine response in the ALVAC/HPV group, whereas either TNF-α, IFN-γ, or IL2 was produced in the HPV group.

**Systemic and mucosal gp120-specific Abs induced by vaccination**

ALVAC-SIV priming induced gp120-specific IgG in the blood, but by the end of the vaccination regimen, both groups had similar levels of high-titer binding Abs (Fig. 3A). To assess Abs in mucosal secretions, we collected vaginal swabs after the last vaccination. Equivalent levels of gp120-specific IgG were found in the ALVAC/HPV and HPV groups presented as titer per microgram of total IgG to normalize for the levels of total IgG isolated from each animal (Fig. 3B). Before normalization by total IgG, we directly compared the gp120-specific titers in each animal’s blood and vaginal mucosa and observed that, on average, Env-specific IgG was approximately one log lower in the vaginal mucosa than in blood. Low levels of gp120-specific IgA were detected in the vaginal secretions of both vaccinated groups (Fig. 3B). A similar frequency of Env-specific memory B cells was measured in both groups 1 wk prior to SIV challenge (Fig. 3C). Although both vaccine regimens induced measurable IgG+ gp120-specific B cells, no IgA+ gp120-specific B cells were detected in blood (data not shown).

The functional capacity of Abs induced by the two vaccine regimens was determined in the blood, owing to the limited quantity of protein extracted from vaginal swabs. The two vaccine regimens induced serum Abs that mediated similar levels of ADCC, measured as % maximum granzyme activity and ADCC titer (Fig. 3D). In contrast, the ALVAC/HPV group had significantly greater neutralization titers for the tier-1–like SIVmac251.6 virus compared with the HPV group \( p = 0.0023 \) (Fig. 3E). Neither vaccine regimen induced Abs that neutralized the tier-2–like SIVmac251.30 isolate (data not shown) or the SIVmac251 challenge stock (Fig. 3E).

Abs to the V1/V2 loop of gp120 were found to be a correlate of reduced HIV risk in the RV144 Thai trial, using ALVAC-HIV and gp120 immunogens (9). In another study involving ALVAC-SIV/ gp120 vaccination, we found that animals that resisted SIVmac251 infection had high-avidity Abs directed to the V1/V2 region (39).

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**FIGURE 3.** Vaccine-induced binding Abs and their functional capacity. (A) gp120-specific IgG titer in blood during the vaccination phase, with the ALVAC/HPV group represented as circles and with a large dashed line; the HPV group, squares with a small dashed line; and controls, triangles with a solid line. Vertical lines indicate the time when a vaccine was given: ALVAC, weeks 0 and 4; HPV-PsVs, weeks 6, 10, and 24; and gp120/adjuvant, weeks 10 and 24. (B) Gp120 binding Ab titers in mucosal secretions per microgram of total IgG/IgA measured after the last vaccination, week 25. IgG is shown on the left and IgA on the right. The ALVAC/HPV group is represented by white bars and the HPV group by hatched bars. (C) Percent gp120-specific IgG memory B cells measured by B cell ELISPOT in PBMCs at week 27. (D) ADCC in the blood shown as % granzyme B activity (left) or ADCC titer (right) measured at week 26. Circles represent animals in the ALVAC/HPV group; squares, the HPV group; and triangles, the control group. (E) Neutralization of an easy-to-neutralize tier-1–like virus SIVmac251.6 (left) and the SIVmac251 challenge stock (right) measured at week 26 after the last vaccination. A significantly higher level of neutralization was observed in the ALVAC/HPV group, indicated by the asterisk, using the Mann–Whitney–Wilcoxon test, with \( p = 0.0023 \).
We therefore measured vaccine-induced Ab binding to overlapping linear peptides that spanned gp120, including the V1/V2 region, and Ab avidity. Both regimens had an overall similar recognition of overlapping peptides spanning the constant and variable regions of gp120 (Fig. 4A). We compared the average binding of each peptide in the two vaccination regimens, using the Z statistic of the Mann–Whitney–Wilcoxon test (Fig. 4B). After correction for multiple comparisons by the Hochberg method, two peptides, 24 and 29, in the V2 loops had significantly greater Ab recognition in the HPV group (Fig. 4B). The ALVAC/HPV group showed increased binding to peptides 16 and 17 within the C1/V1 region and to peptide 40 in the C2 regions of gp120, but the difference was not statistically significant. The avidity of Abs to the whole gp120 protein of SIVmac239 was evaluated after sodium thiocyanate treatment. On average, Abs from both vaccine regimens had a similar avidity index (Fig. 4C).

To determine the contribution of the V1/V2 region of gp120, the avidity index of vaccine-induced Abs was assessed using a gp120 protein in which the V1/V2 loop was deleted (ΔV1/V2) and a conformational protein containing the entire V1/V2 stem loop of SIVmac239 linked to a tag from the C-terminal of HIV gp120. A significant reduction in the avidity index was observed when the V1/V2 region of gp120 was deleted (p < 0.0001) (Fig. 4C). The average avidity to the entire gp120 was 20.1, whereas the ΔV1/V2 avidity index was 6.4. Furthermore, when the avidity index of Abs to the V1/V2 mini protein was assessed, an average avidity of 30.9 was observed, a significant increase when compared with gp120 protein (p = 0.0011) (Fig. 4C). In some animals, the V1/V2 avidity was greater than 40. Of interest, an avidity index of 35–44 has been observed in other vaccination regimens in macaques protected from SIVmac251 and SIVmac660 infection (15, 39).

Vaccination with ALVAC-SIV/HPV-PsV-SIV/gp120 influences persistent viremia

The efficacy of each vaccination regimen was assessed by challenging animals with up to nine intravaginal low doses of SIVmac251 (250 tissue culture–infective doses, 50%), given every 10 d, beginning 4 wk after the last vaccination. The level of SIV RNA was determined in plasma by nucleic acid sequence–based amplification, 7 d after each challenge; animals that tested negative (50 copies per milliliter) were rechallenged. All three groups acquired SIV at a similar rate (Fig. 5A), and at the end of the challenge phase five vaccinated animals, two in the ALVAC/HPV group and three in the HPV group, remained SIV negative in plasma.

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**FIGURE 4.** Both vaccination regimens induce Abs that target the V1/V2 region of gp120. (A) Average Ab binding to 89 overlapping peptides that span gp120 with the ALVAC/HPV group on the left and the HPV group on the right. Variable regions are represented by colored bars, and dashed vertical lines designate the boundaries of the constant and variable regions. Blue arrows indicate peptides 24 and 29 in the V2 loop. (B) Comparative recognition of overlapping peptides presented as the difference between the median absorbance in the ALVAC/HPV group, relative to the HPV group, shown in red on the right y-axis. The statistical significance of the differences is shown by the Z statistic of the Mann–Whitney–Wilcoxon test in black, on the left y-axis, with the dashed lines marking significance at the p = 0.05 level after the correction for multiple comparisons by the Hochberg method. Increased recognition by the ALVAC group is presented as positive (0 to 5) on the top half of the graph, and increased recognition in the HPV group is presented as negative (0 to −5) on the lower half of the graph. Two peptides in the V2 loop, 24 and 29, demonstrated significantly greater Ab recognition in the HPV group relative to the ALVAC group, denoted by asterisks. (C) Avidity, shown as the avidity index of Abs to the entire gp120 of SIVmac239 on the left. The ALVAC/HPV group is represented by white bars and the HPV group by hatched bars. On the right is the comparison of the avidity of Abs induced by both vaccine regimens to the entire gp120 of SIVmac239, a gp120 protein deleted of the V1/V2 loop (ΔV1/V2), and a conformational mini protein containing the entire V1/V2 step loop of SIVmac239. A significant difference in the avidity index is observed between gp120 and ΔV1/V2-gp120, with p < 0.0001, and between gp120 and the V1/V2 mini protein, with p = 0.0011, indicated by the asterisk, using the Wilcoxon signed rank test.
whereas one control animal remained negative. Because most HIV infections are initiated with a single or few viral variants, we aimed to model this outcome in our mucosal challenge experiment in macaques. The number of transmitted viral variants is an independent analysis of a limiting dose challenge (47). Thus, we quantified the number of variants in all SIV-infected animals that had at least two viral load measurements >10^3 RNA copies per milliliter and created neighbor joining trees for each group (Supplemental Fig. 1). No difference in the number of transmitted variants was observed between the three groups. Each group had a median of one viral variant and a maximum of three (Figure 5B, Supplemental Fig. 1). This finding suggests that our intravaginal SIVmac251 was given at a dose that models HIV heterosexual transmission. Furthermore, neither the intravaginal vaccination nor the progesterone/N9 treatment caused a significant increase in the number of transmitted variants. A similar number of variants (median 1) were also observed in naive macaques that were given a low-dose challenge by the vaginal or rectal route (39) (N. Miller, unpublished observations). No significant associations were observed between vaccine-induced immune responses and the number of transmitted variants. Most infected animals demonstrated high peak (10^6–10^8) and set point (10^5–10^7) plasma virus (Fig. 5C). However, of the vaccinated animals, 8 had no detectable plasma virus or transient plasma viremia that remained below the limit of assay detection: 50 SIV RNA copies per milliliter (Fig. 5C). In total, 16 of 24 vaccinated animals and 10 of 12 controls demonstrated persistent SIV viremia. Persistent viremia was defined as at least two successive plasma viral load measurements >10^4 SIV RNA copies per milliliter. We next compared the viremia over the 16 wk of follow-up in the persistently SIV-infected animals. No significant differences in either peak or set point viremia in vaccinated animals or controls were observed (Fig. 5D). In addition, a similar loss of CD4+ T cells was observed in the blood of all persistently SIV-infected macaques (Fig. 5E). To assess virus levels in mucosal tissues, pinch biopsies were collected from the vagina and rectum during acute SIV infection, and the levels of SIV DNA were determined (Fig. 6A, 6B). Unlike our findings for plasma viremia, significantly less SIV DNA was measured in the vaginal and rectal mucosa during the acute phase in the ALVAC/HPV-vaccinated animals in comparison with controls (p = 0.014 and p = 0.022) (Fig. 6A, 6B). Reduced viral DNA in the mucosa.
during the acute phase of SIV infection was also temporally associated with the expansion of GagCM9\textsuperscript{+}CD8\textsuperscript{T} cells measured 10 d after SIV infection (Fig. 6C), with the vaginal tract having the highest frequency of SIV-specific CD8\textsuperscript{T} cells and the lowest virus DNA levels in the acute phase of infection.

**CD4\textsuperscript{T} cells and Abs to V1/V2 correlate with protection from persistent viremia**

We investigated potential associations between mucosal SIV DNA levels in the acute phase and vaccine-induced responses. We observed an inverse correlation between the level of Env-specific CD4\textsuperscript{T} cell proliferation in blood measured 2 wk after the last vaccination and SIV DNA in the vaginal and rectal tract after SIV infection ($r = -0.5$ and $-0.47$ and $p = 0.014$ and 0.027 for vaginal and rectal tissues, respectively) (Fig. 6D). We did not observe a correlation between vaccine-induced CD8\textsuperscript{T} cell responses and SIV viral loads. However, the temporal expansion of vaginal CD8\textsuperscript{T} cells during the acute phase and association of CD4 helper responses with reduced viremia may indicate that the increased CD4\textsuperscript{T} cell responses induced by the ALVAC/HPV may have helped the development of a secondary CD8\textsuperscript{T} cell response, which in turn affected virus replication in the mucosa.

Next, we investigated the four animals with transient viremia; these animals had virus loads ranging from 50 to $10^4$ copies per milliliter and then controlled viremia during the remaining repeated low-dose challenges and for 14 wk of follow-up. We questioned whether their SIV-specific immune responses were boosted during the successive SIV challenges. We observed a reduction in gp120 binding Abs and IFN-γ ELISPOT responses to Gag in the vaccinated animals, comparing responses before the first SIV challenge with samples collected after the fifth challenge (Supplemental Fig. 2A). In addition, we did not detect a Vif-specific response in the cervicovaginal tract at the end of the challenge phase (Supplemental Fig. 2B). Vif is not in any of the vaccines administered but is abundant in the challenge virus. Furthermore, the levels of gp120 binding Ab titers in the vaginal secretions had also declined following the ninth and final SIV challenge, when compared with pre-SIV levels, consistent with the findings in blood (Supplemental Fig. 2C).

The two distinct outcomes observed in this study, that is, persistent SIV infection versus protection from infection or high virus replication, gave us the opportunity to investigate any associations between the measured immune responses and outcome. A comparison of immune responses in protected animals and persistently SIV\textsubscript{mac251}-infected macaques yielded a significant difference in the levels of Abs with high avidity to the V1/V2 region (Fig. 7A). Furthermore, we found a significant correlation between the number of challenges to attain persistent infection and the avidity index of V1/V2 Abs in blood (Fig. 7B). In all, these data highlight the importance of Env-specific CD4\textsuperscript{T} cells in the containment of virus replication at mucosal sites and of high-avidity Abs targeted to the V1/V2 region of gp120 in the prevention of SIV\textsubscript{mac251} acquisition.

**CD8 T cells contribute to protection from persistent viremia**

At 3 wk after the ninth SIV challenge, SIV-negative animals and animals with transient viremia had $<50$ copies of SIV RNA in plasma and were tested for SIV DNA in the mucosa (Fig. 6A, 6B, arrows). With the exception of one of the control animals that had $24$ copies of SIV RNA per $10^6$ cells in the rectum, the remaining nine animals tested negative for SIV DNA in the vaginal and rectal tract, using an assay that detects $>10$ SIV DNA copies per $10^6$ cells (45). We followed these 10 animals for 14 wk, testing for SIV RNA in blood, and all remained SIV negative.

To investigate in more detail the immune control of viremia, we performed CD8 depletion in nine of the ten animals (one animal

**FIGURE 6.** Reduced SIV DNA in mucosal tissues of ALVAC/HPV-vaccinated animals. (A) Vaginal SIV DNA levels per $10^6$ cells. Circles represent the ALVAC/HPV group, squares the HPV group, and triangles the controls. Significantly less SIV DNA was present in the vaginal tissues of the ALVAC/HPV group compared with the control group, denoted by an asterisk using the Mann–Whitney–Wilcoxon test, with $p = 0.014$ and $0.027$ for vaginal and rectal tissues, respectively. (B) Rectal SIV DNA per $10^6$ cells in vaccinated macaques and controls. Significantly less SIV DNA was present in the rectal tissues of the ALVAC/HPV group compared with controls, denoted by an asterisk using the Mann–Whitney–Wilcoxon test, with $p = 0.022$. (C) Mononuclear cells from the blood and from vaginal and rectal biopsy specimens were obtained 10 d after SIV infection. The frequency of memory (CD95\textsuperscript{+}) GagCM9-specific CD8\textsuperscript{T} cells is shown in the vaccinated animals (white bars) and controls (black bars). (D) An inverse correlation was observed between the levels of SIV DNA in the vaginal tract (left) and the rectum (right), and the Env-specific proliferating memory (CD95\textsuperscript{+}) CD4\textsuperscript{T} cells presented as the square root of the data. The correlation was assessed using a nonparametric Spearman test with $r$ values of $-0.5$ and $-0.47$ and $p$ values of $0.014$ and $0.027$ for the vaginal and rectal tracts, respectively.
was excluded owing to surgical complications), as well as in two persistently viremic macaques, as a control. Treatment with anti-CD8 Ab rapidly depleted CD8+ cells in the blood (Supplemental Fig. 2G) and caused a significant decline in the frequency of CD8+ cells in the lymph nodes (57%) and rectum (62%) (Supplemental Fig. 2E). As expected, in the two animals with persistent viremia, plasma virus levels further increased following CD8 depletion (Supplemental Fig. 2F). We collected vaginal and rectal biopsies before CD8 depletion (pre), 9 d after depletion (during) when CD8+ T cells were undetectable in the blood, and 42 d post-treatment (post) when CD8+ T cells had rebounded, and quantified SIV DNA and RNA using a low-copy ultrasensitive assay (18). Both unimmunized animals tested positive at mucosal sites either pre-, during, or post–CD8+ T cell depletion (Table I). Of the vaccinated animals, four remained negative, and three of them belonged to the HPV/gp120 group (Table I), suggesting early and transient SIV infection were compared with the animals that were protected from SIV infection or had high viremia. A significant difference was observed using a Mann–Whitney–Wilcoxon test with \( p = 0.024 \) (B) A direct correlation was observed between the V1V2 Ab avidity and the number of SIV challenges needed to attain persistent SIV infection, assessed by a Spearman test, with \( r = 0.49 \) and \( p = 0.014 \). Animals that were protected from infection or had only transient viremia are represented at challenge 9.

Discussion
SIV infection of rhesus macaques models key aspects of HIV infection. To date, HIV vaccine candidates tested in the macaque model, using repeated low doses of SIV given across mucosal surfaces, have recapitulated the results of HIV clinical trials (12–14). Furthermore, a low-dose mucosal challenge with an uncloned SIV swarm can be titered to transmit a single or few virus variants in macaques (49), similar to the bottleneck described during most HIV infections (48). Thus, in this study, we used a repeated intravaginal challenge with a SIVmac251 swarm to test the efficacy of a novel mucosal vaccination regimen HPV-PsV-SIV, with and without ALVAC-SIV priming. Neither vaccine regimen significantly altered the rate of SIV acquisition compared with controls, although several animals were either protected from SIV infection or had transient viremia. In all, 16 of 24 (67%) vaccinated animals, and 10 of 12 (83%) of controls, developed persistent infection. Vaccinated animals that became persistently viremic had similar peak and set-point plasma virus levels, which was not surprising, given the low levels of systemic CD8+ T cell responses induced by these vaccines. However, we observed a reduction in viral burden in mucosal tissues in vaccinated animals compared with controls, and this reduction was significant in the ALVAC/HPV group, which had the highest levels of vaginal Gag-specific CD8+ T cell responses. The limited number of mononuclear cells isolated from the vaginal tract precluded our assessment of vaginal Gag-specific responses in all animals and of rev, tat, and nef responses. In addition, we observed an inverse correlation between proliferating Env-specific CD4+ T cells in blood and the levels of systemic SIV DNA. A number of vaccinated animals had a long-lasting control of viremia. Altogether, these data suggest that T cell responses induced by HPV-PsV vaccination exerted early mucosal virus control, but virus expansion likely outpaced T cell expansion, leading to systemic dissemination and uncontrolled viremia. Surprisingly, the mucosal T cell response induced by the HPV-PsV/gp120 regimen did not curtail local virus levels or provide sustained virus control, whereas priming with ALVAC-SIV induced an overall higher Gag and Env proliferative T cell response (Fig. 1C) and better control of mucosal virus levels (Fig. 6A, 6B). This suggests a role for SIV-specific T cells in early protection from virus replication. In a murine model, intravaginal HPV-PsV vaccination induces long-lived CD103+ CD8+ T cells that home to the site of vaccination and intercalate throughout the epithelial layers of the vaginal tract (32). If HPV-PsV vaccination similarly induces tissue-resident CD8+ T cells in humans as it does in mice, these CD8+ T cells would be well positioned to combat HIV at the site of virus entry. The exposed columnar epithelium in the cervix of young women is potentially susceptible to HPV vaccination and a likely site of HIV transmission (51).

Progesterone treatment was used to facilitate vaccine delivery and may have influenced the vaccine-induced immune response, as progesterone has been shown to reduce antiviral responses (52). Intravaginal delivery of vaccines and the disruption of the epithelium used to facilitate delivery are cumbersome, and they introduce several challenges for clinical applications. However, vaccination in the secretory phase may eliminate the need for

Table I. Virus in mucosal tissues before, during, or after CD8 depletion

<table>
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<tr>
<th>Animal</th>
<th>Group</th>
<th>SIV Infected</th>
<th>Vaginal Biopsies</th>
<th>Rectal Biopsies</th>
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<td>–</td>
<td>–</td>
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<tr>
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<td>No</td>
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</table>

Bold text denotes SIV infected animals. –, no SIV DNA or RNA detected; pos, positive for SIV RNA or DNA or both; tx, treatment.
hormonal treatment, and the collection of a cytology specimen as routinely done during a Papanicolaou test causes sufficient microtrauma to facilitate HPV vaccination (53). Thus, intravaginal HPV vaccination could be easily incorporated into a routine gynecologic visit and would potentially confer protection against both HPV and HIV.

Both regimens (ALVAC/HPV and HPV) elicited high-titer gp120-specific IgG in the blood and vaginal secretions, but neither regimen induced appreciable levels of IgA in the vaginal secretions, nor detectable gp120-specific IgA memory B cells in the blood. Thus, IgA was unlikely to have played a role in the outcome of these studies. We found similar levels of ADCC, and neither vaccine regimen induced Abs capable of neutralizing the SIVmac251 challenge stock.

A theme is emerging from nonhuman primate studies that are consistent with the results of the RV144 Thai trial: nonneutralizing Abs mediate protection from lentiviral infection. Indeed, in a vaccine regimen that efficiently primes CD8+ T cells using gp96 as a vaccine carrier, neutralizing Abs mediate protection from lentiviral infection. Indeed, in a vaccine regimen that efficiently primes CD8+ T cells using gp96 to express SIV peptides, protection from SIV infection was achieved only when an Ab-inducing protein boost was added to the vaccination regimen (54). Our results similarly support this concept, as delayed persistent SIV infection was associated with the avidity of Abs directed to the V1/V2 region of gp120 and not with T cells. The functional role of Abs to the V1/V2 region in the efficacy of HIV vaccines remains to be clearly defined, and recent data suggest that mAbs to V2 synergize with other envelope regions to neutralize the virus (55). The studies described underscore the importance of this immunologic target, as we demonstrated that Abs targeting the V1/V2 region were associated with delayed virus acquisition, as in the case of HIV in RV144. These data suggest the relevance of this animal model for the preclinical evaluation of an HIV-1 vaccine efficacy trial. N. Engl. J. Med. 12: 1276–1285.


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SIVmac251 challenge stock.

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