Partner Characteristics Predicting HIV-1 Set Point in Sexually Acquired HIV-1 Among African Seroconverters

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Abstract

Plasma HIV-1 RNA set point is an important predictor of HIV-1 disease progression. We hypothesized that inoculum size and HIV-1 exposure prior to HIV-1 transmission may modulate set point. We evaluated predictors of set point among 141 African HIV-1 seroconverters and their HIV-1-infected study partners. We compared characteristics of seroconverters and their HIV-1-infected partners and HIV-1 set point. Data were from a clinical trial of genital HSV-2 suppression with acyclovir to reduce HIV-1 transmission in HIV-1 serodiscordant couples with HIV-1 transmission linkage assigned through virus sequencing. Our analysis includes data from all transmissions including those with transmission linkage to the HIV-1-infected “source partner” and those that were not linked to their HIV-1-infected study partner. In multivariable analysis, higher plasma HIV-1 in source partners was associated with higher seroconverter set point ($+0.44 \log_{10}$ copies/ml per $\log_{10}$ source partner plasma HIV-1, $p < 0.001$). In addition, bacterial vaginosis (BV) among female source partners near the time of infection was associated with higher set point in their male seroconverters ($+0.49 \log_{10}$, $p = 0.04$). Source partner characteristics associated with lower set point included male circumcision ($-0.63 \log_{10}$, $p = 0.03$) and assignment to acyclovir ($-0.44 \log_{10}$, $p = 0.02$). The proportion of variation in set point explained by plasma HIV-1 RNA of the source partner, after controlling for other factors, was 0.06. Source partner plasma HIV-1 level is the most significant predictor of seroconverter set point, possibly reflecting characteristics of the transmitted virus. Acyclovir use, BV among women source partners, and circumcision among male source partners may alter the set point by affecting transmitted virus inoculum in the source partners’ genital compartment.

Introduction

INCREASED PLASMA HIV-1 set point (i.e., the steady-state concentration of virus in plasma established after acute infection) is strongly associated with increased risk of HIV-1 disease progression and transmission,^{1–3} making factors that predict set point of interest for understanding the pathogenesis of HIV-1 infection. Studies of newly HIV-1-infected individuals have shown that during the process of early HIV-1 infection, a balance between cytotoxic T lymphocyte host responses and virus escape contributes to determining HIV-1

set point,⁴ and that, in this context, specific host genetic characteristics (e.g., alleles in the HLA and KIR loci) also influence set point.^{5–10} Studies evaluating HIV-1-infected source partners in diverse epidemiologic contexts have found that plasma HIV-1 RNA levels of source partners^{11–14} and genetic characteristics of their transmitted viruses¹⁵ are associated with HIV-1 set point in their seroconverting partners. Collectively, these findings have been interpreted as demonstrating that fixed genetic characteristics of the host and transmitted virus define set point in the newly infected partner.¹⁶ However, in stable HIV serodiscordant couples,

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exposure to HIV-1 likely occurs over a prolonged period prior to transmission of the virus. It is possible that this HIV-1 exposure itself may impact host immune responses to the virus and, in turn, modify HIV-1 set point. Therefore, it may be important to include factors associated with HIV-1 exposure of the susceptible partner along with characteristics of the HIV-1 transmitting partner together in the same analysis when evaluating HIV-1 set point.

Prospective studies of HIV-1 serodiscordant couples collect specimens and data from both the prevalent HIV-1-infected partner and their initially seronegative sex partner. For cases of HIV-1 transmission, sequencing of plasma viruses from both partners allows determination of whether the seroconverting partner's virus was likely acquired from the HIV-1-infected partner (linked infections) or may have been acquired through a different partnership (unlinked infection). We used data from linked and unlinked infections in a large prospective study of African HIV-1 serodiscordant heterosexual couples to evaluate the association of HIV-1 exposure to the HIV-1-infected partner and other partner characteristics with HIV-1 seroconverter set point.

Materials and Methods

Study population

We analyzed data from the Partners in Prevention HSV/HIV Transmission Study, a clinical trial designed to assess the efficacy of genital herpes simplex virus type 2 (HSV-2) suppression for reducing HIV-1 transmission (Clinicaltrials.gov # NCT00194519).¹⁷ A total of 3,408 stable heterosexual HIV-1 serodiscordant couples were recruited in 14 sites in East and Southern Africa; HIV-1-infected partners were required to be dually infected with HSV-2 and have a CD4 count ≥ 250 cells/mm³. Prevalent HIV-1-infected partners were randomized to acyclovir 400 mg twice daily or matching placebo, and their HIV-1-exposed, uninfected partners were tested for HIV-1 quarterly. Follow-up was for up to 24 months. Further details on recruitment and follow-up for this trial have been published elsewhere.¹⁷ The study found that acyclovir did not significantly reduce HIV-1 transmission, but did reduce plasma HIV-1 levels in the HIV-1-infected partners by 0.25 log₁₀ copies/ml.^{17,18}

HIV-1 serology and plasma HIV-1 RNA levels

HIV-1 seroconversions were confirmed by ELISA, and HIV-1 transmission linkage assessed by comparing HIV-1 *env* and/or *gag* sequences of plasma viruses from both enrolled partners.^{17,19} Transmissions were classified as "linked" if viruses from both partners were linked by sequence analysis (in which case the HIV-1-infected partner was regarded as the source of the transmitted HIV-1) and "not linked/unable to be linked" if comparison of the virus sequences was more consistent with transmission from a partner outside of the study relationship.

Plasma HIV-1 RNA was measured by real-time reverse transcriptase polymerase chain reaction (RT-PCR) (COBAS AmpliPrep/COBAS TaqMan HIV-1 RNA assay, version 1.0, Roche Diagnostics, Indianapolis, IN), with a limit of detection of 240 copies/ml, on plasma from the HIV-1-infected partner at the 0, 3, 6, and 12-month visits and at the final study visit. HIV-1 RNA quantity was assessed for seroconverters on

plasma collected at the first HIV-1 seropositive visit and at visits <1 month, 3, 6, 9, and 12 months after seroconversion. Plasma from the HIV-1-negative partner at quarterly visits prior to HIV-1 seroconversion was also tested by HIV-1 RNA PCR to more precisely establish the date of HIV-1 infection.

Testing for curable sexually transmitted infections (STI, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*) was performed by transcription-mediated nucleic acid amplification assays (Gen-Probe, San Diego, CA) performed on samples collected at study enrollment. As previously described,²⁰ Gram stain of vaginal secretions was used to define bacterial vaginosis (BV).

Date of HIV-1 infection

We estimated the date of HIV-1 infection of new seroconverters based on a combination of their HIV-1 serology and plasma HIV-1 RNA PCR results. Among the 141 HIV-1 seroconverters included in this analysis, 34 (24%) had a plasma sample from a visit prior to HIV-1 seroconversion that was positive for HIV-1 RNA by real-time RT-PCR; for these 34 individuals, the date of infection was estimated to occur 17 days before the date of the first plasma PCR positive visit, as done by others previously.^{21,22} For the remaining 107 seroconverters (76%), the first detection of HIV-1 RNA by real-time RT-PCR corresponded to the visit at which seroconversion was detected; for these, the date of infection was calculated as the midpoint between the last HIV-1 seronegative and first HIV-1 seropositive visit, or 45 days before detection the first HIV-1 seropositive visit, for individuals who missed a quarterly follow-up visit and thus had a long gap between visits. This latter requirement was imposed to obtain a conservative estimate of the date of infection and minimize the chance that missed visits over extended periods could lead to misclassification of acute infection samples as representing HIV-1 set point.

HIV-1 set point

Plasma HIV-1 RNA measurements taken 4 months or more after the estimated date of infection were considered as having occurred after establishment of HIV-1 set point.³ Because samples were collected six times during the first year after HIV-1 seroconversion was detected, each seroconverter could contribute multiple set point measurements, and the analysis took into account multiple measures per participant. Set point measurements were excluded if the seroconverter reported taking an antiretroviral drug regimen in the preceding interval. The estimated within-person variance in HIV-1 set point was 0.17 log₁₀ copies/ml and the estimated between-person variance was 0.87 log₁₀ copies/ml giving an estimated interclass correlation coefficient of 0.83.

Statistical analysis

Comparisons of characteristics of linked and not linked seroconverting and prevalent HIV-1-infected partners were performed through chi-squared or Wilcoxon rank sum tests, as appropriate. We estimated the relationships between HIV-1 set point and potential risk factors using multivariate linear mixed effects modeling with log₁₀ set point as the outcome and participant characteristic as a random effect. Risk factors in the seroconverting partner examined for possible

associations with set point were gender, partnership duration, any unprotected sex, envelope subtype of acquired virus, HSV-2 serology, BV status among seroconverting women, and status of HIV-1 transmission as linked/not linked with HIV-1-infected partner. Risk factors evaluated in the HIV-1-infected partner were plasma HIV-1 viral load, CD4 count, and acyclovir exposure. Factors examined in both partners were age, curable STI, genital ulcer disease (GUD) by examination or history, circumcision (in men), hormonal contraceptive use (in women), and whether pregnant at time of infection (in women). Although use of any hormonal contraception was included in this analysis, we have previously reported that most (76%) women reporting hormonal contraceptive use used injectable forms.²³ Time-varying factors were assessed for the visit most proximate but prior to the estimated date of HIV-1 infection. The only exception to this was for HIV-1 infections occurring just prior to the baseline visit for which covariate baseline values were used and exposure to acyclovir was considered to have not yet occurred. We included interaction terms between transmission linkage status and characteristics of HIV-1-infected partners in order to assess the impact of those characteristics separately on set point of linked and not linked infections. Effects of seroconverting partner characteristics on set point were assumed not to vary depending on linkage. Factors kept in the multivariate model encompassing partnerships with linked and unlinked infections were plasma HIV-1 RNA, randomization to acyclovir suppression, linkage (and appropriate interaction terms), gender (necessary for interpretation of other variables in the model such as female hormonal contraceptive use), and any risk factors with unadjusted $p \leq 0.1$. Transmission linkage status and gender were included as variables in the adjusted model, regardless of their association in the unadjusted analysis, to permit all partners to be evaluated in a single model.

We calculated the proportion of variation in set point explained by plasma HIV-1 RNA using the coefficient of determination (R^2). All analyses were performed in SAS v9.0.

Human subjects research

The study protocol was reviewed and approved by human subjects research committees at the University of Washington and all local study site and affiliated institutions.

Results

Characteristics of cohort, and plasma HIV-1 set point

A total of 151 HIV-1 seroconversions were observed; of these, 141 (93%) were followed for at least 4 months after seroconversion and had ≥ 1 plasma HIV-1 RNA result available for analyses of HIV-1 set point (Fig. 1). In total, these 141 seroconverters contributed 502 plasma HIV-1 measurements after set point, including 109 (22%), 143 (28%), and 250 (50%) measurements taken 4 to 5 months, 5 to 8 months, and 9 to 18 months after the estimated date of infection, respectively. A median of four [interquartile range (IQR) three to four] plasma HIV-1 RNA set point measurements were obtained per seroconverter. The median of all set point measurements was 4.60 $\log_{10}$ copies/ml (IQR 3.83–5.09). Among 86 seroconverting men, 302 set point measurements yielded a median 4.62 $\log_{10}$ copies/ml (IQR 3.88–5.05), while the median of

FIG. 1. Flowchart of seroconverters with HIV-1 set point data. Breakdown of the 151 seroconverters from the Partners in Prevention HSV/HIV Transmission study showing those included in this analysis indicating viral genetic linkage status and availability of set point data.

200 measurements in 55 seroconverting women was 4.58 $\log_{10}$ copies/ml (IQR 3.74–5.20).

Among these 141 transmissions, 101 (72%) of seroconverters had viruses that were genetically linked to the prevalent HIV-1-infected partner's virus, while 40 (28%) were not linked. Table 1 provides partner characteristics of these 141 couples. Among 61 (43%) seroconverters, the HIV-1 envelope of the infecting virus was determined to be subtype A in 61 (43%), subtype C in 52 (37%), subtype D in 19 (13%), and another subtype or subtype not identified in 9 (6%).

Statistically significant differences between characteristics of linked versus not linked partners were evident only for plasma HIV-1 RNA level of prevalent HIV-1-infected persons (median 4.9 for linked and 4.1 $\log_{10}$ copies/ml for not linked infections, respectively; $p < 0.001$), and gender of seroconverting partners (54% of linked infections and 78% of not linked seroconverting partners occurred in men, $p = 0.01$).

Predictors of plasma HIV-1 set point

In the unadjusted analysis, plasma HIV-1 RNA level and BV in female source partners (HIV-1-infected partner in couples with transmission linkage) had the strongest associations with set point in seroconverters (0.41 mean increase in seroconverter set point per $\log_{10}$ increase in plasma HIV-1 RNA, $p < 0.001$, and 0.69 mean increase in seroconverter set point, $p < 0.001$, respectively) (Table 2).

In a multivariate analysis, higher plasma HIV-1 levels in the source partner and BV in female source partners were associated with increased seroconverter set point (+0.44 $\log_{10}$ copies/ml in set point per $\log_{10}$ increase in plasma HIV-1 RNA, $p < 0.001$, and +0.49 $\log_{10}$ copies/ml in set point, $p = 0.04$, respectively), while acyclovir use and male circumcision in source partners were associated with reduced set point (−0.44 $\log_{10}$ and −0.63 $\log_{10}$ copies/ml set point, with $p = 0.02$ and 0.03, respectively).

We saw some evidence that among seroconverters whose virus was not linked to their study partners' virus, set point HIV-1 levels were higher with increased plasma HIV-1 RNA in their HIV-1-infected partner (+0.32 $\log_{10}$ copies/ml in set point per $\log_{10}$ increase in plasma HIV-1 RNA in the not linked partner, $p = 0.02$ in unadjusted analysis); in the adjusted analysis the point estimate remained similar (+0.24 $\log_{10}$

TABLE 1. CHARACTERISTICS OF HIV-1 SEROCONVERTERS AND THEIR HIV-1-INFECTED PARTNERS

Partner characteristic	Linked HIV-1 transmissions (n = 101)	Not linked HIV-1 transmissions (n = 40)
Seroconverting partners		
Female gender	46 (46%)	9 (23%)
Age ^a (years)	30 (26, 38)	32 (26, 42)
Years in relationship ^a	3.8 (1.6, 8.3)	3.6 (1.7, 8.5)
Frequency of sex in prior month ^b	4 (2, 8)	4 (1, 8)
Any reported unprotected sex ^b	35 (35%)	15 (38%)
Proportion reporting outside partnership ^b	2 (2%)	11 (28%)
HSV-2 serology ^a	80 (79%)	35 (88%)
Genital ulcer disease (GUD) by exam or history ^b	11 (11%)	2 (5%)
Curable STI ^{a,c} (data available for n = 126)	20/90 (22%)	6/36 (17%)
Bacterial vaginosis (BV) at time of partner infection (women only)	19 (45%)	4 (44%)
Time (days) from enrollment to infection, median (IQR)	136 (45, 316)	314 (229, 423)
Number of plasma RNA levels ≥ 4 months postinfection	4 (3, 4)	4 (3, 5)
CD4 count ^d	479 (350, 633)	503 (369, 597)
Envelope subtype of acquired virus		
A	43 (43%)	18 (45%)
C	37 (37%)	15 (38%)
D	14 (14%)	5 (13%)
G	1 (1%)	1 (3%)
Other or unknown ^e	6 (6%)	1 (3%)
Circumcised ^a (men only)	33/55 (60%)	18/31 (58%)
Hormonal contraceptive use ^b (women only)	9/46 (20%)	0/9 (0%)
Pregnant at time of partner infection (women only)	8/46 (17%)	2/9 (22%)
HIV-1-infected partners		
Female gender	55 (54%)	31 (78%)
Age ^a (years)	30 (26, 36)	31 (24, 39)
GUD by exam or history ^b	18 (18%)	4 (10%)
Curable STI ^{a,c} (data available for n = 126)	20/91 (21%)	9/35 (24%)
BV at time of partner infection (women only)	32 (62%)	17 (55%)
CD4 count ^b cells/μl	374 (287, 524)	394 (305, 497)
Log ₁₀ plasma HIV-1 ^b	4.9 (4.4, 5.3)	4.1 (3.5, 4.9)
Time (months) between plasma HIV-1 RNA evaluation and estimated date of infection of seroconverter ^f	1.8 (1.5, 4.0)	3.7 (1.7, 5.0)
On acyclovir at time of partner infection ^g	37 (37%)	20 (50%)
Circumcised (men only) ^a	11/46 (24%)	3/9 (33%)
Hormonal contraceptive use ^b (women only)	14/55 (25%)	6/31 (19%)
Pregnant at time of partner infection (women only)	6/55 (11%)	5/31 (16%)

^aAt enrollment.^bMost recent assessment before estimated date of infection.^c*N. gonorrhea*, *C. trachomatis*, *T. vaginalis* by transcription-mediated amplification.^dFirst measure ≥ 4 months after infection.^eOne recombinant of subtype C and D, the others could not be subtyped.^fAmong those infected after enrollment (38 not linked, 79 linked).^gAs defined by study protocol.

Proportion [N (%) or median (IQR)] of HIV-1 seroconverting and HIV-1-infected partners with the indicated HIV-1 transmission linkage phenotype having the indicated characteristics among N=141 couples associated with HIV-1 seroconversion in the Partners in Prevention HSV/HIV Transmission Study. Linkage was assessed based on plasma HIV-1 sequence characteristics of HIV-1-infected and seroconverting partners as previously reported.¹⁹

copies/ml increase) but was no longer statistically significant ($p=0.1$).

We did not find that any characteristics of the seroconverters themselves were associated with set point HIV-1 levels, including age, gender, duration of partnership, envelope subtype of the acquired virus, circumcision status (for male seroconverting partners), and hormonal contraceptive use or pregnancy (for female seroconverters). HIV-1 set point was not associated with whether the seroconverter acquired HIV-1 from their study partner (linked infection) or from an outside partner (not linked infection). After controlling for other variables, the proportion of variation in seroconverters'

set point explained by their partners' plasma HIV-1 RNA (R^2) was 0.06.

Discussion

Our analysis of 141 prospectively identified African heterosexual seroconverters found that plasma HIV-1 level in the source partner was strongly associated with set point RNA level in their seroconverting partner. Furthermore, we found that independent of plasma HIV-1 levels, genital compartment factors in source partners also modified set point in seroconverters: circumcision of male source partners and

TABLE 2. ASSOCIATIONS OF PARTNER CHARACTERISTICS TO SEROCONVERTER PLASMA HIV-1 SET POINT

Partner characteristics	Mean difference in set point (95% CI)	p-value	Adjusted mean difference in set point (95% CI)	p-value
Seroconverting partner characteristics	502 observations on N=141		492 observations on N=138	
Age ^a				
<25	Referent	0.04	Referent	0.3
25–39	0.26 (–0.17, 0.69)		–0.11 (–0.59, 0.36)	
40+	0.66 (0.14, 1.17)		0.15 (–0.46, 0.76)	
Male gender	–0.03 (–0.36, 0.30)	0.84	^e	^e
Partnership duration (per year) ^a	0.02 (0.00, 0.04)	0.07	0.01 (–0.01, 0.03)	0.3
Any unprotected sex ^b	–0.34 (–0.67, 0.00)	0.05	–0.12 (–0.44, 0.19)	0.4
Curable STI ^{a,c}	–0.15 (–0.57, 0.27)	0.5		
HSV-2 serology ^a	0.02 (–0.40, 0.43)	0.9		
GUD by exam or history ^b	–0.24 (–0.62, 0.13)	0.2		
Linked with index partner ^d	–0.12 (–0.47, 0.24)	0.5	^e	^e
Envelope subtype of acquired virus				
A	Referent	0.7		
C	–0.10 (–0.45, 0.27)			
D	–0.16 (–0.66, 0.34)			
G	–0.84 (–2.20, 0.52)			
Other	–0.23 (–1.00, 0.55)			
In men				
Circumcision	0.18 (–0.23, 0.59)	0.4		
In women				
Hormonal contraceptive use ^b	–0.89 (–1.57, –0.22)	0.01	–0.30 (–0.97, 0.36)	0.3
Pregnant at time of infection	–0.07 (–0.77, 0.62)	0.8		
BV at time of infection	–0.08 (–0.64, 0.48)	0.8		
Source partner characteristics	352 observations on 101 subjects			
Age ^a				
<25	Referent	0.2		
25–39	–0.04 (–0.44, 0.52)			
40+	0.46 (–0.14, 1.07)			
Male gender	–0.01 (–0.40, 0.38)	0.9		
Curable STI ^{a,c}	–0.23 (–0.72, 0.26)	0.4		
GUD by exam or history ^b	–0.24 (–0.75, 0.26)	0.4		
Plasma HIV-1 RNA, per 1 log increase ^b	0.41 (0.18, 0.64)	0.0005	0.44 (0.19, 0.69)	0.0006
CD4 count, per 100 cell decrease ^b	0.08 (–0.04, 0.20)	0.2		
Acyclovir exposure, per study protocol	–0.31 (–0.71, 0.09)	0.1	–0.44 (–0.82, –0.06)	0.02
In men				
Circumcision ^a	–0.59 (–1.29, 0.11)	0.1	–0.63 (–1.21, –0.05)	0.03
In women				
Hormonal contraceptive use ^b	–0.12 (–0.70, 0.46)	0.7		
Pregnant at time of infection	–0.39 (–1.20, 0.43)	0.4		
BV at time of infection	0.69 (0.21, 1.17)	0.005	0.49 (0.03, 0.95)	0.04
Not linked prevalent HIV-1-infected partner characteristics	150 observations on 40 subjects			
Age ^a				
<25	Referent	0.4		
25–39	0.07 (–0.61, 0.76)			
40+	0.54 (–0.28, 1.36)			
Male gender	0.32 (–0.37, 1.01)	0.4		
Curable STI ^{a,c}	–0.04 (–0.75, 0.66)	0.9		
GUD by exam or history ^b	0.01 (–0.97, 0.98)	0.99		
Plasma HIV-1 RNA, per 1 log increase ^b	0.32 (0.05, 0.58)	0.02	0.24 (–0.05, 0.52)	0.10
CD4 count, per 100 cell decrease ^b	0.00 (–0.14, 0.15)	0.94		
Acyclovir exposure, per study protocol	–0.35 (–0.93, 0.22)	0.22	–0.30 (–0.91, 0.31)	0.3
In men				
Circumcision ^a	–0.36 (–1.34, 0.63)	0.47	–0.47 (–1.59, 0.64)	0.4
In women				
Hormonal contraceptive use ^b	–0.04 (–0.96, 0.87)	0.9		
Pregnant at time of infection	0.37 (–0.69, 1.44)	0.5		
BV at time of infection	–0.39 (–1.10, 0.33)	0.28	–0.32 (–0.89, 0.25)	0.27

^aAt study enrollment.^bMost proximate, but prior to, the estimated date of infection.^cNucleic acid amplification for *N. gonorrhea*, *C. trachomatis*, or *T. vaginalis* performed in a subset of *n* = 126 (89%) seroconverters.^dTransmission linkage based on plasma virus sequencing of both partners.¹⁹^eLinkage status and gender were included in the adjusted analysis to permit evaluation of all participants in a single model.

The mean difference in set point is shown for the indicated partner characteristics both as an unadjusted analysis and adjusted for all the variables in the multivariate model.

genital herpes suppression with acyclovir both reduced seroconverter set point, while BV in female source partners increased set point in their male seroconverting partner. Thus, our analysis is the first to demonstrate that a diverse set of source partner characteristics may modulate set point in seroconverters.

The magnitude by which plasma HIV-1 RNA of the source partner increased set point in our analysis (0.44 log₁₀ copies/ml for each log₁₀ increase in plasma HIV-1) was very similar to that previously reported in 115 Zambian HIV-1 serodiscordant couples (0.36 log₁₀ copies/ml increase in set point per log₁₀ increase in source partner's plasma HIV-1 RNA level).¹¹ Similar findings reported for other cohorts^{12-14,16} have been interpreted to suggest that characteristics of the transmitted virus predict set point in the seroconverter. After controlling for other variables, we found that plasma HIV-1 RNA level explained a small proportion (0.06) of the overall variation in seroconverter set point, which was also similar to that reported for Zambian serodiscordant couples ($R^2 = 0.04$).¹¹ The Zambian analysis also found that HLA allele sharing between seroconverting partners contributed only modestly to defining HIV-1 set point,¹¹ a conclusion we are evaluating in this diverse cohort of East and southern African serodiscordant couples.

Our analysis also suggests that source partner genital tract factors also contribute to determining the set point in seroconverters. Previous analyses (including of this cohort) have reported that genital factors strongly influence risk of HIV-1 acquisition: circumcised compared to uncircumcised men have lower HIV-1 transmission rates to their female partners,²⁴⁻²⁶ and BV in female source partners in this cohort was recently shown to increase risk of HIV-1 transmission to male partners.²⁰ However, our analysis is the first to suggest that male circumcision and BV in the source HIV-1-infected partner may also influence plasma viral set point in seroconverters. The mechanism behind this association is not clear, but it may be mediated through changes in HIV-1 inoculum in the genital compartment. High levels of CD4⁺/CCR5⁺ T cells are present in foreskin,²⁷ and similarly accumulation of CD4⁺/CCR5⁺ T cells in the cervical mucosa has been reported among women with BV.²⁸ Thus, increased levels of HIV-1-infected activated T cells associated with uncircumcised HIV-1-infected men or HIV-1-infected women with BV could mediate increased levels of genital HIV-1. Some studies^{20,29-31} have demonstrated that women with BV have higher genital levels of HIV-1 RNA in comparison to women with normal vaginal flora; however, this has not been consistently reported.^{32,33} Studies have also demonstrated that male circumcision can reduce the prevalence of proinflammatory anaerobes on the penis,³⁴ and can impact infections, such as human papilloma virus³⁵ and symptomatic GUD.^{36,37} Further investigation is needed to determine if these factors may influence the observed differences in seroconverter HIV-1 set point.

Finally, we have also demonstrated that acyclovir treatment in the source partner was associated with a reduction in seroconverter set point. Acyclovir is generally thought to impact plasma HIV-1 levels of HIV-1-infected partners indirectly through suppression of HSV-2 replication.³⁸ Recent *in vitro* studies have suggested that acyclovir can directly suppress HIV-1 replication,^{39,40} but we did not find an increase in the V75I resistance mutations that could have been

interpreted as *in vivo* evidence supporting such a direct anti-HIV effect.⁴¹ However, we have previously reported that acyclovir provided to the HIV-1-infected partners in this cohort reduced plasma HIV-1 levels by 0.25 log₁₀ copies/ml,¹⁷ and genital HIV-1 levels by 0.3 log₁₀ copies/ml,⁴² when compared to placebo. Plasma and genital HIV-1 levels are correlated in this cohort ($r = 0.56$),⁴² and the modestly higher reduction in genital compared to plasma HIV-1 levels by acyclovir may have mediated our finding that acyclovir reduced seroconverter set point, after controlling for plasma HIV-1 RNA level of the source partner.

Taken together, these data support the idea that male circumcision, BV, and acyclovir use may influence genital tract HIV-1 inoculum. The mechanism for how the source partner's inoculum might modify seroconverter set point is not completely clear, particularly given recent studies suggesting that most transmission events involve a single virus,⁴³ and those with transmission of multiple variants are not associated with significantly increased plasma HIV-1 set point.⁴⁴ In this regard it is intriguing that prior studies have found a relationship between plasma HIV-1 levels in the source partner and levels of activated T cells in peripheral blood of exposed partners.⁴⁵ Given recent nonhuman primate studies underscoring the importance of levels of HIV-1 target cells (such as activated CD4⁺ T cells) in the genital mucosa for early HIV-1 replication,⁴⁶ we speculate that the source partner virus inoculum may elicit increased levels of activated T cells in the genital mucosa of exposed partners, thus leading to increased virus replication when a transmitted virus actually begins replicating. Although protective adaptive immune responses elicited through recurrent exposure to the HIV-1-infected partner's virus may modify this effect, consistent evidence of such protective responses has been elusive.^{47,48} We may also speculate that our finding of infected partner plasma HIV-1 level in unlinked partners having a trend to association with seroconverter's set point could be another manifestation of HIV-1 exposure inducing host immune activation in the genital tract of HIV-1-exposed partners even if virus to which the seroconverter was previously exposed from their enrolled partner is genetically distinguishable from the acquired virus. Alternative explanations for this trend in the not linked prevalent HIV-1-infected partner include that the association is by chance, that there are unmeasured factors (such as dietary, stress-related, and infectious diseases) shared between individuals in a partnership that influence HIV-1 set point, or that some seroconversions classified as not linked were actually linked but the linked viruses were present at a level not detectable by either single genome amplification or pyrosequencing analysis.¹⁹ Our linkage classifications were supported by epidemiologic correlates showing that seroconverting partners whose viruses were not linked to their enrolled partners had a higher frequency of sex with nonenrolled partners compared to those whose viruses were linked.¹⁹ Nevertheless, the possibility of some level of misclassification bias cannot be completely eliminated. Further study, particularly of the characteristics of host responses to HIV-1 exposure in the genital mucosal, is needed to better evaluate these speculations.

In contrast to other reports, we did not find that age,^{13,49} GUD,^{21,50} hormonal contraceptive use,²¹ or gender^{49,50} was associated with HIV-1 set point in the seroconverting partner. Associations of set point with age and gender have been

inconsistent across previous studies, so our findings may reflect cohort differences or the impact of controlling for other factors in the analysis (e.g., plasma HIV-1 level of the infected partner). Furthermore, GUD and hormonal contraceptive use were not prevalent among seroconverters in our study (9% and 16%, respectively), so our statistical power was limited to detect influences of these variables on HIV-1 set point. We also did not find any influence of viral subtype on set point.

In conclusion, our findings support the association between plasma HIV-1 levels of source partners and HIV-1 set point in seroconverting partners. However, we also found that factors related to the genital tract of the source partner also modify seroconverter set point independent of plasma HIV-1 levels. We hypothesize that this may be mediated through changes in the HIV-1 inoculum from the source partner's genital tract.

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