Cerebrospinal fluid (CSF) samples were examined from 7 patients infected with human immunodeficiency virus type 1 (HIV-1) who had progressive multifocal leukoencephalopathy (PML). Samples were obtained both before and after 35–365 days of highly active antiretroviral therapy (HAART). By polymerase chain reaction, JC virus (JCV) DNA was found in 6 of 7 patients at baseline but in only 1 patient after HAART. In contrast, in 25 historical control patients from whom sequential CSF specimens were obtained, no reversion from detectable to undetectable JCV DNA was observed. By use of enzyme-linked immunosorbent assay, intrathecal production of antibody to JCV-VP1 was shown in only 1 of 4 HAART recipients at baseline but in 5 of 5 patients after treatment. The neuroradiological picture improved or had stabilized in all patients after 12 months of HAART, and all were alive after a median of 646 days (range, 505–775 days). Prolonged survival after HAART for PML is associated with JCV clearance from CSF. JCV-specific humoral intrathecal immunity may play a role in this response.

Highly active antiretroviral therapy (HAART), i.e., a combination of 3 anti-HIV drugs, including at least 1 protease inhibitor [7–14]. In this study, changes in CSF markers of JCV and HIV infections in patients with PML receiving HAART were analyzed and compared with those observed in historical control patients, who were patients with PML who did not receive HAART.

Patients and Methods

Patients and samples studied. The study involved 7 HIV-1–infected patients with PML from whom CSF samples were drawn before and after HAART. The patients were admitted to San Raffaele Hospital (Milan) or Spedali Civili (Brescia, Italy) from January to July 1997 because of the recent onset of neurological problems. Upon admission, the cliniconeurological picture was suggestive of PML, and the diagnosis was confirmed by CSF PCR for JCV DNA (6 patients) or brain biopsy (1 patient). HAART was started between 26 days before and 14 days after the start of HAART (table 1). None of the patients had ever received protease inhibitors.

A second CSF specimen was drawn 45–365 days after the initiation of HAART (table 1). For 1 patient (patient 7), HAART was discontinued after 3 months, and the second CSF sample was taken 9 months after its withdrawal. Patients 2 and 4 started receiving cidofovir 85 and 265 days, respectively, after the start of HAART.

As control patients for the PCR CSF analyses, we included 25 additional HIV-1–infected patients with PML observed during 1992–1996 either at San Raffaele Hospital or at other northern Italian clinics for infectious diseases that sent serial CSF specimens to our laboratory for diagnostic examinations. The clinical and
radiological characteristics of all of these patients were consistent with PML, and the diagnosis was confirmed by CSF PCR (24 patients) or at postmortem examination (1 patient).

These patients were treated with antiretroviral monotherapy, cytarabine, or both, or they received no specific therapy. Of the control patients for whom sufficient clinical details were available, 7 were matched for CD4+ cell counts to the HAART-treated patients and were selected for comparative purposes. Two of these patients received zidovudine, 1 received didanosine, and 4 received cytarabine and were selected for comparative purposes. Two of these patients who received HAART were assessed every 3 months by a specialist neurologist. CT scanning or MRI of the brain was performed every 3–6 months. CD4+ cell counts and plasma HIV-1 RNA load were determined periodically according to the current guidelines [16].

Results

**CSF and clinical findings at baseline.** JCV DNA was found in the CSF of 6 of the 7 patients before the start of HAART. Concurrent opportunistic brain infections of the brain were not found in any of these patients by clinical, radiological, or CSF examination. At baseline, a JCV-specific intrathecal immune response was revealed in 2 of 4 patients. HIV-1 RNA was detected in the CSF of 6 of the 7 patients (table 1). The CD4+ cell counts, plasma HIV-1 RNA loads, and clinicoradiological features are summarized in table 2.

**CSF and clinical findings at follow-up.** After HAART, JCV DNA was no longer detectable in the CSF of 5 of the 6 originally positive patients, but it was still detectable in 1 patient whose CSF was drawn 35 days after the start of HAART. Intrathecal JCV-specific IgG synthesis was observed in all 5 patients examined. CSF HIV-1 RNA levels decreased or remained low in all of the patients (table 1). Three months after the start of HAART, clinical and neurological conditions had substantially improved for 5 patients and had worsened for 2, whereas a progression of MRI lesions was observed in all of the 5 patients examined.

A response in terms of plasma HIV-1 RNA load and CD4+ cell counts was observed in all evaluable patients. After 1 year of HAART the neurological conditions and MRI lesions of all

### Table 1. CSF markers of JC virus (JCV) and HIV infection in HIV-infected patients with progressive multifocal leukoencephalopathy (PML) receiving highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex, age (y)</th>
<th>Risk factor</th>
<th>PML diagnostic procedure</th>
<th>Therapya</th>
<th>Days from therapy</th>
<th>JCV DNA in CSF</th>
<th>Antibody indexb</th>
<th>HIV-1 RNA in CSF, copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M, 39</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>AZT, 3TC, IDV</td>
<td>6</td>
<td>ND</td>
<td>544,000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>M, 35</td>
<td>IDU</td>
<td>Brain biopsy</td>
<td>d4T, RTV, SQV</td>
<td>27</td>
<td>0.13</td>
<td>8300</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M, 36</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>AZT, 3TC, IDV</td>
<td>26</td>
<td>ND</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F, 32</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>AZT, RTV, SQV</td>
<td>5</td>
<td>0.55</td>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M, 36</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>3TC, d4T, RTV</td>
<td>1</td>
<td>0.94</td>
<td>3500</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M, 32</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>AZT, 3TC, RTV</td>
<td>1</td>
<td>ND</td>
<td>34,300</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>F, 33</td>
<td>IDU</td>
<td>CSF PCR</td>
<td>AZT, 3TC, IDV</td>
<td>1</td>
<td>6.96</td>
<td>760</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Findings of standard CSF analyses were normal for all patients except patient 1 at baseline (36 cells/μL) and patient 7 at follow-up (protein level, 99 mg/dL). AZT, zidovudine; d4T, stavudine; ddI, didanosine; F, female; IDU, intravenous drug use; IDV, indinavir; M, male; ND, not determined; RTV, ritonavir; SQV, saquinavir; 3TC, lamivudine; +, positive; −, negative.

* All patients were receiving 2 reverse transcriptase inhibitors at onset of PML, except for patient 1, who was antiretroviral therapy-naive.

* Antibody index ≥1 indicates intrathecal antibody synthesis.

* In patients 2 and 4, administration of cidofovir was started 85 and 265 days, respectively, after start of HAART.

* For patient 7, therapy was discontinued after 3 months.
Table 2. Clinical, radiological, and laboratory findings in HIV-infected patients with progressive multifocal leukoencephalopathy (PML) receiving highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Neurological symptoms and signs</th>
<th>Brain MRI findings</th>
<th>CD4⁺ cells/µL</th>
<th>HIV RNA, copies/mL</th>
<th>Days survivedᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Month</td>
<td>BL</td>
<td>3 12</td>
</tr>
<tr>
<td>1</td>
<td>Cognitive impairment, aphasia, R hemiparesis</td>
<td>Multifocal lesions (L fronto-parietal lobes, corpus callosum)</td>
<td>ND</td>
<td>ND</td>
<td>8 136</td>
</tr>
<tr>
<td>2ᵈ</td>
<td>Cognitive, visual, and speech impairment, L hemiparesis</td>
<td>Multifocal lesions (R fronto-parietal lobes, brain stem, cerebellum, thalamus, corpus callosum)</td>
<td>W</td>
<td>I</td>
<td>397</td>
</tr>
<tr>
<td>3</td>
<td>Dysarthria, abnormal gait</td>
<td>Multifocal lesions (centrum semiovale, corona radiata)</td>
<td>ND</td>
<td>ND</td>
<td>14</td>
</tr>
<tr>
<td>4ᵈ</td>
<td>Cognitive impairment, 7th cranial nerve deficit, dysarthria, abnormal gait</td>
<td>Multifocal lesions (L cerebellum, L temporal lobe)</td>
<td>W</td>
<td>I</td>
<td>162</td>
</tr>
<tr>
<td>5</td>
<td>Speech impairment, 7th cranial nerve deficit</td>
<td>Multifocal lesions (L frontal lobe)</td>
<td>W</td>
<td>S</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>Cognitive impairment, abnormal gait</td>
<td>Multifocal lesions (R cerebellum)</td>
<td>W</td>
<td>S</td>
<td>22</td>
</tr>
<tr>
<td>7ᵉ</td>
<td>Speech and visual impairment, 7th cranial nerve deficit, abnormal gait</td>
<td>Multifocal lesions (L frontal lobe, thalamus, corpus callosum, R cerebellum)</td>
<td>W</td>
<td>NE</td>
<td>13</td>
</tr>
</tbody>
</table>

NOTE. BL, baseline; I, condition improved; L, left; ND, not determined; NE, not evaluable; R, right; S, stable; W, condition worsened compared with previous assessment.

ᵃ At month 3, an increased volume of preexistent lesions was observed in all patients; new cerebral and brain-stem lesions were observed in patients 6 and 7, respectively.
ᵇ As of 1 February 1999.
ᶜ CT scan was performed on patients 1 and 3. All lesions were located in the white matter and showed no mass effect; a dyshomogeneous contrast-enhancement was observed in patient 3.
ᵈ For patients 2 and 4, administration of cidofovir was started 85 and 265 days, respectively, after start of HAART.
ᵉ For patient 7, therapy was discontinued after 3 months. One year after starting HAART, she developed toxoplasmosis, and clinico-neurological picture of PML was not clearly evaluable at that time.

The widespread introduction of HAART has been followed by a sharp decline in both mortality and morbidity due to opportunistic infections [17]. This study demonstrates a beneficial effect of HAART, not only on neurological status and survival but also on the virological markers of JCV infection in AIDS patients with PML. At the same time, reduced CSF and plasma HIV-1 loads and increased CD4⁺ cell counts were observed in most patients.

In most of our patients, the diagnosis of PML was established by detection of JCV DNA in the CSF, which subsequently became negative for all of the patients receiving HAART for

of the patients had improved or remained unchanged in comparison with month 3, and HAART still had a sustained effect on HIV-1 RNA plasma levels and/or CD4⁺ cell counts (table 2). The patients’ clinical conditions and MRI lesions remained substantially unchanged at subsequent follow-up, and all were still alive 505–775 days (median, 646 days) after the onset of symptoms (table 2).

CSF and clinical findings in control patients. In the whole group of 25 patients with PML who did not receive HAART, the CSF was JCV DNA–positive for 12 at the time of the onset of symptoms and for 24 at follow-up (median interval between first and second sample, 48 days; range, 7–250 days). No patient showed any PCR reversion from positive to negative. There were no substantial differences between the patients who received HAART and the 7 control patients matched for the CD4⁺ cell counts, with regard to PML as first-related illness (5 patients and 6 control patients), presence of multifocal CNS lesions (7 patients and 7 control patients), and time interval between first and second CSF sampling (35–365 days for patients and 45–134 days for control patients). JCV DNA was noted in the first CSF specimen of 6 patients who received HAART and 2 control patients but in the CSF of only 1 patient and 6 control patients at follow-up. The duration of survival was significantly longer among the patients than among control patients (median, 646 vs. 134 days; range, 505–775 vs. 90–156 days; P = .0017 by Mann-Whitney test).

Discussion

The widespread introduction of HAART has been followed by a sharp decline in both mortality and morbidity due to opportunistic infections [17]. This study demonstrates a beneficial effect of HAART, not only on neurological status and survival but also on the virological markers of JCV infection in AIDS patients with PML. At the same time, reduced CSF and plasma HIV-1 loads and increased CD4⁺ cell counts were observed in most patients.
In contrast, the analysis of sequential CSF specimens drawn from historical control patients (patients with PML not treated with HAART) showed either the persistence or the reversion from undetectable to detectable JCV DNA. To the best of our knowledge, clearance of JCV DNA from the CSF of HIV-infected patients with PML has been reported only once for a patient treated with cytarabine before HAART became available [18].

As in other forms of viral encephalitis or meningitis, the presence of viral genomes in the CSF probably reflects replication of virus in the brain [2]. It is therefore conceivable that the clearance of JCV DNA from the CSF of our patients was due to the inhibition of JCV replication in the brain after HAART. Previous studies have demonstrated that PCR analysis of the CSF is negative in approximately one-third of patients with PML and that both a positive finding and a high JCV DNA load are associated with shorter survival [15, 19–22].

In line with these observations, we found that clearance of JCV genome from the CSF during HAART is associated with extraordinarily long durations of survival, compared with those observed before HAART became available [4]; JCV was still detectable in the CSF of a patient after 35 days of HAART. Since the duration of this patient’s survival was not shorter than that of the others, this finding was probably due to the relatively short interval between first and follow-up sampling and indicates that the clearance of JCV from the CSF may take weeks after HAART.

Clearance of JCV DNA was also recently described in reports of a few additional HIV-infected patients with PML; clearance occurred after 5–25 months of HAART [13, 19, 20]. While corroborating the existing data by the study of a substantial number of patients, our PCR findings add information regarding the association with other CSF markers. In this regard, we found increased intrathecal synthesis of JCV-VP1–specific IgG in most of our patients who received HAART, which paralleled JCV DNA clearance from the CSF. Although these findings need to be extended to a larger number of patients, as well as to control patients, they suggest a possible relationship between virological and immunologic events in the brain.

We also observed that the persistence of a JCV-specific humoral response in 1 patient (patient 7) was associated with an absence of JCV DNA in CSF for as long as 9 months after HAART withdrawal, which suggests that, once established, immunologic surveillance might control JCV replication in the brain for months. In addition, CSF HIV-1 RNA levels decreased in our patients who received HAART at the same time as JCV was cleared from the CSF. There is experimental evidence that HIV-1 may upregulate JCV protein expression in glial cells [23] and that both HIV-1 and JCV can infect the same cells, i.e., astrocytes [5, 24]. Therefore, it is also possible that the effects of HAART on the JCV lytic cycle were locally mediated by HIV-1.

Abatement or stabilization of neurological symptoms, in parallel with JCV clearance from the CSF, was also evident weeks to months after the initiation of HAART. However, it is remarkable that MRI revealed a worsening of existent brain lesions and, in some instances, the appearance of new white matter lesions, even months after the start of HAART. As in cases of other opportunistic infections in patients who received HAART [25], it is possible that this was an effect of a post-treatment inflammatory reaction. In this regard, it is noteworthy that in patients with PML who did not receive HAART, a more benign prognosis is associated not only with high CD4+ cell counts [26] but also with the presence of an inflammatory response in the brain tissue [27].

In conclusion, our observations support the view that reduced JCV replication in the brain may eventually lead to stabilization or regression of disease. This effect may be mediated by a JCV-specific humoral and cellular immune response. Beyond the mechanisms underlying these events, our findings may have practical implications for both diagnosis and treatment of PML. It is conceivable that the proportion of patients with PML whose CSF is negative for JCV DNA at diagnosis will increase as an effect of HAART, which would suggest that both brain biopsy and other diagnostic CSF markers might be required in the future. On the other hand, clinical trials evaluating new compounds that are possibly effective on PML should be designed to take into account the effects of HAART on the clinical and virological outcome of PML.

References