Cerebrospinal Fluid β Chemokine Concentrations in Neurocognitively Impaired Individuals Infected with Human Immunodeficiency Virus Type 1

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Macrophages express the chemokine receptor CCR-5, a coreceptor for human immunodeficiency virus (HIV) entry. This receptor is ligated by β chemokines, which influence HIV type 1 (HIV-1) replication in CCR-5–bearing cells in vitro and could influence the course of infection in the central nervous system. Cerebrospinal fluid (CSF) samples from 73 HIV-infected men were assayed for macrophage inflammatory protein-1 α (MIP-1α), MIP-1β, and regulated upon activation, normal T cell expressed and secreted (RANTES). Distributions of all three were positively skewed. CSF chemokine concentrations were correlated with each other and were higher in demented patients. In a multivariate analysis, demented subjects were more likely to have detectable CSF MIP-1α, elevated CSF HIV RNA levels, and lower CD4+ cell counts. However, among those with detectable CSF MIP-1α, levels were lower in demented patients. CSF β chemokine elevation is consistent with the macrophage activation known to occur in dementia and with studies of β chemokine mRNA expression in the brain. Low, but detectable, levels of CSF MIP-1α were strongly associated with dementia, suggesting that higher levels may have neuroprotective effects.

Elevated plasma human immunodeficiency virus (HIV) RNA levels predict immunologic progression of HIV type 1 (HIV-1) infection [1–3]. Viral penetration into the central nervous system (CNS) occurs early in the course of HIV infection, and elevated cerebrospinal fluid (CSF) HIV RNA levels correlate with neurologic dysfunction [4–8]. Macrophages, which are derived from either microglia or circulating monocytes, are the primary brain cells infected by HIV-1 [9–12]. Such cells can both replicate HIV and secrete soluble factors, such as chemokines, that may mediate chemotraction and neuropathogenesis [13–15].

Chemokine receptors are a family of membrane proteins, two of which (CCR-5 and CXCR-4) are coreceptors for the entry of HIV into cells [16–19]. Macrophage-tropic virus uses the chemokine receptor CCR-5 along with CD4 for cell entry [20–22]. The β chemokines have been identified as the natural ligands of CCR-5 and the major soluble factors secreted by CD8+ T cells in response to HIV [23]. These chemokines include macrophage inflammatory protein-1 α (MIP-1α), MIP-1β, and regulated upon activation, normal T cell expressed and secreted (RANTES). These chemoattractionts might protect susceptible cells from HIV in vivo [24], because they inhibit HIV entry in vitro [25] and other proinflammatory cytokines protect cultured human brain cells from HIV-1 [26]. On the other hand, β chemokines are produced by HIV-1 and simian immunodeficiency virus (SIV)–infected macrophages in vitro [27, 28] and in vivo [29] and paradoxically may increase HIV replication in these cells [30–32]. Autopsy studies have demonstrated that β chemokine mRNA expression in the brain is elevated in those with HIV or SIV encephalitis [33, 34], thus arguing against a protective effect. Moreover, Kelder et al. [35] recently demonstrated that CSF levels of monocyte chemotactic protein-1 and RANTES, but not MIP-1α or MIP-1β, were elevated in patients with HIV-associated dementia (HAD).

Because β chemokines in CSF may reflect levels in the brain that influence the pathogenesis of neurocognitive impairment in HIV infection, we measured their concentrations in samples from patients who had previously been neurocognitively characterized. We correlated these levels with other viral and immunologic markers of brain damage and with clinical measures of cognitive impairment.

Methods

Seventy-three CSF specimens from patients with a wide spectrum of cognitive impairment were selected from the Multicenter AIDS Cohort Study (MACS) specimen bank for this pilot study. Specimens were collected over a 9-year period (September 1986 through
November 1995). In the MACS, regular neurologic examinations and neuropsychologic tests were administered to identify individuals with HAD and to classify the severity of dementia, using the Memorial Sloan-Kettering scale [36]. HAD was defined by use of the American Academy of Neurology criteria: all individuals had significant neurologic and neuropsychologic deficits affecting function in the absence of CNS opportunistic infections [37]. Participants were then assigned consensus diagnoses as normal (n = 25), impaired (n = 34), or demented (n = 14) on the basis of both clinical and neuropsychologic findings. Classification as impaired corresponded to a diagnosis of minor cognitive-motor disorder.

MIP-1α, MIP-1β, and RANTES were measured by ELISA (Quantikine: R&D Systems, Minneapolis). These immunoassays have coefficients of variation of ≤8% and detection sensitivities of 5 (RANTES), 7 (MIP-1α), or 11 (MIP-1β) pg/mL. They were validated for CSF by spiked samples and storage for 18 h under different conditions (−70°C, −20°C, and 25°C) and were found to accurately determine β chemokine concentrations in this fluid (data not shown). Sixty matched plasma specimens were also assayed for MIP-1α and MIP-1β. Plasma specimens were not assayed for RANTES, because platelets were not removed prior to freezing. Specimens were coded to blind the investigator to the subjects’ neurologic status. They were then diluted to a volume sufficient to perform all three assays in duplicate, and assay sensitivities were adjusted accordingly. Plates were scanned at dual wavelengths (450 and 540 nm), and output was analyzed by use of computer software (DeltaSoft II; BioMetallics, Princeton, NJ), using log-log interpolation and correcting for dilution. The chemokine concentration was determined from the mean absorbance of the duplicate wells.

Most specimens had been previously assayed for other markers of potential significance, including CSF HIV RNA (73), blood CD4+ lymphocyte counts (72), CSF leukocyte counts (72), CSF β2 microglobulin (68), and plasma HIV RNA (65). HIV RNA levels were measured in plasma and CSF specimens by use of nucleic acid sequence–based amplification methods (NucliSence; Organon Teknika, Durham, NC), with a lower limit of quantitation of 100 copies/mL (2.0 log10 copies/mL). CSF β2-microglobulin methods were reported elsewhere [38]. Antiretroviral use was recorded for 64 (88%) of 73 subjects and was coded as “current use,” “remote use,” or “never used.” Consistent with the sampling dates, almost all treated patients were prescribed only one nucleoside analogue reverse transcriptase inhibitor.

Statistical analyses were done by use of JMP and SAS software (SAS Institute, Cary, NC). At the time the study was performed, data on levels and variation of chemokines in CSF were unavailable, and it was not possible to estimate the power-to-detect associations. Patients were grouped as either “abnormal” (impaired + demented) or “nonimpaired” (normal + impaired). CSF-to-plasma (CSF/plasma) ratios of several factors were designated as CSF/plasma MIP-1α, CSF/plasma MIP-1β, and CSF/plasma HIV RNA. To normalize the distributions, we applied data transformations to HIV RNA levels (log10), CD4+ lymphocyte counts (square root), and CSF β2-microglobulin levels (reciprocal). Each CSF β chemokine distribution demonstrated a high proportion of undetectable values, resulting in skewing and precluding symmetrization of the distributions. Therefore, a nonparametric measure of correlation, Spearman’s ρ, was used to assess the relationships between continuous variables, and the Kruskal-Wallis procedure was used to evaluate the univariate relationships between cognitive function category and the independent variables. Odds ratios (ORs) were calculated for statistically significant univariate associations and were considered undefined if their denominator equaled zero. Because multiple correlations were performed, the significance limits were also adjusted to reduce the risk of type I errors. The Bonferroni adjusted level of statistical significance for the correlational analyses was .002. Adjusted, unadjusted, and marginal (0.05 < P < .10) significance levels are shown, since this is an exploratory analysis.

The nonlinear relationship observed between the CSF β chemokine distributions and the dementia status was assessed by use of the Kolmogorov-Smirnov procedure, using S PLUS (MathSoft, Cambridge, MA). This procedure tested goodness of fit, using the null hypothesis that the true CSF β chemokine distribution had low- to midrange levels associated with dementia and either undetectable or high levels associated with its absence. The algorithm of Kim and Jennrich [39] was used to calculate the exact distribution of the two-sided test for various sample sizes.

Multivariate regression methods were used in the analyses of dementia status (logistic) and CSF HIV RNA levels (linear). The logistic regression analysis was limited by missing values for plasma HIV RNA, CSF β2 microglobulin, plasma MIP-1α, and plasma MIP-1β. Either plasma HIV RNA levels or CSF β2-microglobulin levels were available for only 9 demented subjects and plasma β chemokine levels for 3. Only covariates that had values for at least half of the demented patients were included in the multivariate analysis of dementia status.

Results

Demographic comparability of subjects across levels of cognitive functioning. Patients in each category were similar in age and ethnicity, but demented subjects had significantly fewer years of education (see table 1). Since HAD occurs most commonly in advanced stages of disease, the demented group had lower CD4+ cell counts and higher plasma HIV RNA levels. Consistent with prior reports from this cohort, demented subjects had higher CSF levels of HIV RNA [8] and β2 microglobulin [38]. The rate of current antiretroviral use did not differ across groups. The MACS cohort is composed exclusively of homosexual men.

CSF β chemokine distributions and correlations with other plasma and CSF markers. CSF chemokine concentrations (see figure 1) were positively skewed because of the high proportions of undetected values (CSF MIP-1α, 62%; CSF MIP-1β, 33%; CSF RANTES, 20%). The age of the specimen did not correlate with chemokine concentrations, which argues against significant time-related protein degradation. CSF chemokine levels also did not differ by the patient’s use of antiretroviral agents.

Correlations between levels of β chemokines and other viral and immunologic markers are listed in table 2. CSF β chemokine levels were strongly correlated with each other but not with plasma concentrations, supporting separate sources of
these chemokines in the CNS and systemic compartments. Both the CSF levels and the CSF/plasma ratios of MIP-1β and HIV RNA were correlated. The latter correlation suggests that a chemokine gradient may partially determine the relationship between plasma and CSF levels of HIV RNA. CSF levels of RANTES were correlated with both the CSF level and the CSF/plasma ratio of HIV RNA. Since plasma levels of RANTES were correlated with both the CSF level and the CSF/plasma ratio of HIV RNA, this is not typical of cohort and is an artifact of sampling. NS, not significant; WBCs, white blood cells.

Since the risk of HAD is markedly increased in advanced stages of disease, the correlations were analyzed within strata of immunosuppression (CD4 cell count <200 or ≥200 cells/mm³). Correlations between several chemokines were stronger in patients with less-advanced disease: CSF MIP-1α and CSF RANTES (ρ = 0.54, P < .001), plasma MIP-1α and plasma MIP-1β (ρ = −0.44, P = .004), and plasma MIP-1α and CSF MIP-1β (ρ = 0.33, P = .03). In contrast, levels of several chemokines were more strongly correlated with viral markers in those with <200 CD4+ cells/mm³: CSF RANTES and CSF HIV RNA (ρ = 0.73, P < .001), CSF RANTES and the CSF/plasma ratio of HIV RNA (ρ = 0.66, P = .002), and CSF MIP-1β and CSF HIV RNA (ρ = 0.55, P = .004). Figure 2 displays these relationships between CSF levels of HIV RNA and either MIP-1β or RANTES. The strong correlations between CSF MIP-1β and the other CSF chemokines were unaffected by CD4 stratiﬁcation.

Association of neurocognitive impairment with single plasma and CSF constituents. In addition to those markers noted in Table 1, neurocognitive impairment was associated with higher CSF levels of MIP-1α and RANTES (see Table 3). Most of the variance of the CSF β chemokines across stages of cognitive function was attributable to demented subjects. In other words, normal and impaired subjects had similar CSF β chemokine levels, and both differed signiﬁcantly from those diagnosed as demented. When these groups were collapsed to compare demented and nondemented (normal + impaired) subjects, CSF levels of all three β chemokines were higher in demented patients (see Figure 1).

Samples with CSF chemokine levels below the level of detection were almost entirely from nondemented patients: 42 (93%) of 45 samples for CSF MIP-1α, 23 (96%) of 24 samples for CSF MIP-1β, and 14 (93%) of 15 samples for CSF RANTES. Although the overall mean and median CSF MIP-1α concentrations were lower for nondemented patients than for demented patients, these summary statistics were heavily inﬂuenced by the large number of undetectable values. In men with detectable CSF MIP-1α values, the relationship between CSF MIP-1α and dementia reversed. Nondemented patients had higher median concentrations of CSF MIP-1α (497 pg/mL vs. 283 pg/mL, P = .01). The distributions of both CSF MIP-1β and RANTES were also skewed, but the effect was not as pronounced and did not reverse the direction of the relationship between these chemokines and the diagnosis of dementia when limited to the detectable range.

In consideration of the skewed CSF β chemokine distributions, two dichotomous expressions were analyzed for their relationships to dementia. First, the CSF β chemokine variables were dichotomized for detectability. Detectable CSF MIP-1α and MIP-1β were both associated with dementia (OR, 9.1, 95% confidence interval [CI], 2.5–43.9; and OR, 8.3, 95% CI, 1.5–155.9, respectively); RANTES was not associated with dementia. Second, since the CSF chemokine levels of many demented patients clustered in restricted ranges of values (CSF MIP-1α, 7–300 pg/mL; CSF MIP-1β, 451–625 pg/mL; and CSF RANTES, 115–225 pg/mL), the distributions were also dichotomized to account for this nonlinear effect. All three clustering variables (within or outside the noted range) were associated with dementia (MIP-1α: OR undefined, P =

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**Table 1.** Demographic and clinical comparisons of human immunodefiency virus (HIV)-infected subjects by cognitive category.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal (n = 25)</th>
<th>Impaired (n = 34)</th>
<th>Demented (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years a</td>
<td>38</td>
<td>39</td>
<td>39</td>
<td>NS</td>
</tr>
<tr>
<td>Race, % white b</td>
<td>83</td>
<td>70</td>
<td>71</td>
<td>NS</td>
</tr>
<tr>
<td>Median years of education a</td>
<td>18</td>
<td>16</td>
<td>13</td>
<td>.03</td>
</tr>
<tr>
<td>Current antiretroviral use, % c</td>
<td>33</td>
<td>36</td>
<td>43</td>
<td>NS</td>
</tr>
<tr>
<td>Mean CD4 cell count, cells/mm³ c</td>
<td>559</td>
<td>345</td>
<td>122</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Median CSF WBCs, cells/mm³ c</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Median plasma HIV RNA, log copies/mL c</td>
<td>3.67</td>
<td>4.30</td>
<td>4.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Median CSF HIV RNA, log copies/mL</td>
<td>2.00</td>
<td>2.00</td>
<td>3.15</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Median CSF β microglobulin, mg/L c</td>
<td>1.54</td>
<td>1.88</td>
<td>3.60</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

NOTE. Consistent with previous studies, several markers differed across stages of clinical neurocognitive function. Although subjects classified as “demented” had fewer years of education, this is not typical of cohort and is an artifact of sampling. NS, not significant; WBCs, white blood cells.

a Kruskal-Wallis rank sums test.
b χ² test.
c Values are missing for some patients (see Methods section).
Figure 1. Distributions of dementia group by cerebrospinal fluid (CSF) \( \beta \) chemokine strata. CSF \( \beta \) chemokine distributions were positively skewed. Levels of all 3 chemokines were higher in demented (black bars) than nondemented (white bars) patients. Levels for many demented subjects fell in restricted ranges of values compared with levels for nondemented subjects (see text for ranges). \( P = .02 \) (A, C) and .05 (B), by Kruskal-Wallis rank sums test.

\( 2 \times 10^{-4} \), Fisher’s exact test; MIP-1\( \beta \): OR, 4.8, 95% CI, 1.3–17.8; RANTES: OR, 13.8, 95% CI, 3.4–65.3). To further assess a hypothesis of nonlinearity, we performed a Kolmogorov-Smirnov analysis. This analysis supported the finding that the associations between dementia and levels of CSF \( \beta \) chemokines, especially MIP-1\( \alpha \), were nonlinear. Those with either undetectable or high detectable levels were more likely to be nondemented, whereas the opposite was true for those with low- to midrange detectable levels.

Since patients who had detectable levels of CSF MIP-1\( \alpha \) were more likely to be demented, correlations between this chemokine and other markers were reexamined. When CSF MIP-1\( \alpha \) levels were detectable, they were correlated with levels of blood CD4\(^+\) lymphocytes (\( \rho = 0.58, P = .001 \)), CSF MIP-1\( \beta \) (\( \rho = 0.52, P = .005 \)), plasma MIP-1\( \alpha \) (\( \rho = 0.47, P = .056 \)), and CSF HIV RNA (\( \rho = -0.36, P = .056 \)), but not with CSF RANTES. Figure 2A shows the relationship between detectable levels of CSF MIP-1\( \alpha \) and levels of CSF HIV RNA.

Multivariate analysis of neurocognitive impairment. To model the complex relationships among these variables, we constructed a series of multivariate logistic regression models, in which cognitive groups were combined to compare either normal patients with all impaired patients (impaired = demented) or demented patients with nondemented patients (normal = impaired). In a model discriminating cognitively normal patients from all others, those with abnormal cognitive function...
had lower CD4+ cell counts (OR, 3.5, 95% CI, 1.6–8.9, for a decrease of 10 × cells/mm³, and higher CSF HIV RNA levels, OR, 8.1, 95% CI, 1.8–58.9, for a level >2.5 log copies/mL). Although this model explained 39% of the variability between normal and abnormal patients (model P < .001), CSF β-chemokine concentrations did not contribute significantly to it. These differences are consistent with previous studies [7, 8] and with the finding that CSF chemokine levels do not distinguish cognitively normal patients from those with mild impairment.

In contrast, demented patients were more likely to have detectable CSF MIP-1α concentrations (OR, 10.2, 95% CI, 1.8–82.5, for a detectable level), as well as higher CSF HIV RNA levels (OR, 12.9, 95% CI, 2.3–112.6, for a level >2.5 log copies/mL) and lower CD4+ cell counts (OR, 4.5, 95% CI, 1.6–17.5, for a decrease of 10 × cells/mm³) when compared with values for nondemented men. This model explained 64% of the variability between demented and nondemented patients (model P < .001). Thus, detectable CSF MIP-1α levels were associated with dementia status independently of CSF HIV RNA levels and CD4+ cell counts. Because of missing values for plasma HIV RNA and CSF β2-microglobulin levels, we reduced to 9 the number of demented patients in screening models that included these markers. Despite this reduction, the independent contribution of CSF MIP-1α on dementia was maintained in models that included levels of either of these markers.

The relationship of dementia to CSF levels of MIP-1α and HIV RNA levels is illustrated by dividing patients into 3 groups: Group 1 were those with CSF RNA levels <2.55 log copies/mL and with CSF MIP-1α levels >300 pg/mL, group 2 were those with undetectable CSF MIP-1α levels, and group 3 were all others (see figure 3). Patients in group 3 were much more likely to be demented (OR, 38.2, 95% CI, 8.1–298). These groups also differed in levels of blood CD4+ cells and CSF β2-microglobulin. Thus, this graph demonstrates the findings of the logistic regression models: detectable CSF MIP-1α levels, elevated CSF HIV RNA levels, and diminished blood CD4+ cell counts along with elevated CSF β2-microglobulin levels combine to distinguish demented subjects from those with only mild or no cognitive impairment.

Multivariate analysis of CSF HIV RNA levels. Since chemokines may exert part of their effect on the brain by modulating HIV replication, the relationship between CSF HIV RNA and CSF β chemokines was investigated further. In a linear regression model, higher CSF HIV RNA levels were associated with low- to midrange detectable levels of CSF MIP-1α (P = .03), higher levels of CSF MIP-1β (P = .05) and plasma HIV RNA (P = .01), and CD4+ cell counts <200 cells/mm³ (P = .01). Combined, these variables accounted for 36% of the variance in CSF HIV RNA levels (model P < .001). CSF levels of RANTES, β2-microglobulin, and white blood cells were associated with CSF HIV RNA in other models, but they did not demonstrate independent effects in this best model. This model supports the hypothesis that MIP-1α inhibits and MIP-1β promotes HIV replication in the CNS.

Discussion

Many viral and immunologic factors have been implicated in the pathogenesis of HIV-associated cognitive impairment. Two cross-sectional studies have correlated CSF HIV RNA levels with dementia [7, 8]. Earlier studies correlated cognitive deficits with elevated levels of proviral DNA [40], β2-microglobulin [38, 41], quinolinic acid [42], and neopterin [43] in CSF. This study examined the relationships among three β chemokines in CSF and their relationship to other viral and immunologic correlates of cognitive impairment to assess their role in the pathogenesis of HIV dementia. We found that levels of β chemokines in CSF, like levels of HIV virions and other molecules that are indicative of immunologic activation in the CNS, are associated with dementia, which indicates that they may reflect HIV-induced brain damage.
Figure 2. Correlations of cerebrospinal fluid (CSF) β chemokine levels with CSF human immunodeficiency virus (HIV) RNA. In subjects with detectable levels, CSF macrophage inflammatory protein-1α (MIP-1α) was inversely correlated with CSF HIV RNA (A). CSF MIP-1β (B) and regulated upon activation, normal T cell expressed and secreted (RANTES) (C) were strongly correlated with CSF HIV RNA levels, especially in those with <200 CD4+ cells/mm³. Correlations did not differ by dementia group. One outlier is not displayed in C for graphical clarity. Solid line represents correlation line for each plot.
Table 3. Concentrations of β chemokines (pg/mL) in cerebrospinal fluid (CSF) and plasma of human immunodeficiency virus-infected subjects grouped by cognitive category.

<table>
<thead>
<tr>
<th>Category</th>
<th>Entire cohort</th>
<th>Normal</th>
<th>Impaired</th>
<th>Demented</th>
<th>( P^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF MIP-1α</td>
<td>&lt;7 (&lt;7–913)</td>
<td>&lt;7 (&lt;7–913)</td>
<td>&lt;7 (&lt;7–671)</td>
<td>254.0 (&lt;7–681)</td>
<td>0.01</td>
</tr>
<tr>
<td>CSF MIP-1β</td>
<td>579.9 (&lt;11–1701)</td>
<td>576.8 (&lt;11–1701)</td>
<td>449.1 (&lt;11–1021)</td>
<td>630.2 (&lt;11–1141)</td>
<td>NSb</td>
</tr>
<tr>
<td>CSF RANTES</td>
<td>95.4 (&lt;5–1442)</td>
<td>85.2 (&lt;5–1442)</td>
<td>68.6 (&lt;5–1135)</td>
<td>119.2 (&lt;5–345)</td>
<td>0.04</td>
</tr>
<tr>
<td>Plasma MIP-1α</td>
<td>172.3 (60–820)</td>
<td>188.9 (60–820)</td>
<td>155.6 (96–225)</td>
<td>121.1 (116–209)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma MIP-1β</td>
<td>314.8 (47–1434)</td>
<td>274.9 (150–1170)</td>
<td>298.6 (47–1454)</td>
<td>432.2 (226–1052)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE. Data are median (range). CSF macrophage inflammatory protein-1 (MIP-1α) and regulated upon activation, normal T cell expressed and secreted (RANTES) levels differed significantly across cognitive categories; however, normal and impaired patients had similar CSF chemokine levels, as did those with less severe and more severe dementia (Memorial Sloan-Kettering [MSK] stage 1 \( n = 9 \) vs. MSK stage 2 \( n = 5 \); data not shown). MIP, macrophage inflammatory protein-1; NS, not significant.

a Kruskal-Wallis rank sum test.
b 0.05 < \( P < 0.10 \).
c Only 3 demented patients had plasma available for testing.

The degree of immunosuppression appeared to modify these relationships profoundly. Although CSF β chemokines generally correlated only with each other in earlier stages of HIV disease, these correlations weakened, and the chemokines were instead correlated with other markers in AIDS patients. For example, CSF levels of RANTES correlated with those of MIP-1α but not with HIV RNA in patients with >200 CD4+ cells/mm\(^3\). These relationships were reversed in subjects with AIDS (i.e., CSF RANTES correlated with CSF HIV RNA but not with CSF MIP-1α). This suggests that the normal immunologic relationships between chemokines is disturbed in patients with advanced immunosuppression. In addition, plasma and CSF MIP-1α levels were correlated in those with less immunosuppression, suggesting that the systemic circulation may contribute to CSF levels in earlier stages of disease. Levels of this chemokine in plasma and CSF were not correlated in those with AIDS, which suggests that the brain is the source for CSF MIP-1α in those with more advanced disease.

Although demented patients had higher concentrations of all three CSF β chemokines tested, levels did not differ between the mildly impaired and cognitively normal groups. Of the three chemokines, MIP-1α exhibited the most significant relationship to cognitive status. Whereas almost 90% of patients with either undetectable or high levels of CSF MIP-1α were nondemented, all of those with low- to midrange detectable levels were demented. Although this lower range of CSF MIP-1α values was associated with elevated CSF HIV RNA levels, the relationship between CSF MIP-1α and dementia was independent of this association. Thus, very high levels of MIP-1α may both inhibit HIV replication and protect the brain by other mechanisms. The complexity of this relationship may be explained by the presence, in the brain, of monocyte-derived macrophages that can both secrete MIP-1α and robustly replicate HIV [44].

CSF MIP-1β levels were also associated with dementia, but less strongly than MIP-1α. CSF levels of MIP-1β were independently associated with CSF HIV RNA, which suggests that MIP-1β may up-regulate HIV replication within the CNS. Like MIP-1α, CSF RANTES levels were higher in demented subjects and were strongly correlated with CSF HIV RNA levels. Moreover, both CSF RANTES levels and the CSF/plasma ratio of MIP-1β were correlated with the CSF/plasma ratio of HIV RNA, supporting a role for these chemokines in either modulating the integrity of the blood-brain barrier or recruiting transcriptionally active cells into the CNS. The failure of CSF RANTES to contribute independently to regression models may be due to its strong correlations with levels of other chemokines and CSF HIV RNA.

Our findings suggest that the relationships among levels of CSF chemokines, HIV in the CSF, and brain injury are complex. β Chemokine secretion is a component of the host immune response to HIV, which includes both inductive and suppressive factors [15, 45]. Since brain MIP-1α and MIP-1β levels are elevated in HIV encephalitis [27] and CSF levels are elevated in HIV dementia, CSF levels probably reflect those in the brain and may originate there. CNS β chemokine secretion could impact the encephalitic process by three mechanisms.

First, the chemotactic signals of the β chemokines may recruit macrophages to areas of chronic infection in the brain. Such uninfected brain macrophages not only secrete additional chemokines in these chronically infected areas [33] but also serve as a ready pool of HIV-susceptible cells. The presence of a large pool of such cells in the brain is a strong predictor of clinical dementia [46].

Second, β chemokines may directly modulate HIV replication in the brain. Although β chemokines can inhibit HIV replication in vitro [25], the net chemokine effect on replication varies under different experimental conditions [30]. In vitro, this variability depends on factors such as the cellular state of activation, exposure to β chemokines prior to infection [32], and the cell surface density of CCR-5 [47, 48]. On the basis of our findings, high concentrations of MIP-1β and RANTES may stimulate HIV replication in the CSF, whereas high levels of MIP-1α may inhibit it. The concentration-dependent effects of chemokines on HIV replication in vitro may partially explain the nonlinear relationship between these factors and dementia.

Last, chemokines may be directly neurotoxic. Some neurons express CCR-5 [49] and others undergo β chemokine–induced calcium mobilization [50]. Chemokines may both damage neu-
Figure 3. Cerebrospinal fluid (CSF) macrophage inflammatory protein-1α (MIP-1α) by CSF human immunodeficiency (HIV) RNA by dementia group. Groups 1 and 2 did not differ in neurocognitive category, but group 2 subjects with CSF HIV RNA levels >2.5 log copies/mL were more likely to have abnormal cognitive status ($P = .004$, Fisher’s exact test). Group 3 subjects were almost 40× more likely to be demented than the other 2 groups combined.

The associations of high CSF levels of MIP-1β and RANTES with dementia further support a role for direct neurotoxicity in HIV infection.

Kelder et al. [35] recently measured β chemokine concentrations, including monocyte chemotactic protein-1, in the CSF of patients with HAD and compared them with 4 other groups: HIV-infected but neurologically normal patients, HIV-infected patients with other CNS infections, and HIV-uninfected patients with neurologic diseases characterized by either a mononuclear pleocytosis or a lack of inflammation. Similar to our findings, CSF levels of RANTES were higher in demented patients than in the neurologically normal group. These elevated levels were also higher than the levels observed in HIV-seronegative patients with noninflammatory neurologic disorders but similar to levels in those with neurologic disorders characterized by mononuclear inflammation, such as multiple sclerosis or Lyme disease. CSF levels of MIP-1α and MIP-1β did not differ between the groups. A difference in power may explain the difference in findings: 130 CSF specimens from HIV-infected patients were assayed for RANTES in the Kelder study, but far fewer were assayed for MIP-1α (35) or MIP-1β (28).

Our study was limited by being cross-sectional and by the lack of HIV-seronegative controls. In addition, unmeasured factors, such as stimulant drug use, HIV env genotype, and host chemokine and chemokine receptor genotype, may have influenced the results.

Nuovo and colleagues [33] hypothesized that the two key elements in AIDS dementia are productive virus infection and concomitant stimulation of cytokine transcription [33]. This study supports this model of pathogenesis and suggests that the role of some chemokines, such as MIP-1α, in the CNS are complex and nonlinear. Specifically, the absence of β chemokine secretion in the CSF may signify a low risk of HAD, whereas very high concentrations of MIP-1α may be neuroprotective.

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