Increased Macrophage Chemoattractant Protein–1 in Cerebrospinal Fluid Precedes and Predicts Simian Immunodeficiency Virus Encephalitis

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Macrophage chemoattractant protein–1 (MCP-1) may be a key trigger for the influx of macrophages into the brain in human immunodeficiency virus (HIV) encephalitis. In this study, simian immunodeficiency virus–infected macaques that developed moderate-to-severe encephalitis had significantly higher MCP-1 levels in cerebrospinal fluid (CSF) than in plasma as early as 28 days after inoculation, which was before the development of brain lesions. In contrast, CSF:plasma MCP-1 ratios remained constant at preinoculation levels in macaques that developed minimal or no encephalitis. Abundant MCP-1 protein and mRNA were detected in both macrophages and astrocytes in the brain. Macaques with increased MCP-1 in CSF had significantly greater expression of markers of macrophage and microglia activation and infiltration (CD68; \( p = .003 \)) and astrocyte activation (glial fibrillary acidic protein; \( P = .019 \) and \( P = .031 \) in white and gray matter, respectively). The results suggest that the CSF:plasma MCP-1 ratio may be a valuable prognostic marker for the development of HIV-induced central nervous system disease.

Activated macrophages may contribute to the development of HIV dementia by releasing a number of potent neurotoxins, including tumor necrosis factor–\( \alpha \), nitric oxide, and platelet-activating factor. In addition, HIV-infected macrophages release toxic viral gene products, such as Tat and gp120 [12–17]. These toxins contribute to the neuronal damage and loss that are seen in HIV-infected individuals with dementia. To understand the pathogenesis of HIV encephalitis and to develop better treatment modalities, it is critical to establish which factors initiate activation and infiltration of macrophages in the CNS.

Macrophage chemotactic protein–1 (MCP-1), a CC chemokine produced by macrophages, microglia, activated astrocytes, and endothelial cells, is a potent activator of macrophage function and plays a crucial role in the recruitment of monocytes into several organs and tissues, including the brain [18–22]. Elevated CSF levels of MCP-1 have been detected in patients with HIV-associated dementia and HIV encephalitis and in clinically healthy individuals with high HIV-1 RNA levels in the CSF, which suggests that this chemokine is a key player in inflammatory processes in the brains of HIV-infected individuals [23–25]. Serial analysis in a small study of individuals with HIV dementia showed that CSF MCP-1 levels increased before the development of clinical neurologic disease [25].

To explore the relationship between MCP-1 and HIV encephalitis in detail, a rapid, reproducible model of AIDS and CNS disease in simian immunodeficiency virus (SIV)–infected macaques was used [26]. MCP-1 in CSF and plasma was measured during infection, and the results correlated with the se-
verity of encephalitis. Brain tissues from macaques with and without SIV encephalitis were examined by immunohistochemistry and in situ hybridization to detect MCP-1 protein and mRNA, respectively.

Materials and Methods

**Viruses.** SIV/DeltaB670 was obtained originally by coculturing lymph node tissue from SIV-infected monkey B670 with primary human phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PHA blasts) and was never passed in cell lines. Macaques inoculated with this virus swarm develop the full range of SIV-associated diseases, including immunosuppression, opportunistic infections, pneumonia, and encephalitis [26, 27]. SIV/17E-Fr is a cloned recombinant neuroviral virus that was obtained by inserting the entire env and nef genes and the 3’ long terminal repeat of SIV/17E-Br, a virus isolated from the brain of a macaque with fulminant encephalitis, into the backbone of SIVmac239, the virus from which SIV/17E-Br had been derived by serial passage in rhesus macaques [28–31]. Recombinant virus stock was obtained by transfecting the DNA from molecularly cloned SIV/17E-Fr into CEMx174 cells and by using the virus to infect primary rhesus macaque peripheral blood lymphocytes.

**Animal experiments.** Twelve macaques were inoculated intravenously with SIV/DeltaB670 (50 animal infectious dose [AID]₅₀) and SIV/17E-Fr (10,000 AID₅₀), as described elsewhere [27, 32]. An additional 3 macaques were mock inoculated and served as virus-negative controls for the detection and quantitation of MCP-1 in CSF, plasma, and brain and for the quantitation of markers of activation and cellular infiltration in the brain. Macaques were observed daily for clinical signs of illness, such as inappetence, inactivity, or depression. Blood and CSF were sampled on postinoculation (pi) days 3, 7, 10, 14, and 28 and every 2 weeks thereafter. All manipulations were done while the monkeys were anesthetized with ketamine-HCl (Parke-Davis). Eleven of the 12 macaques were euthanized on pi days 84–94; 1 died unexpectedly of AIDS at pi day 72. At euthanasia, animals were perfused with sterile PBS to remove virus-containing blood from the vasculature.

**CD4⁺ T cell counts.** Complete blood cell counts with differentials were done on every blood sample, and the absolute number of lymphocytes was determined by use of a hematology analyzer (CellDyne 3200; Abbott). Mononuclear cells were separated on Percoll discontinuous gradients and were labeled with fluorochrome-conjugated monoclonal antibodies (CD3, clone PR34; BD PharMingen; CD4, Leu3a, CD8, Leu2; Becton Dickinson) to identify CD4⁺ lymphocytes, as described elsewhere [33]. Absolute CD4⁺ lymphocytes were determined by multiplying the percentage of CD4⁺ cells by the absolute lymphocyte count.

**Histopathology.** Sections of CNS, including frontal, parietal, temporal, and occipital cortex, basal ganglia, thalamus, midbrain, medulla, cerebellum, and cervical spinal cord, were examined by microscope in a blinded fashion by 2 pathologists (M.C.Z. and J.L.M.). To quantitate the severity of lesions, sections of frontal and parietal cortex, basal ganglia, thalamus, midbrain, and cerebellum were each given numerical scores of 1 (minimal), 2 (moderate), or 3 (severe), using the following semiquantitative system. Sections with >30 perivascular macrophage-rich cuffs were given a score of 3, sections with 10–30 perivascular cuffs were given a score of 2, and those with <10 perivascular cuffs were given a score of 1. The scores for all sections were totaled and were divided by 6 (6 regions were graded for each brain), to give a mean score (of a maximum of 3) for severity of CNS lesions.

**MCP-1 in plasma and brain.** MCP-1 levels in CSF and plasma from the 12 infected macaques throughout infection and from 3 mock-inoculated control macaques were measured by ELISA (R&D Systems) at each time point. To determine whether there was higher MCP-1 expression in the brain than in the plasma, indicating a gradient of chemokine expression that would result in the net influx of macrophages into the brain, MCP-1 levels also were expressed as the ratio of MCP-1 in the CSF versus that in the plasma.

To measure MCP-1 concentrations in the brain parenchyma, homogenates were prepared from fresh frozen sections of parietal cortex. About 300 mg of fresh frozen tissue, from which gross myelin was dissected, was washed in PBS and then was homogenized for 15 s with an adapted battery-operated homogenizer (KONTES Glass) in 500 μL of buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 0.2 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol). Samples then were triturated through a 19-gauge needle and were incubated for 20 min on ice. Samples subsequently were spun at 16,000 relative centrifugal force at 4°C for 20 min, and supernatants were saved at −70°C for analysis by ELISA. Protein concentrations in the homogenates were determined by a modified Bradford assay (Bio-Rad Protein Assay).

To determine which cells in the brain were expressing MCP-1, sections of frontal cortex at the level of the basal ganglia were immunohistochemically stained with an antibody to human MCP-1 (10-M21; Fitzgerald Industries International). Samples were stained by use of an automated cell stainer (Optimax Plus; BioGenex). In brief, Streck-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated and then were postfixed in Streck tissue fixative for 20 min. For antigen retrieval, tissues were rinsed in water and were heated in a microwave, using antigen unmasking solution (Vector), for 8 min, followed by a 20-min cooling at room temperature. Endogenous peroxidase was quenched with 3% H₂O₂ in water, and then sections were blocked with buffered casein. Primary antibody was applied to the tissues for 3 h at room temperature, the tissues were washed in wash buffer, and secondary biotinylated goat anti–mouse antibody (BioGenex) was applied for 20 min. The tissues were washed again, and streptavidin–horseradish peroxidase was added for another 20 min. The sections then were washed, and diaminobenzidine tetrahydrochloride in buffer containing H₂O₂ was applied to the sections for 10 min. The sections were counterstained with hematoxylin and were washed, dehydrated, and mounted.

Immunohistochemical staining with anti–MCP-1 detects MCP-1 that is being produced by cells, as well as MCP-1 that is bound to CCR2 (the MCP-1 receptor) on the surface of cells. Thus, to identify the cells in the brain that were producing MCP-1, we used in situ hybridization to detect MCP-1 RNA in tissues, as described elsewhere [34].

**Quantitative immunohistochemical analysis.** Monoclonal anti-
body KP-1 (DAKO), which recognizes the macrophage marker CD68 in both humans and macaques, was used to identify macrophages/microglia in the brain parenchyma. A bovine polyclonal antibody to glial fibrillary acidic protein (GFAP, DAKO) that recognizes human GFAP and that is highly cross-reactive between species was used to detect macroke GFAP. To ensure uniformity of staining, a 200× magnification encompassing a 2.8-mm² area of subcortical white matter adjacent to the cingulate gyrus. Images were captured using a digital camera (Sensys 2; Photometrics), and were analyzed by use of imaging software (IP-lab; Scanalytics). Each pixel in the image was converted to a value of 1 (positive) or 0 (negative), and the total area occupied by positive pixels was calculated. This provides a quantitative measure of the total area occupied by positively stained cells or portions of cells in the area evaluated.

Quantitation of immunohistochemical staining on tissues was done on 20 adjacent fields of tissue examined at 200× magnification encompassing a 2.8-mm² area of subcortical white matter adjacent to the cingulate gyrus. Images were captured using a digital camera (Sensys 2; Photometrics), and were analyzed by use of imaging software (IP-lab; Scanalytics). Each pixel in the image was converted to a value of 1 (positive) or 0 (negative), and the total percentage of area occupied by positive pixels was calculated. This provides a quantitative measure of the total area occupied by positively stained cells or portions of cells in the area evaluated.

Statistical analysis. A Mann-Whitney 2-sample rank test was used to test the equality of the medians of the 2 populations (CSF: plasma MCP-1 ratios of macaques with moderate or severe encephalitis, [n = 9] vs. CSF: plasma MCP-1 ratios in uninfected macaques and macaques with minimal encephalitis [n = 6]).

Results

Elevated CSF and brain MCP-1 in macaques with encephalitis. Macaques that were coinoculated with the immunosuppressive virus (SIV/DeltaB670) and the neurovirulent molecularly cloned virus (SIV/17E-Fr) developed AIDS, as indicated by rapid, sustained declines in peripheral blood CD4⁺ T cell counts, and sustained high levels of viral RNA in plasma, ranging from 10⁶ to 10⁷ copy equivalents of viral RNA per milliliter of plasma [26]. Each of the 12 coinoculated macaques in this study showed the same pattern of CD4 cell decline and plasma virus load. Microscopic evaluation of brain sections demonstrated that 9 macaques had moderate or severe encephalitis, whereas the remaining 3 had minimal (2 animals) or no (1 animal) CNS lesions.

MCP-1 levels were measured in the CSF and plasma of all 15 macaques throughout infection (table 1). Infected macaques had elevated levels in both CSF and plasma at pi days 7–10, after which MCP-1 levels declined. In the macaques that developed moderate or severe encephalitis (n = 9), CSF MCP-1 levels had increased again by pi day 28 and continued to increase throughout the remainder of the infection period. In contrast, macaques that had no or mild encephalitis (n = 6) had continued low CSF MCP-1 levels. After pi day 28, plasma MCP-1 levels stayed relatively stable in both groups of animals.

To determine whether there was a gradient of chemokine expression between the brain and periphery, which would result in an influx of macrophages to the brain, MCP-1 levels were expressed as the ratio of MCP-1 in the CSF versus that in the plasma. MCP-1 ratios of all 12 SIV-infected macaques increased during acute infection, peaked at pi day 10, and then declined. As early as pi day 28, CSF:plasma MCP-1 ratios clustered into 2 overtly distinct patterns. The first group, which consisted of macaques that developed moderate or severe encephalitis, had significantly higher median MCP-1 ratios (P < .05) at pi day 28, and the median MCP-1 ratios of this group continued to increase throughout infection (figure 1). The other group, which consisted of uninfected macaques and infected macaques that had no or mild encephalitis terminally, had median MCP-1 ratios that increased slightly at pi days 10–14 but then declined.

Table 1. Macrophage chemoattractant protein–1 (MCP-1) levels in cerebrospinal fluid (CSF) and plasma from macaques with different severity levels of encephalitis.

<table>
<thead>
<tr>
<th>Time of testing</th>
<th>Severe or moderate encephalitis</th>
<th>No or mild encephalitis</th>
<th>Severe or moderate encephalitis</th>
<th>No or mild encephalitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after inoculation</td>
<td>3176 (548–7349)</td>
<td>849 (363–1742)</td>
<td>1317 (707–2249)</td>
<td>909 (153–2454)</td>
</tr>
<tr>
<td>7</td>
<td>6290 (532–29,523)</td>
<td>1759 (462–6690)</td>
<td>541 (209–943)</td>
<td>342 (158–486)</td>
</tr>
<tr>
<td>10</td>
<td>742 (361–1433)</td>
<td>302 (129–466)</td>
<td>305 (172–437)</td>
<td>195 (114–283)</td>
</tr>
<tr>
<td>14</td>
<td>985 (480–2302)</td>
<td>404 (190–564)</td>
<td>250 (180–438)</td>
<td>221 (176–282)</td>
</tr>
<tr>
<td>28</td>
<td>1885 (731–5495)</td>
<td>336 (96–500)</td>
<td>323 (239–457)</td>
<td>238 (186–295)</td>
</tr>
<tr>
<td>42</td>
<td>2080 (784–3925)</td>
<td>517 (183–792)</td>
<td>330 (249–491)</td>
<td>198 (109–281)</td>
</tr>
<tr>
<td>56</td>
<td>4627 (1676–18,381)</td>
<td>342 (108–791)</td>
<td>330 (223–677)</td>
<td>254 (114–386)</td>
</tr>
<tr>
<td>70</td>
<td>3957 (1672–10,224)</td>
<td>484 (141–1141)</td>
<td>358 (239–543)</td>
<td>297 (178–437)</td>
</tr>
</tbody>
</table>

NOTE: Data are mean (range). SIV, simian immunodeficiency virus.

a Mean values, as detected by ELISA, for 9 SIV-infected macaques that developed severe or moderate encephalitis by postinoculation day 84.

b Mean values, as detected by ELISA, for 3 uninfected macaques and 3 SIV-infected macaques that had no or mild encephalitis by postinoculation day 84.

c Mean for 3 different samples obtained at 2-week intervals before inoculation.
Macaque with no or mild encephalitis are also shown. The 25th, 50th (median), and 75th percentiles of all data points for the group of uninfected macaques and in the CSF than in plasma (expressed as the ratio of MCP-1 in CSF:plasma) as early as postinoculation (pi) day 28. At pi day 42, the difference in MCP-1 ratios between the 2 groups of macaques was highly significant (p < 0.05). The 25th, 50th (median), and 75th percentiles of all data points for the group of uninfected macaques and macaques with no or mild encephalitis are also shown. Q, quartile.

Figure 1. Median ratios of macrophage chemoattractant factor–1 (MCP-1) in cerebrospinal fluid (CSF):plasma of macaques with moderate or severe encephalitis (●, n = 9) and uninfected macaques and macaques with no or mild encephalitis (▲, n = 6). Simian immunodeficiency virus (SIV)-infected macaques with moderate or severe encephalitis 3 months after inoculation had significantly higher MCP-1 in the CSF than in plasma (expressed as the ratio of MCP-1 in CSF:plasma) as early as postinoculation (pi) day 28 (p < 0.05). At pi day 42, the difference in MCP-1 ratios between the 2 groups of macaques was highly significant (p < 0.005). The 25th, 50th (median), and 75th percentiles of all data points for the group of uninfected macaques and macaques with no or mild encephalitis are also shown. Q, quartile.

and remained low. These data suggest that, although MCP-1 expression in the brain increases during acute infection in all SIV-infected animals, macaques that will develop SIV encephalitis experience a second increase and sustained high levels of MCP-1 expression in the brain.

To determine whether there was any evidence of inflammation in the brain coincident with this second increase in expression of MCP-1 in the CSF, sections of brain from SIV-infected macaques euthanized at pi days 21, and 56 were examined. Of interest, 0 of 6 SIV-infected macaques euthanized at pi day 21 and only 1 of 6 SIV-infected macaques euthanized at pi day 56 had evidence of encephalitis in the brain. Inflammation in the single macaque at pi day 56 consisted of only rare small perivascular cuffs. These data suggest that the increase in MCP-1 expression in CSF:plasma occurs well before the development of overt inflammatory changes in the brain.

To further examine the relationship between MCP-1 expression in the brain and the severity of encephalitis, MCP-1 was measured by ELISA on brain homogenates from the 12 SIV-infected macaques euthanized 3 months after inoculation and from the 3 uninfected control macaques. Homogenates were produced from parietal cortex, a target site for SIV encephalitis. In this region of brain, all 9 macaques with moderate or severe encephalitis had detectable levels of MCP-1 in brain homogenates, ranging from 52 to 1637 pg/mL (table 2). None of the uninfected macaques or the SIV-infected macaques with no or minimal encephalitis had detectable MCP-1 in brain homogenates. There was no direct correlation between MCP-1 levels in CSF and MCP-1 detected in this single area of brain at necropsy. The wide range of MCP-1 levels in brain homogenates may reflect real differences between animals or, more probably, local differences in MCP-1 expression due to sampling variability. The lesions in SIV encephalitis are multifocal and cannot be visualized during gross dissection. MCP-1 levels were measured in homogenates made from small (3 mm³) samples of brain, which may or may not have included areas with large numbers of MCP-1–producing cells. In contrast, the CSF contains MCP-1 in the extracellular fluids that have drained from all parts of the brain. Thus, the level of MCP-1 in CSF would more accurately reflect MCP-1 production in the brain as a whole.

MCP-1 in brain macrophages and astrocytes. To determine which cells in the brain were expressing MCP-1, we stained sections of frontal cortex immunohistochemically, using an antibody to MCP-1. There was abundant MCP-1 expression in the brains of macaques with SIV encephalitis, particularly in perivascular cuffs and in microglial nodules. These MCP-1–expressing cells had eccentric nuclei and abundant cytoplasm and were morphologically consistent with macrophages (figure 2). There also was abundant MCP-1 expression in cells evenly distributed throughout the brain that had large eccentric vesicular nuclei, abundant cytoplasm, and branching cell processes. These cells were morphologically consistent with astrocytes (figure 2). Since immunohistochemical staining may potentially detect MCP-1 that is bound to CCR2b, the MCP-1 receptor,

<table>
<thead>
<tr>
<th>Infection status,</th>
<th>Brain MCP-1 level, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain lesion severity, macaque identification no.</td>
<td>Brain MCP-1 level, pg/mL</td>
</tr>
<tr>
<td>Uninfected None</td>
<td>0</td>
</tr>
<tr>
<td>18013</td>
<td>0</td>
</tr>
<tr>
<td>18130</td>
<td>0</td>
</tr>
<tr>
<td>18180</td>
<td>0</td>
</tr>
<tr>
<td>Infected None or mild</td>
<td>0</td>
</tr>
<tr>
<td>713</td>
<td>0</td>
</tr>
<tr>
<td>715</td>
<td>0</td>
</tr>
<tr>
<td>17834</td>
<td>0</td>
</tr>
<tr>
<td>Moderate 397</td>
<td>524</td>
</tr>
<tr>
<td>394</td>
<td>52</td>
</tr>
<tr>
<td>708</td>
<td>502</td>
</tr>
<tr>
<td>18301</td>
<td>1637</td>
</tr>
<tr>
<td>18242</td>
<td>756</td>
</tr>
<tr>
<td>Severe 389</td>
<td>426</td>
</tr>
<tr>
<td>17850</td>
<td>60°</td>
</tr>
<tr>
<td>18033</td>
<td>1364</td>
</tr>
<tr>
<td>18292</td>
<td>708</td>
</tr>
</tbody>
</table>

NOTE. MCP-1 levels, as detected by ELISA, on homogenates of parietal cortex from macaques with encephalitis of varying severity.

° This macaque died unexpectedly, resulting in a postmortem interval of ~12 h before the brain was sampled.
we also performed in situ hybridization to detect MCP-1 mRNA being produced by cells in the brain. As in the immunohistochemically stained sections, in situ hybridization demonstrated MCP-1 mRNA in macrophages in perivascular cuffs and microglial nodules, and in astrocytes throughout the brain parenchyma (figure 2).

**Increased macrophage activation and infiltration in the brains of macaques with high CSF MCP-1.** A high CSF:plasma MCP-1 ratio would be expected to result in an influx of monocytes from the blood into the brain in response to the chemokine gradient. We therefore measured the expression of a macrophage marker in the brains of the 12 SIV-infected macaques and 3 uninfected control macaques to quantitate macrophage activation and infiltration. Coronal sections of brain at the level of the caudate nucleus of the basal ganglia were immunohistochemically stained with the monoclonal antibody KP-1, which binds to CD68, a marker specific for macrophages and microglia. The total area that stained positively for CD68 in a 2.8-mm² segment of subcortical white matter dorsal to the caudate nucleus was calculated by quantitative digital image analysis. Macaques with high CSF:plasma MCP-1 ratios had significantly greater CD68 expression \( (P = .002; \text{table 3}) \) than macaques with low MCP-1 ratios. Microscopic examination of sections revealed that the increase in expression of CD68 in the brains of macaques with encephalitis reflected both microglial activation and an increased influx of macrophages into the brain (figure 2). Since macrophages themselves produce MCP-1, this influx of macrophages into the brain may set up a vicious cycle of MCP-1 production and macrophage infiltration and activation.

**Astrocyte activation in macaques with elevated CSF MCP-1.**

Astrocytes, the most numerous cells in the brain, are prolific producers of MCP-1 when activated [35, 36]. Therefore, we measured GFAP expression as a marker of astrocyte activation in the brains, using digital image analysis on immunohistochemically stained sections of brain. There was significantly greater GFAP expression in both white and gray matter in the subcortical white matter \( (P = .019 \text{ and } P = .031, \text{respectively}) \) 3 months after inoculation in the macaques with higher CSF:plasma MCP-1 ratios (table 3) than in the group of macaques with low CSF:plasma MCP-1 ratios. Thus, activated astrocytes may be an important source of MCP-1 in the brains of macaques with SIV encephalitis.

**Table 3.** Comparison of expression of macrophage and astrocyte markers in the brains of macaques with high and low macrophage chemoattractant protein–1 (MCP-1) ratios.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Low MCP-1 ratio</th>
<th>High MCP-1 ratio</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>1071</td>
<td>25,574</td>
<td>.002</td>
</tr>
<tr>
<td>GFAP (white)</td>
<td>11</td>
<td>131,140</td>
<td>.019</td>
</tr>
<tr>
<td>GFAP (gray)</td>
<td>&lt;1</td>
<td>83,455</td>
<td>.031</td>
</tr>
</tbody>
</table>

**NOTE.** The total area that was stained positively for CD68 (a marker of macrophage activation and infiltration) or glial fibrillary acidic protein (GFAP; a marker of astrocyte activation) was measured on digital images of 20 adjacent sections encompassing a 2.8-mm² area of frontal cortex in the area of the cingulate gyrus. Mean of the 20 sections was determined for each animal. Values represent the median of means for all animals from each group. Data represent CD68 and GFAP expression of brains from macaques euthanized 3 months after inoculation. SIV, simian immunodeficiency virus.

a Measured in 3 uninfected macaques and 3 SIV-infected macaques with no or minimal encephalitis.

b Measured in 9 SIV-infected macaques with moderate or severe encephalitis.

c From a left-tailed hypothesis test.
Discussion

In this study, SIV-infected macaques that developed moderate or severe encephalitis had elevated levels of MCP-1 in the CSF as early as pi day 28, which was before the development of encephalitis. MCP-1 levels in the CSF of these macaques continued to increase throughout infection; however, levels remained low in macaques with no or mild encephalitis. MCP-1 protein and mRNA were detected in both macrophages and astrocytes. Elevated MCP-1 levels correlated with increased expression of markers for macrophage and astrocyte activation.

HIV and SIV infections frequently result in productive infection of the CNS and subsequent encephalitis. However, it remains difficult to diagnose such infections in the early or preclinical stages of infection, without directly sampling the brain. CSF analysis provides a valuable technique by which cells, virus, and soluble factors produced in the brain parenchyma can be sampled. Since MCP-1 is present constitutively in both plasma and brain, in this study we measured and compared MCP-1 in both compartments to determine whether there was a gradient of MCP-1 expression between the CNS and the periphery that would result in a net influx of macrophages into the brain. Our results showed that the increases in the ratios of MCP-1 in CSF versus plasma seen in macaques that developed moderate or severe encephalitis was accounted for mainly by increases in CSF MCP-1.

CSF is composed both of plasma components that are filtered through the choroid plexus and of interstitial fluid and cells from the brain parenchyma that drain into the perivascular (Virchow-Robin) spaces and enter the meningeal spaces [37, 38]. It is not known whether MCP-1 passes into the CSF through the choroid plexus. However, if MCP-1 passes freely from the blood through the choroid plexus and into the CSF, the level of MCP-1 in CSF should never be greater than that in plasma. Thus, CSF:plasma MCP-1 ratios >1 are indicative of MCP-1 production by cells in the brain parenchyma.

For MCP-1 expression to result in an influx of macrophages to the brain, there must be a gradient of chemokine expression, with the highest levels in the site to which most macrophages are attracted. This gradient of MCP-1 expression may initially be created by production of MCP-1 by activated astrocytes, since we routinely see up-regulation of GFAP expression as early as 21 days after inoculation, which occurs before any microscopic evidence of inflammation in the brain. Activation of astrocytes may result from exposure to soluble factors secreted by trafficking-activated lymphocytes and monocytes during acute infection. Activated astrocytes may signal events in endothelial cells, including MCP-1 production and activation, to increase cell trafficking. Macrophages in the circulation then would respond to the chemotactic gradient by binding to the endothelial cells and by passing into the brain parenchyma. This hypothesis is supported by the significantly increased expression of macrophage markers in macaques with high CSF:plasma MCP-1 ratios. Infiltrating macrophages would form inflammatory foci, secreting more MCP-1 into the brain parenchyma in addition to myriad neurotoxic and proinflammatory factors. Furthermore, a percentage of infiltrating macrophages would carry the virus, thus up-regulating virus load.

CSF MCP-1 is elevated in HIV-infected individuals with dementia and increases over time in patients who develop dementia [23-25]. MCP-1 is abundantly expressed by astrocytes in active demyelinating lesions of multiple sclerosis (MS) and in chronic active MS lesions, although it is not detected in perivascular and parenchymal macrophages in these individuals [39, 40]. Additional studies have demonstrated significant increases in MCP-1 in the brains of rats with experimental autoimmune encephalomyelitis [41]. A recent study, however, reported that CSF MCP-1 levels were significantly lower in individuals with acute MS, compared with those with stable MS, which suggests that the role of MCP-1 in the inflammation that characterizes MS remains to be elucidated [42].

The data presented here suggest that MCP-1 is an early predictor of encephalitis after SIV infection. MCP-1 is thus an excellent candidate for a surrogate marker of early infection and inflammation in the CNS of HIV-infected individuals, especially if combined with the predictive value provided by measurement of viral RNA in the CSF [26]. Such early predictors of CNS inflammation could be used to design therapeutic agents that, if used in the early stages of infection, might interrupt the development of CNS inflammation. Although only a small number of currently available antiretroviral drugs penetrate the brain to effective levels, these and newly developed CNS-targeting antiretroviral drugs could be used selectively for patients that are at risk for CNS infection and inflammation as indicated by high MCP-1 ratios. It will be important to examine the prognostic value of CSF:plasma MCP-1 ratios in HIV-infected patients who are followed longitudinally, using virus, immunologic, and cognitive and/or behavior tests. Such studies would complement studies in animal models in which the direct assessment of CNS infection and disease can be made.

Acknowledgments

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NOTICES

GlaxoSmithKline Drug Discovery and Development Award 2002

GlaxoSmithKline (GSK) is offering $500,000 in unrestricted awards for innovative human immunodeficiency virus (HIV)/AIDS drug research. Applications are now being solicited for the 2002 awards and must be submitted by 31 July 2002. The one-time awards are from $25,000 to $150,000 and are intended to further the development of inventive treatments for HIV/AIDS, including therapies aimed at treating infection and prophylactic vaccines or microbicides designed to prevent transmission of the virus. Award recipients will be chosen by 3 independent AIDS researchers, and awards will be announced in September 2002 at the Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in San Diego. The awards will be paid on 1 November 2002. For detailed information about the GSK Drug Discovery and Development Award, as well as an application, please call (888) 527-6935.

Charles E. Culpeper Scholarships in Medical Science

The Rockefeller Brothers Fund is currently accepting applications for its 2003 Charles E. Culpeper Scholarships in Medical Science Program, designed to support the career development of academic physicians. Up to 4 awards of $100,000 per year for 3 years will be made to US medical schools or equivalent US educational institutions on behalf of candidates who are US citizens or aliens who have been granted permanent US residence (proof required); who have received the MD degree from a US medical school or who have the equivalent of an MD degree from an educational institution equivalent to a US medical school in 1994 or later (except under extraordinary circumstances, as approved by the Fund before submittal); and who are judged worthy of support by virtue of the quality of their research proposals. All scientific research relevant to human health is eligible for consideration. No institution may nominate more than 1 candidate.

In selecting awardees, emphasis will be on identifying young physicians with clear potential for making substantial contributions to science as academic physicians. Since January 1988, 49 physicians have been selected as Charles E. Culpeper Medical Scholars.

The deadline for applications is 15 August 2002. Awards will be announced in January 2003, for activation on or about 1 July 2003. Application forms and instructions may be obtained at http://www.rbf.org or by contacting the Rockefeller Brothers Fund, 437 Madison Avenue, 37th Floor, New York, NY 10022-7001; phone, (212) 812-4200; fax, (212) 812-4299.
ERRATA

In an article in the 15 October 2001 issue of the Journal (Zink MC, Coleman GD, Mankowski JL, et al. Increased macrophage chemoattractant protein–1 in cerebrospinal fluid precedes and predicts simian immunodeficiency virus encephalitis. J Infect Dis 2001;184:1015–21), the following should have been included in the financial support footnote: National Institute of Mental Health (MH-61187) and National Institutes of Health/National Institute of Neurological Disorders and Stroke (NS-35751, NS-36911, and NS-35334).

In an article in the 15 November 2001 issue of the Journal (Walzer PD, Ashbaugh A, Collins M, Cushion MT. Anti–human immunodeficiency virus drugs are ineffective against Pneumocystis carinii in vitro and in vivo. J Infect Dis 2001;184:1355–7), there is an error in the first paragraph of the Discussion section. In the sixth sentence, the phrase “the Spinner flask cell culture system counts” should read “the Spinner flask and cell culture systems count.”

In an article in the 15 May 2002 issue of the Journal (Skorowski DM, De Serres G, MacDonald D, et al. The changing age and seasonal profile of pertussis in Canada. J Infect Dis 2002;185:1448–53), there are errors in the legend and panel B of figure 2. The first sentence of the legend should read “Proportion (A) and incidence (B) [not ‘Incidence (A) and proportion (B)’] of pertussis notifications, by age group . . . .” Also, in panel B the activity bars for 1990 for age groups 5–9 and 10–14 are missing. A corrected version of panel B is shown below.