A peripheral monocyte interferon phenotype in HIV infection correlates with a decrease in magnetic resonance spectroscopy metabolite concentrations

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Abstract

Objective—In spite of effective antiretroviral therapy (ART), cognition is impaired in upwards of 35% of the HIV-infected population. We investigated a possible link between peripheral immune activation and brain metabolite concentrations.

Design and methods—Thirty-five HIV-seropositive (HIV+) and eight HIV-seronegative adults were recruited to this cross-sectional study. All HIV-positive patients were on ART or a treatment interruption. Participants were evaluated for monocyte gene expression, cognitive status, and brain metabolite concentrations using 4-Tesla short echo-time proton magnetic resonance spectroscopy. Absolute concentrations of brain metabolites in the frontal white matter (FWM), anterior cingulate cortex (ACC), and basal ganglia were derived and related to monocyte gene expression and global deficit scores.

Results—Analysis of monocyte gene arrays revealed an interferon (IFN)-\(\alpha\)-induced activation phenotype. Fourteen genes having the greatest fold increase in response to HIV were IFN genes. Monocyte activation as measured by gene expression profiles strongly correlated with lower N-acetylaspartate (NAA) in FWM. The IFN response gene Interferon-gamma inducible protein-10 (IP-10) was activated in monocytes from HIV individuals and strongly correlated with plasma protein levels. Plasma IP-10 correlated significantly and inversely with ACC NAA, which was lower in HIV-positive patients with mild compared to no cognitive impairment.

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Conflicts of interest

There are no conflicts of interest.
Conclusion—Chronic peripheral immune activation driven by a type 1 IFN correlates with neuronal injury in FWM and ACC and cognitive dysfunction. Easily measured IFN-induced blood markers may be clinically significant in following early neural cell damage.

Keywords
cognitive symptoms; gene array; interferon; IP-10; magnetic resonance spectroscopy; monocyte; neuroimaging

Introduction
In spite of antiretroviral therapy (ART), individuals chronically infected with HIV-1 (HIV+) continue to show immune activation and neurocognitive dysfunction [1]. Central nervous system (CNS) involvement in the ART era has transitioned from a life-threatening HIV-associated dementia to milder forms of cognitive impairment, which have been collectively named HIV-associated neurocognitive disorder (HAND) [2]. Neurological impairment in chronically infected HIV-positive individuals on ART is associated with measures of brain chemistry obtained by $^1$H magnetic resonance spectroscopy (MRS) [3,4]. Many years of HIV research show that a decrease of the neuronal marker N-acetylaspartate (NAA) together with an increase in one or more glial activation-related metabolites [i.e., myoinositol (mI) or choline-containing metabolite (Cho)] is a hallmark of HAND with larger metabolic abnormalities generally related to cognitive deficits [3–6]. Recent studies also show that measures of lower cerebral glutamate (Glu) or glutamate and glutamine (Glx) correlate with impaired cognitive performance [4,7,8].

We previously reported a type 1 interferon (IFN)-α-driven monocyte phenotype in individuals with chronic HIV infection that correlated with viral load [9,10]. Here, we show that a monocyte IFN-α activation profile and IFN-gamma-inducible protein-10 (IP-10, CXCL10) in the plasma of HIV-positive individuals correlated with lower concentrations of NAA in the frontal white matter (FWM) measured by $^1$H MRS.

Methods
Research participants
Participants were recruited from the San Francisco VA Medical Center (SFVAMC) based on their HIV status and viral load; neuropsychological status was not a consideration for enrollment. All participants provided written consent previously approved by the University of California, San Francisco Committee on Human Research. Forty three participants, 35 HIV-positive and eight HIV-negative controls, completed neuroimaging, neuropsychological testing, and blood draws for monocyte gene expression and plasma assays. All HIV-positive individuals were on stable ART or treatment interruption. Neuropsychological testing assessed seven domains of cognitive function and cognitive impairment was defined as more than 1.5 SD below the norm in at least two domains [9]. A global deficit score (GDS) was calculated with 0 being normal and a GDS at least 0.5 defined as mildly impaired [11].
**Image acquisition**

MRS data were obtained on a Bruker MedSpec 4T system with Siemens Trio console. Three-dimensional imaging (T1-weighted sagittal MP-RAGE with TR/TE = 3000/1200/3.4 ms and T2-weighted spin-echo with TR/TE = 5000/1900/355 ms) covered the entire head at 1 mm isotropic resolution. Short echo-time (TE) single volume STEAM spectra (TR/TE/TM = 2000/12/10 ms, spectral width = 2000 Hz, 128 scans = 4 : 24 min) were obtained from three volumes of interest (VOIs) corresponding to different tissue types: mid-frontal centrum semiovale (FWM, 15 × 25 × 20 mm$^3$); anterior cingulate cortex (ACC, cortical gray matter, 20 × 20 × 20 mm$^3$) and basal ganglia (deep gray matter, 17 × 35 × 15 mm$^3$). A STEAM spectrum without water signal suppression was also acquired from the same VOI to aid in calculating absolute metabolite concentrations.

**Spectral data processing and metabolite quantitation**

All processing methods have been previously described including quantitative spectral processing methods using software tools developed in-house [12]. Metabolite quantification used an approach that has been extensively described, validated, and used in many magnetic resonance laboratories. Cerebrospinal fluid (CSF) contributions to the VOIs were derived from VOI positions overlaid on segmented T1-weighted images, allowing absolute molar quantitation of individual metabolite levels expressed in institutional units (IU).

**Monocyte gene microarray**

Monocyte isolation, gene expression microarrays, and statistical methods have been previously described [9,10]. Total RNA was isolated and hybridized to Codelink Human Whole Genome Bioarrays (Applied Microarrays, Tempe, Arizona, USA). Microarray data were normalized with Loess normalization using R [13] and Bioconductor package [14]. Differential gene expression, significance, fold changes, and patterns were determined using GeneSpring GX 7.3 software package (Agilent, Santa Clara, California, USA). $P$ values of two-sample $t$-tests for differential genes of group comparisons were adjusted using Benjamini and Hochberg’s false discovery rate method [15] in GeneSpring.

**Plasma IP-10 assay**

Plasma IP-10 concentration was quantified using a Bio-Plex assay (Bio-Rad, Luminex, Hercules, California, USA). Data were analyzed using Student’s $t$-test in SAS 9.1 (SAS Inc., Cary, North Carolina, USA).

**Statistical analysis**

Two-tailed $t$-tests with equal variance were used to compare plasma and clinical variables for control and HIV-positive group comparisons. One-tailed $t$-tests were used for MRS group comparisons of brain metabolite levels as we postulated directionally specific concentration changes with HIV infection based on abundant literature. Spearman’s correlation coefficients were used to describe the relationships between gene expression, metabolite concentrations, and plasma variables. $P$ values were adjusted using false discovery rate multiple testing corrections described by Benjamini and Hochberg [15]. All statistical analyses were performed using SAS 9.1 or R.
Results

Study population and clinical variables

All 43 participants were men, aged 30–66 years (mean 50.2 × 7.5), predominantly white (56%) and African–American (26%), and well educated (mean 14.5 years, range 11–20). The 35 HIV-positive individuals were chronically infected for a mean of 17 years and were on ART or in treatment interruption. Nineteen of the individuals in HIV-positive group had undetectable (<1.7 log_{10} RNA copies/ml) or low viral loads (LVLs; <4 log_{10} RNA copies/ml) and 16 had high viral loads (HVLs; ≥4 log_{10} RNA copies/ml). Based on neuropsychological tests, 16 of the 35 HIV-positive individuals were mildly impaired (mean GDS = 0.49 ± 0.42), whereas 19 of them (54%) showed no impairment [9]. There was no difference in impaired versus nonimpaired groups with respect to current categories of CNS-penetrating antiretrovirals [9,16].

HIV infection alters brain metabolite levels

When comparing HIV-positive individuals with HIV-negative controls, we found infection was linked to higher Cho (P = 0.008) and mI (P = 0.05) concentrations in FWM largely consistent with previous reports (Table 1) [3,5,17]. There was a trend toward reduced Glu concentration in the basal ganglia (P = 0.05), and this reduction did not parallel a corresponding decrease in NAA. As a group, HIV-positive individuals did not show significant NAA decreases in any brain region tested.

Peripheral immune activation correlates with brain metabolite concentrations

The top 14 differentially expressed monocyte genes with a greater than five-fold increase in activation were all IFN-responsive transcripts. We compared the 14 genes individually with the five metabolite concentrations from the three brain regions. In the FWM, lower NAA correlated significantly with seven IFN genes following correction for multiple comparisons (Fig. 1a).

IP-10 gene expression and plasma levels correlate with brain metabolite levels

In addition to identifying a peripheral mechanism that might correlate with brain metabolites, a goal of this study was to determine whether any IFN-associated blood marker(s) might be related to brain metabolite dysregulation. We chose plasma IP-10 as it was a gene that was overexpressed on monocytes in HIV-positive individuals (elevated 3.8-fold), can be easily measured in blood from HIV-positive individuals [18], and is associated with HIV disease progression [19], neurotoxicity [20], and possibly HIV dementia [19,21]. IP-10 monocyte gene expression correlated with plasma levels (R = 0.61, P < 0.001; Fig. 1b). IP-10 plasma concentrations showed a highly significant inverse correlation with ACC NAA concentrations (R = −0.69, P < 0.001; Fig. 1c) and FWM Cho (R = −0.55, P = 0.004; Fig. 1d). Of all three regions examined and five metabolites, only these two correlations remained significant after corrections for multiple comparisons.
**Cognitive function and brain metabolite levels**

When comparing HIV-positive individuals for ACC NAA based on GDS, despite a relatively high missing data rate due to MRS acquisition, 11 mildly impaired individuals (mean GDS = 0.91 ± 0.42, mean ACC NAA = 7.15 ± 0.76 IU) had a trend toward lower ACC NAA versus 15 nonimpaired individuals (mean GDS = 0.19 ± 0.16, mean ACC NAA = 7.81 ± 0.98 IU; n = 26, P = 0.031, one-tailed t-test on ACC NAA levels). None of the other metabolite concentrations differed as a function of GDS status.

**Discussion**

Our results demonstrated a strong association between high IFN-α-driven peripheral monocyte transcripts and lower concentrations of the neuronal marker NAA in the FWM. This has also been observed in simian immunodeficiency virus (SIV)-macaque models in which immune responses in the periphery directly influence CNS damage [22]. In humans, an activated peripheral phenotype correlated with neuronal dysfunction related to lower NAA levels in the cortex [23]. Several studies including our own are consistent with IFN-α driving immune activation in the periphery [10,24,25] and the brain [26]. These reports underscore the significance of cognition and NAA loss in the FWM and the correlations with several blood and CSF markers. Our findings suggest that an increase in peripheral activation that accompanies an increase in viral load correlates with a decrease in NAA.

The IFN-driven plasma protein IP-10 has previously been reported to be elevated in HIV and SIV infection [10,21,27]; however, this is the first report of an inverse correlation with ACC NAA concentrations. In a reported post-hoc analysis, higher CSF levels of IP = 10 correlated with lower FWM NAA/Cr ratios (Spearman’s correlation $R = -0.43$ and $P = 0.003$, not adjusted for multiple comparisons) [28]. Our study shows higher IP-10 in the plasma inversely correlating with ACC NAA ($R = -0.69$, $P < 0.001$ with adjustment for multiple comparisons) suggesting that the peripheral IFN response is a robust measure of neuronal changes.

Whereas we examined the relationship between periphery and brain metabolites, others have compared metabolites with CSF markers. Macrophage colony-stimulating factor (M-CSF) was followed as a marker of activation before and during ART and correlated with MRS [29]. At 3 months after treatment initiation, reduced CSF M-CSF and viral load were observed concurrent with increased NAA in several brain regions. In another study, CSF macrophage chemoattractant protein (MCP-1, CCL2) levels inversely correlated with MRS-derived Glx (glutamate and glutamine together) levels in the basal ganglia [30]. Also, higher CSF MCP-1 levels correlated with cognitive impairment that returned to normal after therapy; however, sustained high CSF MCP-1 correlated with a glial response [31].

In a recent study, low Glu in the FWM or parietal gray matter was associated with cognitive deficits [8] or HIV dementia [4] and may indicate mitochondrial and glial injury as well as altered glutamate neurotransmission [7,8]. Here, we report a trend to lower Glu in the basal ganglia of HIV-positive individuals versus HIV-negative controls. Taken together, glutamatergic dysregulation appears to be present in anterior brain regions and may be of clinical significance.
Our finding that monocyte gene expression and IP-10 levels in the periphery are linked to FWM NAA suggests these would be useful biomarkers for individuals at risk for cognitive impairment. Also, by targeting IFN-α activation with adjunctive therapies, it might be possible to reduce neurocognitive impairment associated with infection in today’s aging HIV population.

Acknowledgments

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References


Fig. 1. Correlations between interferon-α genes, IP-10 plasma levels, and brain metabolites from monocytes of HIV-positive individuals

(a) Correlation between the top seven overexpressed interferon (IFN)-α genes on monocytes that correlated significantly with a decrease in frontal white matter (FWM) N-acetylaspartate (NAA). (b) Significant correlation between IP-10 monocyte gene expression intensity and its plasma levels from HIV-positive individuals. Cross indicates IP-10 levels (mean ± SD) from controls. (c) Significant inverse correlations were found between plasma IP-10 levels and anterior cingulate cortex (ACC) NAA and (d) FWM Cho. C to the right of panels (c) and (d) are HIV-negative controls (mean ± SD) for corresponding metabolite levels. Spearman’s correlation coefficient [R] and P values are presented in each panel. All correlations remained significant after multiple comparison adjustments.
**Table 1**

Brain metabolite concentrations by magnetic resonance spectroscopy (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>Glu</th>
<th>Cho</th>
<th>mI</th>
<th>Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frontal white matter</strong></td>
<td></td>
<td></td>
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<tr>
<td>HIV-negative (n = 7)</td>
<td>5.68 (± 0.30)</td>
<td>5.00 (± 0.21)</td>
<td>1.13 (± 0.22)</td>
<td>4.90 (± 0.61)</td>
<td>5.66 (± 0.48)</td>
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<tr>
<td>HIV-positive (n = 30)</td>
<td>5.55 (± 0.69)</td>
<td>4.91 (± 0.76)</td>
<td>1.33 (± 0.19)</td>
<td>5.24 (± 0.50)</td>
<td>5.63 (± 0.65)</td>
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<tr>
<td>P value</td>
<td>0.224</td>
<td>0.299</td>
<td>0.008</td>
<td>0.053</td>
<td>0.454</td>
</tr>
<tr>
<td><strong>Anterior cingulate cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-negative (n = 5)</td>
<td>7.30 (± 1.24)</td>
<td>4.68 (± 0.86)</td>
<td>1.51 (± 0.30)</td>
<td>5.03 (± 0.33)</td>
<td>5.85 (± 1.02)</td>
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<td>HIV-positive (n = 26)</td>
<td>7.53 (± 0.93)</td>
<td>4.90 (± 0.80)</td>
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<tr>
<td>P value</td>
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<td>0.292</td>
<td>0.093</td>
<td>0.153</td>
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<td><strong>Basal ganglia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HIV-negative (n = 6)</td>
<td>5.73 (± 0.70)</td>
<td>5.37 (± 0.67)</td>
<td>1.63 (± 0.22)</td>
<td>2.73 (± 0.81)</td>
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<td>HIV-positive (n = 28)</td>
<td>5.65 (± 0.89)</td>
<td>4.86 (± 0.68)</td>
<td>1.65 (± 0.20)</td>
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<tr>
<td>P value</td>
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<td>0.051</td>
<td>0.380</td>
<td>0.389</td>
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</tr>
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</table>

Values are presented as absolute metabolite concentrations (institutional units). P values represent the difference between the HIV-negative controls and HIV-positive groups using one-tailed t-tests. Cho, choline-containing metabolite; Cr, creatine; Glu, glutamate; mI, myoinositol; NAA, N-acetylaspartate.