In Vitro Exposure to PC-1005 and Cervicovaginal Lavage Fluid from Women Vaginally Administered PC-1005 Inhibits HIV-1 and HSV-2 Infection in Human Cervical Mucosa

Guillermo Villegas,^a Giulia Calenda,^a Shimin Zhang,^a Olga Mizenina,^a Kyle Kleinbeck,^a Michael L. Cooney,^a Craig J. Hoesley,^b George W. Creasy,^a Barbara Friedland,^a José A. Fernández-Romero,^a Thomas M. Zydowsky,^a Natalia Teleshova^a

Population Council, New York, New York, USA^a; University of Alabama Birmingham School of Medicine, Birmingham, Alabama, USA^b

Our recent phase 1 trial demonstrated that PC-1005 gel containing 50 μ M MIV-150, 14 mM zinc acetate dihydrate, and carrageenan (CG) applied daily vaginally for 14 days is safe and well tolerated. Importantly, cervicovaginal lavage fluid samples (CVLs) collected 4 or 24 h after the last gel application inhibited HIV-1 and human papillomavirus (HPV) in cell-based assays in a dose-dependent manner (MIV-150 for HIV-1 and CG for HPV). Herein we aimed to determine the anti-HIV and anti-herpes simplex virus 2 (anti-HSV-2) activity of PC-1005 in human cervical explants after *in vitro* exposure to the gel and to CVLs from participants in the phase 1 trial. Single HIV-1_{BaL} infection and HIV-1_{BaL}–HSV-2 coinfection explant models were utilized. Coinfection with HSV-2 enhanced tissue HIV-1_{BaL} infection. *In vitro* exposure to PC-1005 protected cervical mucosa against HIV-1_{BaL} (up to a 1:300 dilution) in single-challenge and cochallenge models. CG gel (PC-525) provided some barrier effect against HIV-1_{BaL} at the 1:100 dilution in a single-challenge model but not in the cochallenge model. Both PC-1005 and PC-525 at the 1:100 dilution inhibited HSV-2 infection, pointing to a CG-mediated protection. MIV-150 and CG in CVLs inhibited HIV (single-challenge or cochallenge models) and HSV-2 infections in explants in a dose-dependent manner ($P < 0.05$). Stronger inhibition of HIV-1 infection by CVLs collected 4 h after the last gel administration was observed compared to infection detected in the presence of baseline CVLs. The anti-HIV and anti-HSV-2 activity of PC-1005 gel *in vitro* and CVLs in human ectocervical explants supports the further development of PC-1005 gel as a broad-spectrum on-demand microbicide.

In 2014, there were 2 million new HIV infections, the majority of them transmitted via sexual intercourse (1; <http://www.unaids.org>). Although the risk of vaginal HIV transmission is estimated to be 1 in 1,250 exposures (2), preexisting infection with herpes simplex virus 2 (HSV-2) is a significant cofactor of HIV-1 transmission, increasing HIV-1 acquisition by severalfold (3–7). In populations like sub-Saharan Africa, where HSV-2 is highly prevalent, nearly half of the HIV-1 infections are attributed to HSV-2 coinfection (7, 8). Several HSV-2-induced immunomodulatory effects possibly underlying increased HIV-1 transmission have been described (9–11). Furthermore, increased HIV-1 reverse transcription, DNA integration, and RNA expression and increased p24 release in ectocervical tissue following cochallenge with HIV-1_{BaL} and HSV-2 have been reported (10). Thus, it would be beneficial for a microbicide to have potent activity against both viruses. The CAPRISA004 study demonstrated that the vaginal 1% tenofovir gel reduced HIV-1 acquisition by 39% and HSV-2 acquisition by 51% (12, 13). However, decreased potency of low tenofovir concentrations (1 μ g/ml) *ex vivo* against both HIV-1 and HSV-2 in the coinfection scenario (coincident with an increased number of CD4⁺ CCR5⁺ CD38⁺ T cells) was reported (10).

The Population Council's candidate microbicide gel, PC-1005, which contains the nonnucleoside reverse transcriptase inhibitor (NNRTI) MIV-150, zinc acetate, and carrageenan (CG), has broad anti-HIV activity independent of clade, tropism, or phenotype and against multidrug-resistant strains or clones containing multiple drug resistance mutations (DRMs) in cell-based assays (14). PC-1005 protects macaques against chimeric simian-human immunodeficiency virus expressing HIV-1 HxB2 reverse transcriptase (SHIV-RT) and partially reduces HSV-2 infection when applied vaginally up to 8 h before single high-dose SHIV-RT or

SHIV-RT–HSV-2 cochallenge (15–17). In a repeated-dosing SHIV-RT–HSV-2 cochallenge model, PC-1005 and CG gel (PC-525) significantly reduce the frequency and level of HSV-2 shedding (18). PC-1005 inhibits vaginal and anorectal HSV-2 infection of mice (14, 19). Moreover, the anti-HIV-1 and anti-HSV-2 activities of the gel are unaffected in the presence of human seminal plasma (SP) (J. Kenney, M. Aravantinou, N. Derby, S. R. Ugaonkar, A. Wesenberg, J. Wilk, L. Kizima, A. Rodriguez, S. Zhang, O. Mizenina, K. Levendosky, M. L. Cooney, S. Seidor, R. Singer, P. Mawson, A. Gettie, B. Grasperge, J. Blanchard, M. Piatk, Jr., J. D. Lifson, J. Fernández-Romero, T. M. Zydowsky, and M. Robbiani, unpublished data). We recently demonstrated that PC-1005 is active against cell-free and cell-associated SHIV-RT infection of macaque vaginal and rectal explants *ex vivo* (19–21; G. Calenda, G. Villegas, P. Barnable, C. Litterst, K. Levendosky, A. Gettie, M. L. Cooney, J. Blanchard, J. A. Fernández-Romero, T. M.

Received 18 February 2016 Returned for modification 25 May 2016

Accepted 26 June 2016

Accepted manuscript posted online 5 July 2016

Citation Villegas G, Calenda G, Zhang S, Mizenina O, Kleinbeck K, Cooney ML, Hoesley CJ, Creasy GW, Friedland B, Fernández-Romero JA, Zydowsky TM, Teleshova N. 2016. *In vitro* exposure to PC-1005 and cervicovaginal lavages from women vaginally administered PC-1005 inhibits HIV-1 and HSV-2 infection in human cervical mucosa. *Antimicrob Agents Chemother* 60:5459–5466. doi:10.1128/AAC.00392-16.

Address correspondence to Natalia Teleshova, nteleshova@popcouncil.org.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AAC.00392-16>.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

Zydowsky, and N. Teleshova, submitted for publication), and its activity is not affected by SP (20). Furthermore, PC-1005 gel provides CG-mediated activity against HPV, and this activity is not compromised in the presence of SP (22, 23).

The first phase 1 clinical trial of PC-1005 demonstrated that vaginal administration of the gel is safe, well tolerated, and leads to low MIV-150 absorption (24, 39). Cervicovaginal lavage fluid samples (CVLs) collected at 4 and 24 h after gel application showed MIV-150 and CG dose-dependent inhibition of HIV-1 and HPV in cell-based assays. However, the anti-HSV-2 activity of these CVLs could not be tested in cell-based assays because of the anti-HSV-2 activity of baseline CVLs in this particular setting (24; Friedland et al., in press).

The aim of this study was to evaluate the *in vitro* activity of PC-1005 and CVLs collected at 4 h and 24 h after PC-1005 application in a phase 1 trial against single HIV-1 infection and HIV-1–HSV-2 coinfection in human ectocervical explants. We demonstrated that PC-1005 applied *in vitro* and CVLs from participants administered PC-1005 effectively protected against single HIV-1 challenge and HIV-1–HSV-2 challenge in explant models.

MATERIALS AND METHODS

Viruses. For the generation of HIV-1_{BaL}, peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats of anonymous donors (New York Blood Center, Long Island City, NY) using Ficoll-Hypaque density gradient centrifugation, depleted of CD8⁺ cells using anti-CD8⁺ magnetic beads (Miltenyi Biotec, Inc., San Diego, CA) and activated for 6 days with 10 nM retinoic acid (RA) (Sigma-Aldrich, St. Louis, MO), 20 U/ml interleukin-2 (IL-2) (NCI BRB Preclinical Repository, Frederick, MD), and 50 ng/ml of anti-CD3 monoclonal antibodies (MAbs) (clone OKT3; e-Bioscience, San Diego, CA) in RPMI 1640 (Cellgro Mediatech, Manassas, VA) containing 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY) (25, 26). Activated cells were then challenged with 10³ 50% tissue culture infectious doses (TCID₅₀) of HIV-1_{BaL} (ABI, Eldersburg, MD) per 10⁶ cells and cultured for 7 days in the presence of 10 nM RA and 20 U/ml IL-2. The TCID₅₀ was determined in TZM-bl cells (27). A single HIV-1_{BaL} stock was used in the study. The HSV-2 G strain was propagated in Vero cells (ATCC), and the titer was determined by plaque assay (28). Both virus stocks were aliquoted and stored at –80°C.

To determine whether HIV-1_{BaL} stock harbors NNRTI DRMs, the RT gene was sequenced. RNA was extracted from an aliquot of HIV-1_{BaL} with the QIAamp UltraSens virus RNA kit (Qiagen, Valencia, CA) and reverse transcribed using SuperScript III First-Strand Supremix and random hexamer primer (Invitrogen, Carlsbad, CA) as previously described (29). PCR amplification was carried out to amplify the RT-RNase H region of the *pol* gene with primers *Pol* Fwd (5' AGAGCCAACAGCCCCACCA 3') and *Pol* Rev (5' CACCTGCCATCTGTTTCCCA 3'). PCR products were purified with the QIAquick gel extraction kit (Qiagen) and sent for sequencing (GeneWiz, South Plainfield, NJ) with primers specific for HIV-1 RT. Gene sequences were analyzed by Lasergene 10 (DNASar, Madison, WI). No NNRTI DRMs were detected.

Gels. PC-1005 was used *in vitro* (lot 130605A1005TR) and *in vivo* in the phase 1 trial (lot 131118A1005MC). The gel contains 2.8% (wt/vol) CG, 0.002% (wt/vol) MIV-150 (50 µM), and 0.3% (wt/vol) zinc acetate dihydrate (ZA) (14 mM). PC-525 gel, a CG placebo, containing 2.6% (wt/vol) CG (lot 130613A525TR), was used as a control in the *in vitro* studies. Hydroxycellulose (HEC; universal placebo gel) was used in the phase 1 study. All gels were manufactured and packed at the Population Council. MIV-150 was developed by Medivir AB (Sweden) and licensed to the Population Council.

CVLs. CVLs were available from 15 participants administered gels vaginally once a day for 14 days in a PC-1005 phase 1 trial (24; Friedland et al., in press). CVLs were collected using 10 ml of saline at the baseline

and 4 h (PC-1005, *n* = 6; and HEC, *n* = 2) or 24 h (PC-1005, *n* = 6; HEC, *n* = 1) after the last gel administration.

MIV-150 and CG concentrations in CVLs. Concentrations of MIV-150 and CG in CVLs were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and enzyme-linked immunosorbent assay (ELISA) (16), respectively.

Ectocervical tissue. Human ectocervical tissues without gross pathological changes were obtained from routine hysterectomies through the National Disease Research Interchange (NDRI [Philadelphia, PA]) and transported overnight in RPMI medium.

Ectocervical tissue viability. Ectocervical tissue was processed and 3- by 3-mm explants were prepared as we previously described for macaque vaginal and cervical tissues (20, 30). Tissue viability following ~18 h of incubation with PC-1005 and PC-525 at 1:100 and 1:300 dilutions and CVLs at 1:2 dilution was tested using the MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide] assay (31).

***In vitro* anti-HIV-1_{BaL} and anti-HSV-2 activities of PC-1005 in ectocervical tissue.** The design of *in vitro* experiments testing PC-1005 activity is summarized in Fig. 1A. Ectocervical tissues were challenged with 500 TCID₅₀ HIV-1_{BaL} or 500 TCID₅₀ HIV-1_{BaL}/10⁶ PFU HSV-2 per explant (three explants per condition) in the presence of 1:100 and 1:300 (184.2 ng/ml and 61.4 ng/ml of MIV-150, respectively) diluted PC-1005 or PC-525, 10 µM lamivudine (3TC) and/or 100 µg/ml acyclovir (3TC/Acy) or culture medium. These concentrations of MIV-150 are within the range of concentrations detected in vaginal fluids 4 h and 24 h after the last gel application in the phase 1 PC-1005 study, adjusting for dilutions during CVL collection. After incubation overnight for ~18 h at 37°C in 5% CO₂, tissues were washed to eliminate input virus and cultured in cD-MEM (Dulbecco's modified Eagle's medium containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin with 100 µM minimal essential medium [MEM] nonessential amino acids) for 14 days. Supernatants were collected at 0 (after washes), 3, 7, 11, and 14 days of culture. Infections were monitored with HSV-2 quantitative PCR (qPCR) and HIV *gag* reverse transcription-quantitative PCR (qRT-PCR). Endpoint "soft" (SOFT) and cumulative (CUM) (days 3 to 14 as day 0 represents carryover input virus) analyses were performed (30, 32, 33).

Anti-HIV-1_{BaL} and anti-HSV-2 activity of CVLs in ectocervical tissues. The design of experiments testing activity of CVLs is summarized in Fig. 1B. Ectocervical explants were incubated with diluted (1:2) CVLs in cDMEM for 4 h. Following washout, tissues were challenged with 500 TCID₅₀ HIV-1_{BaL} or 500 TCID₅₀ HIV-1_{BaL} and 10⁶ PFU HSV-2 per explant (three explants per condition), washed, and cultured, and infections were monitored as described above. Paired (baseline and post-gel application) CVLs were tested in ectocervical tissues from the same donor. Controls included tissues not exposed to CVLs (medium) and challenged with HIV-1_{BaL} or HIV-1_{BaL}–HSV-2 or challenged with HIV-1_{BaL}–HSV-2 in the presence of 3TC/Acy. Eight surgical ectocervical tissues were used to test the activity of 15 CVL pairs.

To determine the contribution of CG to anti-HIV activity of MIV-150 in CVLs, tissues were challenged with HIV-1_{BaL} after exposure to baseline pooled CVLs spiked with the range of CG concentrations (three explants per condition) detected in the post-gel application CVLs (*n* = 3 experiments).

HSV-2 qPCR, HIV qRT-PCR, and p24 ELISA. HSV-2 infection was analyzed by HSV-2 *pol* qPCR in supernatants (5 µl per reaction). Briefly, primers (IDT, Coralville, IA) that amplify the UL30 region of the HSV-2 polymerase gene were used: forward primer 5' GCTCGAGTGCAGAAA AACGTTTC 3' and reverse primer 5' TGCGGTTGATAAACGCGCAGT 3'. qPCR was performed using the Kapa SYBR Fast qPCR kit (Kapa Biosystems, Wilmington, MA). The cycling conditions were 1 cycle at 95°C for 3 min followed by 40 cycles at 95°C for 3 s and 60°C for 20 s. The standard curve was generated using 10-fold dilutions of the plasmid TOPO UL30 (9; Calenda et al., submitted). HIV-1_{BaL} infection in tissue culture supernatants was measured by p24 ELISA (ZeptoMetrix, Buffalo, NY) and by HIV-1 *gag* qRT-PCR using SYBR FAST One-Step qRT-PCR

FIG 1 Designs of experiments testing activity of PC-1005 *in vitro* and activity of CVLs collected from subjects in the PC-1005 phase 1 trial. Two designs were chosen. (A) Ectocervical tissues were challenged with HIV-1_{BaL} or HIV-1_{BaL} and HSV-2 in the presence of PC-1005 versus controls. Following challenge, tissues were washed and cultured for 14 days (D14). (B) Alternatively, ectocervical tissues were preincubated with CVLs and then washed and co-challenged with HIV-1_{BaL} or HIV-1_{BaL} and HSV-2. After challenge, tissues were washed and cultured for 14 days.

(5 μ l of supernatant per reaction; Kapa Biosystems, Wilmington, MA). The primers were forward primer 5' GGTGCGAGAGCGTCAGTAT TAAG 3' and reverse primer 5' AGCTCCCTGCTTGCCCATATA 3'. Results were analyzed using the standard curve method, using pNL(AD8) (11346; NIH AIDS Research and Reference Reagent Program, Germantown, MD). The cycling conditions were 1 cycle at 42°C for 5 min, 1 cycle at 95°C for 5 min, and 40 cycles at 95°C for 3 s and 60°C for 20 s. Dissociation curves were generated to verify the absence of nonspecific amplification. Both HSV-2 *pol* qPCR and HIV-1 *gag* qRT-PCR were performed using the ViiA 7 real-time PCR system (Applied Biosystems, Carlsbad, CA). Data were analyzed using the ViiA 7 software (Applied Biosystems).

Statistics. (i) **Tissue viability.** A lognormal generalized linear mixed model predicted the weight-normalized optical density at 570 nm (OD_{570}) of each tissue replicate postexposure to gels or to CVLs. A random intercept and treatment slope were included by tissue subject identification (ID) number. Significance was determined by the type 3 *F* test for overall treatment effect.

(ii) **HIV-1_{BaL} infection in a single-infection and coinfection models.** A paired *t* test on log-transformed values was used to test whether levels of infection of HIV-1_{BaL} were different between the single-infection and coinfection settings.

(iii) **Activity of PC-1005 and PC-525 against tissue HIV-1_{BaL} and HSV-2 infection.** SOFT and CUM HIV *gag* and HSV-2 *pol* values (pooled replicate values) were analyzed using lognormal generalized linear mixed models with treatment as the only predictor. A random intercept for each sample was included. Pairwise tests were performed with Tukey's adjusted *t* tests. For CUM and SOFT endpoint analyses, HIV *gag* and HSV-2 *pol*

copy numbers of pooled values that were greater than or equal to the lower limit of quantification ($\geq$ LLOQ [2,000 copies/ml]) were assumed lognormal. Any value of <LLOQ at 3 to 14 days of culture was set to $2,000^{1/\sqrt{2}} = 215.86$, a common substitution for lognormal data. The CUM value from 3 to 14 days for replicates with pooled values that were <LLOQ for all time points corresponds to 863.44 copies/ml.

(iv) **Activity of CVLs against tissue HIV-1_{BaL} and HSV-2 infection.** The PC-1005 phase I trial was not designed for prediction. The fact that there are replicates per tissue sample in each experiment in addition to there being few independent tissue samples led to a choice between the minimizing type I (probability of a false-positive result) and type II (probability of a false-negative result) error rates. The choice to use linear mixed models was predicated as that minimizing the type I error is paramount, by accounting for variability within tissue sample replicates and the variability between different tissue samples. It is important to note, moreover, the reduction of the type II error rate will be minimal at best, as the aforementioned trial was not powered to evaluate any hypotheses. Mixed models are the only method that does not treat tissue replicates of each experiment as an independent measurement, which indirectly leads to a false-positive result by artificially suppressing the overall model variance. SOFT and CUM HIV *gag* and HSV-2 *pol* (individual replicate values) results were analyzed using lognormal generalized linear mixed models with treatment (PC-1005 versus HEC placebo), time point (preexposure or 4 or 24 h postexposure to gels), and the treatment-time point interaction as predictors. A random intercept and time point slope by subject were included with either a Cholesky root-parameterized unstructured covariance matrix or, if that failed to converge, a heterogeneous com-

FIG 2 Single HIV-1_{BaL} infection and HIV-1_{BaL}-HSV-2 coinfection of ectocervical tissue. Ectocervical tissues were challenged with (A) 50 to 2,000 TCID₅₀ HIV-1_{BaL} or (B) cochallenge with 500 TCID₅₀ HIV-1_{BaL} and 10⁴ to 10⁶ PFU HSV-2 per explant (three explants per condition) versus 3TC or 3TC/3TC-Acy controls for ~18 h. After washout, tissues were cultured for 14 days, and infections were analyzed in tissue culture supernatants by p24 ELISA, HIV qRT-PCR, and HSV-2 qPCR. Shown are (A) HIV p24 (picograms per milliliter) (mean of replicates; *n* = 1 experiment) and (B) HIV gag and HSV-2 pol copy numbers (mean ± standard error of the mean [SEM]; *n* = 3 experiments).

pound symmetric or then a compound symmetric structure. Tests of effects were done with *F* tests. Pairwise tests of least-squares means were performed with *t* tests adjusted by stepdown simulation for multiple comparisons. The association of MIV-150 and CG concentrations with anti-HIV and anti-HSV-2 activity in CVLs, respectively, was analyzed using lognormal generalized linear mixed models on the individual replicate values to predict the SOFT and CUM values. The analysis was done including baseline and placebo gel data. The MIV-150 or CG concentration was the only predictor. MIV-150 and CG values that were <LLOQ (100 pg/ml for MIV-150 and 58 ng/ml for CG) were set to 100^{1/√2} = 0.025 ng/ml and 58^{1/√2} = 0.017 μg/ml, respectively. A random intercept was included by subject.

Ethics review. The phase 1 trial (Population Council protocol 558) was approved by the Institutional Review Boards of the Population Council and University of Alabama Birmingham School of Medicine. All subjects provided written informed consent for specimen collection, storage, and use in this study.

RESULTS

PC-1005 inhibits HIV-1_{BaL} and HSV-2 in ectocervical tissue.

Optimal HIV-1 single-infection and HIV-1-HSV-2 coinfection conditions were first established. Tissues were challenged with titrated doses of HIV-1_{BaL} (~50 to 2,000 TCID₅₀ per explant). After virus washout, explants were cultured for 14 days, and infection was monitored by p24 ELISA. A dose of 500 TCID₅₀ was selected as a middle challenge dose resulting in productive infection (Fig. 2A). Next we cochallenge tissues with 500 TCID₅₀ of HIV-1_{BaL} and titrated doses of HSV-2 (10⁴ to 10⁶ PFU per explant). Infection was monitored with HIV-1 gag qRT-PCR and HSV-2 pol qPCR. Based on reproducible infection, a challenge dose of 10⁶ PFU HSV-2 was selected (Fig. 2B). Challenge with HIV-1_{BaL} led to robust and reproducible infection in all experiments (10 out of 10 [data not shown]). Seven of these experiments included the HIV-1_{BaL}-HSV-2 cochallenge condition, which resulted in HIV-1_{BaL} infection in 4 out of 7 experiments and HSV-2 infection in all experiments (Fig. 3A and B [data from the 4 successful coinfection data sets]). The HIV-1_{BaL} infection level in the cochallenge model was ~10-fold higher than that in the HIV-1_{BaL} single-infection model (SOFT/CUM, *P* < 0.05) (Fig. 3A), and HSV-2 infection was robust (Fig. 3B).

To determine anti-HIV activity of PC-1005, ectocervical explants were challenged with 500 TCID₅₀ HIV-1_{BaL} or 500 TCID₅₀ HIV-1_{BaL} and 10⁶ PFU HSV-2 in the presence of PC-1005 (versus untreated, PC-525, and 3TC/3TC-Acy controls). PC-1005 at nontoxic 1:100 and 1:300 dilutions (Fig. 4) inhibited >98% of HIV-1_{BaL} infection relative to untreated control (SOFT/CUM, *P* < 0.0001

for both dilutions) (Fig. 3C). Similarly, PC-1005 suppressed HIV-1_{BaL} infection (>99% inhibition) at both 1:100 and 1:300 dilutions in the more stringent coinfection model (SOFT/CUM, *P* < 0.001 and *P* < 0.01, respectively; Fig. 3D). PC-525 at the 1:100 dilution (~300 μg/ml of CG) exerted a barrier effect against HIV-1_{BaL} in the single-challenge model; however, this effect was not significant at a 1:300 dilution (~100 μg/ml of CG) of the gel (Fig. 3C). No barrier effect of PC-525 was detected in the cochallenge model (Fig. 3D). PC-1005 and PC-525 significantly (>99%) inhibited HSV-2 infection at a 1:100 (but not at 1:300) dilution of the gels (SOFT/CUM, *P* < 0.05/*P* < 0.01 and *P* < 0.01/*P* < 0.001, respectively) (Fig. 3E).

CVLs from subjects vaginally administered PC-1005 inhibit HIV-1_{BaL} and HSV-2 infection in ectocervical tissue. We aimed to test if PC-1005 administered *in vivo* is active against HIV-1_{BaL} and HSV-2 infection in mucosal cells in the presence of CVLs collected 4 or 24 h after the last PC-1005 gel application (versus baseline and placebo group) in the phase 1 PC-1005 trial. When tissues were challenged with HIV-1_{BaL} in the presence of baseline CVLs (1:2 dilution in culture medium), the infection was inhibited (see Fig. S1A in the supplemental material). Therefore, in CVL activity testing experiments, ectocervical tissues were preincubated with a nontoxic dilution of CVLs (1:2 dilution in culture medium) (Fig. 5) for 4 h, washed, and then challenged with HIV-1_{BaL} or cochallenge with HIV-1_{BaL} and HSV-2. No impact on HIV-1_{BaL} and HSV-2 infection levels was observed under these testing conditions (see Fig. S1B). In 2 out of 15 cochallenge experiments, no productive HIV infection after preincubation with baseline CVLs was observed. These experiments were excluded from the analysis. Comparisons of treatment (PC-1005 versus HEC placebo gel) lacked power due to the low number of placebo samples (*n* = 2 and *n* = 1 in the 4-h and 24-h groups, respectively) and are not reported.

As expected, higher concentrations of MIV-150 and CG were detected in CVLs obtained 4 h after the last gel administration versus 24 h after (Fig. 6, solid circles versus open squares). MIV-150 concentrations in CVLs inversely correlated with HIV-1_{BaL} infection in the tissues in the single challenge (SOFT/CUM, *P* < 0.0001 for both) and cochallenge model (SOFT/CUM, *P* < 0.01/*P* < 0.05) (Fig. 6A and B). With every 10-ng/ml increase of MIV-150, HIV-1_{BaL} infection decreased by >60% and >30% (both SOFT/CUM) in the single and coinfection models, respectively. Infection inhibition was significant in the 4-h (but not in 24-h) post-gel application group versus baseline (SOFT/CUM, *P* <

FIG 3 Coinfection with HSV-2 enhances tissue HIV-1_{BaL} infection. PC-1005 inhibits HIV-1_{BaL} and HSV-2 infection. Ectocervical tissues were challenged with 500 TCID₅₀ HIV-1_{BaL} or cochallenged with HIV-1_{BaL} and 10⁶ PFU HSV-2 per explant versus 3TC/Acy controls as in Fig. 2. Following washout, tissues were cultured for 14 days. Shown are (A) HIV *gag* copy numbers and log₁₀-transformed CUM analysis of HIV *gag* copy numbers in paired single-challenge and cochallenge experiments (mean ± SEM; *n* = 4) and (B) HSV-2 *pol* copy numbers (mean ± SEM; *n* = 4). Explants were challenged as described above in the presence of diluted PC-1005 (versus untreated medium-only control [Med], PC-525, and 3TC/Acy controls). Following washout, tissues were cultured and infections were analyzed as in Fig. 2. Shown are log₁₀-transformed CUM analyses of HIV *gag* copies in (C) single-challenge (*n* = 9) and (D) cochallenge (*n* = 5) experiments (mean ± SEM) and (E) log₁₀-transformed CUM analyses of HSV-2 *pol* copy numbers in the cochallenge (*n* = 5) experiments (mean ± SEM). The LLOQ for CUM HIV *gag* and input HSV-2 *pol* copy numbers (mean; post-washout after challenge) are indicated. Significant *P* values of <0.05 (*), <0.01 (**), and <0.001 (***) are indicated.

0.05/*P* < 0.01) in the single-challenge model. This was not the case in the cochallenge model. We cannot exclude some contribution of CG to the observed anti-HIV activity of CVLs. However, the anti-HIV activity of diluted CVL samples containing ~250 to 500 µg/ml of CG and ~17 to 30 ng/ml of MIV-150 was higher (~77 to 100%) than the activity of 250 to 500 µg/ml of CG in spiking experiments (55 to 83%) (data not shown).

CG concentrations in CVLs were inversely correlated with

HSV-2 infection (SOFT/CUM, *P* < 0.01 for both), resulting in ~30% decrease in HSV-2 infection (SOFT/CUM) per 100-µg/ml increase of CG (Fig. 6C). No significant differences between the 4-h and 24-h groups versus the respective baselines were detected.

DISCUSSION

Consistent with our recent data demonstrating potent activity of PC-1005 against SHIV-RT infection in macaque vaginal and rectal explants (19–21; Calenda et al., submitted), here we demonstrate potent activity of PC-1005 against HIV-1_{BaL} in a single-challenge

FIG 4 Exposure to PC-1005 does not decrease tissue viability. Viability of ectocervical tissues after immersion in medium containing diluted gels versus medium-only (Med) and 1:10 diluted Gynol controls (three explants per condition) for ~18 h was tested by MTT assay. The OD₅₇₀ of the formazan product was measured and normalized by the dry weight of the explants. Each symbol indicates an individual donor, and the mean ± SEM of the log₁₀ OD₅₇₀/g of tissue for each condition is shown. Significant *P* values of <0.001 (***) are indicated.

FIG 5 Exposure to CVLs does not decrease tissue viability. Viability of ectocervical tissue after immersion in 1:2-diluted CVLs versus medium-only (Med) and 1:10 diluted Gynol controls for ~18 h (three explants per condition) was tested by MTT assay as in Fig. 4. Each symbol indicates an individual donor, and the mean ± SEM of the log₁₀ OD₅₇₀/g of tissue for each condition is shown. Significant *P* values of <0.001 (***) are indicated.

FIG 6 MIV-150 and CG in CVLs inhibit HIV and HSV-2 infection in the ectocervical tissue in a dose-dependent manner. Tissues were incubated with paired 1:2 dilutions of CVLs (baseline and post-gel application) for 4 h, washed, and then challenged with 500 TCID₅₀ HIV-1_{BaL} or 500 TCID₅₀ HIV-1_{BaL} and 10⁶ PFU HSV-2 per explant (three explants per condition) for ~18 h. Controls (shown in Fig. S1B in the supplemental material) included tissues not exposed to CVLs (medium only) and challenged with HIV-1_{BaL} or HIV-1_{BaL}–HSV-2 or challenged with HIV-1_{BaL}–HSV-2 in the presence of 3TC/Acy. Following washout, tissues were cultured and infections analyzed as in Fig. 2. Changes in CUM values after exposure to post-gel application CVLs (post) versus baseline CVLs (pre) relative to MIV-150 (A, single-challenge model; B, cochallenge model) and CG (C) concentrations in diluted CVLs are shown. Solid circles, 4-h PC-1005 CVLs; open squares, 24-h PC-1005 CVLs; open diamonds, placebo CVLs.

model and in a stringent HIV-1_{BaL}–HSV-2 cochallenge model in human ectocervical explants.

Similar to the previous report (10), we show that coinfection of ectocervical tissues with HSV-2 enhances HIV-1_{BaL} infection. However, the ~60% frequency of productive HIV-1_{BaL} infection in the presence of HSV-2 (versus 100% following single HIV-1_{BaL} challenge) can potentially be due to apoptosis of T cells induced by exposure to the high HSV-2 challenge dose in this model (34). PC-1005 at 1:100 and 1:300 dilutions (184.2 and 61.4 ng/ml of MIV-150, respectively) strongly inhibited HIV-1 (>99%) in both HIV-1_{BaL} single-infection and coinfection experiments, despite the significantly higher replication of HIV in the latter. PC-1005 also provided significant CG-mediated anti-HSV-2 activity. Although anti-HSV-2 activity was lost at the high gel dilution (1:300), it may still be evident *in vivo* against a more physiologically relevant HSV-2 inoculum (35, 36). Of note, tenofovir at low concentrations (1 μg/ml) was shown to inhibit single infections with HIV-1_{BaL} and HSV-2, but a higher tenofovir dose (100 μg/ml) was required to inhibit infections with both viruses in cochallenge experiments (10).

In support of earlier data demonstrating that anti-HIV-1 (cell-based assays), anti-SHIV-RT (macaque vaginal explants), and anti-HSV-2 (murine model) activity of PC-1005 and anti-HPV activity (murine model) of CG is maintained in the presence of biological fluids (SP) (14, 20, 22, 23; Kenney et al., unpublished), PC-1005 was active against HIV-1_{BaL} and HSV-2 in human ectocervical explants in the presence of vaginal fluids. Infection levels were inversely correlated with MIV-150 levels (HIV-1) and CG (HSV-2) levels in the CVLs. The design of these experiments (tissue preincubation with CVLs followed by washout and then single challenge with HIV-1 or cochallenge with HIV-1 and HSV-2) offered rigorous testing conditions against both viruses. Therefore, here we introduced new models for testing anti-HIV and anti-HSV activity of CVLs in relevant mucosal targets.

This study has several caveats, including caveats associated with the use of cervical explant models (37). Also, the study does not address activity of PC-1005 in human vaginal mucosal targets due to difficulties in obtaining vaginal surgical tissues. As expected, based on the dose-dependent effect of CG against SHIV-RT *ex vivo* (20) in macaque explants and dose-dependent and time-dependent effects *in vivo* (15, 16, 38) in macaques, CG at high concentrations (300 μg/ml, 1:100 dilution of PC-525) inhibited HIV-1_{BaL} infection in the single-challenge

model. This was not the case in the coinfection model (HIV-1–HSV-2), where HIV replication was significantly more robust. Moreover, spiking CVLs with CG provided anti-HIV-1_{BaL} activity; however, the inhibitory activity of CVLs containing PC-1005 components was more potent, pointing to MIV-150-mediated protection. We were not able to test activity of tissue-associated MIV-150 and CG after gel application *in vivo* as vaginal and cervical biopsy specimens in the phase 1 PC-1005 study were prioritized for histological evaluation. Finally, the small number of available CVLs provided little statistical power. In spite of this hindrance, the fact that we observed significant anti-HIV and anti-HSV-2 activity of CVLs is striking, and, as noted earlier, with the decision to minimize the type 1 error rate, hints at the likelihood that this is not a spurious finding.

The results presented in this article are consistent with the extensive *in vitro*, *ex vivo* (explants), and *in vivo* (mice and macaques) data demonstrating efficacy of PC-1005 gel against HIV, SHIV-RT, and HSV-2 (14–21; Calenda et al., submitted). PC-1005 is the only microbicide gel currently being tested in clinical trials that targets HIV and two other main sexually transmitted infections (STIs): HSV-2 and HPV (23). Overall, our data point to the potent anti-HIV and anti-HSV-2 activity of PC-1005 in mucosal targets and endorse the further development of PC-1005 as a broad-spectrum on-demand microbicide.

ACKNOWLEDGMENTS

We thank I. McGowan and D. Katz for critically reading and discussing the manuscript and K. Roberts for insightful discussions of statistical analyses.

FUNDING INFORMATION

This work was supported by the United States President's Emergency Plan for AIDS Relief (PEPFAR) through the United States Agency for International Development (USAID) Cooperative Agreement (GPO-A-00-04-00019-00, <http://www.usaid.gov>). The contents of the manuscript are the sole responsibility of the Population Council and do not necessarily reflect the views or policies of USAID or the U.S. government. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Abdool Karim Q, Sibeko S, Baxter C. 2010. Preventing HIV infection in women: a global health imperative. *Clin Infect Dis* 50(Suppl 3):S122–S129. <http://dx.doi.org/10.1086/651483>.

2. Boily MC, Baggaley RF, Wang L, Masse B, White RG, Hayes RJ, Alary M. 2009. Heterosexual risk of HIV-1 infection per sexual act: systematic review and meta-analysis of observational studies. *Lancet Infect Dis* 9:118–129. [http://dx.doi.org/10.1016/S1473-3099\(09\)70021-0](http://dx.doi.org/10.1016/S1473-3099(09)70021-0).
3. Corey L. 2007. Herpes simplex virus type 2 and HIV-1: the dialogue between the 2 organisms continues. *J Infect Dis* 195:1242–1244. <http://dx.doi.org/10.1086/513570>.
4. Barnabas RV, Wasserheit JN, Huang Y, Janes H, Morrow R, Fuchs J, Mark KE, Casapia M, Mehrotra DV, Buchbinder SP, Corey L. 2011. Impact of herpes simplex virus type 2 on HIV-1 acquisition and progression in an HIV vaccine trial (the Step Study). *J Acquir Immune Defic Syndr* 57:238–244. <http://dx.doi.org/10.1097/QAI.0b013e31821ac6b5>.
5. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. 2006. Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies. *AIDS* 20:73–83. <http://dx.doi.org/10.1097/01.aids.0000198081.09337.a7>.
6. Sobngwi-Tambekou J, Taljaard D, Lissouba P, Zarca K, Puren A, Lagarde E, Auvert B. 2009. Effect of HSV-2 serostatus on acquisition of HIV by young men: results of a longitudinal study in Orange Farm, South Africa. *J Infect Dis* 199:958–964. <http://dx.doi.org/10.1086/597208>.
7. Barnabas RV, Celum C. 2012. Infectious co-factors in HIV-1 transmission herpes simplex virus type-2 and HIV-1: new insights and interventions. *Curr HIV Res* 10:228–237. <http://dx.doi.org/10.2174/157016212800618156>.
8. Weiss H. 2004. Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes* 11(Suppl 1):24A–35A.
9. Goode D, Truong R, Villegas G, Calenda G, Guerra-Perez N, Piatak M, Lifson JD, Blanchard J, Gettie A, Robbani M, Martinelli E. 2014. HSV-2-driven increase in the expression of alpha4beta7 correlates with increased susceptibility to vaginal SHIVSF162P3 infection. *PLoS Pathog* 10:e1004567. <http://dx.doi.org/10.1371/journal.ppat.1004567>.
10. Rollenhagen C, Lathrop MJ, Macura SL, Doncel GF, Asin SN. 2014. Herpes simplex virus type-2 stimulates HIV-1 replication in cervical tissues: implications for HIV-1 transmission and efficacy of anti-HIV-1 microbicides. *Mucosal Immunol* 7:1165–1174. <http://dx.doi.org/10.1038/mi.2014.3>.
11. Zhu J, Hladik F, Woodward A, Klock A, Peng T, Johnston C, Remington M, Margaret A, Koelle DM, Wald A, Corey L. 2009. Persistence of HIV-1 receptor-positive cells after HSV-2 reactivation is a potential mechanism for increased HIV-1 acquisition. *Nat Med* 15:886–892. <http://dx.doi.org/10.1038/nm.2006>.
12. Abdool Karim SS, Abdool Karim Q, Kharsany AB, Baxter C, Grobler AC, Werner L, Kashuba A, Mansoor LE, Samsunder N, Mindel A, Gengiah TN. 2015. Tenofovir gel for the prevention of herpes simplex virus type 2 infection. *N Engl J Med* 373:530–539. <http://dx.doi.org/10.1056/NEJMoa1410649>.
13. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, Kharsany AB, Sibeko S, Mlisana KP, Omar Z, Gengiah TN, Maarschalk S, Arulappan N, Mlotshwa M, Morris L, Taylor D. 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science* 329:1168–1174. <http://dx.doi.org/10.1126/science.1193748>.
14. Kizima L, Rodriguez A, Kenney J, Derby N, Mizenina O, Menon R, Seidor S, Zhang S, Levendosky K, Jean-Pierre N, Pugach P, Villegas G, Ford BE, Gettie A, Blanchard J, Piatak M, Jr, Lifson JD, Paglini G, Teleshova N, Zydowsky TM, Robbani M, Fernandez-Romero JA. 2014. A potent combination microbicide that targets SHIV-RT, HSV-2 and HPV. *PLoS One* 9:e94547. <http://dx.doi.org/10.1371/journal.pone.0094547>.
15. Kenney J, Aravantinou M, Singer R, Hsu M, Rodriguez A, Kizima L, Abraham CJ, Menon R, Seidor S, Chudolij A, Gettie A, Blanchard J, Lifson JD, Piatak M, Jr, Fernandez-Romero JA, Zydowsky TM, Robbani M. 2011. An antiretroviral/zinc combination gel provides 24 hours of complete protection against vaginal SHIV infection in macaques. *PLoS One* 6:e15835. <http://dx.doi.org/10.1371/journal.pone.0015835>.
16. Kenney J, Singer R, Derby N, Aravantinou M, Abraham C, Menon R, Seidor S, Zhang S, Gettie A, Blanchard J, Piatak M, Lifson J, Fernandez-Romero JA, Zydowsky T, Robbani M. 2012. A single dose of a MIV-150/zinc acetate gel provides 24 h of protection against vaginal simian human immunodeficiency virus reverse transcriptase infection, with more limited protection rectally 8 to 24 h after gel use. *AIDS Res Hum Retroviruses* 28:1476–1484. <http://dx.doi.org/10.1089/aid.2012.0087>.
17. Hsu M, Aravantinou M, Menon R, Seidor S, Goldman D, Kenney J, Derby N, Gettie A, Blanchard J, Piatak M, Lifson J, Fernandez-Romero JA, Zydowsky T, Robbani M. 2014. A combination microbicide gel protects macaques against vaginal SHIV-RT infection, but only partially reduces HSV-2 infection after a single high-dose cochallenge. *AIDS Res Hum Retroviruses* 30:174–183. <http://dx.doi.org/10.1089/aid.2013.0165>.
18. Kenney J, Derby N, Aravantinou M, Kleinbeck K, Frank I, Gettie A, Grasperge B, Blanchard J, Piatak M, Jr, Lifson JD, Zydowsky TM, Robbani M. 2014. Short communication: a repeated simian human immunodeficiency virus reverse transcriptase/herpes simplex virus type 2 cochallenge macaque model for the evaluation of microbicides. *AIDS Res Hum Retroviruses* 30:1117–1124. <http://dx.doi.org/10.1089/aid.2014.0207>.
19. Jean-Pierre N, Barnable P, Kizima L, Rodriguez A, Seidor S, Cooney ML, Clark MR, Doncel GF, Robbani M, Zydowsky TM, Teleshova N, Fernandez-Romero JA. 2015. Multipurpose prevention approaches with antiretroviral-based formulations. *Antimicrob Agents Chemother* 60:1141–1144. <http://dx.doi.org/10.1128/AAC.02468-15>.
20. Barnable P, Calenda G, Ouattara L, Gettie A, Blanchard J, Jean-Pierre N, Kizima L, Rodriguez A, Abraham C, Menon R, Seidor S, Cooney M, Roberts K, Sperling R, Piatak M, Lifson J, Fernandez-Romero J, Zydowsky T, Robbani M, Teleshova N. 2014. A MIV-150/zinc acetate gel inhibits SHIV-RT infection in macaque vaginal explants. *PLoS One* 9:e108109. <http://dx.doi.org/10.1371/journal.pone.0108109>.
21. Barnable P, Calenda G, Bonnaire T, Menon R, Levendosky K, Gettie A, Blanchard J, Cooney ML, Fernandez-Romero JA, Zydowsky TM, Teleshova N. 2015. MIV-150/zinc acetate gel inhibits cell-associated simian-human immunodeficiency virus reverse transcriptase infection in a macaque vaginal explant model. *Antimicrob Agents Chemother* 59:3829–3837. <http://dx.doi.org/10.1128/AAC.00073-15>.
22. Rodriguez A, Kleinbeck K, Mizenina O, Kizima L, Levendosky K, Jean-Pierre N, Villegas G, Ford BE, Cooney ML, Teleshova N, Robbani M, Herold BC, Zydowsky T, Fernandez Romero JA. 2014. In vitro and in vivo evaluation of two carrageenan-based formulations to prevent HPV acquisition. *Antiviral Res* 108:88–93. <http://dx.doi.org/10.1016/j.antiviral.2014.05.018>.
23. Fernandez-Romero JA, Deal C, Herold BC, Schiller J, Patton D, Zydowsky T, Romano J, Petro CD, Narasimhan M. 2015. Multipurpose prevention technologies: the future of HIV and STI protection. *Trends Microbiol* 23:429–436. <http://dx.doi.org/10.1016/j.tim.2015.02.006>.
24. Friedland BA, Hoesley C, Plagianos MG, Zhang S, Hoskin E, Alami M, Teleshova N, Zydowsky T, Fernandez-Romero J, Creasy G. 2016. A first-in-human trial of PC-1005 (MIV-150 and zinc acetate in a carrageenan gel), abstr 875. *Conf Retrovir Opportunistic Infect (CROI)* 2016, Boston, MA, 22 to 25 February 2016.
25. Nawaz F, Cicala C, Van Ryk D, Block KE, Jelicic K, McNally JP, Ogundare O, Pascuccio M, Patel N, Wei D, Fauci AS, Arthos J. 2011. The genotype of early-transmitting HIV gp120s promotes alphabeta-reactivity, revealing alphabetaCD4⁺ T cells as key targets in mucosal transmission. *PLoS Pathog* 7:e1001301. <http://dx.doi.org/10.1371/journal.ppat.1001301>.
26. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, Wei D, Xiao Z, Veenstra TD, Conrad TP, Lempicki RA, McLaughlin S, Pascuccio M, Gopaul R, McNally J, Cruz CC, Censoplano N, Chung E, Reitano KN, Kottlil S, Goode DJ, Fauci AS. 2008. HIV-1 envelope protein binds to and signals through integrin alpha4beta7, the gut mucosal homing receptor for peripheral T cells. *Nat Immunol* 9:301–309. <http://dx.doi.org/10.1038/ni1566>.
27. Derdeyn CA, Decker JM, Sfakianos JN, Wu X, O'Brien WA, Ratner L, Kappes JC, Shaw GM, Hunter E. 2000. Sensitivity of human immunodeficiency virus type 1 to the fusion inhibitor T-20 is modulated by coreceptor specificity defined by the V3 loop of gp120. *J Virol* 74:8358–8367. <http://dx.doi.org/10.1128/JVI.74.18.8358-8367.2000>.
28. Ashley R. 1995. Herpes simplex viruses, p 375–395. In Lennette EH, Lennette DA, Lennette ET (ed), *Diagnostic procedures for viral, rickettsial, and chlamydial infections*, 7th ed. American Public Health Association, Washington, DC.
29. Hsu M, Keele BF, Aravantinou M, Krawczyk N, Seidor S, Abraham CJ, Zhang S, Rodriguez A, Kizima L, Derby N, Jean-Pierre N, Mizenina O, Gettie A, Grasperge B, Blanchard J, Piatak MJ, Jr, Lifson JD, Fernandez-Romero JA, Zydowsky TM, Robbani M. 2014. Exposure to MIV-150 from a high-dose intravaginal ring results in limited emergence of drug resistance mutations in SHIV-RT infected rhesus macaques. *PLoS One* 9:e89300. <http://dx.doi.org/10.1371/journal.pone.0089300>.

30. Ouattara LA, Barnable P, Mawson P, Seidor S, Zydowsky TM, Kizima L, Rodriguez A, Fernandez-Romero JA, Cooney ML, Roberts KD, Gettie A, Blanchard J, Robbiani M, Teleshova N. 2014. MIV-150-containing intravaginal rings protect macaque vaginal explants against SHIV-RT infection. *Antimicrob Agents Chemother* 58:2841–2848. <http://dx.doi.org/10.1128/AAC.01529-13>.
31. Aravantinou M, Singer R, Derby N, Calenda G, Mawson P, Abraham C, Menon R, Seidor S, Goldman D, Kenney J, Villegas G, Gettie A, Blanchard J, Lifson J, Piatlak M, Fernandez-Romero JA, Zydowsky T, Teleshova N, Robbiani M. 2012. The nonnucleoside reverse transcription inhibitor MIV-160 delivered from an intravaginal ring, but not from a carrageenan gel, protects against simian/human immunodeficiency virus-RT infection. *AIDS Res Hum Retroviruses* 28:1467–1475. <http://dx.doi.org/10.1089/aid.2012.0080>.
32. Richardson-Harman N, Lackman-Smith C, Fletcher PS, Anton PA, Bremer JW, Dezzutti CS, Elliott J, Grivel JC, Guenther P, Gupta P, Jones M, Lurain NS, Margolis LB, Mohan S, Ratner D, Reichelderfer P, Roberts P, Shattock RJ, Cummins JE, Jr. 2009. Multisite comparison of anti-human immunodeficiency virus microbicide activity in explant assays using a novel endpoint analysis. *J Clin Microbiol* 47:3530–3539. <http://dx.doi.org/10.1128/JCM.00673-09>.
33. Richardson-Harman N, Mauck C, McGowan I, Anton P. 2012. Dose-response relationship between tissue concentrations of UC781 and explant infectibility with HIV type 1 in the RMP-01 rectal safety study. *AIDS Res Hum Retroviruses* 28:1422–1433. <http://dx.doi.org/10.1089/aid.2012.0073>.
34. Vanden Oever MJ, Han JY. 2010. Caspase 9 is essential for herpes simplex virus type 2-induced apoptosis in T cells. *J Virol* 84:3116–3120. <http://dx.doi.org/10.1128/JVI.01726-09>.
35. Mark KE, Wald A, Magaret AS, Selke S, Kuntz S, Huang ML, Corey L. 2010. Rapidly cleared episodes of oral and anogenital herpes simplex virus shedding in HIV-infected adults. *J Acquir Immune Defic Syndr* 54:482–488. <http://dx.doi.org/10.1097/QAI.0b013e3181d91322>.
36. Tronstein E, Johnston C, Huang ML, Selke S, Magaret A, Warren T, Corey L, Wald A. 2011. Genital shedding of herpes simplex virus among symptomatic and asymptomatic persons with HSV-2 infection. *JAMA* 305:1441–1449. <http://dx.doi.org/10.1001/jama.2011.420>.
37. Anderson DJ, Pudney J, Schust DJ. 2010. Caveats associated with the use of human cervical tissue for HIV and microbicide research. *AIDS* 24:1–4. <http://dx.doi.org/10.1097/QAD.0b013e328333acfb>.
38. Turville SG, Aravantinou M, Miller T, Kenney J, Teitelbaum A, Hu L, Chudolij A, Zydowsky TM, Piatlak M, Jr, Bess JW, Jr, Lifson JD, Blanchard J, Gettie A, Robbiani M. 2008. Efficacy of Carraguard-based microbicides in vivo despite variable in vitro activity. *PLoS One* 3:e3162. <http://dx.doi.org/10.1371/journal.pone.0003162>.
39. Friedland B, Hoesley C, Plagianos M, Hoskin E, Zhang S, Teleshova N, Alami M, Novak L, Kleinbeck K, Katzen L, Zydowsky T, Fernández-Romero J, Creasy G. A first-in-human trial of MIV-150 and zinc acetate co-formulated in a carrageenan gel: safety, pharmacokinetics, acceptability, adherence and pharmacodynamics. *J Acquir Immune Defic Syndr*, in press.