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Cognitive Performance Related to HIV-1-Infected Monocytes

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Abstract

The effect that HIV type 1 (HIV) has on neurocognition is a dynamic process, whereby peripheral events are likely involved in setting the stage for clinical findings. In spite of antiretroviral therapy (ART), patients continue to be at risk for HIV-associated neurocognitive disorders (HAND), which might be related to persistence of inflammation. In a yearly assessment of HIV DNA levels in activated monocytes, increased HIV DNA copies were found in patients with persistent HAND. Furthermore, activated monocytes from patients with high HIV DNA copies secreted more inflammatory cytokines. Since these activated monocytes traffic to the CNS and enter the brain, they may contribute to an inflammatory environment in the CNS that leads to HAND.

In the era of effective antiretroviral therapy (ART), in spite of achieving suppression of plasma HIV RNA, HIV type 1 (HIV) continues to affect the central nervous system (CNS) as evidenced by persistence of HIV-associated neurocognitive disorders (HAND).^{1–4} Since the first report of circulating activated monocytes (CD14⁺CD16⁺) in HIV-infected individuals with neurocognitive impairment,⁵ this CD14⁺CD16⁺ subset has been characterized as a viral reservoir, with preferential susceptibility to infection by HIV.^{6–8} Activated monocytes may play an important role in HAND pathogenesis because these cells traffic to the CNS and could contribute to the inflammatory environment in the brain, leading to neurocognitive problems. We previously reported an association between HAND and high HIV DNA copies in peripheral blood mononuclear cells (PBMC) in cross-sectional ART-experienced and ART-naïve cohorts.^{9–12} Others have suggested that activated monocytes were involved in HAND pathogenesis and are prone to an inflammatory phenotype;^{5,13} therefore we examined, for the first time, yearly HIV DNA copy numbers in activated monocytes (CD14⁺CD16⁺) in patients from the Hawaii Aging with HIV Cohort (HAHC). We then characterized HIV-infected activated monocytes to translate the clinical findings to ex-vivo and in-vitro models.

METHOD

Patients and Specimens

The study was approved by the University of Hawaii Institutional Review Board, with adherence to the appropriate informed-consent process. The HAHC was a longitudinal study that characterized HAND in HIV-seropositive individuals living in Hawaii from 2001 to 2006. Details of enrollment and clinical characterization were published elsewhere.^{14,15}

Briefly, subjects enrolled in HAHC were HIV-infected individuals 50 years old and 20–40 years old, and excluded individuals with a history of head injury, learning disability, major neurologic/psychiatric disease, or brain-opportunistic disease. Baseline and annual evaluations included neurologic examination, neuropsychological testing, medical intake with demographic data, risk-behavior, HIV-1 laboratory parameters (viral load, CD4 count, and nadir CD4 count), medication histories, and comorbid illnesses. The inclusion criteria for the study were limited to participants who had an adequate number of peripheral blood mononuclear cells (PBMC; 20×10^6 cells per year) stored in the specimen repository for at least 3 years in order to perform the required observations.

Neurocognitive Evaluations

Assessments included a macroneurologic examination, medical history questionnaire, medication/adherence history, Diagnostic and Statistical Manual of Mental Disorders–IV–based substance abuse/dependence inventory, immunologic and virologic laboratory tests, and neurocognitive testing.^{14,15} The neuropsychological battery included Choice and Sequential Reaction Time (CalCap; CRT/QRT), Rey Auditory-Verbal Learning Test (RAVLT), Rey-Osterreith Complex Figure (RCF) Copy and Recall, Trail-Making Tests A and B (TMA/TMB), WAIS–R Digit Symbol (SYD), Grooved Pegboard Dominant/Non-Dominant (GPD/GPN), Timed Gait (TG), Odd Man Out, fetal alcohol syndrome (FAS), Animal Naming, Boston Naming Test (BNT), and WAIS–R Digit Span (Forward and Backward). Test results were transformed to whole-number z-scores, using appropriate normative sets, as previously described.^{14,15} A sum score of the following tests was used to categorize degree of neuropsychological impairment: RAVLT, delayed-recall, and delayed-recognition; RCF immediate recall and copy; Odd Man Out; FAS; Digit Symbol; CalCap choice and sequential reaction times; and Grooved Pegboard dominant and non-dominant hands. Changes in neuropsychological test z-scores, grouped by cognitive domain, as noted by changes in NPZ–8 score (average of norm-adjusted z-scores for TG, GPD, GPN, TMA, TMB, SYD, CRT, and QRT), were used in the analyses.¹⁶ For the ex-vivo experiments described below, patients were categorized with research-based neurocognitive diagnoses, using the American Academy of Neurology 1991 criteria (HIV-associated dementia [HAD]; normal cognition [NC]).^{14,15,17}

Cell-Separation and HIV DNA Assessment From the Longitudinal Cohort

Frozen PBMCs from patients who had at least 20×10^6 cells per year were separated into monocyte and non-monocyte fractions, using the Human Monocyte Enrichment Kit without CD16 Depletion (Stem Cell Technologies, Vancouver, BC, Canada) as per manufacturer's guidelines, which is a negative selection, leaving the CD14 cells untouched. The CD14 monocyte fractions were then separated into CD16⁺ (activated) and CD16[−] (non-activated fractions). Activated monocytes (CD14⁺CD16⁺) were labeled with the alpha CD16-biotin antibody (BioLegend; San Diego, CA) and magnetically separated by streptavidin magnetic particles (Chemicell). DNA was extracted from cell fractions and PBMC, using the QIAamp DNA Micro Extraction kit (Qiagen; Valencia, CA), with DNA quality assessed with the ND-1000 Spectrophotometer (NanoDrop Technologies; Wilmington, DE).

DNA from PBMC and PBMC subsets were assessed for HIV DNA copy numbers. Multiplex real-time PCR using HIV *gag* (forward 5'-GAC ATC AAG CAG CCA TGC AA-3'; reverse 5'-CTC ATC TGG CCT GGT GCA AT-3') and β -globin (forward 5'-AGG GCC TCA CCA CCA ACT TC; reverse 5'-TCA CTA GCA ACC TCA AAC AGA CAC C-3') primers were designed with Primer Express 3.0 software (Applied Biosystems; Foster City, CA) to amplify a 109 bp and a 111 bp PCR product, respectively. VIC-labeled HIV *gag* (5'-ACC ATC AAT GAG GAA GCT GCA GAA TGG GA-3') and FAM-labeled β -globin (5'-CTC CTG AGG AGA AGT CTG CCG TTA CTG CC-3') probes were used along

with standard reference plasmids with either one copy of the β -globin housekeeping gene (GenBank accession #2253431) or one copy of the HIV *gag* gene (GenBank accession #NC_001802) for the real-time PCR assay of HIV DNA copies. Multiplex real-time PCR master mixes were prepared with both HIV *gag* and β -globin primer and Taqman probe sets: 1x Taqman® Gene Expression Master Mix (Applied Biosystems; Foster City, CA), unknown, and water-to-final volume 10 μ L. Plasmid dilutions from 10^6 to 10^1 gene copies were made for the standard reference curves. Non-HIV-infected Ramos β -cell DNA and water were used as negative-control DNA and HIV-infected cell DNA (OM10.1, 8E5, ACH-2; NIH AIDS Research and Reference Reagent Program; Rockville, MD) were used for positive-control DNA for inter-assay calibrations. Samples were run in triplicate on a 7900HT Real-Time PCR System (Applied Biosystems; Foster City, CA): 95°C for 10 minute; 40 cycles of 95°C for 1 minute, and 60°C for 1 minute. Analyses were completed using SDS 2.3 software (Applied Biosystems; Foster City, CA). The copy numbers of each sample gene were analyzed against the standard curves and the HIV DNA copy number per 1×10^6 cells determined.

In-Vitro and Ex-Vivo CD16⁺ and CD16⁻ Monocyte Cytokines

Ex-vivo and in-vitro experiments were designed to characterize cytokine profiles of cellular subsets, which included PBMC and activated monocytes with HIV DNA.

Ex-vivo experiments were set up with specimens from three groups of patients whose PBMC were previously analyzed for HIV DNA copies as well as from HIV-negative control volunteers: 1) patients with high (log HIV DNA > 2) PBMC HIV DNA copies; 2) patients with undetectable PBMC HIV DNA copies; and 3) HIV-seronegative control volunteers.⁹ Activated and non-activated monocytes (CD14⁺16⁺ and CD14⁺16⁻) from patients in each group were isolated as noted above and plated at a density of 3×10^6 cells per cm² and maintained at 37°C with 5% CO₂. Cells were washed 24 hours after plating to purify for adherent monocytes and maintained for 10 days in media (25% RPMI Media; Sigma, Inc., St. Louis, MO; Isocove's modified Dulvecco's medium; Irvine Scientific, Santa Ana, CA; 4 mM *L*-glutamine, Sigma, Inc.; 100 units/ml penicillin; 100 μ g/ml streptomycin; Sigma, Inc.; 50 U/ml MCSF, Sigma, Inc.; 1 ng/ml IL3; 10% human serum; Sigma, Inc.). Equal numbers of cells were plated (96 or 48 well-plates; 9×10^5 cells/well and 3×10^6 cells/well) depending on the number of cells recovered. After 7 days, the media components were changed (excluding MCSF and IL3) and continued in culture for 3 more days. On Day 10, supernatants were obtained from CD16⁺ and CD16⁻ monocyte cultures analyzed for inflammatory cytokines. Quantikine ELISA kits (R&D Systems, Inc.; Minneapolis, MN) were used according to manufacturer's instructions to assay for IL6, IL8, IL10, TNF α , sFAS, sTNFR1, sTNFR2, and MCP1. For the in-vitro experiments, monocytes were separated from commercially-available PBMC (All Cells; Emeryville, CA) using the Easy Sep Human CD14 Positive Selection Kit (Stem Cell Technologies; Vancouver, BC, Canada). After separation, approximately 24×10^6 monocytes were seeded in a 10-cm Hydro Cell Surface plate (Nunc; Rochester, NY) in 12.5 ml of Isocove's Modified DMEM (Mediatech; Manassas, VA) supplemented with 10% fetal bovine serum (Sigma-Aldrich; St. Louis, MO), 1% penicillin/streptomycin, 1% sodium pyruvate, and 1% *L*-glutamine. After 2 days in culture, approximately 12×10^6 monocytes were exposed to Human M-CSF (Miltenyi Biotec; Auburn, CA) at a concentration of 50 U/ml in a 6-cm Hydro cell surface plate in 8.5 ml of Isocove's Modified DMEM, as previously outlined, in order to yield activated (CD14⁺16⁺) monocytes, which were previously confirmed by flow cytometry on an aliquot of cells from the plate. After a 1-day activation period, the activated monocytes were washed and resuspended in Isocove's Modified DMEM without M-CSF. The other 12×10^6 monocytes were continued in suspension without the addition of M-CSF to yield non-activated (CD14⁺16⁻) monocytes. Each culture (in triplicate) of activated and non-activated

monocytes was exposed to different strains of HIV consisting of R5– (BaL, JR–CSF), X4– (MN, LAI), and dual-tropic (p89.6) HIV with a multiplicity of infection of 0.05 in Isocove's Modified DMEM, supplemented with 2% fetal bovine serum. After an 8-hour exposure, respective viruses were washed from the cell suspensions, and the remaining viable cells were maintained in culture with Isocove's Modified DMEM, supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin for a 1-day period. After recovery in DMEM supplemented with 20% FBS, both activated and non-activated monocyte cell cultures were maintained in culture for a total of 21 days post-infection with Isocove's Modified DMEM, supplemented with 10% fetal bovine serum and additional supplements as previously outlined. HIV p24 levels were assayed (ExpressBio; Westbury, NY) daily to determine optimum HIV-infected days to begin measuring cytokines. Supernatants were collected on Days 11, 15, and 19, with residual cells lysed for HIV DNA assay, had cytokines (TNF α , IL6, IL8, GMCSF, and IL1 β) measured by Luminex analysis, using a 5-plex Invitrogen plate as per manufacture's guidelines. The supernatant volumes were limited; therefore, the cytokines were measured by Luminex instead of using ELISA. Supernatant cytokines from infected cell-lines were analyzed in triplicate and normalized to cytokines measured from concordant cell line cultures set up in parallel without virus.

Statistical Analyses

Pearson correlation coefficients were used to measure the associations between the NPZ8 score and each laboratory parameter at entry visit. To determine the association between HIV DNA level and NPZ8 score longitudinally, the general modeling approach included both repeated-measure models and linear mixed-effect models. One of the aims was to estimate the HIV DNA impact on NPZ8 longitudinally. To account for correlated responses for a given subject, a linear mixed-effect model was utilized to impose covariance structure of responses, to account for individual difference, and to estimate the magnitude of HIV DNA effect. In the linear mixed-effect model, NPZ8 score was regressed on log₁₀ HIV DNA level and other covariates where unstructured covariance was assumed. Also, based on a cutoff point of 400 copies/10⁶ cells in either PBMC HIV DNA or activated monocyte HIV DNA levels, we dichotomized our cohort into two groups (i.e., high DNA level and low DNA level-groups) and analyzed the response profiles over time. Both repeated-measure and linear mixed-effects models were used to determine whether the shapes of the mean response profiles differed among groups. Thus, the interest was focused on whether the patterns of change in mean response were similar among groups.^{18–20} Cytokine measurements and analyses were performed on small numbers of specimens; therefore the data reported were descriptive.

RESULTS

Patient Demographics and Specimens

Sixty-eight patients (61 men, 7 women) were identified; their median age was 49 years. The majority of the patients' risk factors for HIV exposure were categorized as "men who had sex with men" (Table 1). Of the 84% patients on ART, 60% has undetectable HIV RNA levels at the time of entry into the cohort. The nadir CD4 cell counts ranged from 0 to 784 cells/mm³ (median: 160 cells/mm³; Q1–Q3: 40–300 cells/mm³).

Longitudinal HIV DNA Assessment

HIV DNA copies from PBMC, CD14⁺CD16⁺ (activated monocytes), CD14⁺CD16[–], and CD14[–] subsets were calculated from each annual visit; and NPZ8 was also calculated from neuropsychological batteries annually. The standardized response variable, NPZ8, was assumed to be normally distributed. Pearson coefficients of correlation between NPZ8 and log copy numbers of PBMC and activated monocytes were –0.55 (p <0.001) and –0.57 (p

<0.001), respectively (Figure 1A and Figure 1B). In contrast, no linear relationship with HIV DNA copies in CD14⁺ (NS) or CD14⁺CD16⁺ (NS) was found with NPZ8 score. By a linear mixed-effects model, the HIV DNA copy number in activated monocytes was strongly associated with NPZ8 ($p < 0.001$; Figure 1A), but not CD4 cell count, CD4 nadir, gender, and education level. Given that HIV RNA level was fixed, a one-unit increase in $\log_{10}$ HIV-activated monocyte DNA copy resulted in a decrease of NPZ8 score by 0.34. A similar modeling procedure was performed for PBMC, showing that after adjusting for HIV RNA level, the HIV DNA copy number in PBMC was also strongly associated with NPZ8 score ($p < 0.001$; Figure 1B); with a one-unit increase in $\log$ PBMC HIV DNA copy number resulting in a decrease of 0.32 in NPZ8 score. However, linear mixed-effect models didn't reveal significant effects of HIV copy numbers in CD14⁺ (NS) or CD14⁺CD16⁺ ($p = 0.09$; Figure 1C and Figure 1D). Also, we validated such association between HIV DNA copy numbers and NPZ8 score by generalized estimating equation (GEE). In the GEE model, estimated effects of activated monocyte and PBMC were -0.33 ($p < 0.001$) and -0.31 ($p < 0.001$), respectively, which were similar to the linear mixed-effects estimates. The time effect in a linear mixed-effects model was not significant at α level 0.05. Thus, this suggested that with HIV DNA and RNA levels fixed, NPZ8 scores did not change significantly over time. To visualize this relationship of HIV DNA and NPZ8 score longitudinally, patients were divided into two groups, based on the HIV DNA copies in activated monocytes and PBMC at entry: High HIV DNA (≥ 400 copies/ 10^6 cells; $N = 32$) and Low HIV DNA (< 400 copies/ 10^6 cells; $N = 32$; Figure 2A and Figure 2B). Patterns in mean NPZ8 over time seemed to be parallel between High HIV DNA and Low HIV DNA groups, with NPZ8 scores remaining stable over time (Figure 2A and Figure 2B). Both repeated-measure and linear mixed-effect models were used to test this hypothesis and confirmed that interactions between High- and Low- activated-monocytes DNA groups and time were not significant. In the linear mixed-effect model, the activated-monocytes DNA group effect was significant ($p < 0.0001$), whereas the time effect was not ($p = 0.06$), suggesting that mean NPZ8s were different between High- and Low-activated monocyte groups longitudinally, but no significance was observed between different time-points for a fixed HIV DNA group. The same procedure was performed by using dichotomized PBMC HIV DNA groups and showed similar results. Changes in NPZ8 over time were not statistically different for either High- or Low-PBMC groups ($p = 0.06$), but mean NPZ8 was significantly different between PBMC DNA groups longitudinally ($p < 0.0001$).

We were also interested in whether these findings could be applied to patients with undetectable HIV RNA levels. Thus, there were 32 patients who had undetectable HIV RNA levels at every annual visit. By linear mixed-effects models, we found that HIV DNA copy numbers in PBMC (coefficient: -0.2091 ; $p < 0.0001$) and activated monocytes (coefficient: -0.2043 ; $p < 0.0001$) were still highly associated with NPZ8 but not CD14⁺ (NS) or CD14⁺CD16⁺ (NS). Also, interaction effects between time and High/Low HIV group effect were not significant at α level 0.05 (activated-monocyte HIV groups: NS; PBMC HIV groups: NS), suggesting that mean NPZ8 profiles seemed to be parallel between High and Low HIV DNA groups. A linear mixed-effects model demonstrated that mean NPZ8s were statistically different between Low- and High-activated monocytes groups ($p = 0.0008$) as well as Low- and High- PBMC groups ($p = 0.003$); but no statistical significance between Low- and High-DNA groups that NPZ8 increased over time was observed for either activated monocytes or PBMCs.

Ex-Vivo and In-Vitro CD16⁺- and CD16⁺- Monocyte Cytokines

Ex-vivo cultures of activated and non-activated monocytes from patients diagnosed with HAD ($N = 5$) and NC ($N = 6$) and HIV-seronegative control volunteers ($N = 4$) were established to characterize the phenotype of activated monocytes with HIV DNA isolated from HAHC

patients. Activated (CD14⁺CD16⁺) and non-activated (CD14⁺CD16⁻) monocytes were placed in culture and assessed for supernatant cytokines and HIV DNA. Supernatants from activated monocytes from patients with HAD contained higher TNF α (80 [7] pg/ml), IL6 (10 [5] pg/ml), and MCP1 (998 [3] pg/ml) levels than supernatants from CD16⁺ monocytes isolated from NCs (TNF α : 28 [6] pg/ml; IL6: 2.8 [4] pg/ml; and MCP1: 425 [9] pg/ml) and HIV-1-seronegative controls (TNF α : 8 [2] pg/ml; IL6: 0.9 [0.1] pg/ml; and MCP1: 208 [10] pg/ml; Figure 3A and Figure 3B). Also, from the same patients with HAD, the CD16⁺ monocyte supernatants had higher TNF α , IL6, and MCP1 levels than the respective CD16⁻ monocyte supernatants (Figure 3A and Figure 3B). There appeared to be no differences in CD16⁻ monocyte TNF α , IL6, and MCP1 levels among patients with HAD, NC, or HIV-seronegative controls (Figure 3A and Figure 3B). There were also no differences in IL8, IL10, sFAS, sTNFR1, or sTNFR2 levels from either CD16⁺ or CD16⁻ monocytes. The patients with HAD were previously identified as having high PBMC HIV DNA⁷⁻⁹; and, when placed in culture, the CD14⁺CD16⁺ cells had higher HIV DNA than the CD14⁺CD16⁻ cells. Because the number of specimens was small, these descriptive results provided preliminary data for the in-vitro experiments.

From the in-vitro experiments, supernatants from monocytes (activated and non-activated) infected with R5- (BaL, JR-CSF); X4- (MN, LAI); and dual-tropic (p89.6) virus were collected from Days 1–21 and assayed for HIV p24 expression, which showed infection detectable beginning on Day 11. Thus, cytokines and HIV DNA were measured on Days 11, 15, and 19. Activated monocytes infected with R5-tropic viruses (BaL and JR-CSF) had increasing TNF α (Day 19: 69.2 [2.7] and 45.7 [1.2] pg/ml, respectively) and IL6 (Day 19: 60.3 [2.1] pg/ml and 35.1 [1.1] pg/ml levels, respectively) as compared with non-activated monocytes exposed to BaL and JR-CSF (Day 19: TNF α : 25.3 [0.6] and 19.7 [0.4] pg/ml, respectively) and IL6 (Day 19: 17.8 [1.1] pg/ml and 0.0 pg/ml, respectively; Figure 3C and Figure 3D); no differences were noted in the other cytokines. The cells infected by BaL and JR-CSF on the days that cytokines were assessed also had higher HIV DNA copies in activated monocytes, as compared with non-activated monocytes (Figure 3C and Figure 3D). In contrast, X4-tropic virus (MN and LAI) cultures appeared to show no differences in TNF α or IL6 levels (nor in the other cytokines) between activated and non-activated monocytes (Figure 3E). Similar to the ex-vivo experiments, these results are descriptive because of the small number of samples analyzed.

DISCUSSION

The current study expands upon previous work demonstrating high HIV DNA copies in PBMC in patients with HAND.⁹⁻¹² We have now identified the activated monocytes as the unique PBMC cellular subset that might be contributing to an inflammatory milieu. The data showed that patients with HAND have high HIV DNA primarily in the activated monocytes, not only at each annual visit, but patients with HAND continued to have high HIV DNA over the course of years while on ART. The results are important in understanding that patients continue to have HAND even when the HIV RNA levels remain undetectable on therapy.

In an attempt to understand mechanisms that explain the clinical findings, we then set up an ex-vivo model showing that activated monocytes isolated from patients on ART secrete more inflammatory cytokines and that the patients with HAND are those with high HIV DNA. In separate in-vitro experiments, activated monocytes infected with macrophage-tropic (CCR5) virus, versus lympho-tropic (CXCR4) virus, produce more inflammatory cytokines. These findings support a hypothesis that activated monocytes could be a vehicle for HIV. Since monocyte trafficking, particularly activated monocytes, to the brain has been postulated to lead to neurocognitive problems,^{13,21-23} the high HIV DNA levels found in

activated monocytes from patients with lower NPZ8 scores is consistent with theories about HAND neuropathogenesis. The NPZ8 score has been compared with an HIV dementia scale.¹⁶ As a point of reference, an NPZ8 cutoff point was established at -2.0 standard deviations from the mean according to the AAN guidelines.¹⁷ Thus, individuals scoring below the cutoff were considered to be cognitively impaired or to have dementia, whereas scores within the cutoffs were considered to be cognitively normal.

The in-vitro and ex-vivo studies showed that activated monocytes infected with R5-tropic virus had an inflammatory cytokine profile with higher levels of TNF α and IL6. The results are similar to previous studies showing CD16 upregulation and enhanced HIV infected with MCSF; however, the present study expands upon preferential infectivity by R5-tropic virus.^{24–27} In circulation, the release of inflammatory cytokines could activate newly-released monocytes from the bone marrow and thus make them more prone to infection by HIV, thus setting up a chronic inflammatory state that is consistent with what is observed clinically.^{22,28} Our data demonstrated that activated monocytes from patients with HAND not only had higher HIV DNA copies, but produced more inflammatory cytokines than patients without HAND or HIV-1-seronegative control individuals. Others have also demonstrated that activated monocytes may play an active role, with a characteristic cytokine profile.^{6,7} One of the limitations of the study is that the in-vitro and ex-vivo experimental results are descriptive and require further validation to show significance.

In summary, the data showed that individuals with continued neurocognitive deficits over time are distinguished by high HIV DNA in their activated monocytes. The significance of these findings lies in the fact that, in spite of effective ART, as shown by undetectable HIV RNA levels, these patients continued to have neurocognitive deficits, with associated high HIV DNA copies in their activated monocytes. The clinical findings were consistent with the inflammatory profile that was demonstrated by activated monocytes from patients with HAND who had high HIV DNA copies. Furthermore, we showed that a similar inflammatory phenotype was found from activated monocytes that were exposed and infected by macrophage-tropic virus. The new findings, illustrating continued high HIV DNA in activated monocytes in patients with HAND, suggests a need to re-look at the current paradigm in treatment modalities. Novel therapeutic targets may need to be investigated that include monocytes and other viral reservoirs to prevent or treat HAND in patients infected with HIV.

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FIGURE 1. NPZ8 Scores and HIV DNA Copy

Activated monocyte (A), PBMC (B), non-activated monocyte (C), and lymphocyte (D) HIV DNA (log) from Years 1–5 and NPZ8 score, using a mixed-effects model to adjust for repeated measurements.

For both activated monocytes and PBMC, HIV DNA copies per million cells were strongly associated with NPZ8 scores ($p < 0.001$) independent of baseline/nadir CD4 cell counts, gender, or education. A one-unit increase in log HIV DNA copy in activated monocytes and PBMC resulted in 0.34 and 0.32 decrease in NPZ8 score, respectively. The estimated lines assumed that HIV RNA level was 50 at baseline. No linear relationships with HIV DNA

copies were found in lymphocytes (CD14⁻; NS) or non-activated monocytes (CD14⁺CD16⁻; NS).

A)

B)

FIGURE 2. Longitudinal NPZ8 Scores for High- Versus Low-Activated Monocytes (A) and PBMC (B) HIV DNA Copy Groups

Patients were divided into High (≥ 400 HIV DNA copies/ 10^6 cells; N=32) and Low (<400 HIV DNA copies/ 10^6 cells; N=32) and NPZ8 scores averaged over Years 1–5.

FIGURE 3. In-Vitro and Ex-Vivo Monocyte Cytokines

In-vivo cytokines from activated and non-activated monocytes isolated from patients diagnosed with HIV-associated dementia (HAD; N=5), normal cognition (NC; N=6), and HIV-seronegative controls (NEG; N=4); TNF α (A) and IL6 (B). Supernatants from activated monocytes from patients with HAD contained higher TNF α and IL6 than supernatants from non-activated monocytes isolated from those with NC and HIV-1-seronegative controls. In-vitro monocyte cytokine secretions showing TNF α and IL6 levels from activated and non-activated monocytes infected with R5 viruses, BaL (C) and JR-CSF (D); and X4 virus, LAI (E), on Days 11, 15, and 19. For BaL and JR-CSF infections, high cytokine levels from activated monocytes were associated with high HIV DNA copies

(N=3), as compared with LAI infection (N=3). Results were descriptive because the number of specimen analyzed was small.

TABLE 1**Baseline Demographics and Characteristics**

Men:Women	61:7
Age, years; Median (range)	51.6 (24.4–70.8)
Mean (standard deviation)	49.0 (9.9)
Education, median years (Q1–Q3)	13 (12–16)
Median CD4 nadir, cells/ μ L (Q1–Q3)	160 (40–300)
Median CD4 cell count, cells/ mm^3 (Q1–Q3)	415 (281–590.5)
Undetectable viral load	41 (60%)
On antiretroviral therapy (at entry)	59/65 (90.7%)