Published in final edited form as:
AIDS. 2014 July 17; 28(11): 1619–1624. doi:10.1097/QAD.0000000000000306.

HIV DNA in CD14+ Reservoirs is Associated with Regional Brain Atrophy in cART-Naïve Patients

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Abstract

Objective—To examine associations between regional brain volumes and HIV DNA in peripheral CD14+ cells (monocytes) among HIV-infected individuals naïve to combination antiretroviral therapy (cART).

Design—A prospective study of HIV-infected Thai individuals who met Thai national criteria for cART initiation. Enrollment was stratified by HIV DNA in a blinded fashion.

Methods—CD14+ cells were isolated from peripheral mononuclear cells to high purity (median 91.4% monocytes by flow cytometry), and HIV DNA was quantified by multiplex real-time PCR. Baseline regional brain volumes obtained by T1-weighted 1.5-Tesla magnetic resonance imaging were compared between HIV DNA groups using analysis of covariance (ANCOVA).

Results—We studied 60 subjects with mean (SD) age of 34.7 (7.0) years, CD4 T-lymphocyte count of 232 (137) cells/mm^3, and $\log_{10}$ plasma HIV RNA of 4.8 (0.73). Median (IQR) HIV DNA copy number per 10^6 CD14+ cells was 54 (102). Using our previously determined optimal cut-point of 45 copies/10^6 cells for this cohort, a threshold value above which CD14+ HIV DNA identified HIV-associated neurocognitive disorders (HAND), we found that CD14+ HIV DNA ≥ 45 copies/10^6 cells was associated with reduced volumes of the nucleus accumbens ($p = 0.021$), brainstem ($p = 0.033$) and total gray matter ($p = 0.045$) independently of age, CD4 count and intracranial volume.

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Conflicts of interest: The authors declare that no conflicts of interest exist.

Conclusion—HIV DNA burden in CD14+ monocytes is directly linked to brain volumetric loss. Our findings implicate peripheral viral reservoirs in HIV-associated brain atrophy and support their involvement in the neuropathogenesis of HAND, underscoring the need for therapies that target these cells.

Keywords

HIV DNA; reservoir; monocytes; gray matter; nucleus accumbens; brainstem; HIV-associated neurocognitive impairment

INTRODUCTION

Potent antiretroviral therapy has transformed HIV/AIDS to a chronic disease by effectively reducing plasma viral load (HIV RNA)[1], reconstituting the immune system [2, 3] and inhibiting opportunistic infections.[4] Still, many HIV-infected individuals on combination antiretroviral therapy (cART) harbor detectable HIV DNA in peripheral blood mononuclear cells (PBMCs). Elevated PBMC HIV DNA correlates with HIV-associated dementia (HAD) [5] and with milder neuropsychological deficits as well.[6] This PBMC HIV DNA is representative of cellular HIV reservoirs that are established early in infection.[7] Entry of the virus into the brain is believed to occur by way of blood-brain barrier transmigration of monocytes, cells which express CD14 on their surfaces.[8] The burden of HIV DNA within the activated $CD14^+$ monocyte subset has been implicated in cognitive dysfunction.[5, 9] Peripheral HIV reservoirs correlate to disease progression [10, 11] and are a major target of HIV eradication efforts.

Prior work has linked detectable PBMC HIV DNA (≥ 10 copies/10^6 cells) to cortical and subcortical gray matter atrophy in patients on cART who have undetectable plasma HIV RNA levels. [12, 13] However, the relationship between peripheral HIV DNA and brain structure has not been investigated in treatment-naïve subjects with HIV infection; nor have evaluations used HIV DNA isolated from CD14+ monocytes. In the Southeast Asia Research Collaboration with Hawaii (SEARCH) 001 Cohort Study, failure to eliminate HIV DNA in monocytes was associated with continued neurocognitive impairment more than three years after initiating cART.[14] A recent analysis of the cART-naïve HIV+ cohort analyzed in the current paper (SEARCH 011) found that high HIV DNA levels in CD14+ monocytes increased the risk of HIV-associated neurocognitive disorders (HAND).[15] As shown by a receiver operator characteristic (ROC) curve, CD14+ HIV DNA ≥ 45 copies/10^6 cells had a sensitivity of 86% and a specificity of 70% for identifying HAND. Furthermore, proton magnetic resonance spectroscopy (MRS) identified markers of brain neuronal injury and glial dysfunction that were positively linked to CD14+ HIV DNA.[15] We now examine the relationship of CD14+ HIV DNA to regional brain volumes.

METHODS

Subject selection

SEARCH 011 (NCT00782808) was designed as a prospective study to determine the predictive ability of CD14+ HIV DNA for HAND, to assess brain injury by MRS, and to

identify abnormal cytokine profiles in cerebrospinal fluid (CSF) among Thai subjects beginning cART for the first time. Community clinicians referred subjects who met Thai Ministry of Public Health criteria for treatment initiation (CD4 count <350 cells/mm^3 or symptomatic disease).[16] Due to feasibility issues, subjects were screened for levels of PBMC HIV DNA rather than CD14+ HIV DNA, since this required less real-time work at screening and because PBMC HIV DNA had correlated strongly to CD14+ HIV DNA in previous studies.[14, 17] Based on the distribution of our preliminary data, and using a double-blind study design with all clinical staff blinded to HIV DNA levels and laboratory technicians to clinical data, our goal was to enroll 30 cases with more than and 30 cases with fewer than 1000 copies of HIV DNA per 10^6 PBMCs. We further stratified each HIV DNA group by age (greater or less than 35 years) to minimize clustering by age within HIV DNA strata, which could impact cognition.

Subjects were excluded from the study for reasons of head injury, any acute concurrent illness, pre-existing neurologic or psychiatric conditions, learning disability, past substance dependence, and current use of illicit drugs or a positive urine toxicology test completed at two separate points (screening and entry). We aimed for 60 subjects and enrolled 63 in all. Two subjects were excluded during the entry visit because of opportunistic brain infections (toxoplasmosis and tuberculosis), and a third was dropped from analysis on account of unacceptable MRI data quality caused by noise during acquisition. The result was a sample of 60 cases. All subjects signed consent forms approved by the University of California (San Francisco, CA) and the Chulalongkorn Hospital (Bangkok, Thailand) institutional review boards.

Cognitive characterization

Trained nurses performed neuropsychological (NP) testing using a battery developed by the World Health Organization (WHO) for HIV international assessments and modified slightly for feasibility, as previously described.[18, 19] The battery evaluated NP domains found to be important for HIV. The study neurologist conducted an HIV disease-directed neurological examination developed by the AIDS Clinical Trials Group. Physicians and nurses independently interviewed subjects about functional limitations due to cognitive impairment. Proxy informants, when available, also provided information about participants' cognitive and functional status. Cognitive diagnoses were determined by a consensus conference that included the principal investigator (VV), a US HIV neurologist and a US HIV-trained neuropsychologist guided by the 2007 ("Frascati") diagnostic criteria as explained earlier.[15]

Cell separation and HIV DNA quantification

Detailed methodologies for cell separation and HIV DNA assessment are provided elsewhere.[15] Briefly, monocytes were purified by magnetic bead positive separation according to the manufacturer's guidelines (MiltenyiBiotec). The purity of the CD14+ cells was 91.9% (min: 76.9%; max: 98.7%), as determined by multi-parameter flow cytometry on every fifth sample for the first 42 cases. CD14+ separated cells were then frozen in dimethyl sulfoxide (DMSO) and shipped in batches to the University of Hawai'i for HIV DNA assays using standard techniques.[20]

Neuroimaging

Study participants underwent magnetic resonance imaging (MRI) on a GE Signa HDx 1.5-Tesla scanner (GE Healthcare, software v12-M4) with an 8-channel head coil and a standard body coil. For each subject, a high-resolution anatomical volume was acquired with an axial 3D T1-weighted spoiled gradient echo sequence (TE = 7 ms, TR=11.2 ms, flip angle= 25°, 1 mm^3 resolution). Structural (T1-weighted) MRI data were securely transferred and processed by one author (EB) using FreeSurfer (version 5.1.0, http://www.nmr.mgh.harvard.edu/freesurfer).[21–23] The procedures include skull-stripping, intensity normalization, Talairach transformation, segmentation of subcortical white matter and deep gray matter structures, and cortical gray/white matter boundary and pial surface reconstruction. Quality assurance of FreeSurfer data processing was done by visual inspection, and cortical surfaces and subcortical segmentations were checked prior to volumetric group analysis. FreeSurfer's estimate of intracranial volume (ICV) is a standard, reliable measure for regional brain volume normalization.[24]

Statistical analysis

We used the ROC-optimized value [15] to dichotomize our study sample into low (CD14+ HIV DNA < 45 copies/10^6 cells) and high (CD14+ HIV DNA ≥ 45 copies/10^6 cells) HIV DNA groups. Group comparisons of demographic and clinical variables were conducted by t-test or chi-squared test. Analysis of covariance (ANCOVA), controlling for age, CD4 and ICV, evaluated the associations between HIV DNA group and selected volumes of interest: caudate, putamen, thalamus, globus pallidus, hippocampus, amygdala, nucleus accumbens, brainstem, corpus callosum, cortical and subcortical gray matter, cerebral white matter, cerebellar gray and white matter, total gray matter and lateral ventricles. CD4 T-lymphocyte count was log-transformed prior to inclusion in the model. Plasma HIV RNA, a measure of viral control, was dichotomized at 100,000 copies/mL and was tested as a categorical variable both independently and together with CD14+ HIV DNA group in *post-hoc* ANCOVA. The analyses utilized StatView 5.0 (SAS Institute Inc., Cary, NC). We defined statistical significance by $p < 0.05$, and trends toward significance by $0.05 \leq p < 0.1$.

RESULTS

The subjects (34.7 ± 7.0 years old; 34 [57%] female) were enrolled during March 2009 – December 2011. Urine drug screens confirmed that no study participants were using illicit substances or taking psychiatric medications, including methadone, narcotics, and antidepressants. By consensus diagnosis, 32 of 60 (53%) were cognitively normal, 14 had asymptomatic neurocognitive impairment (ANI), 8 had mild neurocognitive disorder (MND), and 6 met criteria for HAD. Plasma HIV RNA levels were < 100,000 copies/mL in 36 subjects (60%). The low (N=24) and high (N=36) HIV DNA groups did not differ at baseline in age, education, gender, CD4 T-lymphocyte count, or plasma or CSF HIV RNA (Table 1). Plasma HIV RNA level showed a moderate negative correlation with CD4 T-lymphocyte count (R^2=0.20, p<0.001) and did not correlate with CD14+ HIV DNA.

Compared to the group with low plasma viral load, subjects with high HIV RNA levels had larger volumes of lateral ventricles (12,573.0 ± 7117.4 ml^3 vs. 8375.0 ± 4632.0 ml^3;

p=0.016) independently of age and ICV. Volumes of other brain regions did not differ between the high and low plasma viral load groups. Regional volumes for the high and low HIV DNA subject groups are presented in Table 2. Higher CD14+ HIV DNA was significantly linked to decreased volumes of nucleus accumbens, brainstem and total gray matter. Subcortical gray matter volume showed a negative trend association with CD14+ HIV DNA. These relationships were independent of age, CD4 count and ICV. There was no statistically significant difference in lateral ventricular volume between high and low HIV DNA groups. CSF HIV RNA did not have a significant effect and was not retained as a covariate in the model. When HIV DNA and plasma HIV RNA groups were simultaneously entered as factors in the ANCOVA, adjusting for age and ICV, plasma HIV RNA had no main effect on volumes of any brain regions except the lateral ventricles (p=0.003 for HIV RNA; p=0.902 for HIV DNA). Interaction effects of CD14+ HIV DNA and plasma HIV RNA groups were not significant.

The three brain regions with significant volumetric differences by HIV DNA group (brainstem, nucleus accumbens, total gray matter) underwent repeated *post-hoc* ANCOVA within the high and low plasma HIV RNA subject groups, covarying for age, CD4 count and ICV. High HIV DNA was associated with smaller nucleus accumbens volume (p=0.006) in subjects with high HIV RNA, and with smaller brainstem volume (p=0.087) in those with low viral load. The association between HIV DNA and total gray matter volume, which was marginally significant (p=0.045) within the entire study sample, did not reach significance in the smaller groups with high or low plasma HIV RNA.

DISCUSSION

Baseline data from this study upheld the hypothesis that the reservoir burden of HIV DNA in peripheral monocytes (CD14+) correlates with damage in brain regions known to be affected by HIV. Moreover, our data add credence to growing concern that the deferral of therapy until progression to symptomatic HIV disease may be accompanied by structural brain changes directly associated with intracellular HIV DNA, although the lack of a comparison group with high CD4 T-lymphocyte counts makes it difficult to draw firm conclusions.

The nucleus accumbens and brainstem were significantly smaller in subjects with CD14+ HIV DNA ≥ 45 copies/10^6 cells than in those with CD14+ HIV DNA < 45 copies/10^6 cells. The nucleus accumbens is of relevance as its volumetric reduction in HIV+ patients has been correlated with apathy.[25] Animal models have linked dysfunction of the nucleus accumbens to attentional deficits [26–28], which are characteristic of HIV-related cognitive impairment. In the current study, our result is subject to the caveat that nucleus accumbens volume is difficult to obtain by automated algorithms, including that of FreeSurfer. When compared to the gold standard of manual segmentation, larger errors have been reported for volumes of nucleus accumbens and globus pallidus due to the disproportionate effect of discrepancies in the boundary segmentation of small brain structures.[29] CD14+ HIV DNA ≥ 45 copies/10^6 cells was also associated negatively with total gray matter volume and with a trend toward decreased volume of subcortical gray matter.

Our findings are broadly consistent with our earlier work associating regional brain atrophy with detectable PBMC HIV DNA in cART-treated, virally suppressed HIV+ study participants in Hawaii. However, some differences are evident. In particular, the previous volumetric study noted smaller subcortical and cerebellar gray matter volumes in subjects with detectable PBMC HIV DNA. Discrepancies between the current and past work are attributable to dissimilar patient characteristics and study methods. The SEARCH 011 and Hawaii cohorts differed in subject ethnicity, viral clade, use of cART, and suppression of HIV RNA. Additionally, because peripheral monocyte HIV DNA reservoirs have been increasingly implicated in the persistence of HAND, SEARCH 011 focused on HIV DNA in CD14+ monocytes whereas our earlier studies included T-lymphocyte HIV DNA in the total PBMC pool.

We considered the possibility that high viral load, low CD4 count, etc. (factors characteristic of an untreated population such as the SEARCH 011 cohort) may confound the impact of CD14+ HIV DNA. In an attempt to mitigate such effects we included analyses that dichotomized HIV RNA levels as high vs. low. This approach had little impact on our findings but does not exclude the possibility that other processes occurring in the absence of cART (e.g., inflammation) alter brain volumes and hence affect the associations we investigated. Because the reported duration of HIV infection is unreliable in the setting of our study, we did not consider this variable in our models. Higher plasma viral load (but not CD14+ HIV DNA) was significantly associated with dilatation of the lateral ventricles, a manifestation of central cerebral atrophy. At the same time, associations of elevated CD14+ HIV DNA with decreased total and subcortical gray matter volumes indicate that HIV DNA, as well, may contribute to central atrophy. As our previous work did identify a significant association between lateral ventricular enlargement and elevated PBMC HIV DNA[13], we surmise that high plasma HIV RNA may mask relationships between HIV DNA and brain structure that are more apparent in treated HIV+ patients. Effects of viral load may contribute to the lack of associations between HIV DNA and volumes of basal ganglia and striatum. Subjects with high CD14+ HIV DNA would perhaps show significant volumetric decreases in multiple brain regions compared to an HIV-negative control group, but such assessments were not made.

In summary, CD14+ HIV DNA ≥ 45 copies/10^6 cells is associated with reduced volumes of selected brain regions in treatment-naive HIV+ Thai subjects, corroborating previous reports linking HIV DNA to HAND and to MRS-derived markers of brain inflammation. Our findings strengthen converging evidence of the role of monocytes in the trafficking of HIV to the brain. Early treatment may be indicated to prevent the establishment and persistence of viral reservoirs that lead to cumulative brain injury.

Acknowledgments

We thank our study participants for their contributions. This study was supported by R01 NS061696 (VV)), U54MD007584 (BS, CS), R01NS053345 (BS), and F31AI081450 (MA). The SEARCH 011 Study Group includes, from UCSF: Lauren Wendelken, Akash Desai, Nicholas Hutchings; from SEARCH: Nitiya Chomchey Supunee Jirajariyavej Pradit Chaisit, Yothin Chinvarun, Phayao Thongkramjaroen, Sumit Thongmuang, Sirichai Jarupittaya, Prapian Khongtia, Tanate Jadwattanakul, Pornpen Methajittiphun, Pravit Mingkwanrungruang, Thep Chalermchai, Nittaya Phanuphak, Eugene Kroon, Nipat Terratakulpisarn, Duanghathai Suttichom, Somprartthana Rattanamanee, Michittra Boonchan.

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Table 1

Demographic and clinical characteristics (mean ± s.d., or N) of study population.

Variable	Low CD14+ HIV DNA (< 45 copies/10^6 cells)	High CD14+ HIV DNA (≥ 45 copies/10^6 cells)	*p*
N	24	36	--
Gender (male)	12	14	0.56
Age (years)	35.2 ± 6.4	34.4 ± 7.5	0.69
Education (years)	11.4 ± 4.6	11.1 ± 4.9	0.80
CD4 (cells/mm^3) (min -max)	239.1 ± 130.3 (24–432)	227.5 ± 142.6 (23–553)	0.76
log_{10} (plasma HIV RNA)	4.71 ± 0.71	4.93 ± 0.73	0.26
log_{10}(CSF viral load)	3.94 ± 0.99	4.08 ± 0.82	0.62
CD14+ HIV DNA (copies/10^6 cells) (min-max)	19.5 ± 9.1 (10.0–37.0)	160.9 ± 158.2 (45.0–724.0)	<0.0001

P-values were computed by chi-squared or t-test. For CSF viral load, *n*=18 for the low HIV DNA group and *n*=25 for the high HIV DNA group.

Table 2

Brain regional volumes (mean ± s.d.) in mm^3 for high and low CD14+ HIV DNA subject groups.

Brain region	Low HIV DNA (N=24)	High HIV DNA (N=36)	*p*
Corpus callosum	3044.96 ± 416.63	2865.06 ± 341.10	0.135
Brainstem	19,211.00 ± 3096.09	17,523.081 ± 1917.51	**0.033**
Thalamus	13,253.58 ± 1392.82	12,604.81 ± 1184.10	0.115
Caudate	6620.46 ± 688.51	6438.56 ± 793.29	0.571
Putamen	10,411.71 ± 1152.89	10,076.28 ± 1113.60	0.520
Globus pallidus	3098.96 ± 437.09	2996.61 ± 337.16	0.534
Hippocampus	8707.83 ± 1082.94	8176.17 ± 955.30	0.127
Amygdala	3139.54 ± 539.36	2915.89 ± 385.02	0.146
Nucleus accumbens	1228.38 ± 286.04	1064.42 ± 195.21	**0.021**
Cerebellar gray matter	96,601.96 ± 14,794.45	90,543.42 ± 11,648.79	0.205
Cerebellar white matter	25,555.42 ± 3435.41	24,194.28 ± 3670.74	0.364
Subcortical gray matter	170,245.88 ± 21,979.89	159,741.67 ± 15,420.27	0.073
Cortical gray matter	430,814.53 ± 42,953.45	410,606.54 ± 37,892.17	0.101
Cerebral WM	448,941.97 ± 62,096.01	424,394.91 ± 44,337.71	0.232
Total gray matter	601,060.40 ± 59,894.84	570,348.20 ± 49,084.61	**0.045**
Lateral ventricles	10,145.75 ± 6351.26	9993.11 ± 5956.19	0.966

Effects of CD14+ HIV DNA on volumetric group differences were assessed by ANCOVA controlling for age, log CD4 and ICV.