A Polyvalent Clade B Virus-Like Particle HIV Vaccine Combined with Partially Protective Oral Preexposure Prophylaxis Prevents Simian–Human Immunodeficiency Virus Infection in Macaques and Primes for Virus-Amplified Immunity

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Abstract

Vaccination and preexposure prophylaxis (PrEP) with antiretrovirals have shown only partial protection from HIV-1 infection in human trials. Oral Truvada (emtricitabine/tenofovir disoproxil fumarate) is FDA approved as PrEP but partial adherence reduces efficacy. If combined as biomedical preventions (CBP), an HIV vaccine could protect when PrEP adherence is low and PrEP could prevent vaccine breakthroughs. The efficacy of combining oral PrEP with an HIV vaccine has not been evaluated in humans. We determined the efficacy of combining a DNA/virus-like particle (VLP) vaccine with partially effective intermittent PrEP in Indian rhesus macaques (RM). Eight RM received intramuscular inoculations of five DNA plasmids encoding four HIV-1 Clade B primary isolate Envs and SIVmac239 Gag (at weeks 0 and 4), followed by intramuscular and intranasal inoculations of homologous Gag VLPs and four Env VLPs (at weeks 12, 16, and 53). At week 61, we initiated weekly rectal exposures with heterologous SHIV162p3 (10 TCID₅₀) along with oral Truvada (TDF, 22 mg/kg; FTC 20 mg/kg) dosing 2 h before and 22 h after each exposure. This PrEP regimen previously demonstrated 50% efficacy. Five controls (no vaccine, no PrEP) received weekly SHIV162p3. All controls were infected after a median of four exposures; the mean peak plasma viral load (VL) was 3.9×10^7 vRNA copies/ml. CBP protected seven of eight (87.5%) RM. The one infected CBP RM had a reduced peak VL of 8.8×10^5 copies/ml. SHIV exposures during PrEP amplified Gag and Env antibody titers in protected RM. These results suggest that combining oral PrEP with HIV vaccines could enhance protection against HIV-1 infection.

Introduction

PREVENTIVE MEASURES AGAINST HIV infection include behavioral and barrier methods, postexposure prophylaxis (PEP), and, more recently, additional biomedical approaches including circumcision, treatment as prevention, and preexposure prophylaxis (PrEP) with antiretroviral drugs.^{1–3} Oral PrEP clinical trials involving Truvada, which is a daily combination dose of emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF), have shown encouraging results, with efficacy rates of 44%, 62%, and 75% being observed in men who have sex with men, heterosexual men and

women, and HIV-1 serodiscordant couples, respectively.^{1–3} A 62% risk reduction was also noted in serodiscordant couples receiving oral TDF alone, and oral TDF reduced HIV-1 incidence in injecting drug users by 49%.^{1,4} The favorable outcomes of these extensive clinical trials culminated in FDA approval of oral Truvada for PrEP in combination with safer sex practices to reduce the risk of sexually acquired HIV infection in high-risk men and women.⁵ The CDC recommended that daily oral Truvada be the preferred PrEP regimen for injecting drug users (IDU), with prevention services targeting both injection and sexual risk behaviors associated with IDUs.⁶

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Nonhuman primate (NHP) studies provided the key proof-of-concept studies that led to the human PrEP trials and are being used to evaluate next generation PrEP, including intermittent dosing, long-acting drugs or formulations, and optimal delivery routes or devices.^{7–9} For example, one study by Garcia-Lerma *et al.* demonstrated a significant reduction in the risk of infection in rhesus macaques (RM) treated orally with daily human-equivalent doses of FTC or a combination of FTC and TDF and challenged rectally with repeat limited doses of simian–human immunodeficiency virus (SHIV).¹⁰ A subsequent study modeled intermittent PrEP regimens and found many to be protective equivalent to daily dosing.^{10,11} Such an intermittent dosing strategy is currently being modeled in humans, as it may overcome some of the problems related to daily dosing.¹² Other modalities including dosing animals with Truvada 2 h before and 22 h after rectal SHIV_{162p3} challenge showed significant, although reduced, protection when compared to daily dosing.¹¹

Despite these promising advances in biomedical preventions, development of a safe, effective, and durable HIV-1 vaccine continues to be a priority for several economic and disease control reasons. Only one HIV vaccine, tested in the RV144 trial and consisting of canarypox vector-based ALVAC-HIV (vCP1521) in a prime-boost combination with AIDSVAX B/E containing HIV-1 gp120, has shown any efficacy, and the modest (31%) efficacy precluded licensure.^{13–16} The poor performance of this and other nonefficacious vaccine candidates in the past 20 years has required new approaches. As for PrEP, nonhuman primate models have also been useful for guiding HIV vaccine development. Partial protection against SIV or SHIV in NHP models has been described for RV144-like regimens and other HIV-1 vaccine strategies.^{17–19}

The optimal preventive HIV vaccine characteristics are not known, but overcoming HIV-1 Env diversity is a key challenge. Several strategies have been used to address this problem. These include polyvalent vaccines, composed of multiple wild-type envelopes with genetic heterogeneity, and monovalent vaccines, using a consensus sequence derived from genetically distinct HIV-1 isolates.^{17,20–23} The Gag protein, which is generally well conserved across HIV-1 clades, is also usually included in vaccine regimens, with SIVmac239 Gag being most commonly used in NHP models. We have previously described a polyvalent Clade B virus-like particle (VLP) vaccine composed of Env from four different Clade B HIV-1 primary isolates, and Gag p55 from SIVmac239.^{18,24} When administered to RM, the vaccine resulted in a 67% reduction in the rate of infection following four repeated vaginal challenges with heterologous SHIV_{SF162p4}.¹⁸ Furthermore, the viral loads in breakthrough infections were two logs lower than that noted in unimmunized RM. Importantly, the level of efficacy conferred by the polyvalent Clade B VLP vaccine was greater than that shown by a monovalent consensus Clade B vaccine, which did not have any protective effects against virus transmission or viral burden.¹⁸ The mechanism of protection with the polyvalent vaccine is not completely understood but appears to be T cell independent and primarily humoral.¹⁸

Since the vaccine and PrEP modalities that have been evaluated to date in humans are only partially effective there is increasing interest in using combination strategies, which have the potential to provide a safety net for those with poor

adherence to PrEP dosing or may result in higher efficacy in groups that are both adherent and vaccinated. Combinations of different approaches to the prevention of HIV infection is not new and is being broadened to include treatment as prevention (TasP) and PrEP, and will likely include a licensed HIV vaccine when available. How HIV vaccines will interact with PrEP to prevent HIV infection is not known.

Testing such a combination in a phase III human clinical trial would be challenging logistically and financially, and difficult to conduct, given the ethical issues around providing only vaccine or PrEP as control arms of a trial.²⁵ Human phase I studies exploring the interaction between an HIV vaccine and PrEP have been proposed. NHP studies can provide proof-of-concept data on the likely interactions of different PrEP regimens and HIV vaccine candidates to evaluate safety and to provide quantitative data regarding synergistic, additive, or other interactions. Moreover, NHP studies can be used to determine potential biomarkers of efficacy, including drug levels or immune correlates that could guide clinical trial design.

One such study was recently reported by Cheng-Mayer *et al.*, where a prime-boost T cell-based vaccine was administered in combination with a topical microbicide containing a suboptimal concentration of an inhibitor against HIV-1 nucleocapsid zinc fingers.²⁶ Although this combination of modalities delayed SHIV acquisition and reduced VL in the breakthrough animals, only 20% of RM were protected after 20 repeat limited-dose vaginal challenges with SHIV_{162p3}.²⁶ One limitation of the study is that the Gag and Pol immunogens in the vaccine are genetically identical to those in the challenge virus, which is not reflective of the scenario in humans who are exposed to variants that are genetically distinct from the HIV-1 vaccine. Barouch *et al.* also evaluated combinations of microbicides and HIV vaccines.²⁷ The partial preventive efficacy of adenovirus-based candidate HIV vaccines combined with a fusion inhibitor T-1249 or the CCR-5 inhibitor maraviroc was demonstrated against SIV or SHIV_{162p3} infection.²⁷ A limitation of these studies was the sample size and lack of human preventive efficacy data of the microbicides. Neither of these studies evaluated oral PrEP, the only FDA-indicated PrEP strategy for HIV-1 prevention.

The approval of oral Truvada for preventing sexual acquisition of HIV-1 in men and women, the availability of precise pharmacokinetics and efficacy data in an RM model of partial PrEP efficacy, and the partial, but significant, efficacy demonstrated by the polyvalent Clade B VLP vaccine prompted the evaluation of the combined biomedical prevention (CBP) strategy described here.^{10,11,22} Our objective was to determine whether combining the polyvalent humoral-based vaccine with a human-equivalent oral PrEP, both having partial efficacy, would interact favorably to achieve an increased level of protection against heterologous SHIV transmission. While the previous polyvalent vaccine study addressed protection from vaginal exposures, we chose to evaluate the efficacy against rectal transmission¹⁸ since rectal transmission of HIV-1 is a key driver of the men who have sex with men (MSM) HIV-1 epidemic and is a significant mode of HIV acquisition in women.^{28–30} The intermittent oral Truvada regimen that was evaluated used the identical protocol against rectal challenges previously shown by Garcia Lerma *et al.* to protect three of six macaques after 14

challenges, thus facilitating a direct comparison between CBP and PrEP alone using historical data.

Materials and Methods

DNA/VLP vaccine plasmids and VLPs

The plasmid backbone for the vaccine, HIV-1 VLP (pHIV-wtVLP_{ADA}), has been previously described.^{18,24} The Env VLPs include *env* from each of four HIV-1 clade B strains, PVO.4, RHPA4259.7, SC422661.8, and AC10.0.29, which were originally isolated from patients located in Italy, the United States, and Trinidad. The ADA Env gene from pHIV-wtVLP_{ADA} was replaced with the Env primary isolates that share between 85% and 87% homology with SF162p3 *env* in the challenge virus. Transmission modes of these strains include male-to-male, female-to-male, and male-to-female transmission, and all were isolated during the acute stage of HIV-1 infection. The SIV Gag VLP includes the codon-optimized SIVmac239 p55 Gag gene as previously described.¹⁸ The production and purification of VLPs were performed as previously described.¹⁸

Macaques and study design

Thirteen adult male rhesus macaque (*Macaca mulatta*) were used. All macaques were housed under approved biosafety level-2 containment conditions at the Caribbean Primate Research Center, University of Puerto Rico (San Juan, Puerto Rico), and later transferred to the Centers for Disease Control and Prevention (CDC). Their diet, care, and maintenance complies with the Guide for the Care and Use of Laboratory Animals (Department of Health, Education and Welfare no. NIH 86-23). All procedures outlined in this study were approved by the CDC Institutional Animal Care and Use Committee.

The RM were split into two study groups, with eight RM receiving a CBP regimen and five RM serving as the untreated control group (Fig. 1). There were insufficient resources to include vaccine only and PrEP only arms in this pilot study. However, since historical data for control RM (no vaccine, no PrEP) and PrEP only arms were available, and partial efficacy was predicted for vaccine only arms,¹⁸ most RM were distributed to the experimental CBP arm. All RM were anesthetized with ketamine prior to procedures and

sample collections. The CBP group was immunized intramuscularly (IM) with DNA plasmids (0.5 mg each) encoding SIVmac239 Gag, and the four Env primary isolates, at week 0 and week 4. Purified HIV Env VLPs and SIV Gag VLP (0.25 mg each) were administered at weeks 12, 16, and 53, via the IM route with Imject Alum adjuvant (Thermo Fisher Scientific Inc., Rockford, IL) and the intranasal (IN) route with CpG oligodeoxynucleotides (Sigma-Genosys, The Woodlands, TX) (Fig. 1). Repeat limited dose rectal challenges with 10 TCID₅₀ SHIV_{162p3} were initiated at week 61 for the CBP group, which is when SHIV_{162p3} challenges for the control group were also started. Virus challenges for both groups continued once weekly for 14 weeks. A 1 ml volume of the SHIV162p3 challenge virus was administered into the rectal vault via atraumatic insertion of a sterile gastric feeding tube of adjusted length connected to a sterile syringe.³¹ The CBP group also received a human equivalent oral dose of Truvada (22 mg/kg TDF, 20 mg/kg FTC) 2 h before and 22 h after each virus exposure.¹¹ PrEP was administered every week for 14 weeks, and was continued in the breakthrough infected RM for 11 weeks until virus challenges for all remaining macaques were completed. All RM were monitored weekly for infection status and virus exposures were stopped when an RM tested SHIV RNA-positive. Animals were considered infected after two consecutive vRNA⁺ results, after which SHIV challenges were stopped.

Blood collection and processing

Blood samples were collected from the CBP group before and after immunization with the DNA vaccine and VLP boost. The animals from both the CBP and control groups were bled before the virus challenge schedule began, followed by blood collections 22 h after each virus exposure. When applicable, samples were processed using standard Hypaque-Ficoll centrifugation to isolate peripheral blood mononuclear cells (PBMCs) and plasma. A second set of blood samples was collected to facilitate serum isolation. Plasma, serum, and PBMCs were stored at -80°C until analysis. Plasma viral RNA (vRNA) load was quantified using real-time reverse transcription polymerase chain reaction (RT-PCR) as previously described.^{10,11,32} The lower limit of detection of this assay is 50 vRNA copies/ml plasma.

FIG. 1. Study design illustrating the components and schedule of the combined biomedical prevention (CBP) regimen and repeat low-dose virus exposures in the CBP [$N=8$ rhesus macaques (RM)] and in naive controls ($N=5$ RM) that did not receive vaccine or preexposure prophylaxis (PrEP). All animals were concurrently exposed to 14 challenges of simian-human immunodeficiency virus (SHIV)_{162p3} and were combined for purposes of analysis with nine historical control RM.¹⁵

Intracellular drug levels

All samples for drug analysis were taken just prior to the +22 h time point after each virus challenge. The concentrations of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP), which are the active drug metabolites of tenofovir (TFV) and FTC, respectively, were determined as detailed previously^{10,11} by high-performance liquid chromatography (Agilent 1100 LC system; Agilent Technologies) with mass spectrometry detection (TSQ Quantum; Thermo Electron). All calibration curves had r^2 values greater than 0.99.

Binding antibody response to immunization and virus exposures

Antibody end-point titers to SIV Gag antigen and the five HIV-1 Env antigens, which include Env from the primary isolates and from the challenge virus, were determined by ELISA-based analysis of serum as previously described.¹⁸ Briefly, 96-well plates were coated with 50 ng of SIV Gag p55 obtained from the NIH AIDS Research and Reference Reagent Program or 50 ng of gp120 purified proteins collected from the supernatant of 293T cells transiently transfected with plasmids expressing the various gp120 proteins. The readings from prebleed sera were subtracted from post-immunization/exposure samples to account for background responses.

Antibody avidity assay

The antibody avidity was determined by a modified SHIV-specific BioPlex assay as described in previous studies.³³ The assay involves a multiplex panel that couples recombinant SIVmac251 p27 and HIV-1 gp41 (MN strain), gp120 (IIIB strain), and gp160 (IIIB strain) (Immunodiagnostics Inc., Woburn, Ma) to Bio-Plex COOH microspheres using the Bio-Plex Amine coupling kit (Bio-Rad Laboratories). Briefly, diluted plasma samples were incubated with the microspheres in appropriate assay buffer in a 96-well plate at room temperature, followed by an additional incubation step of the microspheres with either diethylamine or assay buffer after incubation with the plasma samples. The microspheres were washed, resuspended in assay buffer, and analyzed on a Bio-Plex 200 System (Bio-Rad Laboratories). The antibody avidity index is expressed as a percentage and was calculated using the following formula: (normalized value of diethylamine-treated well/normalized value of buffer-treated well) $\times$ 100. Neutralizing antibodies were also evaluated as previously described.³³

Statistical analysis

Kaplan–Meier curves for the untreated and CBP-treated groups were generated using Graphpad Prism 5 (Graphpad Software Inc., La Jolla, CA). The log-rank (Mantel–Cox) test was performed to determine statistical significance ($p < 0.05$) between the study groups. The Mantel–Haenszel model was used to estimate the risk of infection, as a hazard ratio, of the controls relative to the CBP group or historical PrEP-only group. Where applicable, pairs of data sets were analyzed using the Mann–Whitney t -test to determine statistical significance.

Results

Efficacy of the vaccine and PrEP CBP regimen

The goal of the current study was to determine whether combining the DNA/VLP vaccine and partially effective oral PrEP would result in a high level of protection against rectal virus exposures. Eight RM were in the CBP group and received vaccine and oral Truvada (Fig. 1). Five control RM received no immunization or PrEP. All animals received up to 14 weekly challenges with 10 TCID₅₀ of SHIV_{162P3}. While five of five RM in the control group (solid black line, Fig. 2A) were infected after an average of 4.2 exposures, 87.5% (seven of eight) of the CBP group (red line, Fig. 2A) were protected, and remained uninfected despite 14 exposures. Only one of eight RM in the CBP group became infected, at challenge 3 (assuming a 7-day eclipse period from infection to time of detection) (Fig. 2A). Together with the inclusion of nine historical controls (dotted black line) and six historical PrEP-only treated RM (blue line, Fig. 2A) that received an identical Truvada regimen and subsequent rectal challenges with 10 TCID₅₀ SHIV_{162P3},¹¹ the data show that a significant degree of protection was achieved by the CBP regimen ($p < 0.0001$, hazard ratio = 12.64, log-rank test) and by the PrEP only treatment ($p = 0.0045$, HR = 5.475). While CBP and PrEP-only treatment resulted in the infection of one of eight and three of six

FIG. 2. (A) Survival curve illustrating protection from SHIV_{162P3} infection following a CBP ($N = 8$, red line), PrEP only ($N = 6$, blue line), or untreated regimen ($N = 5$ real time and 9 historical controls,¹⁷ solid and dotted black lines, respectively). Statistical significance ($p < 0.05$, log-rank test), the hazard ratios (HR), and the confidence intervals (CI) are shown. (B) Plasma viral loads in the CBP group ($N = 8$, red lines; breakthrough infection in bold red) and in the control group ($N = 5$, black lines; mean in bold black). Oral Truvada was initiated at the first virus exposure and continued once weekly throughout the 14 week exposure period in all CBP-treated animals. Plasma samples were collected and analyzed 22 h after each exposure. The viral load cut off is indicated by the dotted line.

RM, respectively, this difference was not found to be statistically significant ($p=0.1287$, $HR=0.2026$) but was limited with respect to power.

Virus kinetics in CBP breakthrough and control RM

Previous studies have shown that this vaccine has beneficial effects on the postinfection disease course by lowering virus load in breakthrough animals.¹⁸ Also, oral Truvada as PrEP reduces viral loads in PrEP breakthroughs, and beneficially influences the immune response postinfection, with higher CD4 counts, better preserved memory T cell responses, and less inflammation.^{10,11} The viral RNA (vRNA) in plasma from the eight CBP-treated RM and five control animals was monitored by RT-PCR once weekly over the 14-week exposure period. As shown in Fig. 2B, the peak VL in the five infected RM from the control group (black lines) were in the range of 1.8×10^6 – 5.9×10^7 , with a mean peak VL of 2.73×10^7 copies/ml plasma. The single breakthrough infection in the CBP group (thick red line) showed an almost 2 $\log_{10}$ lower peak viral load of 8.81×10^5 vRNA copies/ml plasma. This difference was maintained over the next 3 weeks, after which point the viral load in the breakthrough RM decreased to undetectable levels. Two of the control animals also had a similar pattern, but the VL in the other three control RM was up to 2 $\log_{10}$ higher. The lower VL noted in the breakthrough RM is consistent with the trend observed in breakthrough animals in our previous PrEP studies in macaques.^{10,11} The time taken for productive infection to occur in the breakthrough CBP-treated RM (3 weeks) was similar to that observed for the control RM (mean of 4.2 weeks). One additional RM (7D9) in the CBP group had transient levels of SHIV_{162P3} vRNA ≤ 250 copies/ml plasma at challenge weeks 2 and 7, but this was not a sustained infection, and the RM was negative for vRNA by the 14th virus exposure, which is when the oral Truvada regimen was also stopped. This animal remained undetectable for vRNA at 2 weeks post last SHIV challenge.

In vivo drug levels

To understand possible reasons for the breakthrough infection in RM 6F9 and the transient infection in RM 7D9, we examined these two animals in further detail. We measured intracellular concentrations of the active metabolites of FTC and TDF in PBMCs and the drug levels in plasma isolated from each animal during the 14-week challenge period. The mean drug levels in PBMCs and plasma seen 24 h after drug dosing are depicted in Fig. 3A and B, respectively. The median intracellular concentrations for TFV-DP and FTC-TP were 51 fmol/ 10^6 PBMCs (range 0–364 fmol/ 10^6 PBMCs) and 1,205 fmol/ 10^6 PBMCs (range 0–4,928 fmol/ 10^6 PBMCs), respectively, over the 14 weeks of exposure, which is consistent with previous reports.^{10,11} As expected, plasma drug concentrations at 24 h after each weekly administration were low (mean=35 ng/ml for TFV and 50 ng/ml for FTC). Importantly, the preinfection intracellular levels of TFV-DP and FTC-TP in the PrEP breakthrough animal (6F9) and in the one animal with transient infection (7D9) were similar to those in the protected CBP-treated RM. These data suggest that low levels of FTC and TFV in PBMCs did not explain the breakthrough or transient infection.

FIG. 3. Drug levels in (A) peripheral blood mononuclear cells (PBMCs) and (B) plasma from CBP-treated RM ($N=7$) during the virus exposure period. Shown are the mean levels of tenofovir diphosphate (TFV-DP) and emtricitabine triphosphate (FTC-TP) (PBMCs) and TFV and FTC (plasma) $\pm$ SD at each time point.

Systemic antibody responses in protected and infected RM

The DNA/VLP vaccine in this CBP has not previously been shown to influence the kinetics or quality of the antibody response in breakthrough SHV infections.¹⁸ However, oral PrEP delays maturation of antibody avidity following SHIV infection.³³ To determine how CBP affected the pre- and postinfection antibody response, we evaluated binding antibodies and antibody avidity in the protected CBP-treated RM, the SHIV-infected CBP breakthrough, and the controls. To determine the breadth of antibody responses generated against Env, an ELISA consisting of a panel of Env from the four clade B viral isolates and the SF162 challenge virus was performed with serial dilutions of sera from each RM prechallenge and at virus exposures 4 and 10. A similar assay was done to measure binding antibody responses generated against SIV Gag_{p55}. It should be noted that the prechallenge time point for the CBP group corresponds to 2 weeks after the third and final VLP boost. Interestingly, many uninfected animals showed large increases in responses to the vaccine and challenge virus antigens in the weeks after challenge with SHIV_{162P3}.

Anti-Gag and anti-Env binding antibodies rose after SHIV_{162P3} exposures, even though no viremia was detected in these animals. As shown in Fig. 4A, the median prechallenge antibody end-point titers for Gag were significantly higher ($p<0.0037$, Mann-Whitney t -test) in CBP-treated

FIG. 4. Binding antibody titers to (A) SIV Gag antigen, and to HIV-1 Env from (B) SF162, (C) sc42, (D) AC10, (E) PVO4, and (F) RHPA in the CBP group ($N=8$, black circles) and in the control group ($N=5$, diamonds). The open blue circles on each graph indicate titers for the breakthrough RM 6F9. The filled green circles indicate titers for RM 7D9, which had transient vRNA expression at weeks 2 and 7. Statistical significance ($p < 0.05$, Mann-Whitney test) is shown where applicable. Bars indicate median titers.

animals than in controls, indicating that the boost raised anti-Gag responses. Following challenge, these responses were also elicited earlier in the CBP group, with as much as 10- to 1,000-fold higher titers being observed at the fourth exposure. In contrast, elevated Gag antibody responses in the control group were observed later at the tenth exposure, several weeks after infection. There were low or undetectable levels of anti-Env antibodies detected pre-challenge in all animals. However, following challenge, similar patterns were shown by the Env antibody responses, with significantly higher median end-point titers against three of four clade B viral isolates (Fig. 4B–E) being noted in the CBP group at exposures 4 and 10 when compared to the control group. Importantly, expansions were greatest for responses to SF162P3 at exposures 4 and 10 in the CBP-treated group, while high SF162 Env titers were seen only at exposure 10 in the control RM (Fig. 4B). Similarly, amplifications of antibody titers after the fourth and tenth challenges were observed to sc42, AC10, PVO4, and RHPA envs in the CBP-treated RM, although the fold increases were not as great as for SF162P3. Moreover, the pre-challenge Env antibody titers in sera from the CBP-treated RM were similar to those observed for the control animals, which is in contrast to what was seen for the Gag antibody responses at the same time point.

Furthermore, the breakthrough infected RM (6F9) in the CBP group consistently showed the lowest Gag and Env

antibody responses (open blue circles) at early and later time points (Fig. 4). Notably, the amplified Gag and Env antibody titers that were observed in the protected CBP-treated RM were also observed in RM 7D9 (green circles), which had a transient expression of vRNA at weeks 2 and 7. There were no neutralizing antibodies (TZM-bl assay) detected in sera from either group up to 4 months postinfection, and ELISpot responses to Gag, Pol, Env, and Nef peptide pools at 0, 2, 4, 6, and 8 weeks were not statistically significant (data not shown).

The effect of CBP on the maturation of the strength of antibody binding, or antibody avidity, was also measured to determine if there were any correlates of protection with maturation of the humoral immune response. The BioPlex assay was used to measure and compare the avidity index for anti-SIV p27, and anti-HIV gp120, gp160, and gp41 antibodies in plasma from the five infected control RM and the single breakthrough infected RM (6F9) from the CBP group. Typically, the primary response to infection generates low avidity antibody, with avidity strength increasing over time as the IgG antibody response matures over the course of the infection. As shown in Fig. 5, this pattern of increasing avidity after becoming positive for SHIV_{162P3} vRNA was observed for the five infected control RM and for two PREP-only infected RM that were evaluated for avidity. Interestingly, the antibody avidity index for the breakthrough animal 6F9 began at a high level, being up to 10-fold that of the

FIG. 5. Maturation of SHIV antibody responses to (A) Gag p27 and to Env (B) gp120, (C) gp160, and (D) gp41 antigens. Avidity index values are shown for RM 6F9, the breakthrough infected RM in the CBP group (red line), for the five infected RM in the control group (black lines), and for two PrEP-only infected RM (green lines) after testing positive for SHIV_{162P3} vRNA. If applicable, statistical significance between RM 6F9 and the infected control RM, or the PrEP-only RM, is indicated in black and green text, respectively.

controls for p27 ($p=0.042$, Mann–Whitney t -test) and gp160 antigens ($p=0.008$) as early as 1 week postinfection (Fig. 5A and C).

A higher avidity index was also observed early for anti-gp41 antibodies in RM 6F9 (Fig. 5D), but the difference in magnitude was not significant when compared to the avidity indices for the control group. A comparison of the CBP breakthrough RM 6F9 with two PrEP-only infected RM showed statistically significant differences in avidity levels of p27 ($p<0.0001$), gp160 ($p<0.0001$), and gp41 ($p=0.0001$). In contrast to the progressive increase in avidity observed for the four antigens in the five infected control RM, the antibody maturation for gp160 and gp41 analytes in the breakthrough infected RM remained relatively constant postinfection, while the avidity index for anti-p27 antibodies showed a moderate decrease. The rate and level of antibody maturation for the gp120 antigen were similar among the control and breakthrough infected CBP RM and PrEP-only infected RM (Fig. 5B).

Discussion

The combination prevention modeled in these studies of a DNA/VLP vaccine followed by intermittent oral Truvada was highly protective with 87.5% of the animals remaining uninfected after repeated SHIV_{162P3} challenges. The data suggest that combining a partially effective HIV vaccine with partially effective PrEP augments efficacy relative to either prevention alone. While this study was not designed or powered to compare the combination against vaccine alone or

PrEP alone, historically the vaccine and the intermittent PrEP had less than 70% efficacy when evaluated separately.^{11,18} The data suggest that when the preventions were combined, their effects were additive. Moreover, evaluation of pre- and post-infection immunity in CBP animals suggests mechanistic explanations for why the interaction of PrEP and vaccine conferred this enhanced protection. The unique aspect of the present study is that a human-relevant PrEP regimen, oral Truvada, was evaluated. The polyvalent Clade B VLP vaccine, which expresses a mixture of VLPs each containing one of four different Env from primary isolates and includes SIV Gag VLP, was administered as two DNA vaccinations followed by three VLP-only boosts with adjuvant, as was previously described. The challenge virus, SHIV_{162P3}, which expresses HIV-1_{SF162} Env, is identical to the strain used in the previous intermittent PrEP study, and is heterologous (85–87% identity) to each envelope in the polyvalent Clade B VLP vaccine used in the study by McBurney *et al.*¹⁸ Thus, every attempt was made to maintain consistency and gain statistical power using the previous data while at the same time ensuring that the stringency of the repeat low-dose macaque model was not compromised.

The CBP regimen resulted in a statistically significant level of protection, with only 12.5% of RM becoming infected after 14 rectal exposures with SHIV_{162P3} as compared to 100% of the controls. No difference in the time to infection in the CBP group was noted compared to the controls. The efficacy of the CBP tested in this study was much greater than and also mechanistically different from that seen in the limited number of previous CBP studies. For example, a

combination of a Zn finger inhibitor and a DNA/Ad5-based vaccine provided only 20% efficacy with a delay in SHIV acquisition.²⁶ These products targeted the viral nucleocapsid and raised primarily T cell responses, respectively. In another study in which an Ad26/Ad5 vaccine was combined with maraviroc, a CCR5 inhibitor, a 67% efficacy was demonstrated.²⁷ In both of these studies, peak viremia was reduced in the CBP breakthrough animals.

Measurements of TFV-DP and FTC-TP in PBMCs from the CBP RM showed that the pre-challenge drug levels in RM 6F9 PBMCs were similar to the seven protected CBP-treated RM, suggesting that other factors may have contributed to susceptibility in this RM. No association with protective MHC type 1 *Mamu* alleles was observed in the protected CBP RM (data not shown). One explanation for the breakthrough animal is weak antiviral immunity as this animal had the lowest levels of pre-challenge anti-Gag and anti-Env antibody titers. Also the early onset of this infection during the challenge series may have precluded amplification of the vaccine-induced responses. We note that rectal immune responses and rectal levels of drug metabolites were not evaluated. In previous studies of this PrEP regimen, rectal levels of TDF and FTC metabolites were higher than those observed in PBMCs and did not explain PrEP breakthroughs.¹¹

The vaccine did, however, raise expected levels and breadth of anti-Gag and Env binding antibodies, which declined following the final VLP boost to low or undetectable levels for Gag and Env. Surprisingly, when the post-challenge antibody responses were evaluated in the seven protected CBP animals, an amplification of the low anti-Gag responses was seen and Env antibody titers rose from undetectable to becoming detectable to the heterologous challenge SF162 Env and to the vaccine Envs. Titers to Gag and Env rose with increasing numbers of challenges. The amplification of the anti-Gag responses was generally similar to the amplification of the anti-Env responses. This amplification was observed in every CBP animal. This phenomenon of virus-induced amplification of vaccine-raised immune responses was not noted in previous vaginal challenge studies of animals that had received the poly B vaccine alone and were challenged with SHIV_{162P4}.¹⁸ Although the challenges TCIDs were not identical and the experiments were carried out in different centers, the assays used to detect the anti-Gag and anti-Env antibodies were the same for both studies, suggesting that these responses were uniquely amplified by viral exposures during PrEP.

These observations suggest mechanistic explanations for why this vaccine PrEP combination provided such high protection, i.e., an interaction of the different modes of action of vaccine and PrEP. Possible explanations include the following: (1) viral challenges resulted in initial rounds of replication that were subsequently prevented by PrEP, but sufficient antigen presentation was allowed that amplified local and systemic B cell responses; (2) inactive virions in SHIV_{SF162P3}, or virion-antibody complexes induced a "vaccine-like" boost of immune responses through dendritic cell antigen presentation to B cells; the fact that this SHIV-boosting phenomenon was not seen in the previous poly B-only study makes this a less likely explanation, although PrEP might have altered virus-antibody kinetics in such a way that boosting occurred; and (3) cryptic infections with SHIV_{SF162P3} are present in the CBP animals, resulting in

amplified B cell responses; against this possibility are previous data suggesting that PrEP-protected NHP do not have cryptic infections after CD8 depletions.³⁴

While there have been a few studies that evaluated CBP, none has reported this antibody-driven phenomenon following viral challenges in the CBP group. The data in the CBP-protected RM, taken together with the unique postinfection binding antibody responses in RM 6F9, suggest that CBP played a role in generating these unique pre- and post-challenge immune responses. Repeated transmissions that are blocked early by PrEP or vaccine may have had a priming or boosting effect on local and/or systemic immunization. Thus, CBP may not only directly inhibit virus infection via pharmacological antiretroviral activity, but also inhibit infection by indirectly facilitating the development of protective immune responses. Lower inoculum doses introduced at mucosal sites in RM have been shown to limit transmitted variants and lengthen the time to achieving productive infection.³⁵

Collectively, our findings show that CBP was highly efficacious with a survival proportion of 87.5%. While each prevention had different mechanisms of action, the high efficacy may have been due, in part, to a novel preventive mechanism resulting from combining the effects of the individual interventions. Further research on the nature of the interactions between the polyvalent vaccine and the PrEP components could generate information useful for improved vaccines and PrEP or for other biomedical preventions. The CBP also resulted in control of viral load in the single breakthrough animal, and may be relevant to consider in research targeting a cure for HIV-1.

While this CBP focused on prevention of rectal transmission, conceptually it should apply to prevention of vaginal or parenteral modes of transmission. This study was specifically designed to evaluate the sequence of vaccination followed by PrEP because approval of an HIV vaccine for prevention of HIV-1 infection will likely have an indication for preteens or young adults prior to onset of sexual activity, as is the indication for human papillomavirus vaccination. PrEP could then be added when approaching a period of sexual activity. PrEP and HIV vaccine combinations may be anticipated in several other settings, such as during an HIV vaccine trial, when PrEP may be provided as part of a prevention package for study participants.^{25,36} Multiple similar trial designs have been discussed, including a two-stage randomization,³⁶ a design similar to that evaluated in the present study. Even after licensure of an HIV vaccine, PrEP could be used to provide protection during immunizations, before a full vaccine series has been administered. Additionally, PrEP may be useful if there is a concern regarding transient enhancement of HIV risk by an HIV vaccine since concurrent PrEP may allow immunity to be raised without the risk of enhancement. Nonhuman primate models can be used to explore all these possibilities and provide useful information to inform human trials and answer questions about implementation.

Acknowledgments

The authors thank members of CDC's DHAP-Laboratory Branch Kelly Curtis and Susan Kennedy for performing the antibody avidity assays and for helpful discussions, and Mian-er Cong for her assistance in the drug dosage calculations and

preparation. This study was supported by funding from CDC and from NIAID R01AI068507 to Ted M. Ross. We thank Debra Hanson for guidance on statistical analysis. We acknowledge the following members of CDC's DHAP-Laboratory Branch/Pre-clinical Evaluation Team for their contributions to our nonhuman primate research: David Garber, James Mitchell, Leecresia Jenkins, Shanon Ellis, and Frank Deyoungs for animal technical assistance as well as Chou-Pong Pao, Chuong Dinh, Amy Martin, and Angela Holder for their assistance with analytical chemistry. The views expressed by the authors do not necessarily reflect those of the Centers for Disease Control and Prevention.

Author Disclosure Statement

No competing financial interests exist.

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