Concentrations of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in Cerebrospinal Fluid after Antiretroviral Treatment Initiated during Primary HIV-1 Infection

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In 6 patients with primary human immunodeficiency virus type 1 (HIV-1) infection, concentrations of HIV-1 RNA and β2-microglobulin were monitored in cerebrospinal fluid (CSF) and in plasma during antiretroviral therapy. Four patients had neurological symptoms. At baseline, the CSF of 5 patients had detectable levels of HIV-1 RNA (median, 3.68 log10 copies/mL; range, <2.60–5.67 log10 copies/mL), and the CSF of 3 patients had elevated levels of β2-microglobulin. After 8 weeks of treatment, the median concentrations of HIV-1 RNA in CSF had decreased to <2.60 log10 copies/mL (range, <1.60–3.00 log10 copies/mL; P = .04) and in plasma to 3.07 log10 copies/mL (range, 2.57–3.79 log10 copies/mL; P = .03). Median concentration of β2-microglobulin in CSF had decreased to 1.2 mg/L (range, 0.9–1.7 mg/L; P = .06) and, in plasma, to 1.7 mg/L (range, 1.1–2.2 mg/L; P = .03). After 48 weeks, HIV-1 RNA concentrations in 1 patient were still 1.97 log10 copies/mL in CSF and 1.51 log10 copies/mL in plasma, although β2-microglobulin concentrations in CSF and plasma had normalized after 8 weeks.

Primary HIV-1 infection is frequently associated with a transient flulike illness that is often undiagnosed or misdiagnosed [1, 2]. Neurological manifestations may occur, ranging from mild viral meningitis to encephalitis [2, 3]. The incidence of symptoms consistent with viral meningitis during primary HIV-1 infection is 9% [2]. Neurological symptoms generally resolve in several weeks [2]. HIV-1 and HIV-1 p24 have been detected in CSF specimens obtained from such patients [3, 4]. Very few longitudinal data are available on CSF findings for patients receiving antiretroviral treatment that was initiated at the time of primary HIV-1 infection [5, 6]. Because the investigation of CSF provides a window on what is happening in the brain parenchyma [7], we longitudinally measured HIV-1 RNA and β2-microglobulin in CSF and plasma from 6 patients who started receiving regimens of 5 or of 6 antiretroviral drugs at about this time.

**PATIENTS AND METHODS**

Since November 1997, 6 patients with primary HIV-1 infection have been enrolled in an open-label trial to evaluate the efficacy of a 5-drug treatment regimen
comprised of zidovudine, 300 mg b.i.d. (or stavudine, 40 mg b.i.d.); lamivudine, 150 mg b.i.d.; abacavir, 300 mg b.i.d.; indinavir, 1000 mg t.i.d.; and nevirapine, 400 mg q.d. [8]. During the study, this drug regimen was adapted as follows: indinavir at 1000 mg t.i.d. was replaced by indinavir at 800 mg t.i.d. (or 800 mg b.i.d.) plus ritonavir at 100 mg b.i.d. Four patients started receiving the 5-drug regimen and later changed to the 6-drug regimen. Two patients received the 6-drug regimen from the start. All patients were asked to participate in a neurological substudy to assess HIV-1 RNA levels and concentrations of β2-microglobulin in the CSF. Lumbar punctures were planned to be performed at baseline and 8, 48, and 96 weeks after the initiation of therapy.

Serum samples were screened for the presence of antibodies to HIV-1 with use of a commercial EIA and confirmed by means of Western blotting, performed according to the manufacturers' instructions (Abbott Laboratories). Levels of HIV-1 RNA in CSF and in plasma were measured by use of the NucliSens HIV-1 QT assay (Organon Teknika). Standard measurements were done on 200 μL of plasma or CSF, resulting in a lower limit of quantification of 400 copies/mL. When HIV-1 RNA levels decreased to <400 copies/mL and a sufficient amount of the sample was available, an initial input volume of 2 mL was used, resulting in a lower limit of quantification of 40 copies/mL; for plasma, an ultrasensitive protocol adaptation was used, resulting in a lower limit of quantification of 5 copies/mL [8].

Concentrations of β2-microglobulin were measured by use of microparticle capture EIA (IMx analyzer; Abbott Laboratories). Normal values are <2.4 mg/L in plasma and <2.2 mg/L in CSF. CSF was analyzed for cell count and glucose and total protein levels. Samples were stored at −70°C until assessment. CSF and plasma samples were available from 16 HIV-1–seronegative control subjects.

Results are reported as medians and ranges for all variables. Differences were tested by use of the Wilcoxon matched pairs signed rank test and Mann-Whitney U test, as appropriate. Correlations were tested by use of Spearman’s rank correlation test. If values were below the lower limit of detection, the cutoff point was used as the subject’s value in all analyses. P ≤ .05 was considered significant.

**RESULTS**

Five patients (patients 16, 18, 20, 21, 23) experienced an acute illness consistent with primary HIV-1 infection and had peak plasma HIV-1 RNA concentrations of ≥5 log10 copies/mL. For 4 of these 5 patients (patients 16, 18, 21, 23), the results of Western blotting were indeterminate during their first visit but evolved toward positive during follow up. One patient (patient 20) had a peak plasma HIV-1 RNA concentration of 6.4 log10 copies/mL, and the HIV-1 antibody level increased 4-fold after the acute illness. For 1 patient (patient 24), results of HIV-1 antibody screening were negative 6 months before study entry, but the patient was found to be HIV-1–seropositive 6 weeks before enrollment.

Two patients (patients 16 and 18) had severe neurological symptoms. Patient 16 had meningoencephaloradiculitis due to primary HIV-1 infection. The first CSF examination (done elsewhere) revealed the following values: leukocyte count, 130 cells/μL; protein level, 2.89 g/L; and glucose level, 2.7 mM/L. The following tests yielded negative results: cultures for bacteria and virus, Ziehl-Neelsen staining, and PCR analysis for herpesviruses. Patient 18 had viral meningitis due to primary HIV-1 infection; symptoms gradually resolved over several weeks. Two patients (patients 20 and 23) had mild neurological symptoms (headache and fever), and 2 patients had no neurological symptoms (table 1).
At baseline, 5 patients had detectable HIV-1 RNA in CSF (median, 3.68 log_{10} copies/mL; range, <2.60–5.67 log_{10} copies/mL); the 2 patients without neurological symptoms had either low (2.79 log_{10} copies/mL) or undetectable levels (figure 1; table 2). In CSF, neither leukocyte level nor protein levels correlated with levels of HIV-1 RNA (data not shown). Concomitant plasma levels of HIV-1 RNA were 5.46 log_{10} copies/mL (range, 4.40–5.97 log_{10} copies/mL). Levels of HIV-1 RNA in CSF were not significantly correlated with plasma HIV-1 RNA levels \((r = .77; P = .07)\). After 8 weeks of treatment, levels of HIV-1 RNA in CSF decreased significantly, to a median of <2.60 log_{10} copies/mL (range, <1.60–3.00 log_{10} copies/mL; \(P = .04\); figure 1; tables 1 and 2).

For 2 patients, data were available for week 48. The patient who had the highest level of HIV-1 RNA in CSF at baseline (5.67 log_{10} copies/mL; plasma HIV-1 RNA level, 5.48 log_{10} copies/mL) still had detectable HIV-1 RNA after 8 weeks (in CSF, 3.00 log_{10} copies/mL; in plasma, 3.52 log_{10} copies/mL) and after 48 weeks (in CSF, 1.97 log_{10} copies/mL; in plasma, 1.51 log_{10} copies/mL). The plasma HIV-1 RNA level remained <0.70 log_{10} copies/mL from week 71 onwards, but follow-up measurement of concentrations in CSF was not planned to be performed until week 96. The other patient’s HIV-1 RNA levels in CSF were <1.60 log_{10} copies/mL at both week 8 and 48. Plasma HIV-1 RNA levels decreased significantly, from 5.46 log_{10} copies/mL (range, 4.40–5.97 log_{10} copies/mL) to 3.07 log_{10} copies/mL (range, 2.57–3.79 log_{10} copies/mL; \(P = .03\)) after 8 weeks of treatment (figure 1).

In addition, the CSF inflammatory response was evaluated during treatment. After 8 weeks of treatment, the median leukocyte count in CSF decreased from 30 cells/μL (range, 4–203 cells/μL; >90% lymphocytes in all patients) to 4 cells/μL (range, 2–13 cells/μL; \(P = .03\)). The median protein level in CSF decreased slightly, from 0.58 g/L (range, 0.32–1.36 g/L) to 0.48 g/L (0.22–0.68 g/L; \(P = .10\)). At baseline, \(\beta_2\)-microglobulin levels in CSF were significantly higher in patients infected with HIV-1 than levels in HIV-1–seronegative control subjects (\(P = .002\)), and they were elevated in 3 patients (table 2).

Baseline levels of \(\beta_2\)-microglobulin in CSF were significantly correlated with the leukocyte count in CSF \((r = .83; P = .04)\) and protein levels in CSF \((r = .83; P = .04)\). Baseline levels of \(\beta_2\)-microglobulin in plasma also were higher in case patients than they were in control subjects \((P = .002)\). Baseline levels of \(\beta_2\)-microglobulin in CSF and plasma were significantly correlated \((r = .78; P < .001)\); 4 patients had levels of \(\beta_2\)-microglobulin in CSF that exceeded the levels in plasma. After 8 weeks of treatment, levels of \(\beta_2\)-microglobulin in CSF decreased from 2.5 mg/L (range, 0.9–5.5 mg/L) to 1.2 mg/L (range, 0.9–1.7 mg/L; \(P = .06\); figure 2).

For 2 patients, data were available for week 48. For patient 16, the \(\beta_2\)-microglobulin level in CSF decreased from 2.0 mg/L to 1.2 mg/L at week 8 and to 0.8 mg/L at week 48; the level in plasma decreased from 1.7 mg/L at baseline to 1.1 mg/L at week 8 and 1.0 mg/L at week 48. At baseline, patient 18 had elevated levels of \(\beta_2\)-microglobulin in CSF and in plasma (5.5 and 3.1 mg/L, respectively), which decreased to 1.4 and 1.9 mg/L, respectively, after 8 weeks and to 0.9 and 1.1 mg/L, respectively, after 48 weeks. After 8 weeks, levels of \(\beta_2\)-microglobulin in plasma had decreased from 2.6 mg/L (range, 1.4–3.1 mg/L) to 1.7 mg/L (range, 1.1–2.2 mg/L; \(P = .03\); figure 2).

**DISCUSSION**

Measuring the level of HIV-1 RNA in CSF may indicate what is happening in the brain [7]. HIV-1 RNA is detectable in the

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**Table 2. Concentrations of \(\beta_2\)-microglobulin and HIV-1 RNA in CSF and in plasma before and after initiation of treatment with 5 or 6 antiretroviral drugs.**

<table>
<thead>
<tr>
<th>Compartment, time relative to initiation of treatment</th>
<th>(\beta_2)-microglobulin, mg/L</th>
<th>HIV-1 RNA, log_{10} copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.8 (0.5–1.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Case patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.5 (0.9–5.5)(^a)</td>
<td>3.68 (&lt;2.60–5.67)</td>
</tr>
<tr>
<td>8 w after</td>
<td>1.2 (0.9–1.7)(^b)</td>
<td>2.60 (&lt;1.60–3.00)(^c)</td>
</tr>
<tr>
<td>48 w after ((n = 2))</td>
<td>0.9 (0.8–0.9)</td>
<td>1.82 (&lt;1.60–1.97)</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>1.3 (0.8–2.2)</td>
<td>NA</td>
</tr>
<tr>
<td>Case patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>2.6 (1.4–3.1)(^a)</td>
<td>5.46 (4.40–5.97)</td>
</tr>
<tr>
<td>8 w after</td>
<td>1.7 (1.1–2.2)(^d)</td>
<td>3.07 (2.57–3.79)(^d)</td>
</tr>
<tr>
<td>48 w after ((n = 2))</td>
<td>1.1 (1.0–1.1)</td>
<td>1.27 (&lt;0.70–1.51)</td>
</tr>
</tbody>
</table>

\(^{a}\) \(P = .002\) compared with HIV-1 seronegative control subjects.

\(^{b}\) \(P = .06\) compared with values before treatment.

\(^{c}\) \(P = .04\) compared with values before treatment.

\(^{d}\) \(P = .03\) compared with values before treatment.
CSF of most untreated asymptomatic HIV-1–infected persons [9–11], and over several years a small but significant increase is seen in the level [11]. The concentration of HIV-1 RNA in CSF varies widely (range, <2.3–5.10 log10 copies/mL), but it is generally lower than the concentration in plasma [9–12]. However, levels of HIV-1 RNA in CSF may surpass levels in plasma [9, 10, 12]. The CSF HIV-1 RNA concentration is correlated with the CSF lymphocyte count but not necessarily with the plasma HIV-1 concentration [9, 10, 12, 13].

Therefore, it is assumed that lymphocytes are the main source of HIV-1 in the CSF of asymptomatic patients. Leakage of HIV-1 from plasma is improbable, because in these patients the integrity of the blood-brain barrier has generally been preserved. However, lymphocyte transport across an intact blood-brain barrier is known to occur [9, 10, 13]. In neurologically symptomatic patients, CSF HIV-1 RNA concentrations correlate with the presence and severity of cognitive impairment, neuropathologic abnormalities, and with high levels of HIV-1 RNA in the brain [14–17]. In these patients, CSF HIV-1 RNA levels are independent of CSF lymphocyte counts, a finding that strongly supports the hypothesis that brain macrophages are the source of HIV-1 RNA in the CSF [7, 18]. In asymptomatic patients, the levels of HIV-1 RNA in CSF and in plasma are seen to decrease at the same rate after the initiation of antiretroviral therapy; whereas in demented persons, the level decreases more slowly in CSF than in plasma, which suggests that HIV-1 replication in the CNS becomes increasingly independent in patients with advanced HIV-1 infection [18].

There is a preliminary report on the monitoring of HIV-1 RNA in CSF in 4 patients who were receiving combination antiretroviral treatment that was started during primary HIV-1 infection, which demonstrated that the level of HIV-1 RNA in CSF had become undetectable after 8 weeks [5]. In 2 patients for whom baseline data were unavailable, HIV-1 RNA was unde-
fection. Eleven patients from our institution started treatment with 2 nucleoside analogues (if necessary, augmented with a protease inhibitor) during chronic HIV-1 infection and had CSF HIV-1 RNA levels of <50 copies/mL after 48 weeks, even when plasma response was not complete [24].

Several explanations can be given for the slow decrease in HIV-1 RNA levels in patient 18. The drug combination used was not the culprit, because it included all of the drugs administered in a previous study [24]. A discordant HIV-1 response in CSF and blood may be explained by divergent resistance patterns in these compartments [25]. There is no reason to doubt that these highly motivated patients adhered to their therapeutic regimens; furthermore, patient 18 eventually had an undetectable plasma HIV-1 RNA concentration. The very high therapeutic regimens; furthermore, patient 18 eventually had an undetectable plasma HIV-1 RNA concentration. The very high concentration of HIV-1 RNA in CSF at baseline may be a contributing factor. Another explanation could be that, at this early stage of HIV-1 infection, the immune response is still immature. Clearance of HIV-1 RNA from the CSF may be slow in patients who start therapy during primary HIV-1 infection. Similar data have been found with regard to levels in plasma [26].

References