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Partner HIV Serostatus Impacts Viral Load, Genital HIV Shedding, and Immune Activation in HIV-Infected Individuals

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Abstract: Studies of seronegative individuals in HIV discordant relationships provide important insights into the effects of HIV exposure on the seronegative partner, but few have examined the impact of partner serostatus on disease progression in seropositive individuals. We investigated the impact of HIV serostatus on clinical and biological factors influencing HIV disease progression in 337 HIV-infected heterosexual individuals in stable long-term HIV-seroconcordant or HIV-serodiscordant relationships. Seroconcordant individuals had significantly higher plasma viral loads (pVLs) than HIV-infected partners in serodiscordant partnerships [4.4 $\log_{10}$ copies RNA/mL (interquartile range 3.7–5.0) versus 3.9 (3.3–4.5), $P < 0.0001$], irrespective of gender. pVLs correlated inversely with CD4$^+$ T-cell counts, although CD4 counts did not differ significantly between seroconcordant and serodiscordant individuals. HIV+ seroconcordant individuals had higher frequencies of CCR5$^+$ CD4 and CD8 T cells ($P = 0.03$ and $P = 0.02$, respectively) than HIV+ individuals in serodiscordant relationships and higher concentrations of plasma IL-1β ($P = 0.04$), TNF-α ($P = 0.02$), and IL-10 ($P = 0.02$). Activated CD4$^+$ T-cell frequencies and TNF-α were the most influential in determining variation in pVLs, independently of CD4 counts. In addition, HIV+ seroconcordant women had significantly higher genital VLs (gVLs) than HIV+ women in serodiscordant relationships ($P < 0.001$), with pVLs correlating significantly with gVLs (Rho = 0.65, $P < 0.0001$). Cervical and blood T-cell activation tended to correlate positively, although partner seroconcordance did not influence genital T-cell activation. We conclude that HIV+ seroconcordant individuals have higher frequencies of activated, CCR5-expressing T cells in blood and higher pVLs and gVLs than their HIV+ counterparts in discordant relationships, which could translate to faster disease progression or larger viral reservoir.

Key Words: HIV, serodiscordance, viral load, genital, immune activation, cytokines, couples

INTRODUCTION

New HIV infections continue to be transmitted between stable couples, especially in countries with both mature

epidemics and limited antiretroviral therapy (ART) uptake.[1,2] Studies in HIV discordant couples have provided insight into factors that influence risk of HIV acquisition.[3–5] The risk of HIV transmission at an individual level is dependent both on the infectiousness of the HIV donor and on the susceptibility of the uninfected recipient.[6] This depends on viral titers within the secretions of the HIV donor, the frequency and nature of exposure, and the existence of cofactors such as genital inflammation and sexually transmitted infections (STIs) that enhance HIV transmission.[7–13] Plasma viral loads (pVLs) strongly predict the amount of HIV that is shed in genital secretions, although other local mucosal factors are likely also important.[14–17]

Properties of the transmitted founder virus may influence VLs in infected recipients,[18] particularly in recipients with the same human leukocyte antigen (HLA) background as the donor.[19] Selective mutations in HIV proteins that allow escape from cytotoxic T-lymphocyte activity, which allow HIV to replicate despite cytotoxic T-lymphocyte pressure but may come at a cost to viral fitness reflected in lower VLs, can be transferred between donor–recipient pairs if the HLA background for the mutation is conserved in the newly infected recipient.[20–23]

Several studies have demonstrated that systemic T-cell activation predicts worse disease course in HIV-infected individuals.[24–28] A network of cytokines affects virtually every step in the life cycle of HIV, from entry into new cells to the budding of a new progeny.[29] These cytokines are also believed to be responsible for localized and systemic inflammatory responses, including the recruitment of HIV target cells to points of infection.[30]

HIV-infected individuals in serodiscordant relationships may be exposed to viral variants from their HIV-infected partners,[31–34] which may influence their rate of disease progression: including their level of viraemia, CD4 T cell counts, and immune activation. To investigate the impact of partner HIV status on HIV disease progression in a large cohort of heterosexual HIV-concordant (both partners are HIV infected) or discordant (one partner is infected, whereas the other is not) black South African couples, in stable long-term relationships,[35,36] we investigated the pVLs, systemic and genital immune cell activation, and cytokine profiles and correlated this to the infectiousness of HIV-infected individuals to their partners by evaluating HIV shedding in genital secretions.

METHODS AND MATERIALS

Cohort Description

A total of 195 HIV-infected individuals in stable HIV-concordant relationships and 142 HIV-infected individuals in HIV-serodiscordant relationships, from Cape Town, South Africa, were enrolled at the Empilisweni Clinic in Gugulethu, between 2006 and 2010.[35,36] All individuals were ART-naive at the time of study. They generally attended the clinic as couples, although interviews regarding demography and sexual behaviors were conducted individually with each participant. A history of symptomatic STIs, genital discharge, ulceration, and genital warts were obtained during clinical examination. Women who were menstruating at the time of sampling, who were postmenopausal, or had undergone a hysterectomy were excluded from the study, and women with clinical symptoms of STIs (such as visible ulcers or vaginal discharge) were not included in the study. Absolute blood CD4 T-cell counts and HIV VLs were measured for each HIV+ individual at enrolment into the study (baseline), at months 12 and 24. The study was approved by the Research Ethics Committee of the University of Cape Town (UCT REC# 258/2006), and informed written consent was obtained from all individuals.

Sample Collection and Processing

Whole blood (~16 mL) was collected from each study participant by venipuncture using ACD vacutainer tubes (BD Biosciences, Plymouth, United Kingdom). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque (Sigma-Aldrich, St Louis, MO) density gradient centrifugation using Leucosep tubes and cryopreserved in liquid nitrogen. Plasma for VL testing and cytokine measurements was stored at −80°C.

In addition, cervical cytobrushes for genital cytokine measurements were collected from all female study participants under speculum examination, as previously described.[36] Of these, a cervical cytobrush-derived T-cell activation was measured in a subset of 18 HIV+ women by flow cytometry (of which, 13/18 were in serodiscordant relationships and 5/18 were in seroconcordant relationships). Cervical samples that had visible red blood cell contamination were discarded. Cervical samples were processed within 4 hours of collection for flow cytometry. The cervical supernatant fraction was stored at −80°C for cytokine and genital VL (gVL) measurement.

Determination of HIV VLs in Plasma and Genital Secretions

VLs were measured in plasma and genital secretions using NucliSENS EasyQ HIV-1 Version 1.2 (National Health Laboratory Services Diagnostic Virology Laboratory, Groote Schuur Hospital, Cape Town, South Africa). The detection limit of the assay was 50 HIV RNA copies/mL. Participants with blood plasma ≤50 HIV RNA copies/mL were considered to be aviremic.

Measuring Immune Activation by Flow Cytometry

We used an 8-colour fow ctometry panel to measure cellular activation and proliferation marker expression (CD38, HLA-DR, and Ki67) on CCR5+ CD4+ and CD8+ T cells, as previously described by Jaspan et al.[37] Briefly, PBMCs (1×10^6) or cervical mononuclear cells (CMCs) were incubated with LIVE/DEAD Fixable Violet Dead Cell Stain (Invitrogen, Carlsbad, CA) for 20 minutes at room temperature (RT) and then washed with 1% fetal calf serum phosphate buffered saline. The pelleted cells were resuspended and stained for 30 minutes at RT with

CD4-PerCP-Cy5.5 [Becton-Dickinson (BD), San Jose, CA], CD8-QDot605 (Invitrogen), CCR5-APC (BD), CD38-PE-Cy7 (E-Biosciences, San Jose, CA), HLA-DR-PE (BD), and CD14-PacBlue (dump marker; BD) and CD19-PacBlue (dump marker; Invitrogen, San Jose, CA). Cells were washed twice with 1% fetal calf serum phosphate buffered saline, centrifuged for 5 minutes at 1500 rpm at RT, fixed and permeabilized with BD CytoPerm/CytoFix for 20 minutes at RT. Cells were then washed with Perm/Wash buffer (BD Biosciences, San Jose, CA) for 5 minutes at 1500 rpm, RT. CD3-APC-H7 (BD Biosciences) and Ki67-FITC (BD Biosciences) were then stained intracellularly. Cells were washed in 2 mL of BD Perm/Wash buffer by centrifugation at 1500 rpm at RT for 5 minutes and fixed with BD Cell Fix (Becton-Dickinson). A BD Fortessa (BD Immunocytometry Systems, San Jose, CA) flow cytometry was used to capture events, and fluorescence minus one was used to distinguish continuous population. Data were analyzed using FlowJo (Tree Star, Ashland, OR). Figure 1, Supplemental Digital Content, http://links.lww.com/QAI/B337 summarizes the gating strategy.

Measurement of Cytokines

High Sensitivity LINCOplex Premixed kits were used to measure the concentrations of interleukin (IL)-1β, IL-6, IL-12p70, tumor necrosis factor (TNF)-α, IL-10, IL-2, interferon (IFN)-γ, IL-7, and granulocyte–macrophage colony-stimulating factor in plasma (LINCO Research, Saint Charles, MO), with sensitivity ranging between 0.01 and 0.48 pg/mL. In cytobrush-collected genital secretions, we used a premixed LINCOplex kit to evaluate IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-12p40, IL-15, IP-10, MCP-1, MIP-1α, MIP-1β, G-CSF, eotaxin, and fractalkine concentrations. Data were collected using a Bio-Plex Suspension Array Reader (Bio-Rad Laboratories, Inc., Hercules, CA), and a 5 PL regression formula was used to calculate cytokine concentrations from the standard curves. Cytokine concentrations below the detection limit were reported as the midpoint between the lowest concentration measured for each cytokine and zero (Masson et al).

Statistical Analysis

Shapiro–Wilk tests for normality were performed to determine the distribution of variables within the data set. Comparisons of unpaired data that did not follow normal distribution were performed using Mann–Whitney U tests. Spearman rank tests were used to test for correlations for data that did not follow normal distribution. Statistical inferences on binary sets of data were performed using the Fisher exact test, and odds ratios (ORs) were calculated. Statistical analyses were performed using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, CA). All tests were 2-tailed, and P values of ≤0.05 were considered significant. Multivariate analyses were performed using STATA (StataCorp, College Station, TX). A multivariate regression model was used to determine the variables that best predicted either VL or CD4 count, as previously

described.[38,39] Predicted gVLs were calculated for each study participant using the standardized β-coefficients of each of genital cytokines included in a regression model for VL, including CD4 counts as a confounder variable.

RESULTS

A total of 337 HIV+ black South African individuals in seroconcordant and discordant relationships with stable long-term heterosexual partnerships were included in this study, of which 62% of discordant couples and 70% of concordant couples were cohabiting with their sexual partners (Table 1). According to South African guidelines at the time of enrollment, all HIV+ individuals were naïve to ARTs at the time the study was conducted (2006–2010). HIV+ serodiscordant individuals reported higher frequencies of condom use in the previous month than did HIV-concordant individuals (75% versus 62%; $P = 0.01$). All women reported being in a monogamous relationship with their sexual partners.

Impact of Partner HIV Status on pVLs and CD4 Counts

At enrollment, individuals in HIV+ concordant relationships had 0.5 log$_{10}$ higher pVLs (14,700 HIV RNA copies/mL) than HIV+ individuals in discordant relationships, with a median of 4.4 log$_{10}$ copies RNA/mL [interquartile range (IQR) 3.7–5.0] for individuals in concordant relationships versus 3.9 log$_{10}$ copies RNA/mL (IQR 3.3–4.5) for HIV+ individuals in discordant relationships (Fig. 1A; $P < 0.0001$). The difference in viremia observed between HIV+ individuals in concordant relationships and those in discordant relationships persisted over time: with a median pVL at 12 and 24 months of follow-up of 4.3 log$_{10}$ copies RNA/mL (IQR 3.7–5.0) and 4.4 log$_{10}$ copies RNA/mL (IQR 3.4–5.1) for individuals in concordant

TABLE 1. Clinical and Sociobehavioural Characteristics of Couples' Cohort

Characteristic	n	HIV Concordant	n	HIV Discordant	P
n		195		142	
Female (%)†		52 (27)		77 (54.2)	<0.0001
Age [yr; median (IQR)]*	195	33 (28–40)	139	34 (28–40)	1.0
Living together with partner (%)†	194	70 (36)	140	62 (43.7)	0.2
Sexual exposure [median years of sex (IQR)]*	194	16 (10.8–22.3)	139	16 (11–24)	0.9
Sex acts in the past month [median (IQR)]*	190	4 (2–10)	131	4 (2–8)	0.4
Condom usage (%)†	191	62 (32.4)	137	75 (52.8)	0.01
Genital ulceration in the past 6 months (%)†	195	13	139	12	0.7
Vaginal discharge in the past 6 months (%)†	195	30	139	29	1.0

*P value calculated using the Mann–Whitney U test.
†P value calculated using the Fischer exact test.
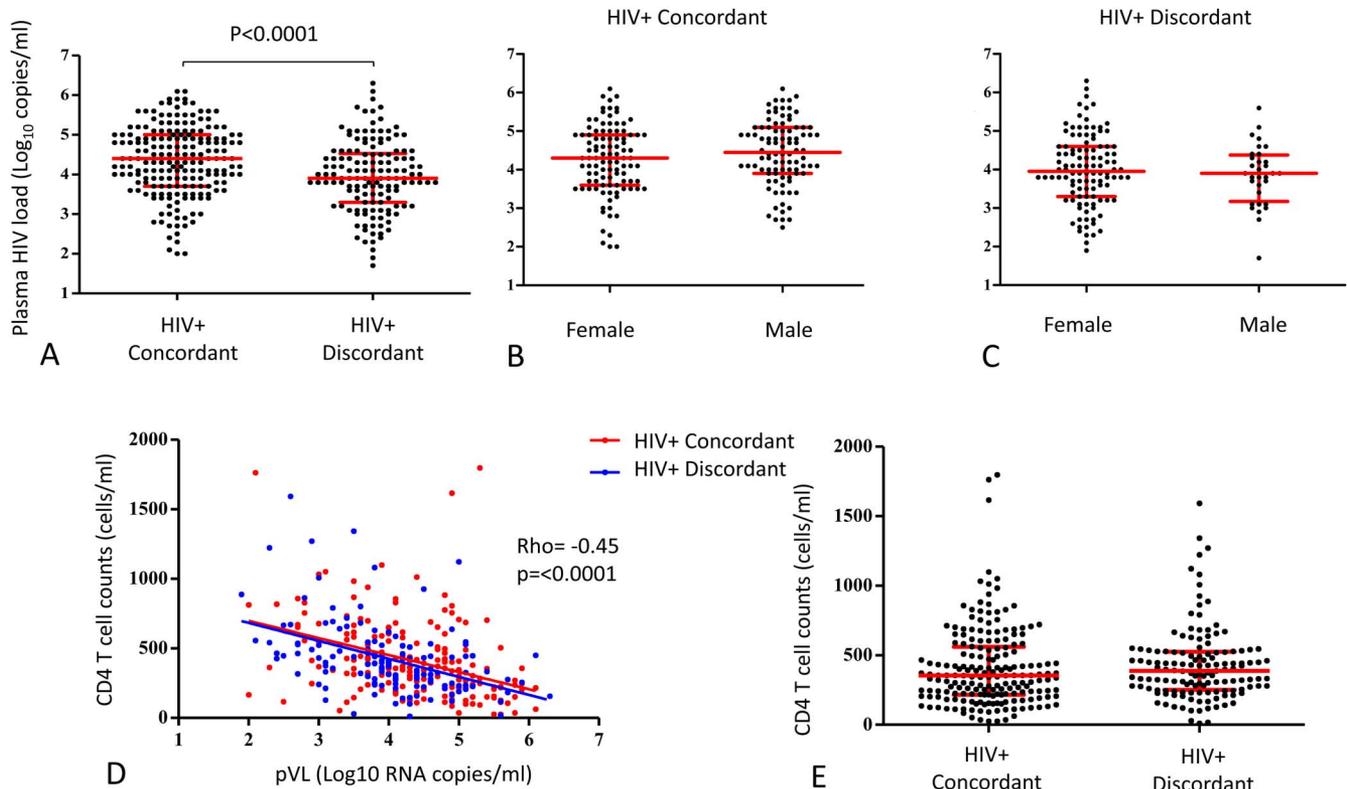
FIGURE 1. Relationship between pVLs and CD4 counts, according to partner HIV status. A, Each data point represents an individual's pVL. The red line indicates the median VL and associated IQR for each group. B and C, Difference in median VLs between women and men in HIV concordant (101 women; 94 men) or discordant relationships (109 women; 33 men), respectively. D, Regression analysis of CD4 decline relative to pVLs in HIV+ concordant (red dots) and discordant (blue dots) individuals. The Spearman ranks test was used to evaluate the relationship. E, Comparison of CD4 counts in HIV-concordant and HIV-discordant individuals. Each data point represents an individual's pVL. The red line indicates the median VL and associated IQR for each group. The Mann–Whitney *U* test was used to compare the pVLs and CD4 T cell counts between HIV-concordant and HIV-discordant individuals. *P* values <0.05 were considered significant.

relationships versus 3.9 $\log_{10}$ copies RNA/mL (IQR 3.3–4.5) and 4.0 $\log_{10}$ copies RNA/mL (IQR 3.3–4.6) for HIV+ individuals in discordant relationships, respectively. Significantly more HIV+ individuals in concordant relationships had pVLs >1500 copies/mL (89/144) than those in discordant relationships (58/118; *P* = 0.003), a threshold of viremia below which no evidence of HIV transmission within couples was noted.[4] There were no differences in VLs by sex, with HIV+ women and men having similar pVLs, irrespective of their partner's HIV status (Figs. 1B, C). Plasma VLs of matched partners within HIV+ concordant relationships did not correlate (Rho = 0.06; *P* = 0.6; data not shown).

pVLs correlated significantly with absolute CD4 T-cell counts (Fig. 1D; Rho = −0.45, *P* < 0.0001), although CD4 counts of HIV+ individuals in concordant relationships were not significantly different to those of HIV+ individuals in discordant relationships (Fig. 1E). No differences in CD4 counts were noted at 12 or 24 months of follow-up either (data not shown). In 83 HIV+ individuals for whom longitudinal data were available, 63.9% (53/83) experienced a decrease in CD4 count from baseline to month 12, corresponding with an increase in pVL. In a univariate analysis, increased pVL

accounted for 35% of the CD4 T-cell depletion in blood (*P* < 0.05; data not shown). Furthermore, a high CD4 absolute count in this group of HIV+ individuals at baseline was highly predictive of the eventual CD4 decrease over the 1-year period (Rho = 0.77, *P* < 0.0001), regardless of partner HIV status. Sensitivity analyses adjusting for gender, sexual practices, and clinical manifestations (ulceration and genital discharge) did not improve the model (data not shown). These analyses suggest that HIV+ individuals who experienced the greatest CD4 decline over the first year of study were those who started with the highest CD4 counts at baseline; that VL was a major contributor to this decline. This analysis also suggests that a possible contributor to the discrepancy between (1) the correlation between pVL and CD4 counts generally; (2) higher VL in concordant versus discordant individuals; but (3) no difference in CD4 T-cells counts at the same time point or longitudinally, may be that individuals enrolled in this study already had relatively low CD4 counts at baseline (354 cells/mL for concordant versus 387 cells/mL for discordant at baseline). Because pVL only explained 35% of the T-cell depletion in this cohort, it may alternatively be other factors that are known to contribute to T-cell decline, including cellular activation.

Impact of Partner HIV Status on T-Cell Activation

To investigate the impact of partner status on T-cell activation in HIV+ individuals, the frequency of CD4$^+$ and CD8$^+$ T cells expressing activation markers CD38, HLA-DR, CCR5, and proliferation marker Ki67 was compared. HIV+ individuals in HIV-seroconcordant relationship had higher frequencies of CD4$^+$CCR5$^+$ T cells and CD8$^+$CCR5$^+$ T cells than those in serodiscordant relationships (Figs. 2A, B, respectively). Although expression of activation markers CD38 and HLA-DR and proliferation marker Ki67 by CD4$^+$ T cells did not differ between groups, HIV-seroconcordant individuals had lower frequencies of activated CD8$^+$ T cells than their serodiscordant counterparts (CD38, P = 0.03; HLA-DR, P = 0.05; Fig. 2B).

HIV+ individuals with pVLs >1500 cps/mL had significantly higher frequencies of CCR5$^+$CD4$^+$ T cells than those with pVLs <1500 cps/mL (P = 0.007), although frequencies of CCR5$^+$CD8$^+$ T cells did not differ by the HIV exposure group (Figs. 2C, D). Significantly higher frequencies of CD8$^+$ T cells from these individuals with pVLs >1500 copies/mL expressed CD38 and CD38 together with HLA-DR (P = 0.01 for both; Fig. 2B) compared with those with pVLs <1500. By contrast, no difference was noted in frequencies of CD4$^+$ T cells expressing CD38 or HLA-DR between pVL categories.

To determine the relationship between CD4$^+$ T cell counts and T cell activation, a univariate quantile regression model was used with CD4 counts as the independent variable. Irrespective of partner HIV status, expression of HLA-DR was associated with lower CD4$^+$ T-cell counts, after adjusting for multiple comparisons and pVLs (data not shown).

**TABLE 2.** Plasma Cytokine Concentrations According to Partner HIV Status

Cytokine	HIV+ Concordant Partnership	HIV+ Discordant Partnership	Unadjusted P
IL-1b	0.72 (0.01–2.85)	0.38 (0.04–1.47)	0.04*
IL-6	5.26 (2.83–11.1)	4.48 (2.57–8.62)	0.2
IL-12p70	0.01 (0.01–0.42)	0.01 (0.01–0.01)	0.5
TNF-a	9.45 (5.61–15.94)	7.64 (4.94–13.07)	0.03*
IL-10	12.6 (7.75–23.44)	10.53 (6.47–15.74)	0.02*
IL-2	0.11 (0.01–0.7)	0.14 (0.01–0.87)	0.6
IFN-g	1.39 (0.27–6.33)	1.09 (0.14–2.8)	0.05
IL-7	1.58 (0.61–3.09)	1.45 (0.72–3.25)	0.9
GM-CSF	0.19 (0.01–0.64)	0.3 (0.01–0.86)	0.1

*Mann–Whitney U tests were applied to compare cytokine concentrations between the concordant and discordant groups. P-values <0.05 were considered significant.
GM-CSF, granulocyte–macrophage colony-stimulating factor.

Relationship Between Plasma Cytokines and HIV Disease Progression

The concentrations of plasma cytokines tended to be higher in HIV+ seroconcordant individuals compared with HIV+ individuals in serodiscordant relationships (IL-1β: median 0.72 versus 0.38 pg/mL; TNF-α: 9.45 versus 7.64 pg/mL; and IL-10: 12.60 versus 10.53 pg/mL), although none of these differences remained significant after adjusting for multiple comparisons (Table 2). Furthermore, TNF-α was positively associated with pVLs in a quantile regression analysis (with pVLs as the predictor variable) in HIV+ individuals in seroconcordant relationships, after adjustments for multiple comparisons, with a β-coefficient of 3.8. A similar relationship between TNF-α and pVLs

FIGURE 2. Impact of partner HIV status and pVLs on T-cell activation in HIV+ individuals with seroconcordant or serodiscordant partners. A and B, Frequencies of activation (CD38, HLA-DR, and CCR5) and proliferation marker (Ki67) expression on CD4$^+$ and CD8$^+$ T cells in HIV+ individuals with concordant (red boxes) or discordant (blue boxes) partners. C and D, Frequencies of activation and proliferation marker expression on CD4$^+$ and CD8$^+$ T cells in HIV+ individuals with pVLs <1500 cps/mL (pink boxes) and >1500 cps/mL (orange boxes). The percentage of activated T cells in each group of individuals is depicted by box-and-whisker plots indicating the median (middle line), 25th (bottom line) and 75th percentiles (top line), and the range (whiskers) of the frequencies of T cells expressing the respective activation markers. Mann–Whitney U tests were applied to compare blood T cell activation frequencies between the 2 groups.
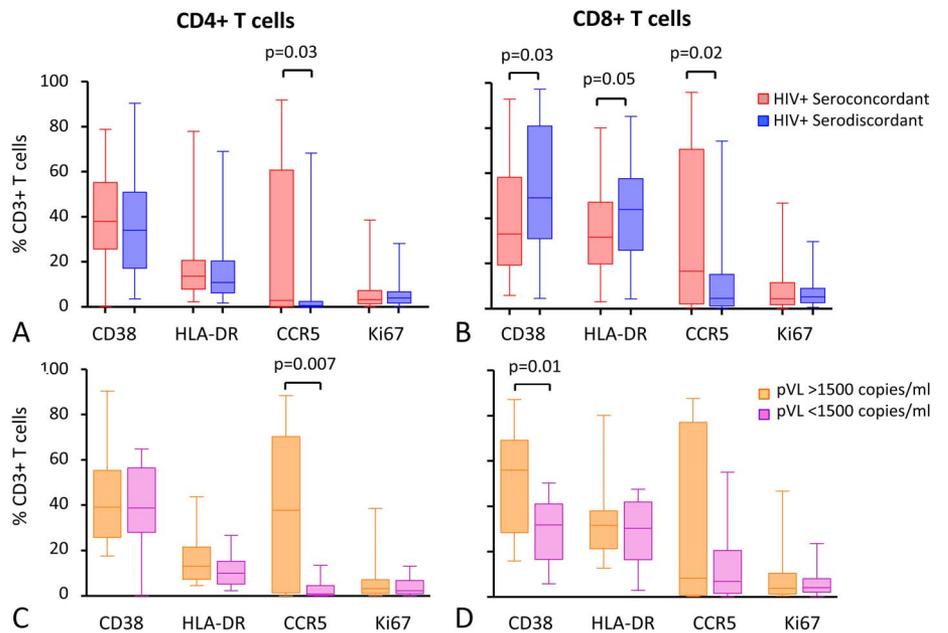
was not found in HIV+ individuals in serodiscordant relationships, who tended to have lower cytokine concentrations generally. None of the plasma cytokines that were measured correlated with CD4$^+$ T-cell counts (data not shown).

Multivariate linear regression was used to evaluate the most important factor/s influencing pVL. The linear regression model included pVL as the dependent variable and the predictor variables that were identified to influence plasma viremia, including T-cell activation (DR/CD38$^+$ CD4$^+$ T cells) and plasma cytokines. pVLs were most strongly associated with plasma TNF-α concentrations {β-coefficient 2.5 [95% confidence interval (CI) 1.06 to 3.95]; $P = 0.001$} and DR/CD38 expression by CD4$^+$ T cells [β-coefficient 1.45 (CI: 95% 0.21 to 2.69); $P = 0.023$].

## Impact of Partner HIV Status on Genital T-Cell Activation and HIV Shedding

Fifty-eight HIV+ women provided genital samples from which gVLs were measured, of which 37 (64%) had detectable HIV RNA. gVLs were positively associated with pVLs (Fig. 3A; Rho = 0.65, $P < 0.0001$). HIV+ women in seroconcordant relationships had 1.6 log$_{10}$ higher gVLs in their genital secretions than women in serodiscordant rela-

tionships (corresponding to a difference of 1450 cps/mL; $P < 0.001$; Fig. 3B).

The concentrations of IL-1α, IL-β, IL-6, IL-8, IL-12p40, IL-10, eotaxin, fractalkine, IP-10, MCP-1, MIP-1α, MIP-1β, IL-15, and G-CSF were measured in genital tract secretions from these HIV+ women (see Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B337). IP-10, IL-6, and G-CSF concentrations tended to be higher in HIV+ women in seroconcordant relationships than those in serodiscordant relationships, although this was not significant. Genital tract concentrations of IL-1α and IL-1β (irrespective of partner HIV status) were positively associated with gVLs, before (Fig. 3C) and after adjusting for pVLs (Fig. 3D; IL-1α: β-coefficient 0.68 for seroconcordant and 0.50 for serodiscordant women; IL-1β: β-coefficient 0.50 for seroconcordant and 0.52 for serodiscordant women). In addition, levels of IL-8 (β-coefficient 0.67) and IL-10 (β-coefficient 0.51) were positively associated with gVLs in HIV+ serodiscordant women, independently of plasma HIV load (Fig. 3D).

Because pVL and markers of genital tract inflammation (including IL-1α, IL-1β, IL-8, and IL-10) predicted the detectability of HIV in genital secretions, the relative predictive value of each of these factors was compared. Although genital IL-1β concentrations positively predicted gVLs [OR 1.4 (95% CI: 1.01 to 1.93); $P = 0.04$], no biological parameter

FIGURE 3. Relationship between VLs in plasma and genital secretions of HIV+ women, and the role of local inflammation. A, Correlation between pVLs and gVLs in HIV+ women. Each data point represents an individual woman's plasma and cervical VL. The Spearman rank test was used to test the association between HIV load in plasma and cervix. B, Comparison of cervical VLs in HIV-concordant and HIV-discordant women. Each data point represents an individual's cervical VL. The red line indicates the median VL and associated IQR for each group. The Mann––Whitney U test was used to compare the pVL between HIV-concordant and HIV-discordant individuals. A P value <0.05 was considered significant. C and D, The association between gVLs and genital cytokine concentrations in HIV+ seroconcordant or HIV+ serodiscordant individuals, by linear regression analyses. C, Association before and (D) after adjusting for plasma HIV load. Regression β-coefficients depicted in red circles represent significant associations (P values ≤0.05). Bars represent standard error. All P values were adjusted for multiple comparisons using the false discovery rate step-down approach. β-coefficients are interpreted as the amount by which cervical VL (HIV RNA copies/mL) increases with every percentage increase in cytokine concentration.
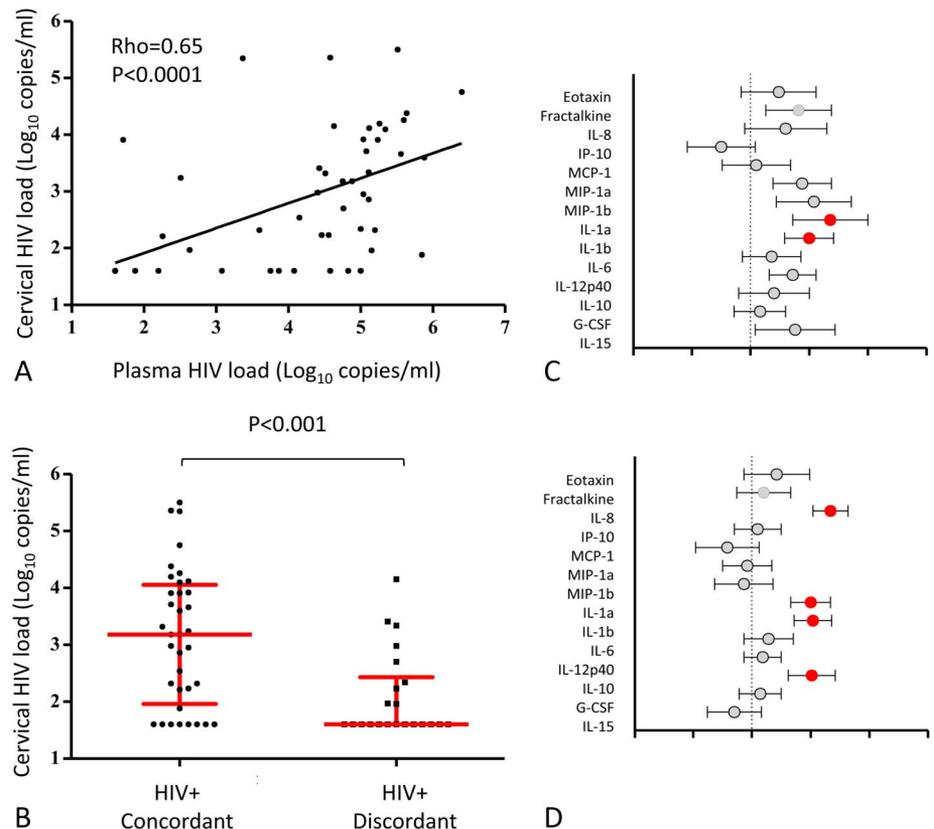
predicted HIV genital shedding as strongly as pVLs [OR 5.8 (95% CI: 1.6 to 20.5); $P = 0.007$].

Cervical T-cell activation data were only available for a subset of 18/58 HIV+ women, of which 13/18 HIV+ women in serodiscordant relationships (72.2%) and 5/18 (27.8%) with HIV+ male partners. For these women, frequencies of T cells expressing activation and proliferation markers were compared in matched CMCs and PBMCs. As observed in blood, frequencies of CCR5-expressing cervical CD4+ T cells (and CD8+ to a lesser extent) tended to be higher in HIV+ CMCs from women in concordant relationships compared with those in HIV-discordant relationships, although this was not significant (Fig. 4). Although frequencies of blood CD38, HLA-DR, and Ki67-expressing CD4 and CD8+ T cells were generally predictive of these populations in the cervix (Fig. 4B),[37] this was not observed for activated CCR5-expressing cells in this subset of HIV+ women.

DISCUSSION

We hypothesized that being in an HIV-seroconcordant stable relationship would influence an HIV-infected individuals' disease course and that prolonged exposure to HIV from an HIV-concordant partner would result in elevated immune activation and more rapid CD4 decline. In a large cohort of black South African heterosexual couples, in which 1 or both partners were HIV+, we show that HIV+ concordant individuals had a 0.5 $\log_{10}$ higher pVL than their HIV+ counterparts in discordant partnerships. Although pVLs were negatively associated with CD4 counts, this 0.5 $\log_{10}$ difference in viremia between groups did not predict faster

CD4 cell decline over a 2-year period of follow-up. Other studies of HIV discordant couples have reported that although HIV+ concordant individuals had an average of 0.34 log higher pVLs than those in HIV-discordant relationships (representing a difference of 71,422 RNA copies/mL), differences in pVLs were associated with greater CD4 decline in the individuals in concordant relationships than in those in discordant relationships.[40] The low starting CD4 counts in HIV+ individuals in this study may have confounded the effects of pVL differences in HIV+ concordant and discordant individuals, although pVL only explained 35% of the variability in CD4 counts in these individuals, who were likely therefore to have other factors playing a bigger role.

Henrard et al[41] showed that clinical HIV disease progression was preceded by an increase in HIV RNA in plasma in untreated individuals. Although CD4 counts have been used as prognostic markers of disease progression,[42,43] CD4 counts alone may not be an accurate surrogate for virological outcome in plasma. Previous studies have confirmed that the variability in CD4 counts does not correlate well with pVL variability,[44] with the study by Rodriguez et al[45] showing that only 4%–6% of the variability in CD4 numbers being explained by pVL. In our cohort, pVLs explained 35% of the variability in CD4 numbers, leading us to focus on investigating other potential determinants influencing CD4 decay, such as systemic inflammation and cellular activation.

The WHO and South African National guidelines now recommend ART for all HIV-infected individuals with CD4 counts <500 cells/mL. UNAIDS aims to ensure that 90% of HIV+ individuals know their status, 90% are initiated on

FIGURE 4. Impact of partner HIV status on cervical T-cell activation in HIV+ women. A, Frequencies of CCR5, CD38, HLA-DR, and Ki67 expression by cervical CD4+ (top) and CD8+ (bottom) T cells in HIV+ individuals with HIV+ (seroconcordant, red boxes, n = 5) or HIV− partners (serodiscordant, blue boxes, n = 13). Box-and-whisker plots indicate the median (middle line), 25th (bottom line) and 75th percentiles (top line), and the range (whiskers) of the frequencies of T cells expressing the respective activation markers. Mann–-Whitney-*U* tests were applied to compare cervical T-cell activation frequencies. B, The association between blood and genital tract CD4+ (top panel) and CD8+ (bottom panel) T cells expressing immune activation markers CCR5, CD38, HLA-DR, Ki67, or their combination was assessed in HIV+ women (n = 18) by univariate quantile regression models. Regression β-coefficients depicted as grey circles represent significant associations (*P* values ≤0.05). Statistical significance, after adjustment for multiple comparisons, is denoted by *.
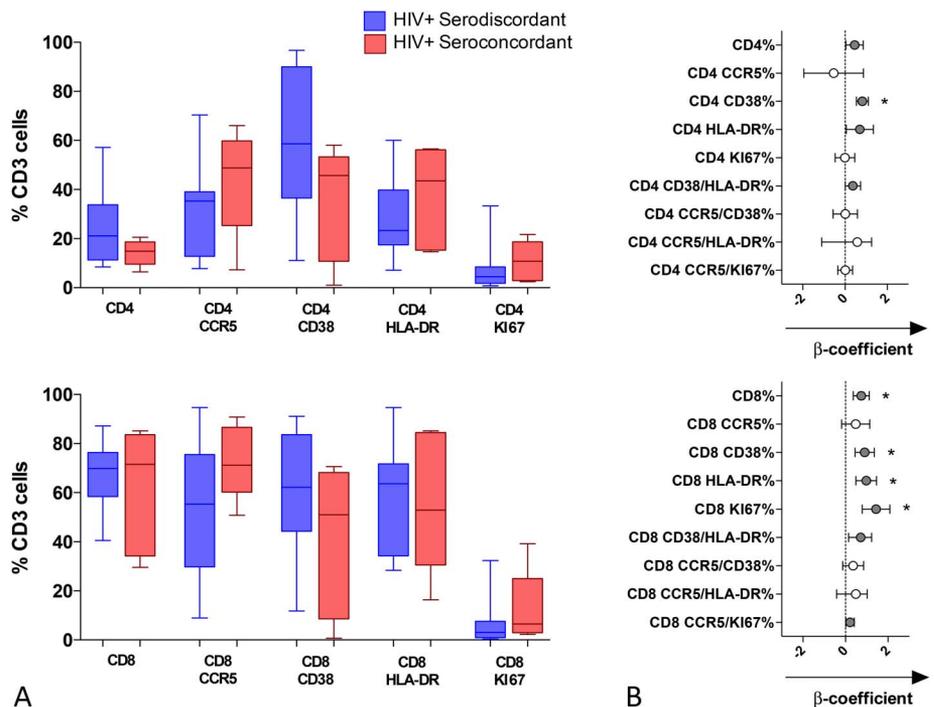
ART, and 90% are virally suppressed by 2020 (90-90-90).[46] Despite these targets and guidelines, in reality, many countries globally and within Africa are falling below these targets and a significant proportion of those infected are not accessing treatment. Untreated HIV infection is characterized by chronic immune activation, leading to accelerated T-cell apoptosis,[47] contributing to CD4 decline,[48] and strongly predicts disease progression.[26] The higher expression of CCR5 on T cells from HIV+ individuals in seroconcordant relationships than on those from individuals in discordant relationships in this cohort provides insights into the mechanism accounting for differences in pVLs between groups. The relationship between HIV susceptibility and immune activation is also reciprocal because binding of HIV to CCR5 receptors on the surface of $CD4^+$ T cells is also known to activate these cells. The HIV Env-mediated signaling pathway through which this activation occurs promotes the expression of CD38/DR and cytokine release, which in turn activate bystander T cells.[49] When HIV+ individuals were categorized according to their pVLs, those with pVLs >1500 cps/mL had higher frequencies of T cells expressing CCR5 than did those with pVLs <1500 copies/mL, suggesting that the increased plasma viraemia in concordant HIV-infected partners has clinical implications.

We questioned whether viral characteristics shared by HIV+ partners might contribute to the maintenance of higher pVLs but found no association between pVLs in matched partners in HIV+ concordant couples. Coinfection of individuals with multiple genetically divergent HIV-1M variants can be an important factor influencing viral set point and the rate of HIV disease progression.[34,50–52] We were not able to determine donor/recipient pairs in the HIV+ concordant couples that would allow us to confirm whether these couples had infected each other, although HIV superinfection resulting from intracouple transmission has previously been demonstrated and should be considered.[52,53] Host genetic characteristics also influence disease course,[54] even if partners share the same virus.

pVLs are considered one of the best predictors of both the amount of HIV being shed in genital secretions[15,55–58] and the risk of HIV transmission to new partners.[59,60] pVLs were found to be the strongest predictor of genital tract shedding of HIV in HIV+ women in this study. Furthermore, HIV+ women in seroconcordant relationships, who had significantly higher pVLs than HIV+ women in serodiscordant relationships, were also shedding more HIV in their genital secretions than women in serodiscordant relationships (with a median difference of 1450 copies/mL).

Local factors, such as genital inflammation, are likely to be important in determining the extent of HIV-infected target cell homing to the mucosa, possibly independently of pVLs, thereby increasing local shedding of HIV.[61] Although plasma inflammatory cytokines were elevated in HIV+ individuals in seroconcordant relationships relative to those in serodiscordant relationships, no significant differences were evident in the genital inflammatory profile between groups. Although the frequency of cervical cytobrush-derived $CD4^+$ T cells expressing CCR5 tended to be higher in seroconcordant women than serodiscordant women (as was observed in blood), these differences were not significant; only a small subset of women provided cytobrushes for this analysis.

Some other limitations need to be acknowledged. HIV+ individuals recruited into this study were enrolled from the general community in Cape Town and were selected based on HIV status at enrollment. As such, we did not have information about the length of time that individuals had been infected for, before enrollment. Furthermore, we acknowledge that the timeframe over which sampling was conducted for this study (including 24 months of follow-up) likely limited our ability to assess disease progression. We focused on investigating T-cell activation and cytokine fluctuations related to pVLs (all HIV+ individuals) and genital HIV shedding (women only) in this study. However, we did not evaluate other cellular innate cells that could similarly have been influenced by pVLs or contribute to differences we observed in cytokine production. Although our study includes a relatively large number of seroconcordant and discordant HIV+ individuals in comparison with pVLs and blood T-cell activation status, only a subset of women consented to having genital samples collected for measurement of cervical T-cell activation status or cytokine concentrations.

Taken together, findings in this study support the hypothesis that the continuous immune activation associated with exposure to an HIV-infected partner leads to continuous activation and differentiation of T cells in a cycle involving immune activation, inflammation, and consequent replication of HIV. This continuous process of immune activation is associated with maintenance of higher VLs in HIV-infected individuals with seroconcordant partners that may influence disease progression, the size of the viral reservoir, and immunological damage.

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