

Antiretroviral treatment alters relationship between MCP-1 and neurometabolites in HIV patients

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Objective: The relationships between neurometabolites and macrophage chemoattractant protein (MCP-1) in serum and cerebrospinal fluid (CSF) were evaluated in HIV patients before and after antiretroviral treatment.

Design: Prior studies found higher CSF MCP-1 levels in patients with HIV-associated dementia compared to those in neuroasymptomatic. We hypothesized that CSF MCP-1 levels would correlate inversely to neuronal metabolites [including N-acetyl compounds, glutamate+glutamine, as assessed by principal component analyses (PCA)] and positively to glial metabolites (including myo-inositol and choline compounds).

Methods: Thirty-nine antiretroviral-naïve HIV patients were evaluated prospectively with proton magnetic resonance spectroscopy (¹H MRS), and serum and CSF MCP-1 measurements prior to highly active antiretroviral therapy (HAART); 31 of these patients completed follow-up studies after 3 months of HAART but only 24 had follow-up CSF studies.

Results: After HAART, brain metabolites and clinical signs showed no change despite improvements in systemic (CD4 counts, plasma viral load, MCP-1) and CSF (viral load and MCP-1) variables. CSF, but not serum, MCP-1 levels correlated inversely with the neuronal component (from PCA) prior to treatment ($r=-0.59$, $P=0.0008$). Conversely, after 3 months of HAART, the glial component (from PCA) correlated positively with CSF MCP-1 levels ($r=0.70$, $P=0.0002$; ANCOVA interaction for treatment status, $P=0.003$).

Conclusions: These findings suggest that higher CSF MCP-1 levels are associated with neuronal dysfunction in untreated patients. After 3 months of HAART, the decreased systemic factors (viral burden, systemically derived MCP-1) no longer associate with neuronal dysfunction, but subjects with the strongest glial response in the brain continue to produce the highest levels of MCP-1.

Introduction

HIV patients with normal-appearing structural brain imaging may demonstrate abnormalities in cerebral blood flow [1-3], cerebral glucose metabolism [4,5], brain activation [6,7] and neurometabolites [8-15]. The neurometabolite abnormalities measured with *in vivo* proton magnetic resonance spectroscopy (¹H MRS) in HIV patients include decreases in the neuronal marker N-acetyl compounds [NA] and elevation of the putative glial markers, myo-inositol [MI] and choline compounds [CHO]. These metabolite abnormalities may be reversible with antiretroviral [16,17] or neuroprotective treatment [18], and thus may serve as clinically useful non-invasive surrogate markers to monitor the effects of treatment on the brain pathology of HIV-infected individuals. However, the relationships between these neuroimaging measures and systemic factors are not well understood. Simultaneous assessments of plasma and cerebrospinal fluid (CSF) viral loads, immune markers and

neuroimaging data in the same individuals should provide valuable insights into the pathogenesis of HIV-associated brain injury.

Several reports have shown strong relationships between several neurometabolites measured by ¹H MRS, and CD4 count, AIDS dementia stage [9,12-14] or neuropsychological tests [19]; however, the relationships between these brain metabolites, and systemic or central nervous system (CNS) chemokine levels have not been evaluated. Since the effect of antiretroviral medications on the immune status in HIV patients may differ depending on treatment regimen, duration and the degree of viral suppression, the assessment of this relationship in medication-naïve patients before and after treatment would be optimal.

In vitro and human studies strongly indicate that a major pathway for neuropathogenesis of HIV dementia is CNS invasion by HIV-infected and uninfected monocytic cells. Monocytes release or transport

neurotoxic proteins (Tat, gp120, cytokines) that may lead to neuronal dysfunction and glial activation. The mechanisms responsible for an increase in the number of monocytes within the CNS are likely to be multiple, including activation of circulating monocytes, as well as monocytic and brain parenchymal cell release of blood–brain barrier degrading proteases [20]. Numerous studies suggest that localized expression of select adhesion molecules and chemokines is also critical, and of the latter, the release of monocyte chemoattractant protein 1 (MCP-1) may be especially important. MCP-1 is a CC chemokine that is released by cells including astrocytes, which are particularly numerous in the CNS, as well as by neurons, monocyte-derived cells and endothelial cells [21]. A variety of stimuli can increase MCP-1 release and interestingly, animal studies have shown that even peripheral blood injection of pro-inflammatory cytokines is followed by a rapid increase in MCP-1 expression by brain astrocytes [22]. MCP-1 regulates the migration of peripheral blood mononuclear cells through the blood–brain barrier [23] and exacerbates the cascade of toxin release that further recruits infected cells into the brain. As compared to other monocyte chemoattractants, which include RANTES, macrophage inflammatory protein-1 α (MIP-1 α), MIP-1 β , MCP-2 β and MCP-3, MCP-1 is the most potent [24]. It has also been suggested that this particular chemokine may be uniquely essential to monocyte recruitment *in vivo* [25,26]. Moreover, elevated levels of MCP-1 may be associated with the Th1 to Th2 shift that favours AIDS progression [25].

MCP-1 is elevated in the brains of AIDS dementia patients [27], and an MCP-1 promoter polymorphism that is associated with increased MCP-1 expression is associated with accelerated HIV disease progression and increased risk for HIV dementia [28]. Elevated CSF MCP-1 in HIV patients also correlates with high CSF, but not plasma, HIV RNA levels [29] and with high CSF-to-plasma HIV RNA ratio [30].

Emerging data indicate that MCP-1 in the CNS leads to selective monocyte recruitment that might exacerbate neuronal injury in a variety of diseases, including stroke [31], Alzheimer's disease [32] and various trauma models [21,33]. Similarly, in HIV patients, CSF MCP-1 levels correlated with clinical staging of dementia [27,34], and in simian immunodeficiency virus (SIV)-infected macaques, elevated MCP-1 levels in CSF preceded and predicted moderate or severe encephalitis [35]. In addition to the monocyte infiltration and repair process by reactive astrogliosis in response to neuronal injury, the neurotoxic protein released by HIV-infected cells can further stimulate astrocytes to release MCP-1 [23,27]. Therefore, higher CSF MCP-1 levels would likely be

related to greater neuronal injury and glial response in HIV-infected individuals.

This study aims to determine prospectively the relationship between MCP-1 levels and cerebral metabolites measured on ¹H MRS in antiretroviral-naive HIV patients before and after 3 months of treatment with highly active antiretroviral therapy (HAART). This study also extends our prior observation that metabolite abnormalities and cognitive deficits persist 3 months after HAART [36]. Since higher MCP-1 levels in the brains of HIV patients likely lead to more neuronal injury and glial response, we hypothesized that CSF MCP-1 levels would correlate inversely with neuronal metabolites (such as, [NA], glutamate+glutamine [GLX]) and positively with glial metabolites (such as, [MI] and [CHO]) in antiretroviral-naive HIV patients. In addition, we expected that MCP-1 levels would decrease, but brain metabolite abnormalities measured on MRS would persist, after 3 months of HAART; hence, MCP-1 would not correlate with brain metabolites after treatment.

Methods

Subjects

Thirty-nine HIV patients naive to antiretroviral medications (36 men and three women, age 34.9 \pm 1.5 years) were recruited for this prospective study. Subjects were recruited from an UCLA-affiliated county hospital, Harbor-UCLA Medical Center, as well as from the local community by word-of-mouth and advertisements. The subjects were ethnically and racially diverse, including 26 Caucasians (16 Hispanic and 10 non-Hispanic), 11 African-Americans (two Hispanics) and two Asians. Prior to enrolment, all subjects were carefully screened by a neurologist and had screening blood and urine tests to ensure they fulfilled the inclusion criteria: 1) age 18–65 years; 2) seropositive for HIV-1; 3) CD4 <500/mm³; 4) naive to antiretroviral medications; 5) negative urine toxicology screen (cocaine, amphetamine, marijuana, benzodiazepine, barbiturates and opiates); and 6) willingness and ability to give informed consent or have it given by a valid representative. In addition, subjects were excluded if they fulfilled any of the following criteria: 1) history of psychiatric illness that might confound the analysis of the study (for example, schizophrenia, major depression); 2) presence of opportunistic brain lesions (for example, toxoplasmosis, lymphoma and progressive multifocal leukoencephalopathy); 3) confounding neurological disorder (for example, multiple sclerosis, Parkinson's disease, degenerative brain diseases, any other brain infections or neoplasms); 4) severe hepatic or renal dysfunction; 5) current or history of drug dependence

(including cocaine, methamphetamine, alcohol, opiates, inhalants and barbiturates); 6) head trauma with loss of consciousness for more than 30 min; 7) for women: pregnant or breast-feeding; 8) metallic or electronic implants in the body (such as, pacemaker, surgical clips and pumps); or 9) inability to read English at 8th grade level.

Once enrolled, each HIV patient had baseline neuropsychological tests, an MRS and a lumbar puncture to determine CSF viral loads and MCP-1 levels. AIDS dementia complex (ADC) staging required both the neuropsychological testing and clinical assessments using the Memorial Sloan Kettering staging for HIV dementia [37]. Each patient was then begun on HAART (defined as three or more antiretroviral medications including at least one protease inhibitor) and was carefully monitored by nurse practitioners weekly to ensure compliance with medications. Three months after stable HAART, each subject was re-evaluated neurologically, neuropsychologically, and had repeat CD4, plasma viral loads and MRS; however, due to an upgrade on the MR scanner and the relocation of the research group, eight of the subjects who participated in the baseline study could not complete the follow-up studies. Therefore, only 31 HIV patients had both repeat MRS and serum MCP-1 measurements, and only 26 of these patients consented to the repeat lumbar puncture (LP) for the CSF MCP-1 measurements, but two of these CSF specimens were mislabelled and had to be discarded. Of the five subjects that did not have the follow-up LP, four had prior post-LP headaches and one could not return due to scheduling conflicts. Subjects who enrolled in this MCP-1 study also participated in a larger study, and their MRS data, neuropsychological tests and comparisons with seronegative subjects have been reported in part previously [19,36]. All subjects signed an informed consent approved by the Institutional Review Board for Human Subjects Research at Harbor-UCLA Medical Centre, which is in accord with the Declaration of Helsinki.

MR studies

The MR studies were performed on a 1.5-Tesla scanner (General Electric Signa, Milwaukee, Wis., USA). Three imaging sequences were performed: 1) sagittal T1-weighted localizer (TE/TR 11/500, 4 mm slice thickness, 1 mm gap, 24 cm FOV); 2) axial fast inversion recovery (TE/TI/TR 32/120/4000, 3.5 mm slices, 24 cm FOV); 3) coronal T2-weighted fast spin echo (TE/TR 102/4000 ms, 5 mm slices, no gap, 24 cm FOV). Three volumes-of-interest (or voxels, 3–5 ml) were selected for MRS: medial frontal grey matter, right frontal white matter and right basal ganglia. Data were acquired using a point resolved spectroscopy (PRESS)

sequence optimized for assessing frontal and subcortical brain regions (TE 30 ms, TR 3 s, 64 averages, 2048 data points and 2.5 kHz bandwidth) [38,39]. Metabolite concentrations of NA, total creatine [CR], [CHO], [MI] and [GLX] were determined using a previously described method [40,41], which includes a correction for the partial volume of CSF (%CSF) in each voxel. A well-validated semi-automatic programme was used to analyze the spectra [40,41].

MCP-1 measurements

Serum and CSF from each patient were kept frozen at -70°C until all samples from baseline and 3 months after HAART had been collected. The MCP-1 assay was then performed on all specimens. MCP-1 levels were measured with a commercial assay using a quantitative sandwich enzyme immunoassay technique (Quantikine, human MCP-1, R&D Systems, Minneapolis, Minn., USA) with a detection limit of 5 pg/ml.

Statistical analyses

Statistical analyses were performed in the Statview program (SAS Institute). All variables other than HIV dementia scale, Karnofsky score and ADC stage showed normality on Kolmogorov-Smirnov tests; viral loads and MCP-1 levels were log-transformed to obtain normality. To minimize the number of statistical tests performed, a principal component analysis (PCA, using varimax rotations) was performed for the 15 MRS variables (five variables in three voxels each). The PCA extracted linear combinations of variables, each of which is associated with the largest possible variance after removing the variance of prior principal components in a stepwise fashion. The final data analysis was performed with the first two principal components, which explained 53% of the variability in the data set. Such an approach has been applied to another larger HIV MRS dataset [42] that yielded similar variables for the components (C Yiannoutsos, personal communication). Simple linear regression analyses were then performed (for variables before and after 3 months of HAART) between log serum and CSF MCP-1 levels and the two principal MRS components, a glial component and a neuronal/basal ganglia component. Additional post-hoc linear regressions were performed between log MCP-1 and those MRS variables that had the strongest contributions to principal components with significant correlations. Non-parametric Spearman correlations were performed between MCP-1 levels and variables that were not distributed normally (ADC stage, Karnofsky score, HIV dementia scale).

Additionally, analyses of covariance (ANCOVAs) were performed, using each principal component as dependent variable, treatment status as a factor and the logarithm of MCP-1 as a covariate, in order to deter-

mine the potential effect of HAART on the relationship between MCP-1 and MRS variables. To evaluate a potential interaction between the glial and neuronal factors with respect to MCP-1, a two-way ANCOVA was performed, using individual differences in MCP-1 before and during HAART as dependent variable, and individual differences in the neuronal and glial factors as independent variables. The effect of HAART (baseline versus 3 months of stable treatment) on all variables (clinical variables, log MCP-1 and principal components) was determined using paired t-tests for normally distributed variables, and paired sign tests for variables that did not follow a normal distribution.

Results

Clinical characteristics

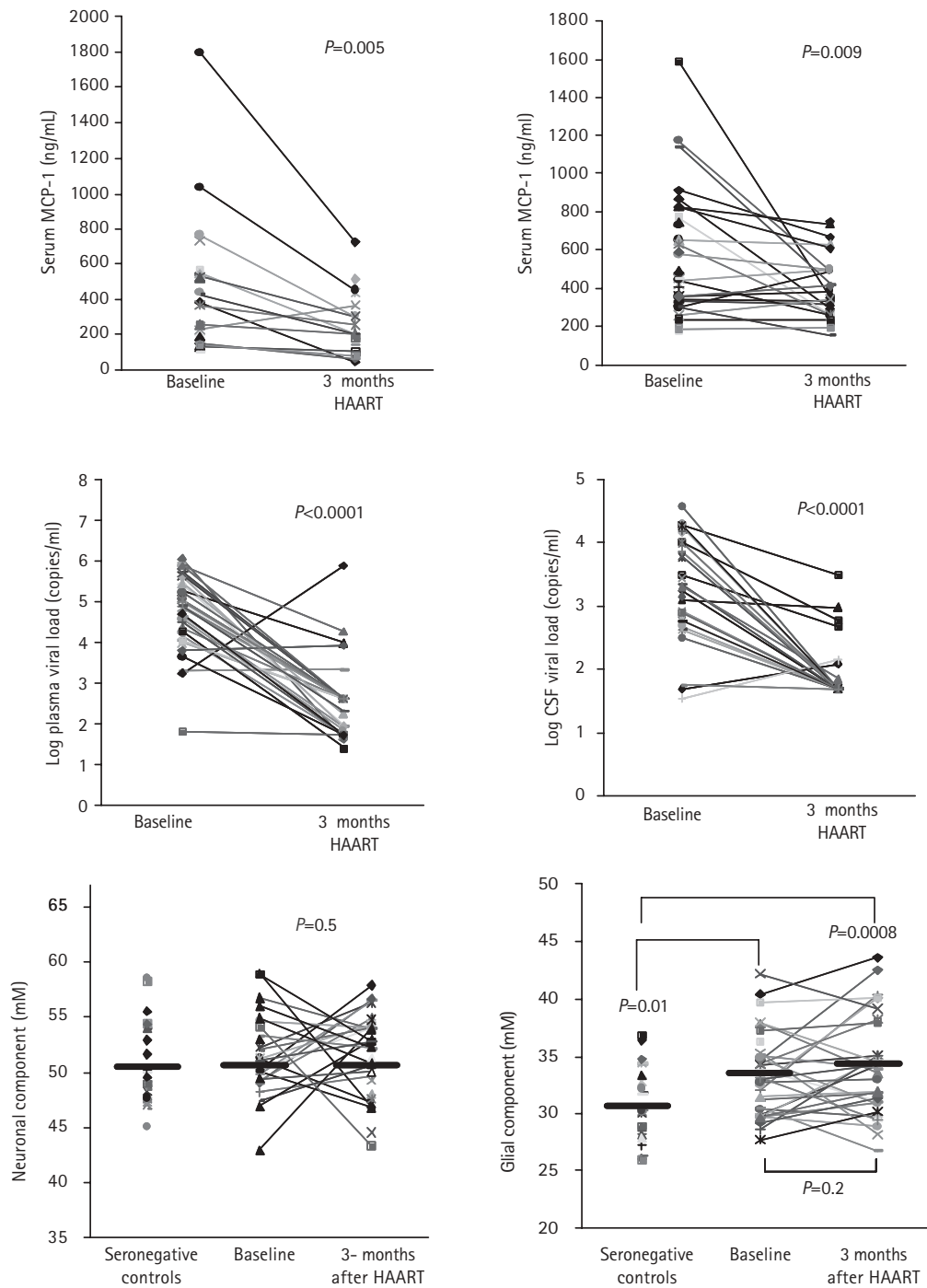
The clinical variables are shown in Table 1. The anti-retroviral-naive HIV patients had mild dementia as a group: 23 of the 39 (59%) HIV subjects fulfilled criteria for dementia (based on clinical and neuropsychological testing; 15 had ADC stage 1, seven had ADC stage 2, one had ADC stage 3) and 16 of 39 (41%) were not demented (seven with ADC stage 0.5 and nine with no significant cognitive deficits, ADC stage 0). These medication-naive subjects also had moderately high levels of plasma and CSF viral loads and mean CD4 count less than 200/mm³. Duration of HIV diagnosis in this group was 12.9 ±3.9 months, and all contracted HIV through sexual contacts (27 were homosexual; three were bisexual and nine were heterosexual).

After 3 months of HAART, ADC stage, Karnofsky score and HIV dementia scale did not improve significantly, although CD4 count rose rapidly, and plasma and CSF viral loads declined dramatically (Figure 1, Table 1). The mean CSF MCP-1 levels were higher than the serum MCP-1 levels before ($P<0.0001$, on log transformed data) and with HAART ($P=0.0007$, on log transformed data). Serum and CSF MCP-1 levels were higher than the values reported for seronegative controls in prior studies (typically <200 pg/ml) [27,34], and both declined significantly after 3 months of HAART (Figure 1, Table 1). Prior to HAART, the patients with ADC stage ≥1 ($n=23$) had higher CSF, but not serum, MCP-1 levels (serum: 474 ±116 pg/ml; CSF: 842 ±162 pg/ml) than those with ADC stage <1 (serum: 466 ±125 pg/ml; CSF: 475 ±51 pg/ml), but the differences were not significant ($P=0.07$). After 3 months of HAART, serum MCP-1 levels in patients with ADC stage ≥1 (279 ±61 pg/ml, log 2.3 ±0.1) were similarly decreased compared to patients without dementia (293 ±82 pg/ml, log 2.3 ±0.1); however, CSF MCP-1 level in those with ADC stage ≥1 (462 ±46 pg/ml, log 2.6 ±0.04 pg/ml) was higher than those with ADC stage <1 (331 ±48 pg/ml, log 2.48 ±0.06 pg/ml), $P=0.05$.

MRS data were evaluated by a PCA, which is a statistical technique to extract major features from multi-dimensional data sets, in order to reduce the dimensionality of the data. The current data set included 15 MRS variables, which were reduced to two major principal components by PCA. The main contributing metabolites (defined as metabolites with loading >0.4; remaining metabolites had low loading values, <0.25) to the first principal component were the CHO and MI concentrations in all three brain regions (positive loading), CR in the frontal grey and white matter (positive loading), and NA in the basal ganglia (negative loading). Since all (MI, CHO and CR) but one (NA) of these metabolites have approximately three-times higher concentration in glia compared to neurons [43], this principal component was defined as the 'glial component'. Major contributors to the second principal component were the NA concentrations in all three brain regions as well as the CR and GLX in the basal ganglia (all with positive loadings). The second principal component was called the 'neuronal component', since it predominantly involves the concentrations of the neuronal marker NA, and since neuronal loss or dysfunction may also be associated with decreased CR and GLX. Although we named these PCA components as 'glial' and 'neuronal' components (based on most of the metabolites that are included in the factor), they do not reflect the exact number of neurons and glia. The components are mathematical linear combinations (sums and differences) of the concentrations of various metabolites that are grouped together by the PCA operation, and happen to group into metabolites that are likely associated with either cell type. In combination, the glial and neuronal principal components explained 53% of the variability in the data set. The third principal component contributed 13% to the overall variance and had major loadings only from mid-frontal CR (negative) and right frontal GLX (positive); therefore, this component cannot be interpreted meaningfully and was not used. Compared to baseline MRS measurements (prior to treatment), neither the neuronal component nor the glial component changed significantly after 3 months of HAART (Figure 1, bottom); however, both the baseline and follow-up glial components were significantly higher compared to those in the controls (baseline: $P=0.01$; follow-up: $P=0.0008$).

Next, linear regressions were performed between MCP-1 levels and the two major principal components (Figure 2). Prior to initiation of HAART, log CSF MCP-1 correlated inversely with the neuronal principal component ($r=-0.59$, $P=0.0008$), but not with the glial principal component ($r=0.19$, $P=0.27$) (Figure 2). Conversely, after the patients were on stable HAART

Figure 1. Changes in serum and CSF MCP-1 levels, plasma and CSF HIV RNA (plasma viral loads), and the neuronal and glial components (derived from principal component analyses of the metabolite concentrations, see legend in Figure 2) after 3 months of HAART



for 3 months, log CSF MCP-1 correlated with the glial principal component ($r=0.70$, $P=0.0002$), but not with the neuronal principal component ($r=-0.07$, $P=0.60$). This reversal in the relationship with log CSF MCP-1 was statistically significant for the glial component ($P=0.003$, interaction between treatment status and log

CSF MCP-1 on ANCOVA, Figure 2), but not for the neuronal principal component. No interactions between the glial and neuronal principal components with respect to MCP-1 were observed on a combined ANCOVA. Furthermore, serum log MCP-1 levels did not correlate with any of the brain metabolites.

Table 1. Clinical variables and MCP-1 levels in HIV patients (mean \pm SE)

	Antiretroviral naive (n=39)	Naive subjects with follow ups (n=31)	3-months after HAART (n=31)	P-values**
ADC stage (0-4)	0.91 \pm 0.12	0.85 \pm 0.14	0.76 \pm 0.12	n.s.
Karnofsky Score (0-100)	85 \pm 2.1	85 \pm 2.4	89 \pm 1.9	n.s.
HIV Dementia Scale (1-16)	12.6 \pm 0.6	13.0 \pm 0.6	12.5 \pm 0.7	n.s.
CD4 (cells/mm ³)	176 \pm 23	183 \pm 26	310 \pm 34	<0.0001
Plasma viral load (copies/ml)	207532 \pm 44372	187727 \pm 49527	1,163 \pm 629	0.0004
Log plasma viral load (copies/ml)	4.8 \pm 0.1	4.7 \pm 0.2	2.4 \pm 0.1	<0.0001
CSF Viral Load (copies/ml)	6369 \pm 1352	7500 \pm 1626	235 \pm 134	0.0002
Log CSF viral load (copies/ml)	3.3 \pm 0.1	3.4 \pm 0.2	1.9 \pm 0.09	<0.0001
Serum MCP-1 (pg/ml)	471 \pm 84	541 \pm 102	285 \pm 47	0.005
Log serum MCP-1 (pg/ml)	2.5 \pm 0.07	2.6 \pm 0.07	2.3 \pm 0.08	0.0004
CSF MCP-1 (pg/ml)	693 \pm 102	637 \pm 81	410 \pm 36*	0.009
Log CSF MCP-1 (pg/ml)	2.74 \pm 0.05	2.73 \pm 0.05	2.58 \pm 0.04*	0.007

* CSF MCP-1 values were only from 24 of the 26 patients who consented to the repeat lumbar puncture.

** P-values are from paired t-tests of subjects who had both baseline and 3-month data.

Post-hoc regression analyses were performed to determine the relationship between log CSF MCP-1 and each metabolite that contributed to the two major principal components. Prior to HAART, log CSF MCP-1 showed a negative association with the NA in the frontal grey matter ($r=-0.36$, $P=0.03$) and with the GLX concentration in the basal ganglia ($r=-0.5$, $P=0.008$) (Figure 3, top), as well as positive correlation with MI in the frontal white matter ($r=0.35$, $P=0.03$). After initiation of HAART, log CSF MCP-1 was positively correlated with the [MI] in the frontal white matter ($r=0.59$, $P=0.003$) and in the frontal grey matter ($r=0.67$, $P=0.0005$) (Figure 3, bottom).

Correlations between MCP-1 or metabolites with other clinical variables

CD4 counts correlated negatively with log CSF MCP-1 ($r=-0.52$, $P=0.001$) prior to treatment, but the correlation showed only a trend after HAART ($r=-0.34$, $P=0.1$). No correlation between log serum MCP-1 and CD4 were observed before or after treatment. CD4 counts also correlated positively with the neuronal component before ($r=0.38$, $P=0.04$) but not after HAART ($r=0.07$, $P=0.73$); this change in the relationship between CD4 count and neuronal component did not reach significance (interaction with respect to treatment status, ANCOVA, $P=0.07$). Only trends were observed between CD4 and the glial component before ($r=-0.31$, $P=0.06$) or after ($r=-0.32$, $P=0.08$) HAART.

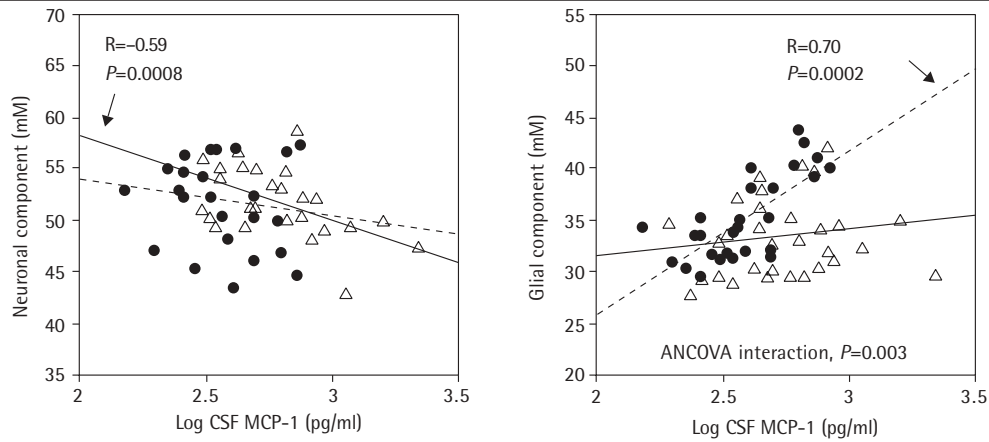
In addition, log CSF MCP-1 correlated with the ADC stage before ($\rho=0.44$, $P=0.008$) and after HAART ($\rho=0.49$, $P=0.02$, Spearman correlations), but not with HIV dementia scale. Log CSF MCP-1 also correlated inversely (non-parametric Spearman correlation) with the Karnofsky scores before ($\rho=0.37$,

$P=0.03$) and after HAART ($\rho=-0.54$, $P=0.01$, Spearman correlation). Log serum MCP-1, however, did not correlate with Karnofsky score, HIV dementia scale or ADC stage. Log CSF MCP-1 level correlated with log plasma viral load, but not log CSF viral load, before ($r=0.37$, $P=0.03$) but not after HAART. Serum MCP-1 level did not correlate with either plasma or CSF viral load measurements (log transformed data).

Discussion

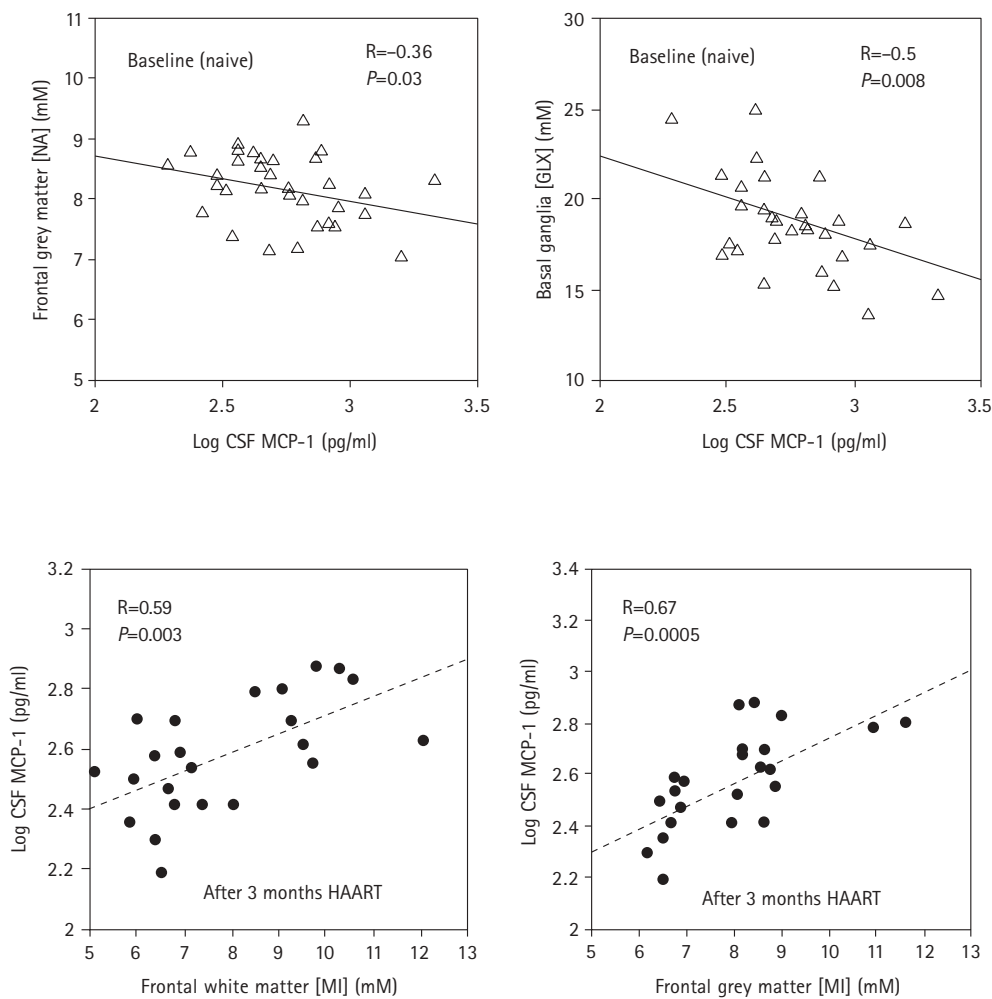
This prospective treatment study in antiretroviral-naive HIV patients has three main findings. First, CSF as well as serum MCP-1 decreased after 3 months of HAART. Although the decline in MCP-1 levels was smaller (-0.3 log for serum and -0.15 log for CSF) than changes in CD4 (+69%) and the suppression of viral loads in both plasma (-2.3 log) and CSF (-1.5 log) after HAART, the changes in the MCP-1 levels were significant and appear to reflect a decreased inflammatory response, both systemically and centrally for the group. This finding is contrary to recent reports that found no influence on CSF MCP-1 levels by HAART, although these studies did not evaluate antiretroviral-naive patients [30] or had a smaller sample size [44]. One study of antiretroviral-naive asymptomatic HIV patients even found further elevation of CSF MCP-1 after 8 weeks of HAART despite undetectable CSF HIV RNA in most patients [45]. This difference in the CSF MCP-1 responses may reflect the different follow-up time points (8-48 weeks) compared to our study (12 weeks), or a more robust CNS immune response in the asymptomatic patients in the prior study. Consistent with prior observations, CSF MCP-1 was higher than serum MCP-1, and HIV patients with lower CD4 counts, higher plasma viral load or neuro-

Figure 2. The graph shows the significant correlation between the neuronal component and log CSF MCP-1 before treatment (left) and the correlation between the glial component and log CSF MCP-1 after 3 months of HAART (right)



Neuronal component = [NA]FGM + [NA]FWM + [NA]BG + [CR]BG + [GLX]BG. Glial component = [CHO]FGM + [CHO]FWM + [CHO]BG + [MI]FGM + [MI]FWM + [MI]BG + [CR]FWM - [NA]BG. FGM, frontal grey matter; FWM, frontal white matter; BG, basal ganglia. ANCOVA demonstrates an interaction effect between CSF MCP-1 and the glial component with respect to treatment status. Solid line and triangles, baseline (naive); dashed line and circles, after 3 months HAART.

Figure 3. Linear regression analyses from post-hoc evaluations showing that HIV patients with higher levels of CSF-MCP-1 levels prior to treatment had lower levels of neuronal metabolites (the neuronal marker [NA] and glutamate+glutamine [GLX]) (top graphs) After treatment, however, HIV patients with higher levels of the glial marker [MI] in frontal grey and frontal white matter might be expressing higher CSF MCP-1 levels (bottom graphs)



logical disorder, had higher CSF MCP-1 levels [29,30]. However, unlike earlier studies, we did not observe significant correlations of serum MCP-1 levels with CD4 count [46], or with plasma HIV-1 RNA levels [46,47], before or after HAART. This lack of correlation may be due to the antiretroviral-naive status of the patient at baseline, as had been observed previously in a smaller sample of antiretroviral-naive HIV patients [45]. The lack of correlation may also be due to variability in patient response to 3 months of treatments or to the limited sample size in our current study.

The second major observation is that in untreated HIV patients log CSF MCP-1 levels correlated inversely, while CD4 correlated positively, with the 'neuronal metabolites'. These findings suggest that high levels of CSF MCP-1 and low CD4 are associated with neuronal dysfunction in untreated HIV patients, which might have resulted from the higher levels of neurotoxic proteins and viral particles that were transported by the monocytes into the CNS. The untreated patients with higher MCP-1 levels also had poorer function (HIV dementia scale, Karnofsky score and ADC stage), which further supports the association with neuronal dysfunction. These findings are also consistent with prior studies that found higher CSF MCP-1 in patients with more severe encephalitis post-mortem [27,29], and in patients with higher CSF, but not plasma, HIV RNA [29,30]. In addition, post-hoc analyses demonstrate associations between log CSF MCP-1 level and the neuronal marker [NA] in the frontal cortex, as well as [GLX] in the basal ganglia, prior to HAART. Since the GLX peak comprises primarily glutamate, which is predominantly in neurons [48], decreased GLX also suggests neuronal loss or dysfunction. Therefore, our findings support the interpretation that elevated CSF MCP-1 levels are associated with neuronal dysfunction or loss in antiretroviral-naive HIV patients. In contrast, with decreased MCP-1 levels and successful suppression of plasma and CSF viral loads after 3 months of HAART, it is likely that much fewer infected monocytes are recruited to the CNS, and hence less neurotoxic proteins that may lead to neuronal injury. Taken together, these findings argue for early antiretroviral treatment to minimize neuronal injury in patients infected with HIV.

The third major finding of this study is that, after 3 months of HAART, CSF MCP-1 correlates with the metabolite concentrations that comprised the 'glial component', as well as with the glial marker MI in the frontal white matter and frontal grey matter on post-hoc analyses. Since astrocytes, the most abundant cells in the brain, can be induced by neurotoxic proteins such as HIV-1 Tat to release MCP-1 [27], elevated glial markers on MRS may be expected to be associated with

higher CSF MCP-1 levels in HIV patients. However, the positive correlation between CSF MCP-1 and the glial component was evident only after 3 months of HAART but not before treatment. The altered relationship between CSF MCP-1 and glial MRS markers may indicate a shift from MCP-1 that is produced by both peripheral cells (that is, macrophages and monocytes) and glia (such as, astrocytes and microglia) prior to treatment, to MCP-1 that is released primarily by activated glia after HAART. Prior to treatment, HIV patients had higher viral loads and more macrophage activity with infected monocytes entering the CNS and contributing to the total MCP-1 release, but do not appear to have a direct relationship to the glial activity. After treatment, despite undetectable viral load and minimal macrophage activity from the periphery, some glial cells continued to express MCP-1, which is demonstrated by the relationship between the MRS glial marker and the CSF MCP-1.

Three months of HAART decreased both grouped serum and CSF MCP-1 levels; however, some patients continued to have elevated CSF MCP-1 and even higher glial activity (on MRS). The high CSF MCP-1 and glial markers may be due to residual viral burden in the brain despite suppression of the virus in the blood and CSF. However, persistently elevated glial activity may also reflect ongoing repair function by the glial cells [49], rather than a 'reactive' inflammatory response to HIV. In such a model, HIV patients with the strongest brain immune response at this early stage of treatment might have the highest glial principal component on MRS and the highest MCP-1 levels. This interpretation is supported by a prior observation that asymptomatic patients with good CSF HIV RNA suppression had elevated CSF MCP-1 during antiretroviral treatments [45]. In addition, MRS studies in patients with progressive multifocal leukoencephalopathy indicated that those with the strongest immune response and who entered clinical remission demonstrated a higher glial response, in the form of higher glial markers [MI] and [CHO], during the early months of recovery [50]. Similarly, patients with multiple sclerosis showed elevated glial markers during the early months of interferon treatment [51]. However, it remains to be determined whether HIV patients with the highest glial component and CSF MCP-1 early in the course of treatment will have the most rapid improvement and recovery.

This study demonstrates the usefulness of combining serological markers with *in vivo* neuroimaging to assess the effects of treatment in HIV brain injury. Although these interesting relationships between MCP-1 and brain metabolite markers may be an epiphenomenon, the shift in the stronger correlation between MCP-1 and neuronal metabolites prior to treatment, to primarily a

correlation between MCP-1 and the glial metabolites could also be interpreted as changing from a brain injury state to a brain repair process. Our findings suggest that in untreated patients, peripheral factors (low CD4 counts and high CSF MCP-1 levels) may lead to neuronal dysfunction. After 3 months of HAART, the suppression of peripheral factors (undetectable viral loads, decreased systemically derived MCP-1 and partial reconstitution of the immune system) no longer leads to ongoing neuronal injury. In contrast, a clear relationship emerges between glial metabolites on ¹H MRS and CSF MCP-1 during HAART, in that patients with the strongest glial response produce the highest levels of CSF MCP-1. This may indicate ongoing glial repair processes in the brain. Altogether, our results argue for early antiretroviral treatment of HIV to avoid brain injury.

Acknowledgements

This work was supported by the NIH [Scientist Development Award for Clinicians for LC (K20-DA00280); R01-NS38834 and GCRC MO1-RR00425]. We are grateful to the HIV patients who volunteered in this study and to Dr Mallory Witt for referring many of these patients to the parent study. We also thank the anonymous reviewers for their helpful suggestions.

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