Highly Active Antiretroviral Therapy and Progressive Multifocal Leukoencephalopathy: Effects on Cerebrospinal Fluid Markers of JC Virus Replication and Immune Response

Barbara Giudici, Beatriz Vaz, Simona Bossolasco, Salvatore Casari, Anna Maria Brambilla, Wolfgang Lüke, Adriano Lazzarin, Thomas Weber, Paola Cinque

Highly Active Antiretroviral Therapy and Progressive Multifocal Leukoencephalopathy: Effects on Cerebrospinal Fluid Markers of JC Virus Replication and Immune Response

Barbara Giudici,[1] Beatriz Vaz,[3] Simona Bossolasco,[1] Salvatore Casari,[2] Anna Maria Brambilla,[1] Wolfgang Lüke,[4] Adriano Lazzarin,[1] Thomas Weber,[3] and Paola Cinque[1]

From the [1]Division of Infectious Diseases, San Raffaele Hospital, Milan, and [2]Clinic of Infectious Diseases, Spedali Civili, Brescia, Italy; [3]Neurology Clinic, Marienkrankenhausen, Hamburg, and [4]Department of Virology and Immunology, German Primate Center, Göttingen, Germany

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.

Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease of the CNS due to JC polyomavirus (JCV) lytic infection of oligodendrocytes. It usually affects immunocompromised patients, including those with AIDS [1]. Over the last few years, CSF examination by PCR for the detection of JCV DNA has been shown to have a diagnostic sensitivity of 70%–80%, with a specificity of virtually 100% [2]. We have recently shown that 76% of patients with PML but only 3% of control patients show a specific intrathecal immune response to the major capsid protein VP-1 of JCV, thus indicating that this test may also be a useful diagnostic tool and a useful means to study JCV-specific humoral immune response within the CNS [3].

Although most cases of PML can now be diagnosed early, the prognosis remains dismal, and death usually occurs within a few months of the onset of symptoms [4]. A number of immunomodulant or antiviral compounds have been given to patients with PML; however, there is still no effective treatment for the disease [5, 6]. Recently, prolonged clinical PML remissions have been reported to occur after the administration of 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 CSF sampling (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

Received 25 March 1999; revised 13 September 1999; electronically published 21 December 1999.

Grant support: Istituto Superiore di Sanità, Italy (grant 50A-0-06).

Reprints or correspondence: Dr. Paola Cinque, Division of Infectious Diseases, San Raffaele Hospital, Via Stamira D'Ancona 20, 20127 Milano, Italy (cinque.paola@hsr.it).

Clinical Infectious Diseases 2000; 30:95–9

1058-4838/2000/3001-0018$03.00

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).

Patient no.	Sex, age (y)	Risk factor	PML diagnostic procedure	Therapy[a]	Days from therapy	JCV DNA in CSF	Antibody index[b]	HIV-1 RNA in CSF, copies/mL
1	M, 39	IDU	CSF PCR	AZT, 3TC, IDV	−6	+	ND	544,000
					35	+	ND	7440
2[c]	M, 35	IDU	Brain biopsy	d4T, RTV, SQV	−27	−	0.13	8300
					70	−	0.65	<400
					213	−	44.40	<400
3	M, 36	IDU	CSF PCR	AZT, 3TC, IDV	26	+	ND	<400
					147	−	ND	<400
4[c]	F, 32	IDU	CSF PCR	AZT, RTV, SQV	−5	+	0.55	2000
					153	−	1.10	1900
5	M, 36	IDU	CSF PCR	3TC, d4T, RTV	−1	+	0.94	3500
					271	−	127	<400
6	M, 32	IDU	CSF PCR	AZT, 3TC, RTV	−1	+	ND	34,300
					270	−	8.20	710
7[d]	F, 33	IDU	CSF PCR	AZT, 3TC, IDV	−1	+	6.96	760
					365	−	15.64	720

NOTE. Findings of standard CSF analysis 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.

[a] All patients were receiving 2 reverse transcriptase inhibitors at onset of PML, except for patient 1, who was antiretroviral therapy–naive.

[b] Antibody index $\geq$1 indicates intrathecal antibody synthesis.

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

[d] For patient 7, therapy was discontinued after 3 months.

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. The median $CD4^+$ cell counts were 53/μL for patients (range, 8–329/μL) and 23/μL for control patients (range, 1–193/μL).

CSF examination. PCR of the CSF for JCV DNA was conducted prospectively for diagnostic purposes by means of a nested assay amplifying a conserved JCV DNA region overlapping the small t and large T antigens and VP-1, as described elsewhere [15]. The CSF was analyzed to determine glucose and protein concentrations and cell counts; and cultures were performed for bacteria, mycobacteria, and fungi. When necessary, the presence of other microbial genomes, including herpes simplex virus type 1 or 2, varicella-zoster virus, cytomegalovirus, and Epstein-Barr virus DNA, was assessed by means of nested PCR assays [15]. For the patients who received HAART, HIV-1 RNA levels were determined in paired CSF and plasma specimens by quantitative reverse transcriptase–PCR (Amplicor HIV-1 Monitor; Roche Diagnostic Systems, Hoffman–La Roche, Basel, Switzerland). Total IgG and albumin concentrations in CSF and plasma were also determined. JCV-VP1–specific intrathecal IgG synthesis was assessed by means of an ELISA using a recombinant JCV-VP1 protein [3].

Clinical follow-up. The neurological conditions of the 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 2. Clinical, radiological, and laboratory findings in HIV-infected patients with progressive multifocal leukoencephalopathy (PML) receiving highly active antiretroviral therapy (HAART).

Patient no.	Neurological symptoms and signs			Brain MRI findings			$CD4^+$ cells/μL			HIV RNA, copies/mL			Days survived[b]
		Month			Month			Month			Month		
	BL	3	12	BL	3[a]	12	BL	3	12	BL	3	12	
1	Cognitive impairment, aphasia, R hemiparesis	I	S	Multifocal lesions (L fronto-parietal lobes, corpus callosum)[c]	ND	ND	8	136	184	ND	<400	<400	775
2[d]	Cognitive, visual, and speech impairment, L hemiparesis	W	I	Multifocal lesions (R fronto-parietal lobes, brain stem, cerebellum, thalamus, corpus callosum)	W	I	397	411	391	8300	<400	<400	505
3	Dysarthria, abnormal gait[c]	W	I	Multifocal lesions (centrum semiovale, corona radiata)[c]	ND	ND	14	ND	40	ND	ND	8000	700
4[d]	Cognitive impairment, 7th cranial nerve deficit, dysarthria, abnormal gait	I	S	Multifocal lesions (L cerebellum, L temporal lobe)	W	I	162	405	458	132,000	<400	4000	530
5	Speech impairment, 7th cranial nerve deficit	I	S	Multifocal lesions (L frontal lobe)	W	S	53	116	209	5600	910	<400	612
6	Cognitive impairment, abnormal gait	I	S	Multifocal lesions (R cerebellum)	W	S	22	69	106	34,300	<400	21,000	703
7[e]	Speech and visual impairment, 7th cranial nerve deficit, abnormal gait	I	NE	Multifocal lesions (L frontal lobe, thalamus, corpus callosum, R cerebellum)	W	NE	13	110	8	330,000	ND	25,000	646

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 cliniconeurological picture of PML was not clearly evaluable at that time.

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 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

⩾4 months. 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 may 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

1. Weber T, Major EO. Progressive multifocal leukoencephalopathy: molecular biology, pathogenesis and clinical impact [review]. Intervirology **1997**; 40: 98–111.
2. Cinque P, Scarpellini P, Vago L, Linde A, Lazzarin A. Diagnosis of central nervous system complications in HIV-1–infected patients: cerebrospinal fluid analysis by the polymerase chain reaction [review]. AIDS **1997**; 11: 1–17.
3. Weber T, Trebst C, Frye S, et al. Analysis of the systemic and intrathecal humoral immune response in progressive multifocal leukoencephalopathy. J Infect Dis **1997**; 176:250–4.
4. Berger JR, Pall L, Lanska D, Whiteman M. Progressive multifocal leukoencephalopathy in patients with HIV-1 infection. J Neurovirol **1998**; 4:59–68.
5. Major EO, Amemiya EE, Tornatore CS, Houff SA, Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus–induced demyelinating disease of the human brain [review]. Clin Microbiol Rev **1992**; 5:49–73.
6. Hall CD, Dafni U, Simpson D, et al. Failure of cytarabine in progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. N Engl J Med **1998**; 338:1345–51.
7. Power C, Nath A, Aoki FY, Del Bigio M. Remission of progressive multifocal leukoencephalopathy following splenectomy and antiretroviral therapy in a patient with HIV-1 infection [letter]. N Engl J Med **1997**; 336:661–2.
8. Elliot B, Aromin I, Gold R, Flanigan T, Mileno M. 2.5-Year remission of AIDS-associated progressive multifocal leukoencephalopathy with combined antiretroviral therapy [letter]. Lancet **1997**; 349:850.
9. Domingo P, Guardiola JM, Iranzo A, Margall N. Remission of progressive

multifocal leukoencephalopathy after antiretroviral therapy [letter]. Lancet **1997**; 349:1554–5.

10. Baqi M, Kurcharczyk W, Walmsley SL. Regression of progressive multifocal leukoencephalopathy with highly active antiretroviral therapy [letter]. AIDS **1997**; 11:1526–7.
11. Cinque P, Casari S, Bertelli D. Progressive multifocal leukoencephalopathy, HIV and highly active antiretroviral therapy [letter]. N Engl J Med **1998**; 339:848–9.
12. Albrecht H, Hoffmann C, Degen O, et al. Highly active antiretroviral therapy significantly improves the prognosis of patients with HIV-associated progressive multifocal leukoencephalopathy. AIDS **1998**; 12:1149–54.
13. Miralles P, Berenguer J, Garcia de Viedma D, et al. Treatment of AIDS-associated progressive multifocal leukoencephalopathy with highly active antiretroviral therapy. AIDS **1998**; 12:2467–72.
14. Clifford DB, Yiannoutsos C, Glicksman M, et al. HAART improves prognosis in HIV-associated progressive multifocal leukoencephalopathy. Neurology **1999**; 52:623–5.
15. Cinque P, Vago L, Dahl H, et al. Polymerase chain reaction on cerebrospinal fluid for diagnosis of virus-associated opportunistic diseases of the central nervous system in HIV-infected patients. AIDS **1996**; 10:951–8.
16. Anderson J, Armstead R, Baker AC, et al. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Ann Intern Med **1998**; 128:1079–100.
17. Palella FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. N Engl J Med **1998**; 338:853–60.
18. De Luca A, Giancola ML, Cingolani A, et al. Clinical and virological monitoring during treatment with intrathecal cytarabine in patients with AIDS-associated progressive multifocal leukoencephalopathy. Clin Infect Dis **1999**; 28:624–8.
19. Taoufik Y, Gasnault J, Karaterki A, et al. Prognostic value of JC virus load in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. J Infect Dis **1998**; 178:1816–20.
20. Garcia de Viedma D, Alonso R, Miralles P, Berenguer J, Rodriguez-Crixems M, Bouza E. Dual qualitative-quantitative nested PCR for detection of JC virus in cerebrospinal fluid: high potential for evaluation and monitoring of progressive multifocal leukoencephalopathy in AIDS patients receiving highly active antiretroviral therapy. J Clin Microbiol **1999**; 37: 724–8.
21. Koralnik IJ, Boden D, Mai VX, Lord CI, Letvin NL. JC virus DNA load in patients with and without progressive multifocal leukoencephalopathy. Neurology **1999**; 52:253–60.
22. Yiannoutsos CT, Major EO, Curfman B, et al. Relation of JC virus DNA in the cerebrospinal fluid to survival in acquired immunodeficiency syndrome patients with biopsy-proven progressive multifocal leukoencephalopathy. Ann Neurol **1999**; 45:816–21.
23. Tada H, Rappaport J, Lashgari M, Amini S, Wong-Staal F, Khalili K. Transactivation of the JC virus late promoter by the *tat* protein of the type 1 human immunodeficiency virus in glial cells. Proc Natl Acad Sci USA **1990**; 87:3479–83.
24. Brack-Werner R. Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis [review]. AIDS **1999**; 13:1–22.
25. Race EM, Adelson-Mitty J, Kriegel GR, et al. Focal mycobacterial lymphadenitis following initiation of protease-inhibitor therapy in patients with advanced HIV-1 disease. Lancet **1998**; 351:252–5.
26. Berger JR, Levy RM, Flomenhoft D, Dobbs M. Predictive factors for prolonged survival in acquired immunodeficiency syndrome–associated progressive multifocal leukoencephalopathy. Ann Neurol **1998**; 44:341–9.
27. Schlitt M, Morawetz RB, Bonnin J, et al. Progressive multifocal leukoencephalopathy: three patients diagnosed by brain biopsy, with prolonged survival in two. Neurosurgery **1986**; 18:407–14.