Macaque studies of vaccine and microbicide combinations for preventing HIV-1 sexual transmission

Macaque studies of vaccine and microbicide combinations for preventing HIV-1 sexual transmission

Dan H. Barouch[a,b,1], Per Johan Klasse[c], Jason Dufour[d], Ronald S. Veazey[d], and John P. Moore[c,1]

[a]Beth Israel Deaconess Medical Center, Boston, MA 02115; [b]Ragon Institute of MGH, MIT, and Harvard, Boston, MA 02114; [c]Department of Microbiology and Immunology, Weill Cornell Medical College, New York, NY 10021; and [d]Tulane National Primate Research Center, Tulane University School of Medicine, Covington, LA 70433

Edited* by Robert C. Gallo, Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, and approved April 12, 2012 (received for review February 22, 2012)

Vaccination and the application of a vaginal microbicide have traditionally been considered independent methods to prevent the sexual transmission of HIV-1 to women. Both techniques can be effective in macaque models, and limited efficacy has been observed in clinical trials for each. Here, we have addressed whether vaccines and microbicides can be used together to provide reinforced protection against virus challenge of rhesus macaques. In two separate experiments, four groups of animals were vaccinated with a T-cell–based adenovirus (Ad) vectored vaccine aimed at reducing postinfection viral loads and/or a partially effective dose of a vaginal microbicide aimed at blocking infection of a high-dose vaginal challenge with SIVmac251 or SHIV-162P3. In the first study, the only two protected animals were in the group that received Ad26/Ad5HVR48 vaccine vectors combined with the fusion inhibitor T-1249 as the vaginal microbicide before SIVmac251 challenge. In the second study, vaccination with Ad35/Ad26 vectors combined with the CCR5 inhibitor maraviroc as the vaginal microbicide led to significant reductions of both acquisition of infection and postinfection viral loads following SHIV-SF162P3 challenge. As expected, the vaccine by itself reduced viral loads but had no acquisition effect, whereas the microbicide had a partial acquisition effect but minimal impact on viral loads. For both measures of protective efficacy, the vaccine–microbicide combination differed more from controls than did either separate intervention. Overall, the data suggest that vaccines and microbicides are complementary techniques that may protect better when used together than separately.

HIV type 1 (HIV-1) continues to spread globally through sexual transmission, particularly among young women in the developing world who have little power to insist on the use of condoms to protect themselves (1, 2). In such circumstances, biology-based interventions become of substantial importance (2). Traditionally, the spread of infectious disease has been most effectively controlled by vaccination, but this approach has had, at best, limited success against HIV-1 in efficacy trials (3, 4). New vaccines based on live viral vectors are under evaluation in the macaque model of HIV-1 infection, with partial success at blocking acquisition of infection and a more consistent ability to reduce postinfection viral loads (5, 6). However, inducing neutralizing antibodies of sufficient breadth and potency to prevent virus transmission remains a major challenge (7).

An alternative approach to HIV-1 prevention involves the application of antiretroviral drugs (ARVs), either systemically as oral preexposure prophylaxis (PrEP) or locally at the mucosal sites of entry of the virus into the body (8). In the latter method, a gel or other formulation containing an ARV is administered vaginally or rectally as a microbicide to prevent the initial stages of entry of HIV-1 into the body (9). Studies with oral PrEP and vaginal microbicides have shown partial efficacy against HIV-1 sexual transmission to humans in the CAPRISA and iPREX trials but not in other studies (10–13).

Within the HIV-1 prevention fields, vaccines and microbicides have been regarded as independent, and in some respects, rival technologies. And yet there is logic to considering the two approaches as complementary. On a very simple level, two partially effective barriers to infection might be superior to either one alone. A more sophisticated argument is that, by reducing the extent to which an incoming virus replicates in mucosal tissues, a microbicide might buy time for the maturation of vaccine-induced immune responses that either eliminate the virus or further counter its expansion such that the viral load set point is reduced (14, 15). Another possibility is that the combined use of a microbicide that partially blocks acquisition of infection with a T-cell–based vaccine that reduces postinfection viral loads will result in both protective effects following virus challenge. We sought to test the underlying hypotheses by carrying out two experiments in the rhesus macaque vaginal challenge model, using inhibitors of HIV-1 entry as the microbicides and adenovirus (Ad)-vector–based vaccines.

Results

Design of Combination Studies. Both studies were of broadly similar design. The rhesus macaques were immunized by the intramuscular route with an Ad-vector–based vaccine. Eight months after the boost immunization, a vaginally applied inhibitor was then given as the microbicide for 30 min before vaginal simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) challenge. For comparison, other animals were not vaccinated or did not receive the microbicide. In both experiments, the macaques were treated with progesterone 30 d before challenge, to ensure that most control animals would become infected after a single exposure to virus (16). We considered other designs, particularly for the second experiment, such as the use of multiple challenges without prior progesterone treatment. However, our experience has been that infection of control animals can be highly inconsistent under such conditions, which adversely impacts how the outcome of the study can be interpreted (17). The Ad-vector–based vaccines were primarily T-cell–based vaccines aimed at reducing viral loads postinfection; they would not be expected to block acquisition of infection based on the high-dose challenge in the SIVmac251 study and the mismatched Env in the SHIV-162P3 study (6, 18). We hypothesized that the acquisition and viral load effects of each individual component would both occur when the two methods were used together. Alternatively, a microbicide-mediated reduction in the infectivity of the incoming virus, or in the rate at which it expanded locally after deposition in the vagina, might increase the protective capabilities of the vaccine.

A critical aspect of the experimental design was to use a partially protective microbicide concentration; too much inhibitor might be so strongly protective as to prevent us from ascertaining any

Author contributions: D.H.B., P.J.K., and J.P.M. designed research; D.H.B., J.D., and R.S.V. performed research; P.J.K. analyzed data; and D.H.B., P.J.K., and J.P.M. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence may be addressed. E-mail: dbarouch@bidmc.harvard.edu or jpm2003@med.cornell.edu.

impact of the vaccine, but too little might have no measurable effect. Hence in preliminary experiments we titrated each microbicide against the challenge virus in an attempt to define a concentration that would protect half of animals when used alone. In both experiments, the animals were divided into four groups: Control (i.e., no intervention), group C; vaccine only, group V; microbicide only, group M; and vaccine + microbicide, group V+M.

Fusion-Inhibitor Microbicide and SIVmac251 Challenge. In the first study, the vaccine was an Ad26/Ad5HVR48 vector regimen expressing Gag-Pol-Env-Nef (18). The microbicide was the fusion inhibitory peptide, T-1249, given at 200 μg/mL (40 μM) in 4 mL of an aqueous gel 30 min before high-dose vaginal challenge with SIVmac251 (19, 20). Each group contained six animals, but one animal was omitted from group V when it was found to be retrovirus infected before challenge. All of the animals in groups C ($n = 6$) and V ($n = 5$) became infected, and postinfection viral loads were consistently ~10-fold lower in vaccinees (group V) as expected (Fig. 1). This magnitude of viral load reduction is comparable with what was observed using the same vaccine in other challenge formats (18). Hence any effect of progesterone treatment on vaccine efficacy and/or local immunity is not substantial (21, 22).

We did not achieve our intended goal of protecting half of the animals in group M. Here, four of the animals became systemically viremic with typical viral load kinetics. However, the other two did so only after a delay of 4–6 wk. In our experience, such a delay is highly unusual and thus likely relates to partially protective effects of the T-1249 microbicide (*Discussion*). Two of the six animals in the V+M group remained uninfected throughout the period of monitoring (Fig. 1). This outcome was not statistically significant for acquisition (V+M vs. C, $P = 0.23$). However, as the only two animals to remain uninfected in this experiment were in the V+M combination, we considered that the data pattern was sufficiently encouraging as to warrant a further exploration of the vaccine–microbicide combination concept.

CCR5-Inhibitor Microbicide and SHIV-SF162P3 Challenge. In the second study, we changed the vaccine to the more potent Ad35/Ad26 vector regimen expressing SIVsmE543 Gag-Pol-Env (6). We also switched the microbicide to the CCR5 inhibitor maraviroc as, unlike T-1249, this drug is used for treating HIV-1–infected people and is currently being evaluated for preventing transmission (23, 24). Our original intent was to continue to use SIVmac251 as the challenge virus; accordingly, the vaccine included an SIV Env component. However, during the period between vaccination and challenge, we concluded that we could not reliably identify a maraviroc dose that provided 50% protection against SIVmac251. In contrast, we obtained a smooth dose–response curve for maraviroc-mediated protection against SHIV-162P3 and decided to use this challenge virus instead (24). We also considered adopting a multiple challenge protocol without progesterone, but were unable to obtain a sufficiently consistent rate of infection of control animals under these conditions (17). Given the necessity to infect almost all of the control animals and approximately half of those in the microbicide-only group, we elected to proceed with a single, high-dose challenge with SHIV-162P3. Thus, the Env component of the vaccine (SIV) and the challenge virus (HIV-1) were mismatched.

As a result, we predicted that the vaccine would reduce viral loads but would not block acquisition of infection following virus challenge (6). The study therefore allowed us to assess whether the combined use of the partially protective microbicide and the T-cell–based vaccine would prove superior to each individual modality by affording both protection against acquisition of infection and improved virologic control. The basic predictions were that: (*i*) V+M would prove superior to V alone for blocking acquisition of infection and (*ii*) V+M would prove superior to M alone for virologic control. A more tangible advantage to the combination would be evident if V+M also protected better against acquisition than M and curbed viral loads more than V.

Each group in this study included eight animals to increase statistical power, although one macaque was omitted from the combination group when it was found to be retrovirus infected

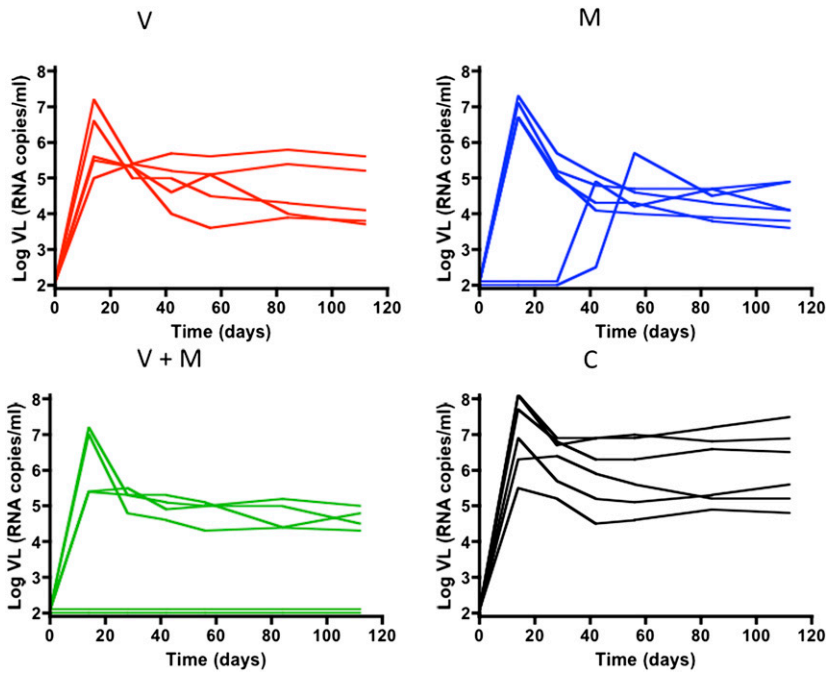

Fig. 1. Viral load profiles for animals given vaccine (V), T-1249 (M), the combination (V+M), or neither (C) before challenge with SIVmac251. The viral load over time after challenge is shown in one diagram for each group ($n = 5$ for V; $n = 6$ for each of the other groups). Baseline values are offset from each other for clarity.

before challenge. The immunogenicity of the Ad35/Ad26 vaccine, as judged by IFN-γ ELISPOT assays, was comparable to that observed previously in experiments involving the same vaccine but different challenge conditions, e.g., intrarectal with SIVmac251 (6).

After the high-dose vaginal SHIV-162P3 challenge (Fig. 2), seven of eight control animals (group C) were infected. Similarly, seven of eight animals in the vaccine-only group (group V) were infected, as expected for this T-cell–based vaccine with no relevant Env component. In the microbicide-only group (group M), four of eight animals were infected. Thus, we achieved our goal of using a microbicide dose that protected half of the animals. In the combination group (group V+M), two of seven animals were infected. The intervention efficacies in groups M and V+M were 43 and 67%, respectively. For V+M compared with V or C, the infection rate was significantly lower ($P = 0.035$), although the rate for group M was not significantly lower than for group C ($P = 0.14$). V+M was, therefore, superior to V for blocking acquisition of infection, in accordance with our first prediction. Although the infection rate was lowest in the combination group, V+M was not, however, significantly different from M for acquisition ($P = 0.38$).

Plasma viral loads were assessed to determine postinfection virologic control in the infected animals. Viral loads in the four groups differed in terms of peak viral loads (Fig. 3*A*; $P = 0.0006$, V vs. C, $P < 0.01$; V+M vs. M, $P < 0.05$; and V+M vs. C, $P < 0.001$), and of viral loads on day 28, which was the first day on which all infected monkeys had detectable viremia (Fig. 3*B*; $P = 0.0001$, V vs. C, $P < 0.01$; V+M vs. M, $P < 0.01$; and V+M vs. C, $P < 0.001$). The log viral load reductions measured as peaks and on day 28 were, respectively, 1.2 and 1.1 for V; 0.70 and 0.45 for M; and 2.4 and 2.3 for V+M. The magnitude of the viral load reduction by the vaccine was again similar to what we have reported in previous experiments with the Ad35/Ad26 vaccine (6). Hence V+M was superior to M for virologic control, in accordance with our second prediction.

Furthermore, the viral loads in the V+M group were the lowest among the four groups in both comparisons and they differed more from controls than did either V or M.

Discussion

In this study, we used a rhesus macaque vaginal transmission model to test the concept that vaccines and microbicides are two prevention methods that might work better together than individually. In both experiments, the fewest infected animals were found in the V+M combination group, and the difference in acquisition between the V and V+M groups was statistically significant in the second experiment. Moreover, the difference in viral loads between groups M and V+M was statistically significant in the second experiment. Thus, these data provide overall support to the concept that combining vaccines and microbicides may be superior to using either by itself. Whether a vaccine containing a relevant Env component combined with a microbicide might provide synergistic protection against acquisition of infection remains to be determined.

We observed a trend toward augmented protection against acquisition of infection in the V+M group, compared with group M, in both studies (two of six vs. zero of six animals uninfected for V+M vs. M in the first study, five of seven vs. four of eight animals in the second). One interpretation of this trend is that the partially protective microbicide dose slowed down the local expansion of the incoming virus in the mucosal tissues of a subset of the animals, and hence the effective size of the inoculum (see below). We note that, in the first experiment, two animals in group M (i.e., T-1249 recipients) become infected only after an unusually long delay of 2–4 wk (Fig. 1). We have only previously seen such a long delay in a single animal, one that was also given a partially protective dose of T-1249 before SIVmac251 challenge. In the combination groups, we hypothesize that the microbicide-mediated delay in virus expansion may have allowed vaccine-elicited immune responses enough additional time to either eliminate the infection entirely or to restrict replication

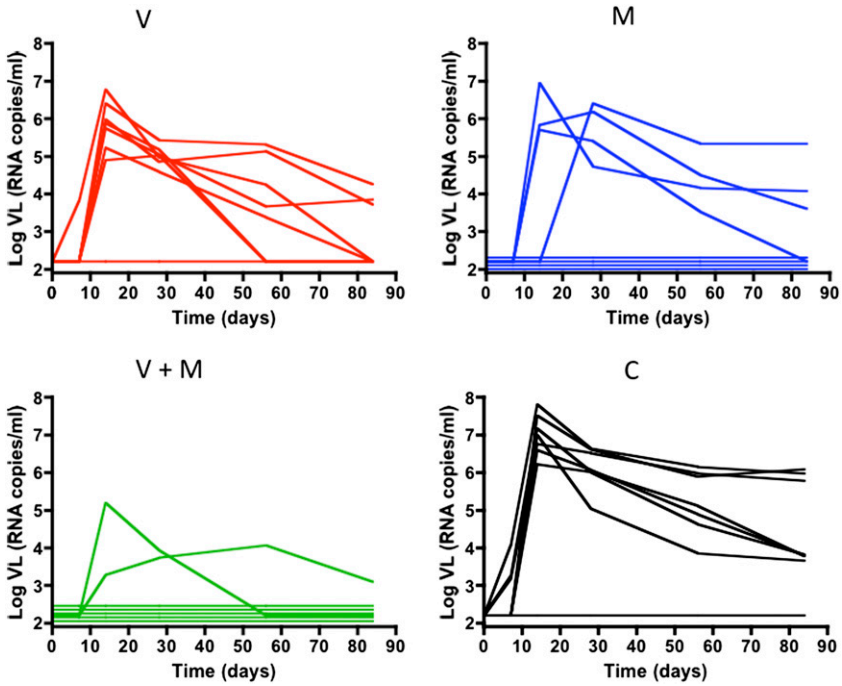

Fig. 2. Viral load profiles for animals given vaccine (V), maraviroc (M), the combination (V+M), or neither (C) before challenge with SHIV-162P3. The viral load over time after challenge is shown in one diagram for each group ($n = 7$ for V+M; $n = 8$ for each of the other groups). Baseline values are offset from each other for clarity.

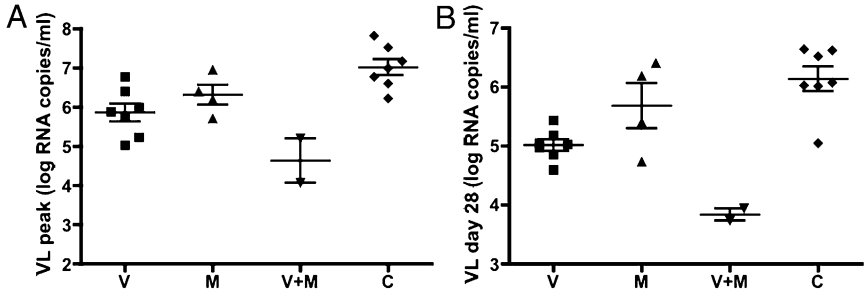

Fig. 3. Peak and set-point viral loads for animals given vaccine (V), maraviroc (M), the combination (V+M), or neither (C) before challenge with SHIV-162P3. Measurements of viral loads are depicted on the *y* axis for each of the four groups as indicated on the category axis. Means are marked by horizontal bars ± SEM for (*A*) the peak viral loads and (*B*) the viral loads on day 28, the first day on which all of the animals that became infected were detectably viremic.

and lower the set point. In the second experiment, maraviroc may reduce the number of target cells in the vaginal mucosa that express sufficient free CCR5 for virus entry, noting that the abundance of $CCR5^+$ target cells is an important influence on the establishment of infection (25). We think it is unlikely that vaccine-induced antibodies are involved in these effects, particularly given the complete mismatch of the Env component of the vaccine and challenge virus in the second study. Instead, cellular immunity, such as $CD8^+$ T-cell responses, is likely responsible (6). Consistent with this possibility is our observation that these Ad vectors, delivered by the intramuscular route to rhesus macaques, elicit potent and durable effector memory $CD8^+$ T lymphocytes at mucosal surfaces, including in the cervicovaginal mucosa (26). We note that if a vaccine–microbicide combination approach were used in humans, the Env components of the vaccine strain and the incoming virus would probably also be mismatched, but not to the same extent as in our experiment (SIV vs. HIV-1). Accordingly, at least some additional protective contribution from the Env component of the vaccine could reasonably be anticipated (6).

The combined effects of the vaccine and the microbicide on acquisition and viral loads are compatible with our emerging understanding of the early events in mucosal SIV/SHIV infection (15). In a simple model, the crucial event is the productive infection of the first target cell, with successive generations of progeny virus then irrevocably colonizing the rest of the organism. However, postexposure prophylaxis with a reverse-transcriptase inhibitor can be started as late as 24–48 h after inoculation and still completely prevent SIV infection (27), and the earliest foci of infection appear during the first few days after intravaginal inoculation (28). A more sophisticated view of mucosal infection is that the events preceding systemic breakout involve a branching process (29); a limited number of replication foci are first established but they can be extinguished in a critical labile phase before they spread the virus to increasingly distant lymphoid tissue sites (15). Sometimes, the initial foci may be eradicated by $CD8^+$ T lymphocytes within the mucosa, leading to a failed infection at the level of the whole organism (30). This beneficial outcome may be more probable when the inoculum size is low or is lowered by an intervention (e.g., a topically or orally delivered ARV). We therefore suggest that, in our experiments, the partially active microbicide doses reduced the infectivity of the inoculum to this labile zone where the outcome of infection could go either way (systemic breakout or extinction), perhaps stochastically. The vaccine-induced immune responses may therefore contribute to reducing acquisition when the number of infectious foci is close to the critical threshold level.

In the only previous study of combining a vaccine (DNA/rAd5) and a microbicide (a zinc-finger inhibitor) in macaques, there were modest differences in the rate of infection and viral loads between the combination and the no-intervention control group (31). However, as neither the vaccine nor the microbicide caused detectable reductions in viral loads or acquisition, respectively, the outcome was inconclusive as to the benefit of the combination; the Kaplan–Meier curves for the vaccine and combination groups were largely superimposed, as were those for the microbicide and control groups (31). Any interpretation is further complicated by two escalations of the challenge virus dose [eventually to 3,000 $TCID_{50}$ (50% tissue culture infectious dose)] to try to overcome inconsistent infections of the control animals (17, 31).

In summary, our data support the concept that vaccines and microbicides might be useful when used in combination for protection against sexual HIV-1 transmission to women by the vaginal route. By extrapolation, the same benefits might apply to rectal microbicides for men and women, although this supposition will need to be confirmed experimentally given that transmission conditions may be tissue dependent. Further research in these areas is important for the HIV-1 prevention field, because if microbicides do become licensed for clinical use they would likely be used in the context of HIV-1 vaccine efficacy trials. Although we used inhibitors of virus-cell entry as the microbicides, blockers of other stages in the HIV-1 replication cycle might have similar effects and thus warrant further investigation (9, 10). Any beneficial effect of a microbicide on vaccine efficacy would, of course, only come into play when the product is actually used but does not fully prevent HIV-1 transmission. Microbicide failures due to nonuse require different solutions such as sustained release devices (vaginal rings) or long-lasting gels (32–34). Whether these coitally independent delivery methods, and also orally delivered ARVs, can be combined with vaccination for reinforced protection remains to be determined. A conceptually related combination approach would be to use a vaccine designed to induce mucosal antibodies of appropriate quality and quantity to block acquisition of infection together with a T-cell vaccine that limits replication postinfection. All of these hypotheses could, in principle, be tested in the macaque model using various challenge formats. The availability of larger numbers of animals would increase the statistical power of tests of vaccine–microbicide combinations to reduce acquisition, particularly when the microbicide itself has, by design, a partial effect by itself, as was the case in our second experiment.

Materials and Methods

Challenge Viruses. The R5 virus SHIV-162P3, derived from the HIV-1 SF162 primary isolate as described elsewhere (35) and propagated in phytohemagglutin (PHA)-activated rhesus macaque peripheral blood mononuclear cells (PBMC), was obtained through the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH (cat. no. 6526; contributors: Janet Harouse, Cecilia Cheng-Mayer, and Ranajit Pal). The SIVmac251 stock we used has been described previously (18).

Microbicides. T-1249 was a gift from Michael Greenberg and Dani Bolognesi who, at the time, worked for Trimeris (Durham, NC) (19, 20). Maraviroc was obtained via John Pottage (ViiV Healthcare, Research Triangle Park, NC) (23, 24).

Vaccine Delivery. Ad26, Ad35, and Ad5HVR48 vectors expressing SIV Gag/Pol/Env antigens were produced as previously described (36, 37). Indian-origin rhesus monkeys were immunized twice by the intramuscular route with 10^{10} viral particles per vector in Ad26/Ad5HVR48 or Ad35/Ad26 heterologous prime-boost regimens (6). Vaccine immunogenicity was confirmed by IFN-γ ELISPOT assays. Viral challenges were performed at least 6 mo following the final vaccination.

Microbicide Delivery and Virus Challenge. A single intramuscular injection of Depo-Provera (progesterone) was given to female Indian rhesus macaques 30 d before challenge, to synchronize the menstrual cycle, thin the vaginal epithelium, and facilitate virus transmission (16). Virus challenge and microbicide delivery protocols are more fully described elsewhere (20, 24, 38). On the day of challenge, 4 mL of the microbicide formulated in a hydroxyethylcellulose (HEC) gel, or a placebo HEC gel, were applied atraumatically to the vagina, 30 min before SHIV-162P3 or SIVmac251 was added in a 1-mL volume containing 500 $TCID_{50}$ or 4×10^7 RNA copies/mL, respectively. Infection status was determined by measuring plasma viral load at 7, 14, 21, 28, 42, 56, and 70 d postchallenge, using a commercially available branched DNA (bDNA) assay with a sensitivity limit of 125 RNA copies/mL (Siemens). All protocols were approved by the institutional animal care and use committees. The animals were housed in accordance with the American Association for Accreditation of Laboratory Animal Care standards. All 55 animals described in Figs. 1 and 2 were experimentally naïve at the start of the studies and were negative for antibodies against SIV and type D retrovirus.

Statistical Analysis. Acquisition frequencies were analyzed by Fisher's exact test. Because the interventions reduced acquisitions in the hypothesized direction, and any marginal increase in acquisition over that for controls (possible only in the second experiment) would have been entirely attributable to random effects, the *P* values given are for one-tail comparisons. The α level was set to $P = 0.05$ for all tests. Measurements of viral loads among infected animals (peak and set points) were compared overall for the four groups by one-way ANOVA, followed by Tukey's posttest for individual group pairs, with Kramer's extension for unequal group sizes. Although the group sizes were too small to test for Gaussian distribution or homoscedasticity, we chose the parametric test because the high frequency of protection by V+M left so few infected animals that a nonparametric approach would have been too blunt to make some important comparisons. In *Results*, the *P* value for the four-group comparison is given first, followed by any significant outcome of Tukey's posttest. Thus, out of the six pairwise combinations of the four groups, three results by Tukey's test turned out to be nonsignificant and are not listed. ANOVA and Tukey tests were performed in Prism (GraphPad).

ACKNOWLEDGMENTS. We thank Dani Bolognesi and Michael Greenberg for the provision of T-1249 and Kelsi Rasmussen, Megan Gardner, Meagan Watkins, and Thomas Ketas for technical support. This work was funded by a supplement to National Institutes of Health (NIH) Cooperative Agreement U19 AI76982 and by NIH Grants AI078526 and AI066924, the Ragon Institute, and the Bill and Melinda Gates Foundation.

1. Quinn TC, Overbaugh J (2005) HIV/AIDS in women: An expanding epidemic. *Science* 308:1582–1583.
2. Padian NS, et al. (2011) HIV prevention transformed: The new prevention research agenda. *Lancet* 378:269–278.
3. Kim JH, Rerks-Ngarm S, Excler JL, Michael NL (2010) HIV vaccines: Lessons learned and the way forward. *Curr Opin HIV AIDS* 5:428–434.
4. Barouch DH, Korber B (2010) HIV-1 vaccine development after STEP. *Annu Rev Med* 61:153–167.
5. Hansen SG, et al. (2011) Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* 473:523–527.
6. Barouch DH, et al. (2012) Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* 482:89–93.
7. Walker LM, Burton DR (2010) Rational antibody-based HIV-1 vaccine design: Current approaches and future directions. *Curr Opin Immunol* 22:358–366.
8. Wainberg MA (2011) AIDS: Drugs that prevent HIV infection. *Nature* 469:306–307.
9. Minces LR, McGowan I (2010) Advances in the development of microbicides for the prevention of HIV infection. *Curr Infect Dis Rep* 12:56–62.
10. Abdool Karim Q, et al.; CAPRISA 004 Trial Group (2010) Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 329:1168–1174.
11. Grant RM, et al.; iPrEx Study Team (2010) Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. *N Engl J Med* 363:2587–2599.
12. Celum C, Baeten JM (2012) Tenofovir-based pre-exposure prophylaxis for HIV prevention: Evolving evidence. *Curr Opin Infect Dis* 25:51–57.
13. van der Straten A, van Damme L, Haberer JE, Bangsberg DR (2012) How well does PREP work? Unraveling the divergent results of PrEP trials for HIV prevention. *AIDS* 26:F13-F19.
14. Haase AT (2005) Perils at mucosal front lines for HIV and SIV and their hosts. *Nat Rev Immunol* 5:783–792.
15. Haase AT (2010) Targeting early infection to prevent HIV-1 mucosal transmission. *Nature* 464:217–223.
16. Marx PA, et al. (1996) Progesterone implants enhance SIV vaginal transmission and early virus load. *Nat Med* 2:1084–1089.
17. Veazey RS, Shattock RJ, Klasse PJ, Moore JP (2012) Animal models for microbicide studies. *Curr HIV Res* 10:79–87.
18. Liu J, et al. (2009) Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature* 457:87–91.
19. Eron JJ, et al. (2004) Short-term safety and antiretroviral activity of T-1249, a second-generation fusion inhibitor of HIV. *J Infect Dis* 189:1075–1083.
20. Veazey RS, et al. (2008) Tropism-independent protection of macaques against vaginal transmission of three SHIVs by the HIV-1 fusion inhibitor T-1249. *Proc Natl Acad Sci USA* 105:10531–10536.
21. Genescà M, McChesney MB, Miller CJ (2010) Depo-provera treatment does not abrogate protection from intravenous SIV challenge in female macaques immunized with an attenuated AIDS virus. *PLoS ONE* 5:e9814.
22. Sanders-Beer B, et al. (2010) Depo-Provera does not alter disease progression in SIVmac-infected female Chinese rhesus macaques. *AIDS Res Hum Retroviruses* 26:433–443.
23. MacArthur RD, Novak RM (2008) Reviews of anti-infective agents: Maraviroc: The first of a new class of antiretroviral agents. *Clin Infect Dis* 47:236–241.
24. Veazey RS, et al. (2010) Protection of rhesus macaques from vaginal infection by vaginally delivered maraviroc, an inhibitor of HIV-1 entry via the CCR5 co-receptor. *J Infect Dis* 202:739–744.
25. Pandrea I, et al. (2012) Mucosal SIV transmission in African Green Monkeys: Susceptibility to infection is proportional to target cell availability at mucosal sites. *J Virol*.
26. Li H, et al. (2011) Durable mucosal simian immunodeficiency virus-specific effector memory T lymphocyte responses elicited by recombinant adenovirus vectors in rhesus monkeys. *J Virol* 85:11007–11015.
27. Tsai CC, et al. (1998) Effectiveness of postinoculation (R)-9-(2-phosphonylmethoxypropyl) adenine treatment for prevention of persistent simian immunodeficiency virus SIVmne infection depends critically on timing of initiation and duration of treatment. *J Virol* 72:4265–4273.
28. Miller CJ, et al. (2005) Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J Virol* 79:9217–9227.
29. Wick D, Self SG (2000) Early HIV infection in vivo: Branching-process model for studying timing of immune responses and drug therapy. *Math Biosci* 165:115–134.
30. Blancou P, et al. (2001) The infiltration kinetics of simian immunodeficiency virus-specific T cells drawn to sites of high antigenic stimulation determines local in vivo viral escape. *Proc Natl Acad Sci USA* 98:13237–13242.
31. Cheng-Mayer C, et al. (2011) Delay of SHIV infection and control of viral replication in vaccinated macaques challenged in the presence of a topical microbicide. *AIDS* 25:1833–1841.
32. Malcolm RK, Edwards KL, Kiser P, Romano J, Smith TJ (2010) Advances in microbicide vaginal rings. *Antiviral Res* 88(Suppl 1):S30–S39.
33. Forbes CJ, et al. (2011) Non-aqueous silicone elastomer gels as a vaginal microbicide delivery system for the HIV-1 entry inhibitor maraviroc. *J Control Release* 156:161–169.
34. Malcolm RK, et al. (2012) Sustained release of the CCR5 inhibitors CMPD167 and maraviroc from vaginal rings in rhesus macaques. *Antimicrob Agents Chemother* 56:2251–2258.
35. Harouse JM, et al. (2001) Mucosal transmission and induction of simian AIDS by CCR5-specific simian/human immunodeficiency virus SHIV(SF162P3). *J Virol* 75:1990–1995.
36. Abbink P, et al. (2007) Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J Virol* 81:4654–4663.
37. Roberts DM, et al. (2006) Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature* 441:239–243.
38. Veazey RS, et al. (2005) Protection of macaques from vaginal SHIV challenge by vaginally delivered inhibitors of virus-cell fusion. *Nature* 438:99–102.