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Severe HIV-1 Encephalitis and Development of Cerebral Non-Hodgkin Lymphoma in a Patient with Persistent Strong HIV-1 Replication in the Brain despite Potent HAART

Case Report and Review of the Literature

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Abstract: A 39 year old patient with HIV-1 infection, who was asymptomatic without antiretroviral therapy (HAART) for ten years, developed severe encephalopathy. Despite therapy with a four drug antiretroviral combination regimen including two protease-inhibitors (PI), plasma viral load could not be suppressed sufficiently with persistence of low level viremia of 3.08-3.40 log copies/ml, even after addition of two other antiretroviral drugs. On therapy the patient showed improvement of clinical symptoms, however with severe persisting cognitive deficits. Repeated parallel measurements of viral load showed a far higher viremia in the cerebrospinal fluid than in the plasma. Resistance testing provided no evidence of relevant PI-mutations and analysis of protease inhibitor levels demonstrated good plasma levels. 17 months after start of HAART, the patient developed a cerebral Non-Hodgkin lymphoma, leading to his death despite radiation therapy. There has been a dramatic reduction in the prevalence of HIV-1 associated CNS events in the post-HAART era. Nevertheless, subgroups of patients are infected with neurotropic viral variants which could cause progressive neurological pathology as they can not be reached sufficiently by the available drugs. These patients require the development of new drugs that achieve a better penetration into the brain.

Key words: HIV-1 encephalitis, Non-Hodgkin lymphoma, HIV-1 replication, HAART

Introduction

Antiretroviral combination treatment has profoundly reduced morbidity and mortality in HIV-1 infection. In the vast majority of antiretroviral-naive HIV-1-infected patients introduced to combination therapy, HIV-1 is rapidly cleared in the plasma [1] and CD4+ cell counts increase. However, despite the potent suppression of plasma viral load and the immune reconstitution on HAART, there have been concerns that insufficient penetration of antiretroviral drugs into the brain could allow persistent HIV-1 replication in the central nervous system (CNS) leading to a progressive encephalopathy in a growing number of patients [2-4]. HIV-1 can be detected in the cerebrospinal fluid (CSF) [5] as well as in brain tissue [6]. In about 20-50% of HIV-infected patients, the disease causes neurological impairment, and dementia develops in up to 66% of patients with AIDS [7]. In the CNS, virus is present during all stages of HIV infection [8-12] and the level of HIV-1 RNA within the CSF has been shown previously to correlate with neurological impairment [8, 10, 13-15]. Fortunately, so far, most studies showed in the majority of patient efficient suppression of HIV-1 viremia in the CSF and improvement of neurological symptoms due to HAART [16-23]. Nevertheless, due to the infection of long-living cells infected cells may persist in the CNS despite the high potency of HAART [24-28].

In this report, we describe an HIV-1 infected patient suffering from severe encephalitis associated with persistent high-level HIV-1 replication in the brain despite therapy with a potent HAART regimen.

Case Presentation

We report on a 39 year old homosexual male patient who was diagnosed with HIV-1 infection in 03/1990. He refused antiretroviral therapy for personal reasons, despite a continuous decrease of the CD4+ T-cell count to below 50/µl. Except for disseminated warts he was asymptomatic until 11/2000 when he developed a severe encephalopathy with confusion, impairment of short-term-memory, disorientation and crying fits. MRI of the brain revealed a massive diffuse leukencephalopathy (Fig. 1). PCR analysis of CSF yielded negative results for CMV, EBV and JC-Virus. His CD4+ count had dropped from 87/µl in 1996 to 9/µl and he showed a high viral load of 5.74 log copies/ml. Start of HAART with lopinavir/r, nevirapine, stavudine and lamivudine achieved a partial clinical improvement, but the patient still suffered from severe cognitive deficits. Despite addition of ritonavir boosted saquinavir, which was later exchanged by amprenavir and tenofovir, the viral load could not be suppressed below the detection limit of 50 copies/ml during the following year, but

persisted at low levels of 2.74 to 3.4 log copies/ml. The CD4+ T-cell count rose to a maximum value of 203/µl in 04/2002. Repeated parallel measurements showed a high viral load of up to 4.86 log copies/ml in the CSF whereas plasma viral load remained stable between 3.08 and 3.4 log copies/ml. Genotypic resistance assays performed from liquor and plasma for Reverse Transcriptase (RT) and Protease in 02/2002 were identical except an amino acid variation at position 182 and 268. Both sequences showed only the I178M substitution as significant RT mutation, in addition to the mutations R211K and L214F. No PI associated mutations were found within plasma and CSF viruses. PCR analysis of CSF in 11/2001 for other viruses yielded a positive result for HHV8 and EBV and negative results for JC-Virus and CMV. Despite several modifications of the HAART regimen and additionaly intravenous treatment with foscarnet, that is supposed to reach good CSF levels, the viral load in the CSF could not be suppressed. Analysis of levels of the protease inhibitors demonstrated good plasma levels for lopinavir, saquinavir and amprenavir. In 04/2002, after 17 months of HAART, the patient was hospitalized with a seizure. MRI revealed a mass in the left frontal lobe of the brain (Fig. 2). Stereotactic biopsy evidenced a high grade diffuse large-cell Lymphoma with positive detection of EBV- and HHV8- DNA within the tumor-tissue. Radiation therapy was delayed by the patient for personal reasons and the patient died 06/2002 from progressive lymphoma one month after irradiation was begun (Fig. 3).

Discussion

Despite a potent antiretroviral 7-drug combination regimen, plasma viral load could not be fully suppressed in this patient. There was no evidence of a poor compliance of the patient, as measured serum drug levels were always in the active range and medication was well tolerated by the patient. In addition, during hospitalisation intake of drugs was monitored. Genotypic resistance analysis showed only the three RT-mutations I178M, R211K and L214F, but no mutation associated with resistance against protease inhibitors or against nevirapine. Thus, the insufficient suppression of plasma viral load could not be explained alone by drug resistance. Analysis of CSF fluid revealed high HIV-1 RNA levels, demonstrating ongoing excessive HIV replication in the brain. Sequence analysis of CSF derived virus revealed the identical pattern of drug resistance mutations as in the plasma. In consideration of this, we assume that the persistent low levels of HIV-1 in the peripheral blood were derived from the ongoing replicating HIV-1 pool in the CSF. There are controversial reports about the correlation between plasma and CSF HIV-1 RNA concentrations. Some studies found no correlation between the plasma and the CSF viral burden [14, 17, 29, 30], whereas in other reports this correlation was observed [10, 26, 31-34], mostly depending on HIV stage and the presence of neurological disease. For example, Antinori et al. [26] showed a significant linear correlation between CSF and plasma viral load in HAART-experienced, but not in drug-naïve subjects.

Still, little data exist about the nature of viral infection and viral replication in the different compartments of the CNS. HIV-1 enters the CNS early in the course of infection [5, 6, 35] and the CNS can remain a viral reservoir for years [36]. HIV antigens and/or genome have been detected in the brains of HIV-infected patients in all stages of infection [37-39]. Systemic infection of the lymph system and blood does not seem to be the direct source of CSF HIV-1. HIV-1 is believed to enter the CNS in different ways, either as free virus [40] or via infected immune cells, crossing the blood-brain barrier and/or the CSF-brain barrier. Recent evidence suggests penetration of brain microvascular endothelial cells (BMVECs) by cell-free virus through macropinocytosis (mitogen-activated protein-kinase dependent) [41]. BMVECs exposed to HIV-1 up-regulate expression of the intercellular adhesion molecule ICAM-1, which in turn may facilitate leukocyte migration across the blood-brain barrier and increase the access of both cell-free virus and infected cells to the CNS [41]. In addition, tumor-necrosis factor-alpha (TNF-α) secreted by infected macrophages [42], increase blood-brain barrier permeability by activation of guanylate cyclase and tyrosine kinase [43]. Apart from CD4+ T-lymphocytes, which are the major targets for infection with HIV-1, cells of the mononuclear phagocyte system play a critical role in the brain, too, both as host cells and as potential reservoir for HIV-1 in vivo. The most accepted model for entry of HIV-1 to the CNS, known as "Trojan horse" hypothesis [44], describes infiltration by infected monocytes that later differentiate into macrophages [45]. This kind of viral transport into the brain has also been described for feline immunodeficiency virus (FIV), simian immunodeficiency virus (SIV), and human T-cell leukaemia virus type I (HTLV-I), and it may therefore be a common mechanism for retroviral and lentiviral penetration of the brain [44, 46, 47]. Furthermore, the choroid plexus, the vascularized structure that makes up the boundary between circulating blood and CSF, seems to have a potent role in spreading the virus. HIV-1 strains derived from the choroid plexus are related to strains isolated from both the brain parenchyma and the periphery [48] and a recent study using the FIV model demonstrated productive infection of the choroid plexus [49]. The origin of CSF HIV-1 RNA is still controversial, whether it derives from endogenous viral production in the CNS, or originates from systemic sources, and is still a matter of investigation. A model including two prototypes of CNS infection by HIV-1 has been proposed: 1. a short-lived, transitory infection in the early stages of the disease, sustained by trafficking across the blood-brain barrier of infected CD4+ cells, rapidly renewed by the bloodstream; 2. a predominantly autonomous CSF infection in the late stages, sustained by long-living cells in the CNS [30, 50]. Subsequent conclusion is the need of blood-brain barrier penetrating drugs in advanced stages of infection with associated neurological disease, while in early stages CSF response to therapy may not necessarily require high-level CSF drug concentrations.

HIV infection is mediated by CD4 as the principal receptor on lymphocytes and macrophages. However, CD4 expression is comparatively low in the brain although brain derived strains of HIV utilize CD4 for infection. Several co-receptors have been reported to exist such as CCR5 on macrophages and CXCR4 on lym-

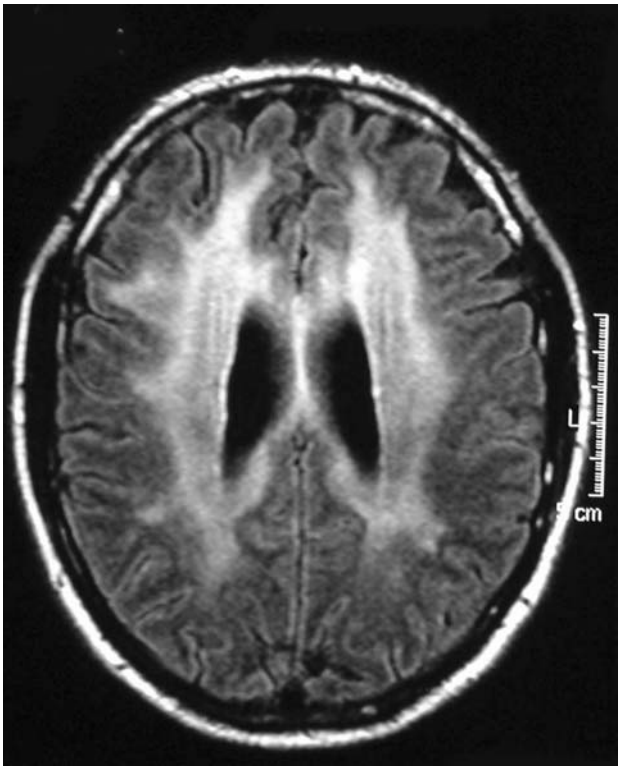

Fig. 1. MRI of the brain, showing massive diffuse white matter hyperintensities as a sign of HIV associated encephalophathy.

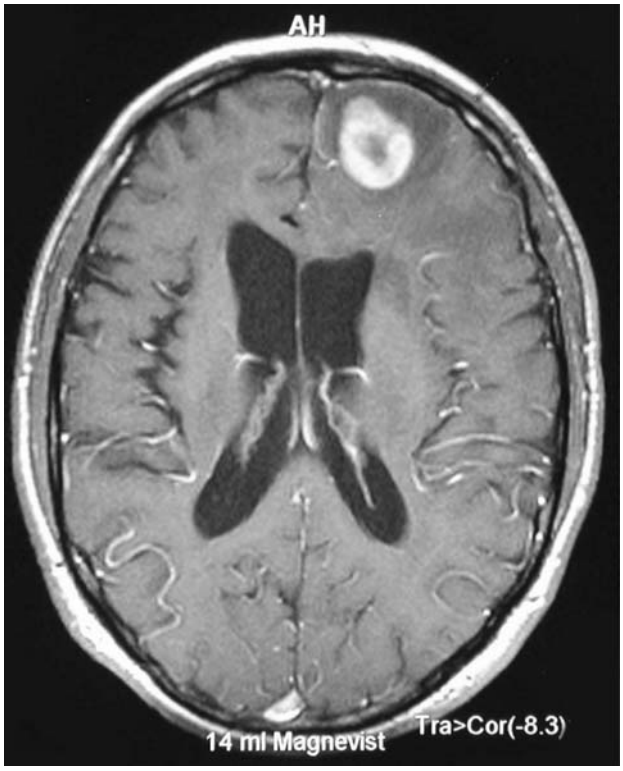

Fig. 2. MRI of the brain, showing a tumor with surrounding edema in the left frontal lobe, revealed as high grade diffuse large-cell Lymphoma.

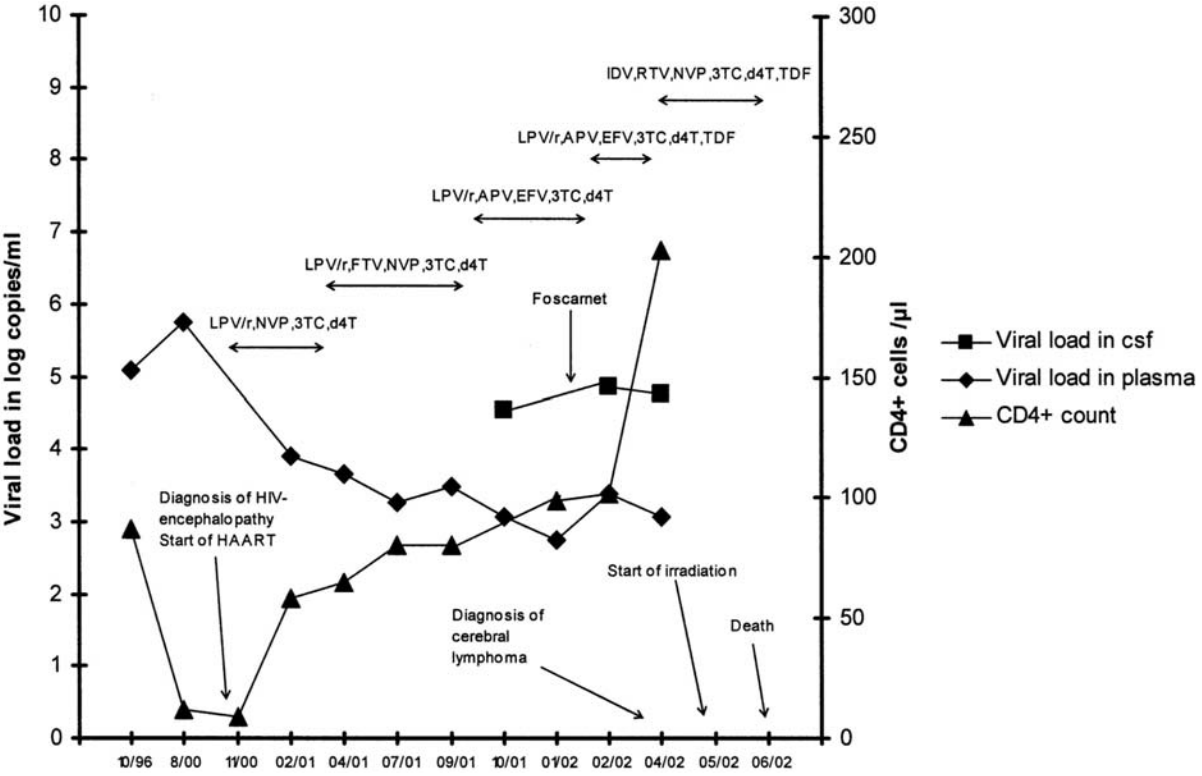

Fig. 3. CD4+ cell count, HIV-1 viral load and selected medication from 1996 to 2002

phocytes. CCR5, the chemokine receptor for RANTES, macrophage inflammatory protein (MIP)-1-α and MIP-1-β, is the primary co-receptor used by most HIV isolates recovered from the CNS, whereas CXCR4 is used by isolates from the periphery [51-54]. Because cells of the macrophage lineage are the only known cells in the

CNS that express both CD4 and CCR5, they seem to be the only productively infected cells within the brain [55]. In contrast, Gorry et al. [56] showed that CXCR4 can mediate efficient virus entry into microglia and suggest that CXCR4 usage in brain may be more prevalent than commonly thought. Moreover, an association between the abilities of primary viruses to replicate in macrophages and microglia is irrespective of co-receptor usage. These findings suggest that M-tropism rather than CCR5 usage predicts HIV-1 neurotropism. However, in patients with severe HIV-1 related neuropathy, astrocytes (expressing CXCR4) and neurons may also become infected [57]. There is evidence suggesting that genetic heterogeneity in HIV-1 may play a role in the infection of brain tissue. The genetic evolution of HIV-1 within the brain is distinct from that in lymphoid tissues and other organs due to the variety of adaptive pressure in different compartments (e.g., different resident cell types, immunologic characteristics and drug concentrations) and viruses infecting the CNS differ from strains prevalent in the systemic circulation [36, 58-64]. The hypervariable V3 of HIV gp120 derived from brain has been shown to be critical for HIV infection of macrophages and microglia [65, 66]. It has been demonstrated that brain-derived HIV-1 V3 sequences mediate the use of CCR5 and CCR3, but not CXCR4 as co-receptors [67]. Repeated passages of HIV in vitro can result in mutations in the envelope that resemble mutations identified in brain-derived sequences, suggesting that the virus may adapt to the brain [68].

HIV-1 can be isolated from the CSF of more than 50% of individuals early in the course of disease, infection of the CNS occurs in only about 20%, despite the preponderance of macrophagetropic strains, most of which can replicate in microglia [68].

As well as the entry of HIV-1 into the CNS, the mechanisms responsible for the development and progression of HIV related neuropathy remain poorly understood. Many studies indicate that viral replication in blood is extremely high in persons with HIV-1 infection and the balance established between viral replication and clearance shown in the viral load set point is predictive of the course of systemic disease [1]. Conflicting results have been found in the correlation between plasma and CSF viral load. In many studies a positive correlation has been noted [10, 31, 69], but in others no correlation was found [14, 29, 70, 71]. Different study populations and the influence of antiretroviral therapy may explain these differences. Interestingly, despite a 1000-fold higher concentration of monocytic cells in the blood, the plasma viral load exceeds the CSF viral load by only 5-10 times and in fact, more than 20% of patients without antiretroviral therapy have higher HIV-1 RNA levels in CSF than in plasma [72]. However, the role of viral load in brain in relation to the development of neurological disease is less clear. In patients with HIV-1 encephalitis, the level of CSF HIV-1 RNA has been correlated with the degree of cognitive impairment [10, 14, 29, 73] and, on the contrary, HIV-1 RNA levels of neurologically asymptomatic patients are usually lower in the CSF than in plasma [9, 32]. In contrast, studies of brain-derived viral mRNA and proviral DNA in brain tissue indicate no significant difference in levels between AIDS patients with and without HIV dementia [74, 75], while viral load in the brain measured by immunostaining or quantitative molecular methods is closely associated with the extent of pathological change accompanying HIV encephalitis [76].

In literature, most studies describe an effective suppression of CSF viral load in the bigger part of patients, parallel to suppression of plasma viremia after administration of antiretroviral therapy with two reverse transcriptase inhibitors or HAART [17, 18, 26, 77]. But different response dynamics between the two compartments CSF and plasma, with slower response in the CSF, have been observed [30, 50, 78], as well as a greater variability between patients in the CSF viral loads than in the plasma viral loads [79]. Even a more pronounced antiretroviral effect in CSF than in blood in some patients was found [80]. Various reasons for slower elimination of viral RNA from the CNS are supposable, like insufficient drug levels, emergence of drug resistance, or different patterns of HIV replication in the CNS. But predicting the response to HAART in the CSF seems not only to depend on viral factors or drug penetration through the blood brain barrier. Data from Eggers et al. [4] indicate that slow virus elimination from the CSF and a high extent of compartmental discordance of viral decay kinetics between plasma and CSF is associated with the presence and the severity of symptomatic CNS involvement. This seems not to be associated with low levels of antiretroviral drugs in the CSF or plasma, or with viral drug resistance, and consistent to the case we describe, an increasing CSF viral load during HAART was found in some patients. Also other groups report on "CNS escape", e.g. patients with neurological symptoms and high CSF HIV-1 RNA levels despite HAART and undetectable plasma viral loads [78, 81]. These observations are endorsed by the data demonstrating persistent immunoactivation in the CSF despite effective antiretroviral therapy, suggesting a remaining low viral replication within the brain parenchyma that cannot be detected with CSF HIV-1 RNA measurement [82]. The CSF levels of neopterin and beta-2-microglobulin, surrogate markers of intrathecal immunoactivation, have been found to be predictive markers of HIV-1 associated dementia (Brew et al. 1996). Whether the CSF viral load and intrathecal immunoactivation correlate during long-term antiretroviral treatment is not yet known.

The CNS is considered to be a distinct pharmacological compartment. The blood-brain barrier restricts the passage of antiretroviral drugs [84] and antiretroviral drugs differ largely in their ability to penetrate the blood-CSF barrier [85]. The therapeutic efficacy of HAART in the CNS is dependent at least in part on its ability to achieve inhibitory concentrations. Among the different classes of available drugs, protease inhibitors (PI) appear to have the lowest CSF levels. The nucleoside reverse transcriptase inhibitors (NRTI) zidovudine, stavudine, lamivudine and abacavir, but not didanosine or zalcitabine, penetrate fairly well into the CSF, where concentrations above the median inhibitory concentration (IC50) levels are reached [17, 86-89]. Also, the non nucleoside reverse transcriptase inhibitors (NNRTI) efavirenz and nevirapine penetrate into the CSF in sufficient concentrations [90]. CSF levels above the IC50

were reached by the PI indinavir, but not by ritonavir, saquinavir, or nelfinavir [91, 92]. The major problem evolving from insufficient CNS drug penetration is the potential harbouring of genotypically different viruses in the CNS with the potential to reseed into the blood plasma. Lanier and colleagues [93] noted that 14 of 21 baseline pair samples (67%) had different CSF and blood plasma genotypes in patients who were randomized to stable background therapy with or without abacavir. Venturi et al. [94] obtained paired CSF and plasma samples from 21 HIV-infected patients, 14 receiving ART and 7 ART naive. Patterns of resistance in both compartments were different in several individuals. Cunningham et al. [95] showed that 10 of 31 patients with a variety of neurological disorders demonstrated clear differences in resistance patterns between their CSF and blood compartments, with a higher occurrence of AIDS dementia complex diagnosis in the discordant resistance profile group compared with that of the patients with identical resistance profiles. In our case, the same pattern of mutations was found in CSF and plasma, with no evidence for significant resistance against the administered antiretroviral therapy. This, and results of other groups, showing fewer exhibitions of mutations in brain-derived viruses associated with drug-resistance than matched blood-derived HIV isolates [36, 96], does not support the credence to "compartmentalization" of drug resistant variants, reflecting poor CNS drug penetration and/or limited replication in the brain.

In the case we describe, the patient developed clinical signs of a severe HIV associated encephalopathy with distinct cognitive deficits and generalized white matter lesion in the MRI. A slight clinical improvement was observed after starting HAART, but repeated MRI showed no significant reduction of the leukencephalopathy. The pathogenesis remains unclear: a direct injury of white matter by HIV-1 is likely, e.g. by damaging oligodendrocytes or brain endothelial cells or by directly injuring myelin. Viral proteins, including the envelope glycoprotein gp160, which is cleaved into gp120 and gp41, and the HIV transactivator protein, Tat, have the potential for neurotoxicity [97-99]. Gp120 has shown to be directly and indirectly neurotoxic in vivo and in vitro [100-102]. At low concentrations, gp120 has been demonstrated to damage cultured neurons by inducting apoptosis mediated by p38 mitogen activated protein kinase [99, 103] or through the activation of JNK and ERK pathways [104]. Specific domains within gp120 have been implicated as especially neuropathogenic, including CD4-binding [105] and the V3 regions [106]. It has also been shown that gp120 affects intracellular signalling that controls the expression of different cell adhesion molecules, cytokines, and perhaps nitric oxide through the JAK-STAT pathway [107]. Other proteins, including Tat, gp41 and Nef have been shown to be neurotoxic in vitro [97, 108]. Tat is also secreted by infected cells and may induce neuronal death through apoptosis directly via increases of intracellular calcium, or indirectly, by stimulating macrophages to produce matrix metalloproteinases that induce neuronal apoptosis and whose expression is upregulated in the brains of patients with HIV associated dementia [98]. Other results suggest that Tat toxicity is dependent upon a polyamine sensitive site on the *N*-methyl-D-aspartate receptor [109]. Additionally, Conant and colleagues [110] have shown that Tat induces the expression of MCP-1 in astrocytes, which may influence macrophage trafficking in the CNS while other data reports increased production of neurotoxic quinolinic acid, a glutamate receptor agonist, by both Tat and Nef [111]. The neurotoxicity of HIV-1 is closely associated with the ability to induce fusion in monocyte derived macrophages, which may result from the increased affinity for CCR5. The immune system can injure white matter by several mechanisms, including the release of pro-inflammatory cytokines like tumor-necrosis-factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6) and tissue growth factor-beta (TGF-β) [112-115]. Although most cells in the CNS can produce cytokines, the chief sources are activated glial cells that include macrophages, microglia and astrocytes. TNF-α is an inflammatory cytokine that has received extensive attention for its potential neurotoxic effects and ability to influence the release of other cytokines. TNF-α is released by micoglia and astrocytes [113, 116] in HIV infection and may be directly toxic to neurons [117]. It has been showed, that TNF-α mRNA and protein levels are increased in the brains of patients with HIV dementia compared to patients without dementia or uninfected controls, furthermore, the level was correlated with the severity of dementia [115].

As mentioned in the introduction, there seems to be an increasing incidence of HIV-associated leukencephalopathy [27, 28] in patients who failed antiretroviral therapy. Prior to the era of HAART, most reports described leukencephalopathy cases that were either associated with opportunistic pathogens, such as JCV, CMV or EBV, or did not specifically exclude them [118-121]. But in the cases described in recent literature, myelinotoxic viruses were not detected, so leukencephalopathy more probably results from HIV, the immune system, or antiretroviral drugs (e.g., certain NRTI, such as stavudine, can inhibit mitochondrial DNA polymerase, resulting in mitochondrial injury and impaired cell metabolism). In the case we describe, analyses of the CSF and the additional brain biopsy yielded positive PCR results for HHV8 and EBV. In addition to their potential myelinotoxic effects, we assume that the inadequate control of these two oncogenic herpes viruses due to HIV-1 associated immunosuppression was an important factor in the development of the cerebral Non-Hodgkin lymphoma.

Most HIV-1 infected patients benefit from antiretroviral therapy and studies investigating viral suppression in the CSF during HAART demonstrate decreasing CSF viral load together with effective suppression of plasma viremia [17, 18, 77]. However, at least in a subgroup of patients on HAART, residual HIV-1 replication in the brain may cause disease and, consequently, the ongoing replication may constitute a major and independent source of HIV-1 RNA, particularly for late stages of the disease [50] and may contribute to systemic failure to HAART [33, 78]. It is important to be aware of the potential risk of continued HIV-1 replication in the brain despite otherwise effective antiretroviral therapy and the menace of subsequent reseeding of virus into the blood plasma, as presumed in the pre-

sented case. These patients require eradication of HIV-1 from these reservoirs may not currently be feasible but a strategy needs to be implemented to identify possible combinations of antiretroviral drugs that can suppress viral replication to the lowest possible concentrations at all sites. That points up the importance of CSF HIV-1 RNA examination in patients with persistent neurological symptoms on HAART. Such patients need more potent antiretroviral drugs with a better CNS penetration than current available drugs.

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