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BRIEF REPORT

Immunological Correlates of the HIV-1 Replication-Competent Reservoir Size

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Understanding what shapes the latent human immunodeficiency virus type 1 (HIV-1) reservoir is critical for developing strategies for cure. We measured frequency of persistent HIV-1 infection after 5 years of suppressive antiretroviral therapy initiated during chronic infection. Pretreatment $CD8^+$ T-cell activation, nadir CD4 count, and CD4:CD8 ratio predicted reservoir size.

Keywords. HIV-1; replication-competent virus; reservoir; T-cell activation.

The greatest challenge to eradicating human immunodeficiency virus type 1 (HIV-1) is the long-lived latent viral reservoir that is seeded early in infection, decays slowly after treatment initiation [1–3], and results in viremia if antiretroviral therapy (ART) is interrupted. There is an urgent need to understand the factors that contribute to the seeding and maintenance of the reservoir to inform strategies for HIV-1 cure.

A range of assays have been developed to quantify the latent HIV-1 reservoir, the reference standard for measuring replication-competent HIV being the quantitative viral outgrowth assay (QVOA) [4]. HIV-1 DNA and cell-associated RNA measures are more easily quantifiable, require lower blood volumes, and are more feasible for population-based studies. However, DNA-based assays overestimate the frequency of infected cells with replication-competent HIV-1 since most infected cells harbor defective proviruses, while QVOA underestimates the true size of the reservoir. Some measures of HIV-1 DNA, such as intact proviral DNA [5], correlate with the infectious units per million resting CD4+ T cells (IUPM) [6] and track with the decay of IUPM on long-term ART. Here, we quantified the latent reservoir using QVOA since it measures only replication-competent virus [7], proviruses that will contribute to viral recrudescence upon ART cessation. The size of the HIV-1 latent reservoir, measured by HIV-1 proviral DNA, correlates inversely with nadir $CD4^+$ T-cell count [8] and CD4:CD8 ratio [9]. Furthermore, pre-ART viral loads (VLs) correlate positively with HIV-1 DNA levels in $CD4^+$ T cells on ART [9]. Early initiation of ART results in a smaller HIV-1 reservoir [2], reducing the cumulative viral burden, preserving $CD4^+$ T cells, and maintaining CD4:CD8 ratios while reducing T-cell activation [10, 11].

There is a paucity of HIV-1 reservoir studies in African cohorts, particularly in African women who bear the greatest burden of disease globally. We measured reservoir size as the frequency of resting $CD4^+$ T cells harboring replication-competent HIV-1 by QVOA and investigated the association between clinical and immunological factors and replication-competent HIV-1 reservoir size in South African women virally suppressed on long-term ART.

METHODS

Participants

We enrolled 20 women from the Centre for the AIDS Programme of Research in South Africa 002 acute infection cohort [12]. Peripheral blood mononuclear cells (PBMCs) were obtained at the following 6 time points/participant, where available: acute infection, 1 year post-infection, and late chronic infection, as well as 2 , 4, and 5 years post-ART initiation (Supplementary Table 1). CD4 and CD8 counts and VL were available for multiple time points from the time of seroconversion.

Measurement of T-cell Activation

PBMCs were thawed, rested overnight, and stained for viability (LIVE/DEAD fixable violet stain), CD3-PE-Cy7, CD4-PE-Cy5.5, CD8-Qdot705, HLA-DR-APC-Cy7, CD38-FITC, CCR7-PE-CF594, and CD45RA-BV650. Data were acquired on a BD Fortessa and analyzed using FlowJo software (TreeStar, v10.5.3). Since naive T cells constitutively express CD38, T-cell activation was assessed on memory cells (CCR7+CD45RA+cells excluded).

Quantitative Viral Outgrowth Assay

The QVOA was performed using resting $CD4^+$ T cells isolated from cryopreserved PBMCs obtained approximately 5 years

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after ART initiation, as previously described [13]. Bias-corrected maximum likelihood estimates for IUPM were calculated in R using the SLDAssay package [14] based on the frequency of HIV-1 p24 capsid-positive wells on day 15 of the assay.

Modeling the Replication-Competent Reservoir Size

We used the model given by the equation [3] in Archin et al [2], where we assumed that the latent reservoir half-life is 25 months before ART and 44 months during ART, to predict reservoir size in our cohort.

Study Approval

The University of Cape Town, University of KwaZulu Natal, University of North Carolina at Chapel Hill, and Los Alamos National Laboratory approved the study. All participants provided written informed consent prior to inclusion in the study. Full details are available in the Supplementary Materials.

RESULTS

In this study, we used samples from 20 women living with HIV-1 (median age, 33 years; interquartile range [IQR], 30–39) who had been infected for a median of 4.4 years (IQR, 3.3–5.2) prior to treatment initiation. The frequency of resting $CD4^+$ T cells harboring replication-competent HIV-1 was determined using QVOA after a median of 5.1 years (IQR, 4.7–5.5) of ART and 4.7 years (IQR, 4.2–5.2) of viral suppression (Supplementary Table 1). The relationship between reservoir size and clinical markers of infection ($CD4^+$ T-cell count and VL) was investigated (Table 1). Reservoir size, represented as IUPM, correlated positively with overall viral burden (area under the curve [AUC] VL) from infection to ART initiation and also with viral burden within the 12 months prior to ART initiation (r = .549, P = .012 and r = .498, P = .026, respectively). Furthermore, reservoir size correlated inversely with nadir $CD4^+$ T-cell count (r = –.597, P = .005) and with the CD4:CD8 ratio at the time of ART initiation (r = –.610, P = .004) and at the time post-ART when the blood sample that was used in the QVOA was collected (r = –.643, P = .007). The inverse relationship between nadir $CD4^+$ T-cell count and IUPM remained significant in a multivariate linear regression model adjusting for AUC VL (Supplementary Table 2).

Using the model in [2] and longitudinal VL and $CD4^+$ T-cell counts from the estimated time of infection, we predicted the frequency of latent cell infection. We observed a correlation between predicted and measured IUPM (Supplementary Figure 1) for VL and $CD4^+$ T-cell counts modeled from acute infection to ART initiation (r = .555, P = .012; Supplementary Figure 1A) and from the year before to ART initiation (r = .552, P = .013; Supplementary Figure 1B). These correlations do not differ when the analyses is extended to include on-ART VL and $CD4^+$ T-cell count (Supplementary Figure 1C and 1D).

Table 1. Demographic, Clinical, and Immunological Factors Associated With Replication-Competent Human Immunodeficiency Virus Reservoir Size [as Log_{10}(IUPM)] (n = 20)

	Spearman Correlation		Univariate Linear Regression	
	Spearman r	*P* Value	Coefficient	*P* Value
Age at QVOA, [a] years	–0.1916	.4185		
Duration, months[b]				
Infection to ART initiation	–0.0361	.8799		
From ART initiation to QVOA	0.0286	.9046		
Virally suppressed on ART before QVOA	–0.1734	.4647		
Plasma VL, log_{10} copies/mL				
At ART initiation[c]	0.3098	.1838		
Cumulative (AUC VL)[d]				
Infection to ART initiation	0.5489	***.0122***	0.3931	***.0220***
1 year pre-ART	0.4977	***.0255***	0.3047	.0528
$CD4^+$ T-cell count, cells/μL[e]				
Nadir	–0.5972	***.0054***	-0.0040	***.0037***
ART initiation	–0.4271	.0604		
At time of QVOA	–0.2947	.2071		
CD4:CD8 ratio[f]				
ART initiation	–0.6102	***.0043***	-1.7576	***.0017***
At time of QVOA	–0.6426	***.0065***	-0.7612	***.0142***
$CD38^+$ $HLA\text{-}DR^+$ $CD8^+$ T cells, %[g]				
Acute infection	0.1622	.7286		
1 year post-infection	–0.0788	.8382		
1 year pre-ART	0.7091	***.0182***	0.0319	***.0288***

Replication-competent HIV-1 reservoir size measured in 20 participants as log10(IUPM). Abbreviations: ART, antiretroviral therapy; AUC, area under the curve; QVOA, quantitative viral outgrowth assay; VL, viral load. Bold, italicized values in the table indicate statistically significant relationships where $P < .05$.

[a]Age of cohort: median, 33 years and interquartile range (IQR), 30.3–38.5.

[b]Time from human immunodeficiency virus infection to ART initiation: median, 52 months and IQR, 38.8–61.3; from ART initiation to the time of sizing by QVOA: median, 61 months and IQR, 56.3–65.8; from viral suppression to QVOA: median, 56 and IQR, 50.3–61.5.

[c]Median VL at ART initiation: 4.7 log_{10} copies/mL; IQR, 4.2–4.9.

[d]AUC VL expressed as log_{10} time copies/mL; from infection to ART initiation (excluding peak VL during acute infection): 6.2 and IQR, 5.8–6.6; over the year prior to ART initiation: median, 5.5 and IQR, 5.0–6.0.

[e]Absolute CD4 + T-cell count at nadir: median, 243 cells/μL and IQR, 182–280; at ART initiation: median, 306 and IQR, 235–396; at time of sizing by QVOA: median, 655 and IQR, 545–881.

[f]CD4:CD8 ratio at ART initiation: median, 0.3 and IQR, 0.2–0.4; at QVOA: median, 1.0 and IQR 0.8–1.3.

[g]Frequency of CD38 + HLA-DR + CD8 + memory T cells at acute infection (2 months post-infection): median, 26.5% and IQR, 17.3–35.0; at 1 year post-infection: median, 21.6 and IQR, 15.9–24.2; at 1 year prior to ART initiation: median, 24.0 and IQR, 17.1–31.4.

Extensive longitudinal sampling enabled investigation into whether memory $CD4^+$ or $CD8^+$ T-cell activation at multiple time points before and after ART initiation correlated with reservoir size. We measured surface expression of CD38 and HLA-DR on memory T cells during acute infection, 1 year post-infection, 1 year pre-ART initiation, and at 2 and 4 years post-ART initiation. Reservoir size correlated positively with the frequency of $CD8^+$ T cells coexpressing HLA-DR and CD38 as well as those expressing HLA-DR alone in the year preceding treatment initiation (r = .709, P = .018 and r = .636, P = .040,

respectively; Table 1), remaining significant after adjusting for multiple comparisons.

Given that nadir $CD4^+$ T-cell count and $CD8^+$ T-cell activation may be influenced by viral burden over the course of untreated infection, we performed multivariable linear regression analyses (Supplementary Table 2) and found that nadir $CD4^+$ T-cell count and memory $CD8^+$ T-cell activation within 1 year prior to treatment initiation were significantly associated with reservoir size, even after adjusting for AUC VL (Supplementary Table 2). These models predict that a 1% increase in the frequency of $CD38^+HLA\text{-}DR^+$ memory $CD8^+$ T cells would result in a 0.033 $\log_{10}$ increase in IUPM ($P = .0422$) after adjusting for CD4:CD8 ratio at ART initiation and AUC VL, while a unit increase in nadir $CD4^+$ T-cell count resulted in a 0.004 $\log_{10}$ decrease in IUPM ($P = .0420$) after adjusting for the frequency of $CD38^+HLA\text{-}DR^+CD8^+$ memory T cells and AUC VL (Supplementary Table 2).

DISCUSSION

Factors that influence HIV-1 reservoir seeding are understudied, particularly in African populations. In this study, we provide insights into the immunological characteristics that correlate with the size of the replication-competent reservoir in a cohort of South African women who initiated treatment in late chronic infection.

We show that the cumulative viral burden measured over the entire course of HIV-1 infection or in the year prior to treatment directly predicted reservoir size. In addition, nadir $CD4^+$ T-cell count and CD4:CD8 ratio before treatment initiation correlated inversely with replication-competent HIV-1 after prolonged viral suppression. Our findings build on previous work that modeled VL and pre-ART $CD4^+$ T-cell dynamics as key predictors of replication-competent reservoir size in the context of early ART [2] and identified the extent of CD4 depletion as shaping the HIV-1 DNA proviral load [8, 9].

We hypothesized that greater T-cell activation pre-ART may enhance seeding of the HIV-1 reservoir by providing a larger pool of target $CD4^+$ T cells for infection. However, the frequency of activated $CD4^+$ T cells was not significantly associated with IUPM at any time point before or after treatment initiation (data not shown). A limitation of this analysis is that $CD4^+$ T-cell activation is highly dynamic due to continual depletion and migration of these cells, possibly limiting our ability to detect a significant relationship between $CD4^+$ T-cell activation and reservoir size.

T-cell activation was measured at too few time points to use in our model predicting IUPM. Nevertheless, our multivariate linear regression analysis found that the link between $CD8^+$ T-cell activation and reservoir size remained significant after controlling for viral burden, providing additional support for a direct link between $CD8^+$ T-cell activation within the year before ART and reservoir size. Of note, in 9 women in this cohort, we showed that 71% of unique viral outgrowth variants were genetically similar to viruses in circulation the year before ART initiation [13]. Together, the results suggest that immunological events late in infection may disproportionately shape the size and composition of the HIV-1 reservoir. We observed that the frequency of activated memory $CD8^+$ T cells in late chronic infection was positively associated with reservoir size. Hyperactivation, a strong predictor of disease progression, drives $CD8^+$ T-cell dysfunction and may explain the relationship between $CD8^+$ T-cell activation and reservoir size [15]. CD38 and HLA-DR coexpression on $CD8^+$ T cells has been linked to an exhausted cell state and reduced cytotoxic ability [16], resulting in diminished clearance of infected $CD4^+$ T cells during the transition to latency upon ART initiation in chronic infection, and potentially a larger latent reservoir.

Despite slight differences in methodology, our measured IUPM falls within the range of those reported in other studies including both males and females [2, 17, 18]. While our study did not compare reservoir size differences between females and males, 2 such studies have been published with discordant findings [17, 1]. However, similar to this study, neither found a correlation between $CD4^+$ T-cell activation and IUPM.

In conclusion, we demonstrate that replication-competent HIV-1 reservoir size during long-term suppressive ART associates with multiple immunological measures prior to ART, underscoring the need for early diagnosis and treatment. This new knowledge may assist with the design of novel cure and/or therapeutic vaccine approaches.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. D. M. M., M. R. A., L. R. M., R. D. S., C. W., N. J. G., and W. A. B. conceived and designed the study. N. J. G ., Q. A. K., and S. S. A. K. contributed samples and designed the study. S. D. I., S. B. J., M. M., and O. D. C. collected experimental data. S. D. I., C. R., N. M. A., A. S. P., T. C., and F. O. analyzed data and performed statistical analysis. S. D. I. drafted the manuscript, and all authors contributed to the final manuscript. All authors discussed the results and approved the final manuscript.

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References

1. Siliciano JD, Kajdas J, Finzi D, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med **2003**; 9:727–8.
2. Archin NM, Vaidya NK, Kuruc JAD, et al. Immediate antiviral therapy appears to restrict resting CD4 + cell HIV-1 infection without accelerating the decay of latent infection. Proc Natl Acad Sci U S A **2012**; 109:9523-8.
3. Crooks AM, Bateson R, Cope AB, et al. Precise quantitation of the latent HIV-1 reservoir: implications for eradication strategies. J Infect Dis **2015**; 212:1361–5.
4. Siliciano JD, Siliciano RF. Enhanced culture assay for detection and quantitation of latently infected, resting CD4+ T-cells carrying replication-competent virus in HIV-1-infected individuals. Human Retrovirus Protocols **2005**; 304:003–16.
5. Bruner KM, Wang Z, Simonetti FR, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. Nature **2019**; 566:120–5.
6. Falcinelli SD, Kilpatrick KW, Read J, et al. Longitudinal dynamics of intact HIV proviral DNA and outgrowth virus frequencies in a cohort of ART-treated individuals. J Infect Dis **2020**; 224:92–100.
7. Ho YC, Shan L, Hosmane NN, et al. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell **2013**; 155:540–51.
8. Boulassel MR, Chomont N, Pai NP, Gilmore N, Sékaly RP, Routy JP. CD4 T cell nadir independently predicts the magnitude of the HIV reservoir after prolonged suppressive antiretroviral therapy. J Clin Virol **2012**; 53:29–32.
9. Martin GE, Pace M, Shearer FM, et al. Levels of human immunodeficiency virus DNA are determined before ART initiation and linked to CD8 T-cell activation and memory expansion. J Infect Dis **2020**; 221:1135–45.
10. Rajasuriar R, Wright E, Lewin SR. Impact of antiretroviral therapy (ART) timing on chronic immune activation/inflammation and end-organ damage. Curr Opin HIV AIDS **2015**; 10:35–42.
11. Ndhlovu ZM, Kazer SW, Nkosi T, et al. Augmentation of HIV-specific T cell function by immediate treatment of hyperacute HIV-1 infection. Sci Transl Med **2019**; 11:eaau0528.
12. van Loggerenberg F, Mlisana K, Williamson C, et al; CAPRISA 002 Acute Infection Study Team. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. PLoS One **2008**; 3:e1954.
13. Abrahams M-R, Joseph SB, Garrett N, et al. The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation. Sci Transl Med **2019**; 11:eaaw5589.
14. Trumble IM, Allmon AG, Archin NM, et al. SLDAssay: a software package and web tool for analyzing limiting dilution assays. J Immunol Methods **2017**; 450:10–6.
15. Perdomo-Celis F, Taborda NA, Rugeles MT. CD8+ T-cell response to HIV infection in the era of antiretroviral therapy. Front Immunol **2019**; 10:1896.
16. Perdomo-Celis F, Velilla PA, Taborda NA, Rugeles MT. An altered cytotoxic program of CD8+ T-cells in HIV-infected patients despite HAART-induced viral suppression. PLoS One **2019**; 14:e0210540.
17. Prodger JL, Capoferri AA, Yu K, et al. Reduced HIV-1 latent reservoir outgrowth and distinct immune correlates among women in Rakai, Uganda. JCI Insight **2020**; 5:e139287.
18. Falcinelli SD, Shook-Sa BE, Dewey MG, et al. Impact of biological sex on immune activation and frequency of the latent HIV reservoir during suppressive antiretroviral therapy. J Infect Dis **2020**; 222:1843–52.