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Severe, demyelinating leukoencephalopathy in AIDS patients on antiretroviral therapy

T. Dianne Langford^a, Scott L. Letendre^b, Thomas D. Marcotte^c, Ronald J. Ellis^d, J. Allen McCutchan^b, Igor Grant^{c,e}, Margaret E. Mallory^d, Lawrence A. Hansen^d, Sarah Archibald^c, Terry Jernigan^{c,e}, Eliezer Masliah^{a,d}, and the HNRC Group*

^aDepartment of Pathology, University of California, San Diego, CA, USA

^bDepartment of Medicine, University of California, San Diego, CA, USA

^cDepartment of Psychiatry, University of California, San Diego, CA, USA

^dDepartment of Neurosciences, University of California, San Diego, CA, USA

^eDepartment of Psychiatry, Veterans Affairs San Diego Health Care System, San Diego, CA, USA

Abstract

Objectives—To describe a severe form of demyelinating HIV-associated leukoencephalopathy in AIDS patients failing highly active antiretroviral therapy (HAART), its relationship to clinical and neuroimaging findings, and suggest hypotheses regarding pathogenesis.

Design and methods—AIDS patients who failed HAART and displayed severe leukoencephalopathy were included. All cases had detailed neuromedical, neuropsychological, neuroimaging and postmortem neuropathological examination. Immunocytochemical and PCR analyses were performed to determine brain HIV levels and to exclude other viruses.

Results—Seven recent autopsy cases of leukoencephalopathy in antiretroviral-experienced patients with AIDS were identified. Clinically, all were severely immunosuppressed, six (86%) had poorly controlled HIV replication despite combination antiretroviral therapy, and five (71%) had HIV-associated dementia. Neuropathologically, all seven had intense perivascular infiltration by HIV-gp41 immunoreactive monocytes/macrophages and lymphocytes, widespread myelin loss, axonal injury, microgliosis and astrogliosis. The extent of damage exceeds that described prior to the use of HAART. Brain tissue demonstrated high levels of HIV RNA but evidence of other pathogens, such as JC virus, Epstein–Barr virus, cytomegalovirus, human herpes virus type-8, and herpes simplex virus types 1 and 2, was absent. Comparison of the stages of pathology suggests a temporal sequence of events. In this model, white matter damage begins with perivascular infiltration by HIV-infected monocytes, which may occur as a consequence of antiretroviral-associated immune restoration. Intense infiltration by immune cells injures brain endothelial cells and is followed by myelin loss, axonal damage, and finally, astrogliosis.

Conclusions—Taken together, our findings provide evidence for the emergence of a severe form of HIV-associated leukoencephalopathy. This condition warrants further study and increased vigilance among those who provide care for HIV-infected individuals.

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Requests for reprints to: E. Masliah, Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093-0624, USA.

*See Appendix.

Note: T. D. Langford and S. L. Letendre contributed equally to this study.

Keywords

HIV leukoencephalopathy; antiretroviral therapy

Introduction

Combination antiretroviral therapy (ART) extends the survival of many HIV-infected individuals and decreases the occurrence of several opportunistic conditions [1,2]. For example, the incidence of cytomegalovirus (CMV) disease, *Pneumocystis carinii* pneumonia, and disseminated *Mycobacterium avium* complex infection has decreased [3]. Despite these improvements, the incidences of fungal infections and Kaposi's sarcoma have not changed while those of bacterial infections and non-Hodgkin's lymphoma have actually increased [2–4]. Similarly, in the central nervous system (CNS), the incidences of many opportunistic conditions have generally decreased but the prevalence of HIV-associated dementia (HAD) and its neuropathologic correlate, HIV encephalitis (HIVE) seems to have increased [3,5,6]. Perhaps even more concerning, new cases of HAD may be occurring in patients with higher CD4 cell counts [7–9].

Neuropathologic changes characteristically associated with HIVE include formation of multinucleated giant cells (MNGC), microglial nodules (MGN) and astrogliosis in the white matter, basal ganglia and neocortex [10]. Diffuse damage to the white matter occurs more frequently in patients with cognitive impairment and is characterized in life by diffuse or focal hyperintensity on T2-weighted magnetic resonance imaging (MRI) [11] and at autopsy by mild to moderate myelin loss and astrogliosis [10,12]. As ART may improve cognitive impairment [7] it may also reverse such white matter damage. However, HIV-infected patients treated with potent ART more frequently have extensive focal white matter lesions on neuroimaging studies, compared to those not taking antiretroviral drugs [13]. Consistent with these findings, we have observed seven cases of severe HIV-associated leukoencephalopathy in patients who have failed combination ART. The three objectives of this study were to describe this condition, its relationship to clinical and neuroimaging findings, and suggest hypotheses regarding its pathogenesis.

Materials and methods

Subjects

Subjects were enrolled in studies at the UCSD HIV Neurobehavioral Research Center (HNRC) and had mild to severe leukoencephalopathy at autopsy. The UCSD Human Subjects Program Office approved all contributing studies and subjects (or their caregivers) gave informed consent for all procedures. Cases were excluded if neurocognitive impairment was attributed to a condition other than HIV infection (e.g., a history of significant head trauma, neurologic infections, or psychiatric conditions) or if autopsy uncovered evidence of CNS infections, neoplasms, or anoxic brain injury. Seven cases met our criteria for inclusion.

The clinical medical records of all cases were reviewed. Subjects also had ante-mortem, prospectively collected, research data, including standardized neuroimaging [14], neuromedical, and neuropsychological evaluations [15,16]. Laboratory data were obtained between 1 and 11 months prior to death, neuropsychological data between 6 and 12 months, and neuroimaging data between 2 and 48 months. The median interval between death and collection of ante-mortem data was 8 months for all three categories. Dates of HIV infection were obtained by self-report, except in two subjects who were dated seroconverters. We classified antiretroviral drugs by the number of classes [nucleoside reverse transcriptase

inhibitor (NRTI), non-NRTI, and protease inhibitor] and the total number taken. Fasting and non-fasting glucose and triglyceride measurements were reviewed and the highest levels recorded.

The HNRC's neuropsychological evaluations classify subjects into one of four levels of neurocognitive functioning: (i) no impairment; (ii) subsyndromic neuropsychologic-impairment; (iii) minor cognitive motor disorder; or (iv) HAD [16–19].

Neuropathologic examination

All subjects died of acute bronchopneumonia and/or sepsis and all autopsies were performed within 24 h of death [3]. After macroscopic examination, tissue blocks from the midfrontal cortex, temporal cortex, parietal cortex, cingulate cortex, hippocampus, basal ganglia, mesencephalon, pons, medulla and spinal cord were obtained, immersion-fixed in 4% formalin and embedded in paraffin. Paraffin blocks were serially sectioned and stained with hematoxylin and eosin (H & E), Luxol fast blue, and Bodian and Prussian blue for ferric iron. Additional paraffin sections were used for subsequent immunocytochemical analysis for HIV-gp41, CD23, CD68, and glial fibrillary acidic protein (GFAP) [20]. RNA and DNA were extracted from frozen tissue blocks for analysis of HIV and other viruses [21].

Immunocytochemical analysis

For HIV immunolocalization in the CNS, paraffin sections were incubated overnight at 4°C with monoclonal antibody against HIV gp41 (1 : 200) (Genetics Systems, Seattle, Washington, USA) [20]. For analysis of macrophage infiltration into the brain, sections were incubated with monoclonal antibodies against CD23 and/or CD68 (1 : 100) (DAKO Laboratories, Carpinteria, California, USA). For analysis of astrogliosis, sections were incubated with a polyclonal antibody against GFAP (1 : 500) (DAKO). Sections were then incubated in biotinylated secondary antibody (1 : 200) (Vector Laboratories, Burlingame, California, USA), followed by Avidin D-HRP (ABC Elite; Vector) and reacted with diaminobenzidine (0.2 mg/ml) in 50 mM Tris buffer (pH 7.4) with 0.001% H₂ O₂.

HIV RNA quantitation and PCR

Regional quantitation of HIV RNA was performed with the QUANTIPLEX bDNA Signal Amplification System (Chiron Corporation, Emeryville, California, USA), according to the method described by Wiley and colleagues [21]. Briefly, tissues were homogenized in TRIzol Reagent (Gibco-BRL, Life Technologies, Gaithersburg, Maryland, USA). Following overnight precipitation at –80°C, the mixture was centrifuged, the supernatant discarded, and the pellet washed in 0.5 ml 75% ethanol, followed by another brief centrifugation for 5 min. The ethanol was decanted and the pellet was air-dried for 5 min. For QUANTIPLEX quantitation of HIV RNA, 0.44 ml of HIV sample working reagent was added to the pellet. The suspension was vortexed and incubated at 53°C for 20 min followed by vortexing for 10 s to ensure complete solubilization of the RNA pellet. Two hundred micro-liters of the solubilized RNA was placed in duplicate HIV capture wells in a standard QUANTIPLEX HIV RNA assay including standards and controls. Samples were incubated overnight at 53°C and the HIV content was measured by luminometry. If duplicate QUANTI-PLEX measurements varied by more than 35%, the test was considered to be invalid.

To screen for other viral sequences, genomic DNA was extracted [22] and amplified in 35 cycles (93°C for 30 s, 50°C for 30 s, 72°C for 1 min) with a final extension at 72 ° C for 5 min. Primer sequences for Epstein–Barr virus (EBV), CMV, JC virus (JCV), human herpes viruses 6 and 8 (HHV-6, HHV-8) and varicellazoster virus (VZV) were obtained from published data [23]. The herpes simplex virus (HSV) 1 and 2 primer sequence was obtained from Sanders *et al.* [24]. The quality of extracted DNA was assessed by amplification of the

glyceraldehyde 3-phosphate dehydrogenase gene [25]. Controls for PCR detection sensitivity included positive control samples from AIDS patients with confirmed EBV, CMV, JCV, HHV-6, HHV-8 and VZV infections. Samples from non-HIV patients without evidence of opportunistic infections or brain alterations were used as negative controls.

Results

Clinical and demographic characteristics are summarized in Table 1 and gross and microscopic findings in Table 2. Six subjects had a history of homosexual activity, one reported heterosexual contacts only, and two used intravenous drugs (Table 1). All subjects were severely immunosuppressed with CD4 cell counts $< 200 \times 10^6/l$, most (4/7, 57%) $< 25 \times 10^6/l$. At the time of their last evaluation, HIV replication was poorly controlled in six patients (86%), with plasma HIV RNA levels greater than the upper limit of quantitation in four. All subjects had high viral loads in brain, including the subject with plasma HIV RNA levels < 400 copies/ml. Ante-mortem cerebrospinal fluid HIV RNA concentrations were measured in six patients (86%) and reflected high brain levels. In two patients, HIV RNA levels in cerebrospinal fluid actually exceeded levels in plasma (Table 2). Most subjects had been diagnosed with HIV-related conditions, including oropharyngeal candidiasis, wasting, Kaposi's sarcoma, viral hepatitis, distal sensory polyneuropathy and disseminated mycobacterial infection (Table 1). Five subjects were diagnosed with HAD, one with subsyndromic neuropsychologic impairment, and one was neurocognitively normal. Cognitive impairment was not explained by psychotropic medications or other conditions, such as CNS opportunistic infections, psychiatric or mood disorders, or a history of head trauma.

T2-weighted MRI revealed multifocal white matter hyperintensities in three of the seven cases (Fig. 1b–d). The other four displayed extensive, diffuse, and more confluent white matter hyperintensity (Fig. 1e–h). Lesions were more abundant in the areas of the centrum semiovale (Fig. 1f–h), corpus callosum (Fig. 1g) and optical radiations. Lesions were observed less frequently in the frontal pole and the parietal white matter (Fig. 1e–h).

Consistent with neuroimaging, gross neuropathologic examination revealed a range of white matter alterations. In mild cases, these alterations included white matter atrophy with focal lesions in the frontal, temporal and parietal cortices (Fig. 2b–d). Cases presenting with more extensive, diffuse white matter damage showed both cortical atrophy and reduced basal ganglia volume (Fig. 2e–h). In the most severe cases, the corpus callosum was diffusely attenuated, the lateral ventricles were dilated, and cavitating lesions were present in the centrum semiovale of the frontoparietal region (Fig. 2g).

Microscopically, cases with milder white matter alterations showed abundant perivascular infiltration by macrophages and lymphocytes, as indicated by labeling with anti-CD23 and anti-CD68, respectively, with occasional formation of MGN and MNGC surrounding blood vessels and along white matter tracks (Fig. 3b–d). HIV-gp41 immunoreactive cells localized in close association with blood vessels in mild cases (Fig. 4a) but were found in brain parenchyma in moderate cases (Fig. 4b and c) and were observed throughout the white matter in the most severe cases (Fig. 4d). In moderate to severe cases, perivascular macrophages frequently contained hemosiderin (Fig. 3g). The most advanced cases exhibited frequent MGN and MNGC, severe disruption of microvessel walls with evidence of endothelial cell damage, expansion of Virchow's space, and hypertrophic glial cell processes (Fig. 3e–h).

Mild cases showed moderate degrees of demyelination and gliosis (Figs. 5b and 5f). In moderate and severe cases, demyelination was considerably more extensive with prominent

astrogliosis and formation of MGN and MNGC (Figs. 5c and 5g). White matter destruction was so extreme in some cases that myelin was completely absent and axons were damaged (Fig. 5d and 5h).

In all cases, characteristic findings of HIVE (e.g., macrophage infiltration, presence of HIV immunoreactive cells, MNGC and formation of MGN) were associated exclusively with white matter microvasculature but not with the cortical ribbon or deep gray matter (Table 2). Lesions were consistently found in the white matter underlying frontal and cingulate cortices, corpus callosum, basal ganglia, substantia nigra, and pons. In each case, lesions found in the different brain regions were similar in appearance.

PCR analysis confirmed the presence of very high HIV levels in the brains of all cases, primarily in white matter rather than other brain regions (data not shown). HIV RNA levels in the brain were exceedingly high. PCR did not detect the presence of other viruses in the brain, including CMV, EBV, JCV, HHV-6 and 8, VZV, and HSV-1 and 2.

Comparing neuroimaging (Fig. 1) and neuropathologic (Table 2, Figs. 3, 4 and 5) findings to ante-mortem cognitive impairment (Table 1), the two non-demented cases had occasional focal and diffuse lesions on MRI and mild demyelination and perivascular cellular infiltration with low to moderately high HIV RNA levels in brain on neuropathologic examination. In contrast, the five cases with ante-mortem dementia had extensive, diffuse white matter hyperintensities by MRI corresponding to intense infiltration by monocytes with more severe demyelination, astrogliosis, and higher brain concentrations of HIV RNA.

Discussion

This study describes a form of severe, HIV-associated leukoencephalopathy in seven patients who failed highly active antiretroviral therapy (HAART). Our observations are consistent with recent reports of an increased incidence of focal white matter lesions in HAART-treated patients [13] and of an unexpectedly high incidence of 'not determined' leukoencephalopathy in AIDS patients [26]. Prior to the HAART era, most reports described leukoencephalopathy cases that were either associated with opportunistic pathogens (such as JCV, CMV or EBV) or did not specifically exclude them [22,27–31]. Berger and colleagues did report leukoencephalopathy cases with multiple sclerosis-like lesions [32] or relapsing and remitting leukoencephalopathy [33]. However, the pathogenesis of our cases may be different for three reasons. First, we found only HIV in the brain, but no other pathogens. Second, the tissue injury in our cases was confined to the white matter, unlike previous cases that also had gray matter abnormalities. Third, the leukoencephalopathy we describe is more severe than that described prior to the use of HAART. In the past, perivascular infiltration by mononuclear cells and myelin loss was less extensive, white matter atrophy was milder, and HIV concentrations in the brain were lower [12]. In comparison, our cases are characterized by intense perivascular macrophage infiltration, extensive demyelination, and evidence of very high levels of HIV replication in the brain.

Leukoencephalopathy in HIV-infected individuals may result from HIV-, immune-, opportunist-, or drug-based mechanisms. HIV itself could injure white matter by damaging oligodendrocytes or brain endothelial cells or by directly injuring myelin (e.g., via myelinotoxic viral proteins, such as gp120 or Tat [34]). The immune system can injure white matter by several mechanisms, including the release of pro-inflammatory cytokines (such as tumor necrosis factor-alpha [35,36]) or the phagocytosis of myelin by macrophages (as in immune-mediated demyelinating diseases, such as acute disseminated encephalomyelitis) [37]. Reactivated infection with opportunistic viruses, such as HHV-6 or JCV [38,39] can also damage the white matter of AIDS patients. Finally, prescribed (e.g.,

HIV protease inhibitors) or illicit (e.g., CNS stimulants) drugs may penetrate the blood–brain barrier and injure myelin [40].

While our study does not directly test these hypotheses, opportunist- and illicit drug-based mechanisms are unlikely. Opportunistic pathogens probably did not damage white matter as evidence of common, myelinotoxic viruses was not detected. Five of the seven subjects did not report the use of illicit drugs and did not show the patterns of dopaminergic neuronal damage reported in HIV patients using stimulant drugs [40,41]. However, we cannot rule out drug abuse as a compounding factor in this syndrome.

Leukoencephalopathy more probably resulted from HIV, the immune system, or antiretroviral drugs. The emergence of this condition in the post-HAART era strongly argues that potent ART plays an important role in pathogenesis. As one of HAART's cardinal effects is the reduction of HIV-related mortality [42], patients whose lives are prolonged by HAART but who also have HIV encephalitis may be susceptible to progressive CNS disease even though HIV replication is suppressed in other tissues [5,43,44]. These seemingly conflicting effects may be attributable to the use of antiretroviral drugs that penetrate poorly into the brain, such as most protease inhibitors, and may explain why HIV encephalitis continues to be observed at a quarter of autopsies [3]. Antiretroviral drugs themselves could be responsible if they directly damaged myelin or oligodendrocytes, causing a toxic leukoencephalopathy [45]. Alternatively, one of several, recently recognized, antiretroviral drug-associated complications might be responsible. Chief among these complications are the emergence of highly adapted HIV strains, immune reconstitution-related tissue injuries, and lipid and metabolic disorders [46–55].

HIV's high replication and mutation rates allow it to readily adapt to different environmental pressures. These adaptive pressures often vary between tissues and include different resident cell types, immunologic characteristics, and antiretroviral drug concentrations. In the brain, HIV commonly infects microglia and brain macrophages, cell-mediated immunity is usually restricted, and concentrations of most antiretroviral drugs, particularly protease inhibitors, are low. These differences can foster the development of virodemes in the CNS that evolve independently from other tissues, particularly when immunosuppression is pronounced [55–57]. In white matter, these combined pressures may yield strains that can both replicate in the presence of antiretroviral drugs and efficiently infect resident cells, such as brain endothelial cells or oligodendrocytes. In fact, strains that infect these cells have been described. Strelow *et al.* described an SIV_{mac} variant that was derived from a neuropathogenic strain and efficiently infected brain microvascular endothelial cells [58]. Albright and colleagues were able to productively infect oligodendrocytes with primary and laboratory strains of HIV [59]. Other SIV studies described strains that can specifically damage white matter [60–62]. Therefore, severe leukoencephalopathy may result from antiretroviral-resistant HIV strains that have adapted to cells that reside in white matter. Notably, all of our subjects had advanced immunosuppression and failed at least one antiretroviral regimen, supporting the presence of an independently evolving CNS virodeme that was resistant to antiretroviral drugs.

While failing antiretroviral therapy can select highly adapted HIV strains, successful therapy can substantially improve CD4 T lymphocyte numbers and function. Unfortunately, tissue injury can occur when the newly reconstituted immune system responds overzealously to clinical or subclinical infections that are present when therapy is initiated [46]. In the CNS, such injury has been described for cryptococcal meningitis [49] and CMV retinitis [63]. We did not detect the presence of other CNS pathogens in our subjects but HIV antigens themselves might induce a reconstituted immune response in white matter, resulting in the

intense infiltration of lymphocytes and monocytes and blood–brain barrier damage that we observed.

Such immune reconstitution injury would result only from therapy that was successful, at least initially. However, if resistance subsequently developed, infiltrating lymphocytes could begin producing HIV virions and proteins, leading to progressive injury. As immune re-deterioration proceeded, peripheral monocytes infected with antiretroviral drug-resistant HIV strains might preferentially and efficiently migrate into deep white matter. In fact, Liu and colleagues postulated that migration of bone marrow-derived monocytes into deep white matter may be accelerated in patients with advanced HIV disease, based on phylogenetic studies of a demented patient who failed HAART [45].

The role of antiretroviral-induced immune reconstitution injury would be supported if disease onset occurred in treated subjects when CD4 cell lymphocyte counts were high. Antinori and colleagues found that most subjects with ‘not determined’ leukoencephalopathy had CD4 counts above $> 200 \times 10^6/l$ [26]. While all of our subjects had advanced immunosuppression at the time of death, all had at least some ante-mortem sampling. Three had sufficient documentation to allow assessment of CD4 lymphocyte trends. The CD4 cell counts of all three reached $< 100 \times 10^6/l$ before improving in response to a new antiretroviral regimen and then subsequently deteriorating (data not shown). This rising–falling pattern is consistent with initial immune reconstitution injury followed by subsequent antiretroviral failure and disease progression.

In addition to adaptive pressures and immune reconstitution injury, treatment with antiretroviral drugs can result in several related, but distinct, metabolic syndromes characterized by insulin resistance, hyperlipidemia, lipodystrophy (or fat redistribution), and lactic acidosis [47,49,52,63,64]. These conditions have resulted in serious complications, including insulin-requiring diabetes, coronary artery disease, and death [52,65].

The pathogenesis of these disorders is still uncertain but at least two mechanisms seem likely. Certain NRTIs, such as stavudine, can inhibit mitochondrial DNA polymerase, resulting in mitochondrial injury and impaired cell metabolism. Protease inhibitors may disrupt normal lipid metabolism because of HIV protease’s homology with proteins such as LRP (low density lipoprotein-receptor-related protein) and CRABP-1 (cytoplasmic retinoic acid binding protein type 1) [51]. Either mechanism could damage the CNS. The brain is frequently damaged in congenital mitochondrial syndromes, suggesting that it is very sensitive to mitochondrial injury. Alternatively, white matter may be particularly susceptible to disruption of lipid metabolism as myelin is composed primarily of fat.

None of our cases had gross evidence of fat redistribution or cardiac complications at autopsy. Table 1 shows that four subjects had evidence of at least transient ante-mortem hyperglycemia (normal < 110). Non-fasting triglyceride concentrations were mildly elevated in three subjects and markedly elevated in three others (normal < 160 mg/dl). Although non-fasting triglycerides are an imperfect marker of antiretroviral-associated lipid disorders, these data support that at least three subjects may have had abnormal lipid metabolism.

In conclusion, our findings support the emergence of a severe form of HIV-associated leukoencephalopathy. Intense perivascular macrophage infiltration, extensive demyelination, and very high levels of HIV replication characterize this condition. We identified three stages of severity, which were correlated with ante-mortem clinical, neuropsychological, and neuroimaging findings. These stages suggest a temporal sequence of pathologic events. In this model, leukoencephalopathy begins with perivascular infiltration by HIV-infected monocytes and blood–brain barrier damage, leading to local HIV replication, moderate myelin loss, but limited gliosis. As disease progresses, more myelin is lost and is eventually

accompanied by axonal damage and extensive astrogliosis. While the cause is unclear, the condition's occurrence in the post-HAART era in highly treatment-experienced individuals strongly argues that ART plays a pivotal role in pathogenesis. HIV-associated leukoencephalopathy may simply be a consequence of ART that extends the survival of patients with HIV without completely controlling HIV replication in the brain. Alternatively, its etiology may be more complex, involving other HIV-, immune-, and drug-mediated effects. Recognition of this condition warrants increased vigilance among HIV care providers and further investigation.

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Appendix

The San Diego HIV Neurobehavioral Research Center (HNRC) group is affiliated with the University of California, San Diego, the Naval Medical Center, San Diego, and the San Diego VA Medical Center, and includes: I. Grant, Director; J. H. Atkinson, and J. A. McCutchan, Co-Directors; T. D. Marcotte, Center Manager; M. R. Wallace, Co-Investigators Naval Medical Center, San Diego; R. J. Ellis, P.I. Neuro-medical Component; R. K. Heaton, P.I. Neurobehavioral Component; T. Jernigan, P.I. Imaging Component; E. Masliah, P.I. Neuropathology Component; D. R. Masys, P.I. Data Management Component; I. Abramson, P.I. Statistics Unit; M. Frybarger, Data Manager.

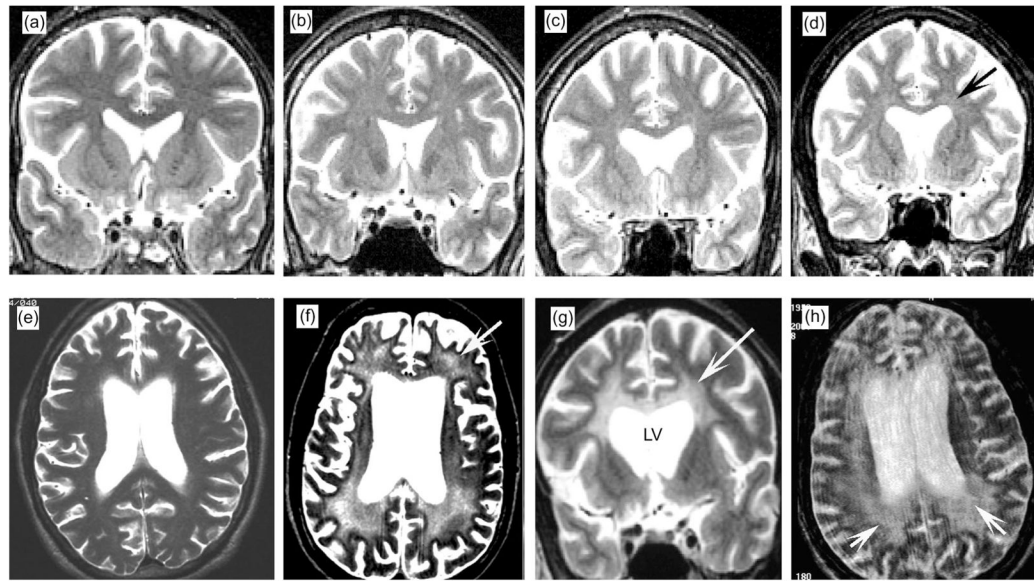


Fig. 1.

White matter alterations by *in vivo* MRI in patients with HIV-associated leukoencephalopathy. All are T2-weighted images obtained within 12 months of death. The available images were coronal in some but horizontal in others. (a) Normal MRI illustrating the expected characteristics of the cortical ribbon and white matter. (b–d) Cases with mild neuropathology had focal and diffuse white matter hyperintensities (arrow). (e–h) Cases with moderate to severe neuropathology had extensive white matter hyperintensities (arrows). Lesions were most prominent in the white matter surrounding the lateral ventricles (LV), corpus callosum, and optic radiations but were also observed in the frontal pole and parietal white matter.

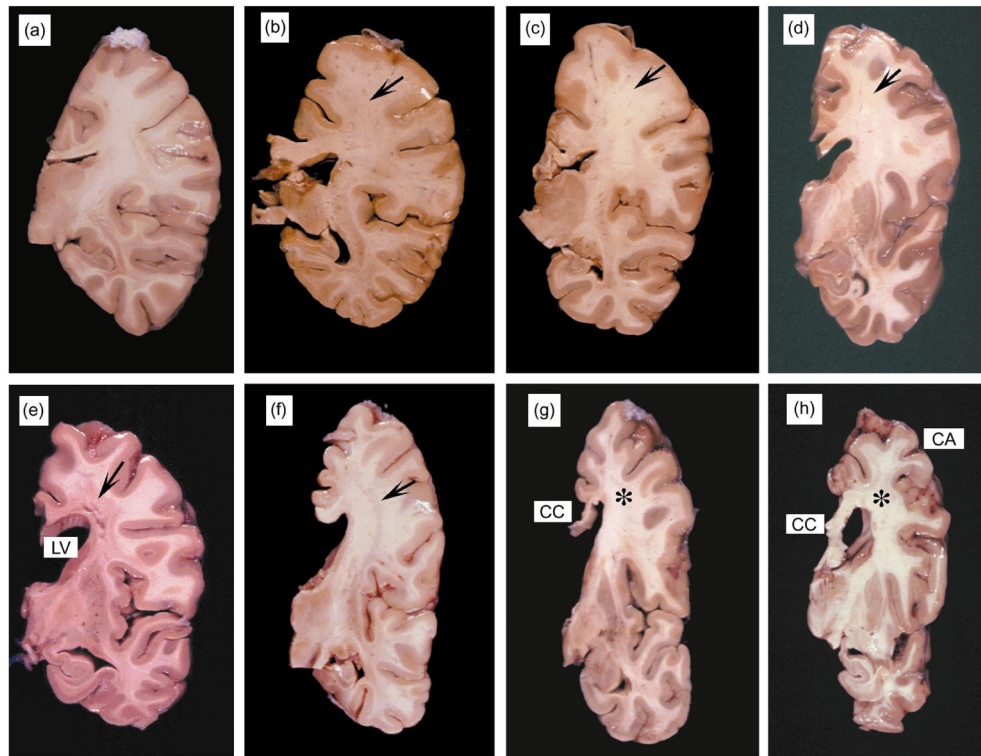


Fig. 2. Gross findings in the white matter in patients with HIV-associated leukoencephalopathy. All images are coronal sections of the right hemibrain at the level of the hippocampus. The order of the images corresponds to the MRI images in Fig. 1. (a) Normal brain tissue illustrating the white matter and cortical ribbon in a fixed specimen. (b–d) Cases with mild neuropathology had focal white matter lesions in the frontoparietal region (arrows). (e–h) In the most severe cases, the corpus callosum (CC) was diffusely attenuated, the lateral ventricles were dilated, and cavitating lesions were present in the centrum semiovale of the frontoparietal region (arrows). Those with extensive white matter damage also showed cortical atrophy (CA) and reduced basal ganglia volume.

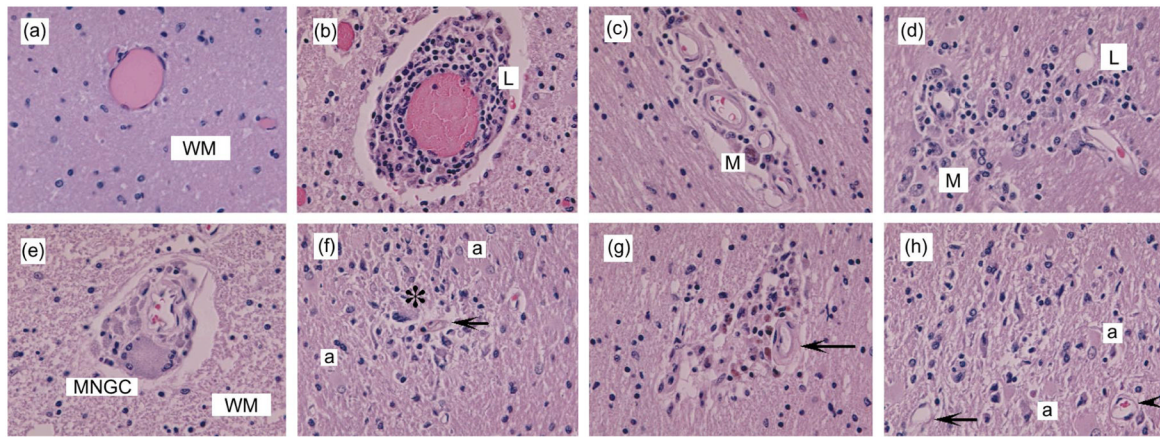


Fig. 3.

Neuropathological alterations in the white matter of patients with HIV-associated leukoencephalopathy. All images are from the white matter of the frontal lobe and corpus callosum, stained with H&E, approximate magnification 400 ×. (a) Normal brain tissue illustrating white matter (WM) and microvasculature. (b–d) Mild cases had intense lymphocytic (L) and macrophage (M) infiltration around blood vessels, with expansion of the perivascular space and white matter rarefaction. (e–h) More severe cases also had disrupted microvasculature (arrows). Multinucleated giant cells (MNGC), microglial cells (*), and reactive astrocytes (a) surrounded vessels. White matter was diffusely fragmented and diminished.

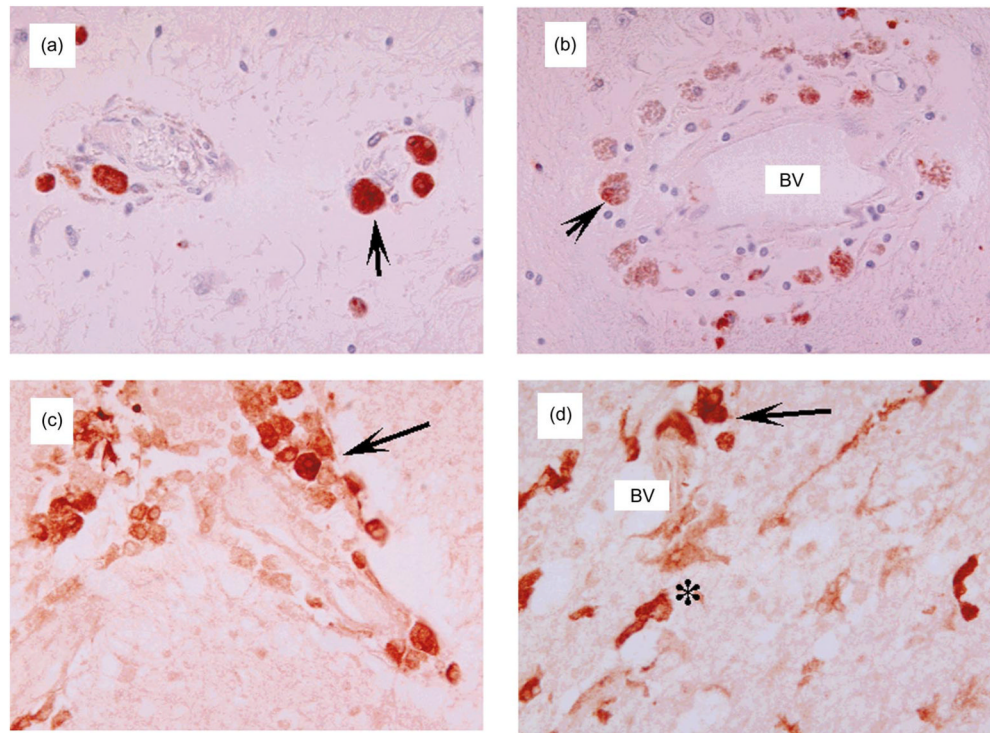


Fig. 4. Patterns of HIV-gp41 immunoreactivity in white matter of patients with HIV-associated leukoencephalopathy. All images are from the white matter of the frontal lobe and corpus callosum. Immunostained sections were developed with diaminobenzidine and counterstained with hematoxylin. Approximate magnification is 400 \times . (a and b) Mild cases had abundant gp41 immunoreactive macrophages (arrows) around blood vessels (BV). (c and d) More severe cases also had abundant gp41 immunostained macrophages and microglia (*) around vessels and in the midst of white matter fibers.

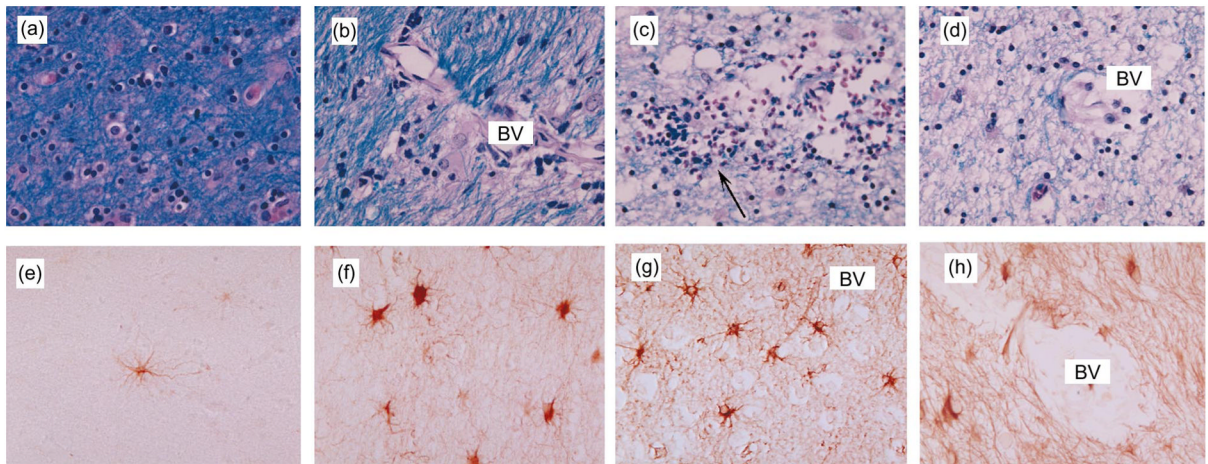


Fig. 5.

Patterns of demyelination and astrogliosis in white matter of patients with HIV-associated leukoencephalopathy. All images are from the white matter of the frontal lobe and corpus callosum. The first sections were stained with Luxol fast blue and the second four panels were immunostained with antibody against GFAP. Approximate magnification is 400 ×. (a) Normal brain tissue illustrating myelin characteristics. (b–c) Cases with mild-to-moderate demyelination, most apparent around blood vessels (BV) infiltrated by mononuclear cells (arrow). (d) Severe case showing diffuse and very extensive loss of white matter. (e) Normal brain tissue illustrating normal astroglia. (f–g) Mild-to-moderate cases showing perivascular (BV) and diffuse astroglia. (h) Severe case showing intense, diffuse astroglia.

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Table 1

Summarized clinical and laboratory data.

Case	Age (years) ^a	Sex	Risk factor	Date of HIV infection	Date of death	Years infected ^b	Cognitive diagnosis ^c	Other ante-mortem diagnoses	ART history ^d	Last sampling ^e	Plasma HIV RNA (copies/ml) ^f	CSF HIV RNA (copies/ml) ^g	CD4 cell count ($\times 10^6/l$) ^h	Glucose (mg/dl) ⁱ	Triglycerides (mg/dl) ^j
1	41	Male	MSM	12/85	11/4/98	12.9	NPI	Disseminated KS	2 Classes 4 Agents	10	< 400	1396	114	103	157
2	48	Male	MSM	09/87	2/27/99	11.5	Normal	KS, CMV, VZV, DSPN, OPC	3 Classes 10 Agents	10	> 750 000	85 800	17	136	1378
3	50	Male	MSM IDU	06/85	2/21/99	13.7	HAD (stage 2)	HBV, HCV, HSV, VZV, DSPN, OPC, CAP, wasting	3 Classes 7 Agents	11	> 750 000	6861	85	109	221
4	41	Male	MSM	02/92	2/7/01	9.0	HAD (stage 4)	HSV, DSPN, PCP, wasting, myositis, salmonellosis	3 Classes 12 Agents	1	423 865	> 750 000	10	238	546
5	34	Male	MSW	07/94	3/15/99	4.7	HAD (stage 4)	Disseminated MAC, HIV retinopathy, seizures	2 Classes 6 Agents	8	> 750 000	NP	24	182	188
6	40	Male	MSM	05/87	9/27/00	13.4	HAD (stage 3)	VZV, OPC, wasting	3 Classes 11 Agents	3	> 750 000	8264	14	92	312
7	39	Male	MSM IDU	06/93	12/27/98	5.6	HAD (stage 4)	HBV, DSPN, wasting	3 Classes 10 Agents	1	313 987	483	90	134	1712

^aMean age was 41.9 years^bMedian duration of HIV infection was 11.5 years.^cSubjects with mild leukoencephalopathy (see Table 2) had only mild or no cognitive impairment while those with more severe disease were also more impaired.^dAntiretroviral drugs were classified by the number of classes [nucleoside reverse transcriptase inhibitor (NRTI), non-NRTI, and protease inhibitor] and the total number taken. Subjects took a median of three classes and 10 antiretroviral drugs prior to death.^eAll subjects had ante-mortem laboratory data available with a mean sampling interval of 6.3 months prior to death.^fHIV RNA levels in plasma approached or exceeded the upper limit of quantitation in six patients (86%).^gHIV RNA levels in cerebrospinal fluid were measured in six patients and were also very high in most, exceeding plasma levels in two.^hThe median CD4 T lymphocyte count was $24 \times 10^6/l$.ⁱMedical records were reviewed for all glucose and triglyceride measurements, fasting and non-fasting; the highest levels are recorded.^jRandom glucose levels were elevated in four out of seven (57%) (normal < 110 mg/dl) and triglyceride levels were elevated in six patients (86%), markedly so in three (43%) (normal < 160 mg/dl). ART, Antiretroviral therapy; MSM, men who have sex with men; NPI, neuropsychological impairment; KS, Kaposi's sarcoma; CMV, cytomegalovirus; VZV, varicellazoster virus; DSPN, distal sensory polyneuropathy; OPC, oropharyngeal candidiasis; IDU, injecting drug user; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; CAP, community-acquired pneumonia; PCP, *Pneumocystis carinii* pneumonia; MSW, men who have sex with women; MAC, disseminated *Mycobacterium avium* complex infection.

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Table 2

Summarized neuropathologic data. Subjects were classified as either mild, moderate or severe stage of white matter neuropathology according to gross brain examination, microscopically by determining demyelination, gliosis, perivascular macrophages (PV), microglial nodules (MG Nod), multinucleated giant cells (MNGC) and Luxol fast blue (LFB) for myelin and iron (Fe) for hemosiderin, where '1' represents least damage and '3' represents most damage. Other findings at autopsy are listed as Kaposi sarcoma (KS), cytomegalovirus (CMV), Epstein-Barr virus (EBV), *Mycobacterium avium* complex (MAC), chronic obstructive pulmonary disease (COPD) or actinomycosis. Regions of the brain most affected were noted: frontal (F), cortical (C), basal ganglia (BG), substantia nigra (SN), pons (P) or occipital (O). Three subjects were diagnosed as neuropathologically normal on gross examination at autopsy.

Patient	Gross	Demyelination	Gliosis	PV Mac	MG Nod	MNGC	LFB	FE	Stage	Regions most affected	Other autopsy diagnoses
1	Normal	2	2	2	1	0	1	2	Mild	SN, P	KS
2	Normal	2	2	2	2	2	1	1	Mild	F, C, P	KS
3	Normal	2	1	2	2	2	1	1	Mild	F, C, P	CMV
4	Mild atrophy, diminished white matter	2	2	1	0	1	1	2	Mild-moderate	F, C, BG, SN	EBV
5	Atrophic, dilated ventricles	3	2	2	2	2	3	1	Moderate	F, C, BG	MAC
6	Atrophic, dilated ventricles, diffuse lesions	3	3	3	3	3	3	3	Moderate-severe	F, C, BG	COPD
7	Atrophic focal lesions	3	3	3	2	2	3	3	Severe	F, C, BG, O	Actinomycosis