

Intact HIV Reservoir in Monocytes Is Associated With Cognitive Function in Virally Suppressed Women With HIV

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Background. Monocytes are susceptible to human immunodeficiency virus (HIV) infection, form HIV reservoirs, and contribute to central nervous system complications (eg, cognitive impairment) in virally suppressed women with HIV (vsWWH). However, it remains unknown if the quality and/or quantity of the monocyte reservoir contributes to cognition in vsWWH.

Methods. Sixty-two vsWWH (mean age = 56.1 years, SD = 7.1; 93% Black, non-Hispanic; all HIV RNA <250 copies/mL) completed a cognitive test battery, blood draw, and whole-blood immunophenotyping. Monocytes and CD4 T cells were isolated from a subset of 53 participants and the HIV reservoir was assessed using cell-specific intact proviral DNA assays (IPDA). Demographically adjusted z-scores were calculated for each outcome using data from participants without HIV in the Women's Interagency HIV Study. Cognitive outcomes of interest included domain-specific and global z-scores.

Results. Thirty-Eight percent of vsWWH had detectable intact HIV genomes in monocytes (median = 21.5 copies/million). Higher levels of intact HIV genomes per million monocytes were associated with poorer verbal memory (delayed recall, $r = 0.55$, $P = .01$; recognition, $r = 0.46$, $P = .04$), fine motor skills ($r = 0.50$, $P = .03$), and global function ($r = 0.47$, $P = .04$). Higher levels of intact HIV genomes in monocytes were associated with percent intermediate monocytes ($r = 0.60$, $P = .008$), and the ratio of intact per intermediate monocyte was associated with worse memory ($r = -0.59$, $P = .008$). There were no associations between CD4 reservoir and cognition.

Conclusions. The number of intact HIV genomes per million monocytes was related to poorer cognition and the percentage of intermediate monocytes. These findings suggest that the presence of HIV genomes in general do not relate to cognitive complications, but intact, and therefore potentially replication-competent HIV, may contribute to cognitive complications in vsWWH.

Keywords. HIV; reservoir; monocytes; cognition; women with HIV.

Cognitive complications persist despite effective antiretroviral therapy (ART) in women with human immunodeficiency virus (WVH), particularly in verbal memory, processing

speed, and motor function [1, 2]. The role of monocytes in HIV central nervous system (CNS) dysfunction is well supported [3, 4]. Monocytes are susceptible to HIV infection [5, 6] and can form HIV reservoirs [7]; however, it has yet to be determined if the quality and/or quantity of the monocyte reservoir may contribute to cognitive outcomes in virally suppressed WWH (vsWWH).

Monocytes can be identified using Toll-like receptor 2 (TLR2) [8] and then divided into 3 subsets, classical (CD14⁺/CD16⁻), intermediate (CD14⁺/CD16⁺), and nonclassical (CD14^{lo}/CD16⁺). Monocytes are targets for HIV infection and linked to numerous HIV-related pathologies including neurologic complications, cardiovascular disease, and opportunistic infections [9]. The ubiquitous distribution of these cells allows HIV to disseminate into tissues and establish compartmentalized reservoirs in the form of macrophages [10]. Evidence from animal models [11–13] and virally suppressed

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people with HIV (vsPWH) [7, 14, 15] demonstrates that HIV persists in monocytes despite long-term ART suppression. Previous studies are limited in assessments of the quality of the monocyte reservoir, as HIV DNA is present at low frequencies and rare populations are difficult to sequence. We recently reported a myeloid adapted intact proviral DNA assay (IPDA) used to assess the quality and quantity of HIV genomes in monocytes from vsPWH [7]. IPDA provides a sensitive assessment of total, defective, and intact HIV genomes, and uses intact genome measurements as an estimate of the replication-competent reservoir [16]. In our previous study, 40% of vsPWH had detectable intact reservoirs, while 100% of individuals had some form of HIV DNA in monocytes [7], providing further evidence that monocytes contain HIV genomes that may contribute to ongoing comorbidities in HIV.

Alterations to monocyte subsets during HIV infection and suppression also contribute to cognitive complications. Increased circulation of HIV-infected intermediate monocytes relate to immune activation and poorer cognition in PWH [17, 18], and are thought to transport HIV to the brain, driving neuroinflammation [19–21]. We reported that higher percentages of intermediate monocytes relate to poorer cognition in vsWWH and are predictive of cognition [22]. However, few studies assess the contribution of the monocyte reservoir to cognition in PWH. Kusao et al reported that larger CD16⁺ monocyte reservoirs, but not classical monocytes or CD4 T cells, relate to poorer global cognition in PWH [23]. However, the CD4 reservoir was not assessed and a subset of PWH were viremic. Another study reported that HIV DNA in monocytes was associated with cognition during viremic infection, and that this association was maintained 48 weeks after ART initiation in PWH with HIV-associated dementia [24]. However, there are no follow-up studies determining if the HIV monocyte reservoir relates to cognition in vsPWH with milder cognitive complications. It remains unknown whether the quality of the HIV genome (intact vs defective) contributes to domain-specific cognition and no study has simultaneously assessed the monocyte and CD4 reservoirs to determine how each reservoir associates with cognition.

Overall, these studies provide evidence that specific monocyte subsets are associated with poorer cognition in PWH [3, 22], and that their role in harboring latent virus may be a contributing factor [17, 23, 24]. Here we examine associations between the HIV reservoir in monocytes and CD4 T cells and cognition in vsWWH. We hypothesized that a higher percentage of intermediate monocytes in blood and larger intact HIV reservoirs in monocytes will relate to poorer cognition.

METHODS

Study Approval

Participant data collection was approved by the Johns Hopkins University Institutional Review Board. Informed written consent

was obtained from participants prior to study enrollment. The study was conducted according to Declaration of Helsinki principles.

Study Participants and Design

Sociodemographic, clinical, behavioral, cognitive, and biospecimen data were obtained from 66 WWH participating in a longitudinal neuroHIV study in Baltimore, MD (Supplementary Table 1). Participants completed a cognitive test battery, questionnaires (including demographic and mental health screeners), a urine toxicology screen, and a blood draw at an initial study visit. Subsequent study visits occurred monthly (for 3 months, no intervention) and included a blood draw at each visit for whole-blood phenotyping. Reservoir data were generated on the same visit or within 1 month of cognitive assessment (Supplementary Figure 1). Inclusion criteria were age 18 to 65 years, female, with HIV, and English speaking. Exclusion criteria were current use of hormone-based contraceptives, currently pregnant, post partum, or lactating, regular steroid use, current untreated hypertension or diabetes, history of closed head injury, psychosis, or dementia or any other neurologic CNS or AIDS-defining disorder, substance use disorder in the past 6 months, positive urine toxicology screen (except marijuana) or breathalyzer, and/or any evidence of acute intoxication or withdrawal.

Cognitive Test Battery

We administered the Women's Interagency HIV Study (WIHS) cognitive test battery [25] that assesses 7 cognitive domains, including verbal learning, verbal memory, attention/working memory, executive function (EF), verbal fluency, motor function, and processing speed. Supplementary Material details the specific cognitive test and their outcomes. Scores for each test outcome were converted to demographically adjusted (age, years of education, Wide Range Achievement Test [reading subtest] score, race/ethnicity) z-scores using data from women without HIV in the WIHS. Details on demographic-norming have been previously published [25, 26]. Domain scores were computed by averaging z-scores for outcome measures within a domain. Global cognitive function was calculated as the average of the demographically adjusted cognitive domain z-scores and the variability in cognitive performance was calculated as the standard deviation of those z-scores across cognitive domains.

Flow Cytometry

Whole-blood samples were stained within 2 hours of collection as previously described [22] with antibody panel TLR2-AF488 (clone 11G7), CD14-BV650 (clone M5E2), CD16-AF700 (clone 3G8), CD3-V500 (clone SP34-2), CD4-PerCP-Cy5.5 (clone L200), CD8a-BV570 (clone RPA-T8), and CD159a-APC (clone NKG2A). TLR2 specificity was previously confirmed

using a matched isotype control [8]. The gating strategy was determined using fluorescence minus 1 controls and has been previously published [22]. Voltage settings were standardized to daily CS&T Research Beads (BD Biosciences) using application settings determined based on fluorescent intensities in FACSDiva. Data were acquired on a BD LSRFortessa (BD Biosciences) within 2 hours of staining and analyzed using FlowJo (version 10.9.0; BD Biosciences).

Intact Proviral DNA Assay

IPDA was performed as previously described to measure intact, defective (3' deleted/hypermethylated and 5' deleted) and total HIV genomes in monocytes and CD4 T cells [7, 16]. TLR2⁺ monocytes were isolated from peripheral blood mononuclear cells (PBMCs) using a biotinylated TLR2 antibody (clone TL2.1) and antibiotin microbeads (Miltenyi). This selection method notably resulted in equivalent selection of all monocyte subsets but fewer contaminating CD4⁺ cells compared to commercially available negative monocyte selection kits (Supplementary Figure 2). Using frozen PBMCs eliminated contaminating granulocytes, which express low levels of TLR2 and all TLR2⁺ cells were CD3⁻ (Supplementary Figure 3). CD4 T cells were isolated from TLR2⁻ flow through using a negative CD4 selection kit (Miltenyi). Selected cells were assessed for purity by flow cytometry (panel TLR2, CD3, CD4, Live/Dead Near IR stain [Invitrogen]) and lysed in AllPrep buffer for DNA isolation using the Qiagen AllPrep DNA/RNA mini kit following manufacturer's recommendations. All primers, probes, and reaction conditions have been previously published [7]. Samples were run in 3–6 replicates until a minimum of 1 million cells, or maximum available, were acquired as determined by the cellular gene *RPP30*. To estimate potential CD4 signal contribution to the monocyte IPDA signal, we assessed CD4 IPDA values and percent CD3⁺/CD4⁺ determined by flow cytometry to calculate the number of CD4 T cells present in 1 million monocytes, and the possible intact, 3' defective, or 5' defective signal contributed by the contaminating CD4 T cells. This value was subtracted from the monocyte IPDA signal. All data were adjusted for CD4 [7] and DNA shearing using the DNA shearing index [16], and shown in Supplementary Table 2.

Statistical Analyses

Pearson's correlations (*r*) were conducted to examine associations between monocytes, CD4 T cells, HIV genomes, and cognition. Adjusted analyses were not necessary as the measured sociodemographic (eg, age), behavioral (eg, substance use), clinical factors (eg, CD4 count; Supplementary Table 1), and female-specific factors (menopause stage) were not related to biomarkers and cognitive outcomes. Analyses were conducted in IBM SPSS Statistics for Windows version 25.0. Significance was set at *P* < .05.

RESULTS

Cohort Characteristics

Sixty-six WWH were enrolled (Supplementary Figure 1 and Supplementary Table 1); of the 66, 3 were excluded due to viral loads >250 copies [cp]/mL and 1 was missing immunophenotyping data. On average, the 62 participants were 56 years of age (SD = 6.9; range = 35–64 years) and 93.5% were Black. Women were on ART an average of 20 years, 84% had viremia <20 cp/mL and 100% had <250 cp/mL for the study duration. Most participants were on an integrase inhibitor-based regimen with 56 (90%) on second-generation integrase inhibitors: 19 (31%) dolutegravir, 24 (39%) bictegravir, and 10 (16%) elvitegravir. Median monocyte subset percentages were 76.7% classical, 8.7% intermediate, and 12.3% nonclassical. Cognitively, 68% of participants were impaired on ≥2 domains (*n* = 42). On average, the cohort demonstrated the greatest difficulty in verbal learning (58%) and memory (71%), fine motor skills (48%), and EF (47.5%; demographically adjusted *z* scores < 1). PBMCs for reservoir analysis were available from 53 of the 62 vsWWH. Demographics, ART, cognition, and monocyte percentages were consistent between the reservoir subset and full cohort.

Higher Percentages of Intermediate Monocytes Relate to Poorer Cognition

We previously reported that a higher percentage of intermediate monocytes was associated with poorer cognition in 25 vsWWH [22]. To confirm this finding, we expanded the initial cohort to include 62 vsWWH and completed the same analysis (Figure 1 and Supplementary Table 3). Additionally, to reduce variability in the flow cytometry measures, the cellular percentages are reported as an average of samples collected at 2–3 visits throughout the longitudinal study. Phenotyping data were collected within 2 months of cognitive assessment. Using flow cytometry data collected over multiple visits results in a high confidence in the percentage reported for cell types, as a single visit can skew the observed trends. The average values across visits were associated with individual visits (*r*s > 0.89, *P*s < .0001; Supplementary Figure 4). A higher percentage of intermediate monocytes was associated with poorer global cognition (global mean, *r* = -0.31, *P* < .05) and greater variability (global standard deviation [SD], *r* = 0.36, *P* < .01) in vsWWH. A higher percentage of intermediate monocytes was also associated with lower verbal learning, memory, recognition, fine motor, and EF (*P*s < .05). Additionally, a higher percentage of classical monocytes was associated with better verbal recognition (*r* = 0.30, *P* < .05). However, CD4 T cells, total, and nonclassical monocytes were not associated with cognitive outcomes (*P*s > .12). These findings are consistent with our previous study and confirm that the prevalence of intermediate monocytes is a marker of cognitive function in vsWWH.

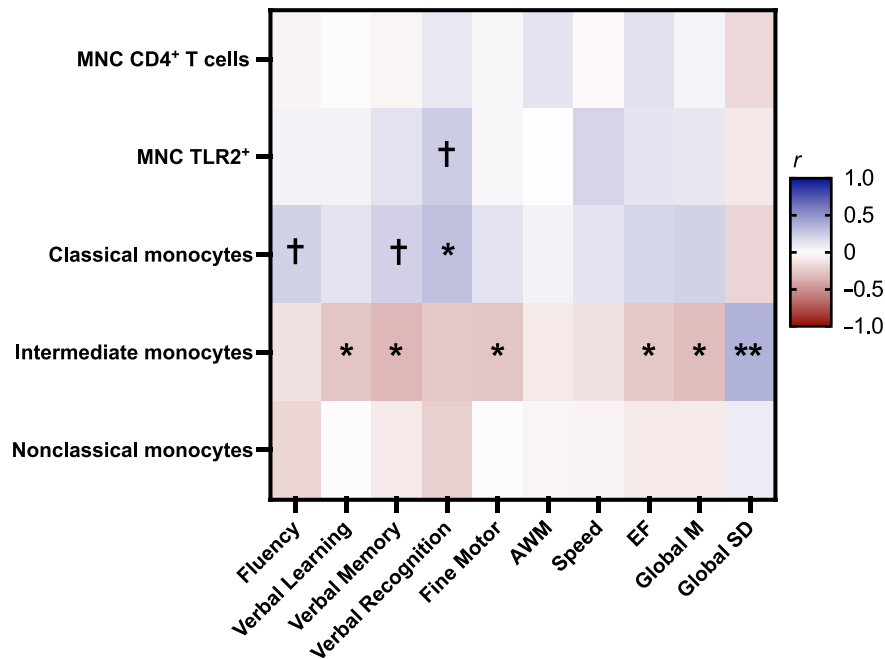


Figure 1. Higher percentages of intermediate monocytes were associated with poorer global and domain-specific cognition. Cell phenotyping data were acquired by flow cytometry on whole blood from 62 virally suppressed women with HIV. Percent CD4 T cells and monocyte MNC, and monocyte subsets were associated with cognition. ** $P < .01$, * $P < .05$, † $P > .05$ and $P < .10$. Abbreviations: AWM, attention working memory; EF, executive function; M, mean; MNC, mononuclear cells; SD, standard deviation.

Thirty-Eight Percent of vsWWH Harbor Intact HIV Genomes in Monocytes

Our group recently reported intact HIV genomes in monocytes from vsPWH using a myeloid adapted IPDA [7]. Using this approach, we assessed the quality and quantity of HIV DNA genomes in monocytes and CD4 T cells isolated from the same blood draw in 53 vsWWH (Figure 2 and Supplementary Table 2). Of the participants assessed, 92% had detectable provirus in monocytes (median 44 cp/million cells) in at least 1 form, 38% had detectable intact (median 21.5 cp/million cells), 72% had detectable 5' defective (median 22 cp/million cells), and 83% had detectable 3' defective (median 19 cp/million cells) proviruses. As expected, 100% of participants had detectable provirus in CD4 T cells (median 480 cp/million cells), 75% had detectable intact (median 56 cp/million cells), 92% had detectable 5' defective (median 292 cp/million cells), and 98% had detectable 3' defective (median 242 cp/million cells) provirus. When comparing the quantity of HIV genomes detected in both cell types, CD4 T cells had significantly higher levels of 3' defective ($P = .02$), 5' defective ($P = .0002$), and total ($P = .001$) versus monocytes. However, when comparing intact genomes, the difference between cell types was not significant ($P < .09$). To confirm that the signal observed was not an artifact of CD4 contamination, we calculated correlations between intact and total HIV genomes in monocytes and CD4 T cells as well as with percent CD4 contamination. No relationships were observed (all P s $> .5$; Supplementary Figure 5). These data provide evidence that monocytes from our cohort of vsWWH contained HIV genomes.

Intact HIV Genomes in Monocytes Correlate With Poorer Cognition

To determine if the quantity and/or quality of HIV genomes was related to cognition, we assessed associations between IPDA and cognition (Figure 3 and Supplementary Table 4). All IPDAs were completed on the same visit as the cognitive assessment or within 1 month, and only detectable signal was used to calculate associations. When assessing total HIV genomes in monocytes, there were no significant associations with cognition. Similar results were observed in CD4 T cells, with the exception of EF ($r = -0.31$, $P < .05$). To assess the quality of the HIV genomes, we used 3' defective, 5' defective, and intact measures. There were no associations between 3' and 5' defective genomes in monocytes with cognition. Similar results were observed in CD4 T cells, with the exception of EF ($r = -0.33$, $P < .05$). Conversely, the number of intact genomes per million monocytes was associated with poorer global cognition ($r = -0.46$, $P < .05$) and greater variability (global SD, $r = 0.48$, $P < .05$). When examining each domain separately, higher levels of intact genomes per million monocytes were associated with lower verbal memory, verbal recognition, and fine motor skills (P s $< .05$). There were no associations between intact genomes in CD4 T cells and cognition. To determine if the presence of intact genomes in monocytes was predictive of poorer cognition, we categorized the cohort into "intact" versus "no intact" and compared z-scores between groups. The presence of intact genomes in monocytes did not predict cognitive performance. Therefore, the quantity of intact genomes

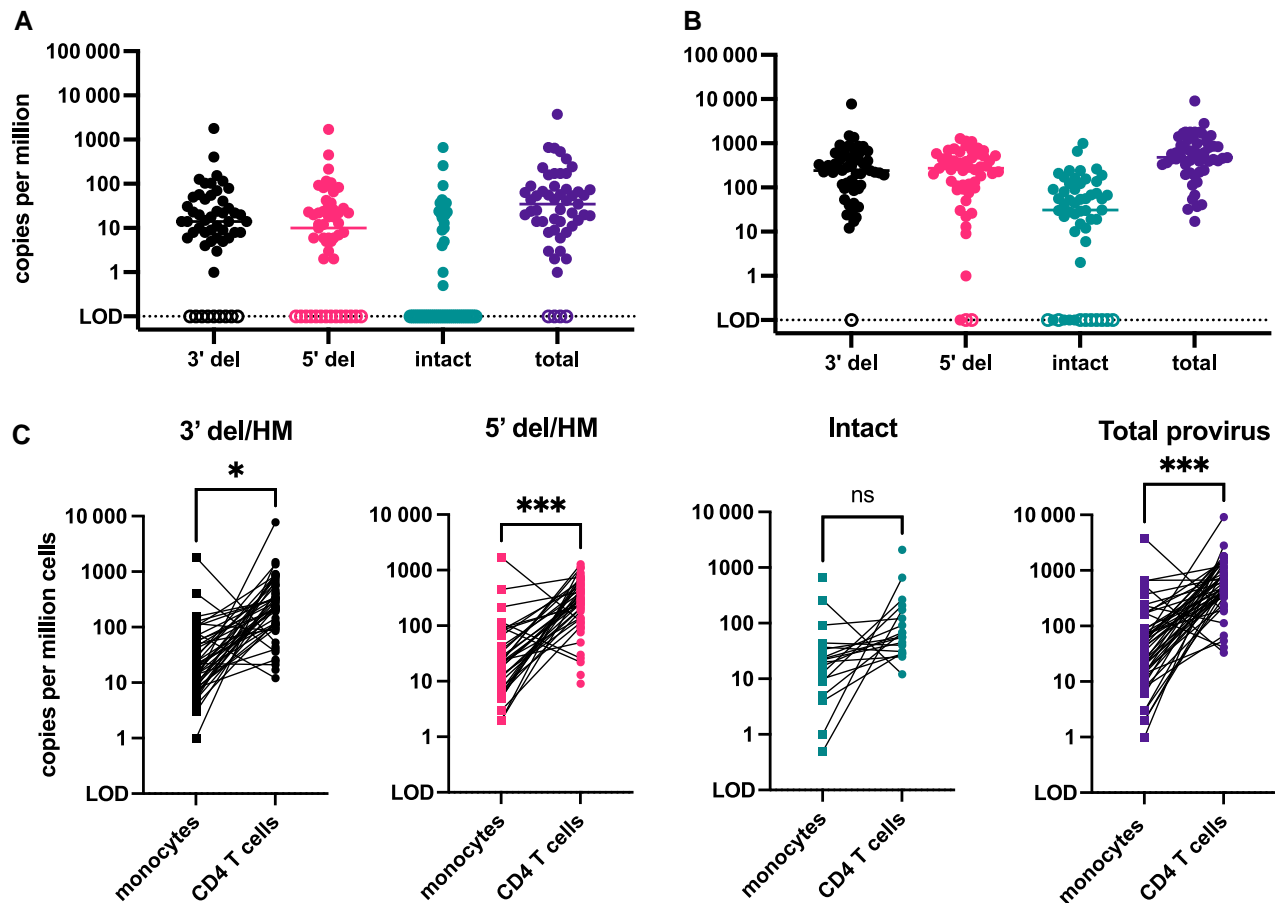


Figure 2. Intact proviral DNA was detected in monocytes from a subset of vsWWH. Monocytes (A) and CD4 T cells (B) were isolated from 53 vsWWH and assessed for HIV proviral DNA using intact proviral DNA assays, line indicates median. C, 3' defective (del/HM), 5' defective (del/HM), intact, and total proviral genome levels per million cells were compared in participants who had measurable DNA levels in both cell types: 3' del/HM $n = 44$, $*P = .02$; 5' del/HM $n = 38$, $***P = .0002$; intact $n = 20$, $ns P = .09$; total $n = 49$, $***P = .001$, paired t test. Abbreviation: del, deleted; HM, hypermutated; LOD, limit of detection; ns, not significant; vsWWH, virally suppressed women with HIV.

in monocytes drove the observed associations. These data suggest that increased levels of monocytes that contain intact HIV genomes is a marker of cognition in a subset of vsWWH.

Intact HIV Genomes in Monocytes Correlate With the Percent of Intermediate Monocytes

To determine if a particular subset was associated with intact genomes in monocytes, we compared the percentages of total monocytes and each subset with the corresponding intact and total HIV genome quantities (Figure 4 and Supplementary Table 5). Total, classical, and nonclassical monocytes did not associate with intact genomes ($P_s > .08$). However, intermediate monocytes related to intact genomes per million monocytes ($r = 0.6$, $P = .008$). To determine if the presence of intact genomes in monocytes was predictive of increased intermediate monocytes, we categorized the cohort into “intact” versus “no intact” and compared the intermediate monocyte percentages between groups. The presence of intact genomes

in monocytes was not predictive of higher percentages of intermediate monocytes, but the quantity of intact drove the association. As a control, the same associations were assessed between total HIV genomes in monocytes and percentage of monocyte subsets; no additional associations were observed. These data suggest that the association between intermediate percentages and intact genomes in monocytes is specific.

Calculated Ratio of Intact HIV Genomes per Intermediate Monocyte Correlates With Verbal Memory

To further probe the association of intact genomes in monocytes with intermediate monocyte percentages and corresponding cognitive outcomes, we calculated a ratio of intact and total HIV genomes per intermediate monocyte (Figure 5 and Supplementary Table 6). Using whole-blood percentages via flow cytometry, we calculated the number of intermediate monocytes per million monocytes. We then divided the number of intact genomes per million monocytes by the number of

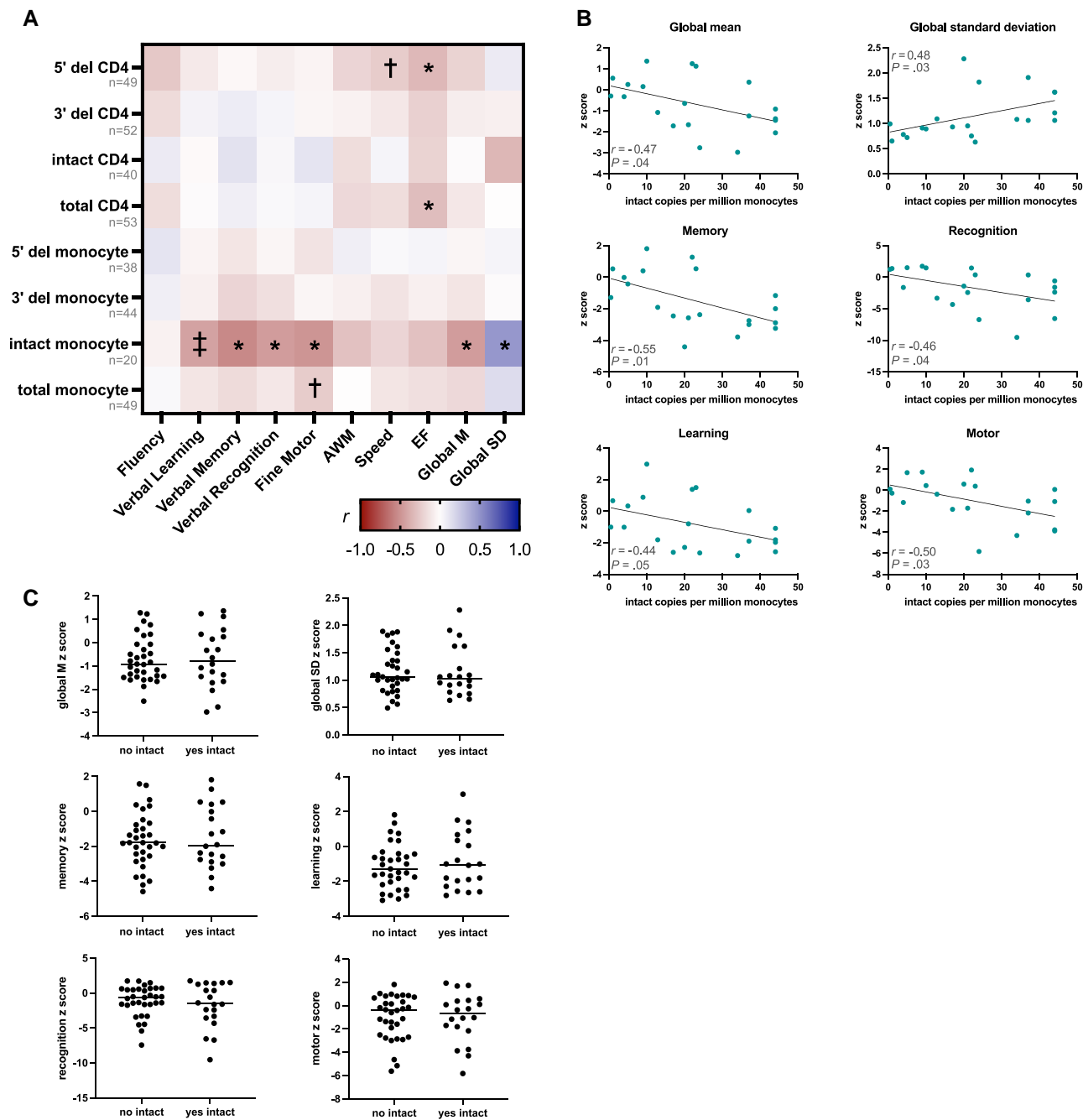


Figure 3. Number of intact HIV genomes in monocytes from vsWWH associated with poorer cognition. **A**, Detectable IPDA signal in monocytes and CD4 T cells from vsWWH were associated with concurrent cognitive outcomes and r values are displayed as a heatmap. Negative associations are shown in red and positive associations in blue; sample number differed for each IPDA measure as each subject had a unique make up of HIV genomes, as shown in the figure under each genome category. * $P < .05$, † $P = .05$, † $P > .05$ and $P < .10$. **B**, Significant associations with intact genomes per million monocytes and each cognitive domain, $n = 20$, r and P values are displayed in each graph; simple linear regression. **C**, The data set was split into categorical variables, no intact ($n = 33$) and yes intact ($n = 20$), to compare cognitive z scores for each category, line indicates median. Abbreviations: AWM, attention working memory; del, deleted; EF, executive function; IPDA, intact proviral DNA assay; M, mean; SD, standard deviation; vsWWH, virally suppressed women with HIV.

intermediates per million monocytes to obtain an estimate of the number of intact genomes per intermediate monocyte (range, $7.5e^{-06}$ to $4.6e^{-03}$; median, $2.3e^{-04}$). The same calculations were completed with total HIV genomes in intermediate monocytes

as a control. The ratios were then related to each cognitive domain that was associated with intact genomes in monocytes in [Figure 3](#). Specifically, the ratio of intact per intermediate monocyte was associated with memory ($r = 0.59$, $P = .008$).

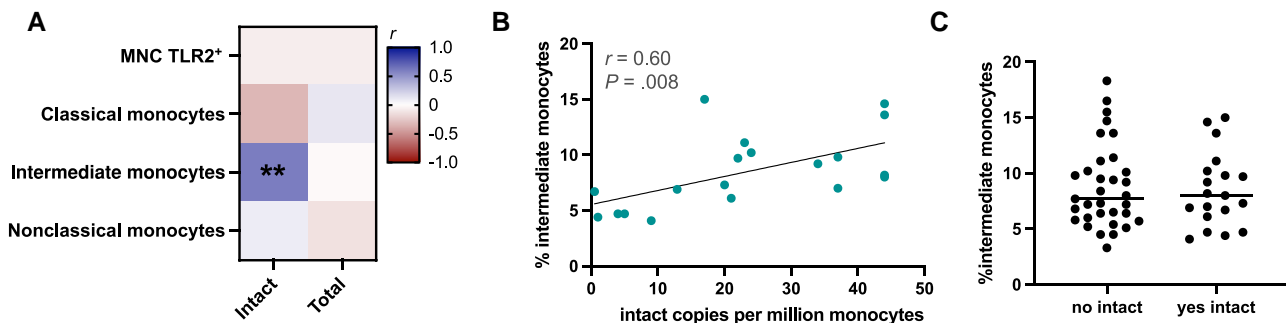


Figure 4. Amount of intact HIV genomes in monocytes were associated with the percent of intermediate monocytes in blood. *A*, Percentage of mononuclear cell (MNC) TLR2⁺, classical, intermediate, and nonclassical monocytes in blood were associated with intact copies per million monocytes, $n = 19$ (1 subject was missing flow cytometry data); negative associations are shown in red and positive associations in blue. ** $P < .01$. *B*, Significant associations between intact genomes per million monocytes and intermediate monocyte percentages; r and P values are displayed; simple linear regression. *C*, Data were split into 2 groups: intact ($n = 19$) and not intact ($n = 33$), to compare group differences in intermediate monocyte percentages, line indicates median.

No associations were observed with total HIV genomes per intermediate monocyte ratio. These data suggest that the association of intact genomes with verbal memory may be driven by intact genomes within the intermediate monocyte subset.

DISCUSSION

We have previously reported that both monocyte [22] and CD4 [27] activation states are associated with cognitive performance in vsWVH. Here, we provide further evidence in a larger cohort of vsWVH that the percentage of intermediate monocytes associates with global cognition and the domains of verbal learning and memory, fine motor skills, and EF, while proportions of total monocytes and CD4 T cells do not contribute.

The shift in monocyte maturation from early classical monocytes towards an intermediate subtype in vsWVH may be caused by ongoing inflammation that is not fully abated by ART [28]. We hypothesized that the HIV reservoir within monocytes may contribute to the intermediate monocyte shift and to poorer cognitive function. Our findings that 92% of vsWVH had detectable reservoirs in monocytes and 38% had detectable intact genomes is consistent with our previously published study [7] and others that have reported HIV DNA in monocytes [10, 14, 15, 29]. The consistency of this finding is important, as this is the largest cohort of vsPWH with monocyte reservoir levels reported. The use of the IPDA method, compared to previous methods, has increased overall sensitivity and ability to measure the HIV reservoir in monocytes. These data provide further evidence that monocytes contain an HIV reservoir, and that a substantial percentage of individuals harbor intact genomes within monocytes.

Our finding that intact genomes in monocytes relate to poorer cognitive function in a subset of vsWVH is significant, as it suggests the presence of HIV genomes in general do not lead to cognitive complications, but intact, and therefore potentially

replication-competent HIV genomes, may contribute to poorer cognition. Importantly, we did not observe associations between the CD4 reservoir and cognition, suggesting that latently infected CD4 T cells do not play a role in ongoing cognitive complications in vsWVH. These findings add to previously published work that reported higher levels of CD16⁺ monocytes containing HIV DNA was associated with poorer global cognition and secretion of higher levels of proinflammatory cytokines [23, 30]. In combination with these previous findings, our study suggests that cognitive complications in a subset of vsWVH may in part be due to the inability to clear the monocyte reservoir despite ART. If the monocyte reservoir is primarily housed within intermediate monocytes, then the lack of clearance and regular traffic to the CNS would result in ongoing injury to the brain. Additionally, these data suggest a mechanism by which continued release of inflammatory cytokines from HIV-infected monocytes could lead to activation of newly released monocytes from the bone marrow, setting up a feed forward loop of activation and injury, and the development of a chronic inflammatory state, as observed clinically [31, 32]. It is also possible that monocytes with intact HIV genomes contribute more to ongoing CNS injury than those with defective HIV genomes. Although we observed associations with global cognition, verbal memory appeared to be the domain that was most affected. Mechanistically, this is well supported as verbal memory can be attributed to the hippocampus and prefrontal cortex [33, 34], an area of the brain where the blood-brain barrier is more susceptible to breakdown [35], and monocyte activation is associated with altered tight junction integrity, subsequent permeability, and poorer cognition [20]. Therefore, intact HIV genomes in monocytes may be predictive of hippocampal function in vsWVH.

Importantly, this study suggests only one mechanism of many that may contribute to cognitive performance in vsWVH as we did not observe intact genomes in monocytes

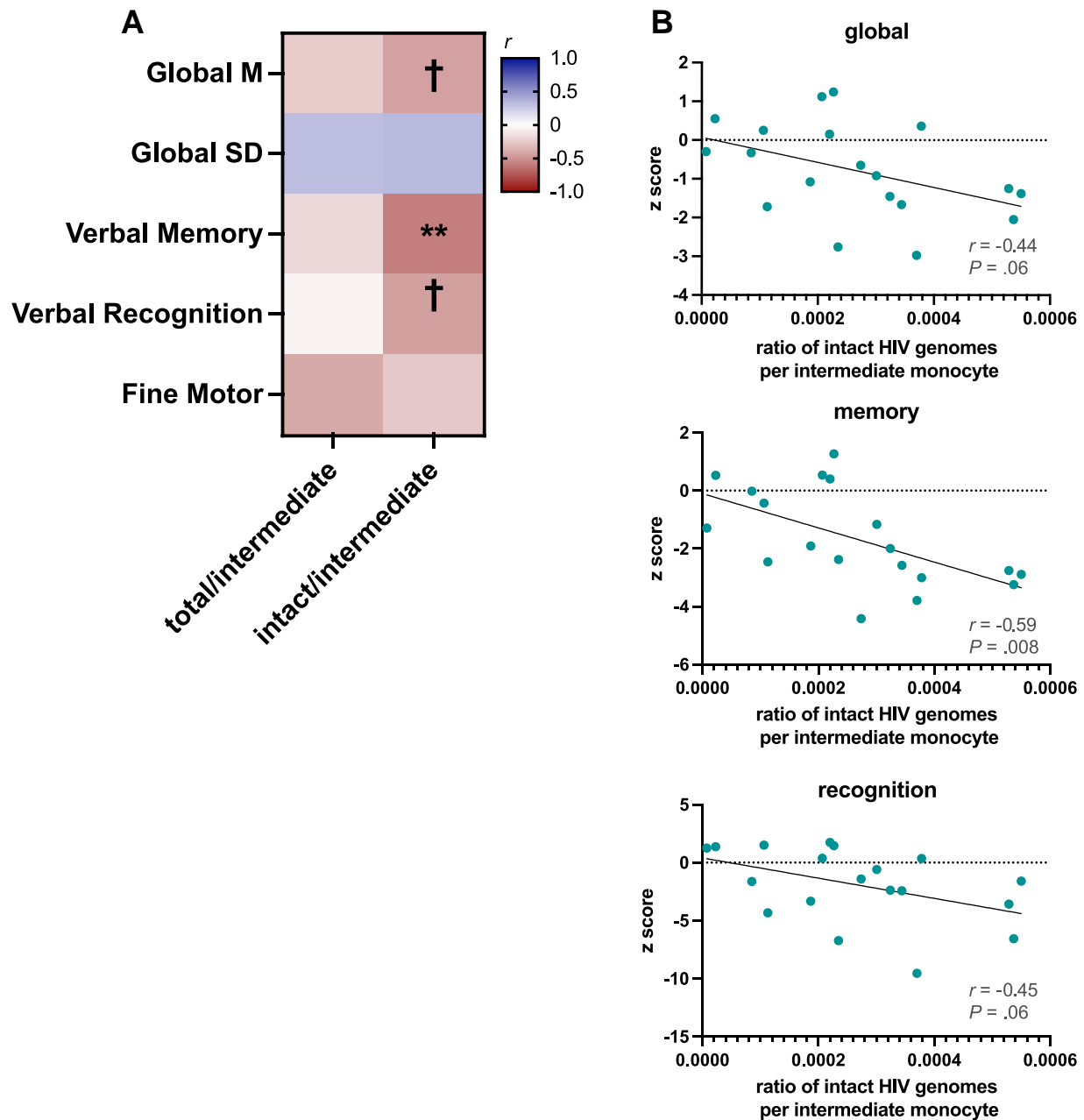


Figure 5. Ratio of intact genomes to intermediate monocytes associated with verbal memory in virally suppressed women with HIV. *A*, Ratio of total HIV genomes and intact genomes per intermediate monocytes were associated with global cognition, global SD, verbal memory, verbal recognition, and fine motor. Negative associations are shown in red and positive associations in blue, $n = 19$. ** $P < .01$, † $P > .05$ and $P < .10$. *B*, Significant and trending associations between the ratio of intact genomes per million intermediate monocytes and cognitive domains, $n = 19$; r and P values are displayed in each graph; simple linear regression. Abbreviations: M, mean; SD, standard deviation.

in all vsWWH with cognitive decline and other mechanisms; for example, increased inflammation, may contribute to the associations observed. Despite the small number of participants with detectable intact genomes within monocytes, we observed associations with cognition that were maintained when associated with intermediate monocyte percentages. Additionally, our lack of findings with the larger numbers of

the other genome categories and CD4 T cells gives weight to the associations observed with intact genomes in monocytes. Due to cell availability, we assessed HIV genomes in all circulating monocyte populations, as we did not have enough cells to specifically sort CD14⁺ or CD16⁺ subsets. This resulted in downstream analysis that relied on associations and calculated ratios rather than a direct detection of HIV DNA in individual

monocyte subsets. Further exploration of intermediate monocytes as a subgroup with a greater likelihood of carrying intact HIV genomes is needed to validate these results. Limited cell availability required analysis by IPDA and the interpretation of intact genomes as representative of the replication-competent reservoir, and therefore may be an overestimate. It is also possible that this approach is an underestimate of the percentage of vsWWH who have intact genomes within monocytes, as it relies on primer matching to a diverse subset of HIV genomes. However, this method allowed for high-throughput screening and simultaneous reservoir assessment in monocytes and CD4 T cells with minimal input. The findings of the present study are limited in generalizability, as our focus was on vsWWH and studies that include men would be informative. Additionally, larger studies are needed to determine the effects of different ART regimens. A strength of the present study is that our cohort was predominately on integrase inhibitor-based regimens.

This study provides evidence that intact HIV genomes within monocytes are associated with cognition in a subset vsWWH. We report that the number of intact HIV genomes per million monocytes relates to poorer cognition and provide further evidence that intermediate monocytes are a suitable marker for cognition. These findings warrant future larger-scale studies to further define characteristics of the monocyte intact reservoir as these cells could become a therapeutic target for cognitive complications in vsPWH.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copy-edited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Author contributions. L. H. R., E. N. S., and R. T. V. contributed conception and design of the study. I. S., J. M. C., and T. T. B. recruited participants and samples. R. T. V., E. N. S., L. P., H. R., and E. R. performed experiments. L. H. R., E. N. S., L. P., R. M. D., and R. T. V. analyzed the data. L. H. R., J. E. C., and R. T. V. provided materials. R. T. V. and L. H. R. wrote the initial drafts of the manuscript. All authors

provided critical review of the manuscript for important intellectual content and contributed to and approved the final manuscript.

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