PAPER

HIV leucoencephalopathy and TNFα expression in neurones

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Background: Human immunodeficiency virus (HIV) leucoencephalopathy (HIVL) is an uncommon and rapidly progressive form of AIDS dementia complex (ADC) that has remained poorly understood. Tumour necrosis factor α (TNFα), which has been implicated in the pathogenesis of ADC, is predominantly localised in macrophages in the HIV infected brain, although in vitro studies indicate that neurones can express this cytokine.

Objective: To examine the clinical/neuroradiological features of HIVL and the expression of TNFα in HIVL.

Methods: Six patients who presented with rapidly progressive dementia within four to 12 weeks of the primary manifestation of their HIV infection were evaluated. Clinical history, treatment regimens, and imaging studies were reviewed, and brain samples from three of the patients were studied by means of immunohistochemistry.

Results: Imaging studies showed diffuse bilateral deep white matter changes in all six patients. Clinical and imaging abnormalities improved in five of the six patients within weeks after initiation of antiretroviral treatment. Brain biopsies of two showed pronounced microglia/macrophage activation, but only scant viral protein (gp41) expression. Staining for TNFα was found in microglia/macrophages, and surprisingly, in neurones also. Postmortem analysis of a third patient also showed TNFα expression in neurones of the frontal cortex and basal ganglia.

Conclusion: This study provides the first demonstration of staining for TNFα in the neurones of the HIV infected brain, and suggests that the process underlying this rapidly progressive form of ADC may reflect indirect mechanisms mediated by host factors, particularly TNFα.

Human immunodeficiency virus (HIV) infection is known to cause brain injury and neurocognitive impairment, variously termed HIV associated dementia or AIDS dementia complex (ADC), and primarily affecting areas of mental processing, behaviour, and motor control.[1–3] Although usually considered a late complication, for reasons that have remained unclear, it can present as the sole or primary manifestation of HIV infection.[4] Onset in most subjects is typically insidious, and the course either slowly progressive or static. Less often, subjects can show rapid progression towards severe neurological impairment, and rarely a rapidly progressive form has been reported to occur in association with extensive deep white matter (DWM) lesions, a poorly understood disorder that has been described as HIV leucoencephalopathy (HIVL).[5]

The pathological features are predominantly localised to the DWM and subcortical grey matter, and include pallor of the white matter, multinucleated cell infiltration in more severe cases, and collections of infected or activated macrophages.[1 3 6] In general, the extent of inflammation correlates with the severity of ADC. Neuronal loss, apoptosis, and synaptic dendritic injury have also been described.[7–10]

The most commonly reported abnormality on cranial magnetic resonance imaging (MRI) or computed axial tomography is the presence of cerebral atrophy.[1 11 12] Volumetric loss in the cortical and subcortical regions, particularly the caudate, has been found to correlate in some reports with the degree of cognitive impairment.[13] Abnormalities in the white matter in HIV encephalitis consist of either patchy or diffuse increases in T_2 signals, but are generally less common.[1 12] It is of interest that similar changes have been seen in reversible posterior leucoencephalopathy, which has been attributed to alterations in the blood–brain barrier (BBB).[14–16]

It is widely accepted that HIV associated brain injury is probably caused by indirect mechanisms, rather than the infection of neurones or glial cells. Recent studies have suggested that the pathogenesis of this disorder may be related to complex interactions of host (TNFα, quinolinate, inducible nitrous oxide synthase, and stromal derived factor 1α) and viral factors (gp120, Tat, and gp41), leading to neuronal injury and apoptosis.[17–22] In fact, the number of activated macrophages has been shown to be a better correlate of ADC than the number of infected cells.[23 24] Among the different host factors believed to contribute to the pathogenesis of the disorder, accumulating evidence indicates that TNFα plays a central role. It is significantly raised in the HIV infected brain and has been shown to correlate with ADC severity and the degree of immunoactivation.[25 26] It is predominantly localised to perivascular microglia/macrophages, but can also be found in astrocytes.[27] In vitro studies have shown that it can lead to neuronal apoptosis, potentiate the neurotoxic effects of HIV-1 proteins (gp120 and Tat), and can be expressed in neurones.[19 20 27 28]

Previous studies have shown that zidovudine or AZT (3'-azido-3'-deoxythymidine) can reverse some of the neurocognitive deficits associated with HIV infection,[29] and more recently, combined potent antiretroviral treatments have been shown to benefit neurocognitive impairment. However, despite adequate viral suppression in the periphery, 30–40% of HIV infected subjects will develop some degree of neurocognitive impairment.[30]

Here, we describe the clinical course, neuroimaging findings, and treatment response of six patients who developed a rapidly progressive dementia associated with

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Abbreviations: ADC, AIDS dementia complex; AZT, 3'-azido-3'-deoxythymidine; BBB, blood–brain barrier; CSF, cerebrospinal fluid; DWM, deep white matter; HIV, human immunodeficiency virus; HIVL, human immunodeficiency virus leucoencephalopathy; MRI, magnetic resonance imaging; PML, progressive multifocal leucoencephalopathy; TNFα, tumour necrosis factor α

diffuse bilateral changes in the white matter, consistent with HIVL. All six subjects responded rapidly to either AZT alone or combined antiretroviral treatments. Furthermore, brain samples from three of these patients showed TNFα staining in the neurones. This is the first report of the localisation of TNFα to neurones in the HIV infected brain.

METHODS

Patients

Between 1993 and 1998, 80 patients were referred to the neuro-HIV services at the Massachusetts General Hospital, Beth Israel Deaconess Medical Center, New England Medical Center (NEMC, Boston, USA) and Lemuel Shattuck Hospital (Jamaica Plain, Massachusetts, USA). Six patients who presented with rapidly progressive dementia as the primary AIDS defining illness and diffuse T_2 signal changes in the DWM on MRI studies were identified (table 1). All patients were evaluated for other potential causes of changes in mental status. The case histories of two patients are described below.

Radiological studies

MRI studies (T_1, T_2, and proton density sequences) at the onset of clinical symptoms and after onset of treatment were reviewed. In five of the six cases, MRI scans performed before and during treatment were available.

Immunohistochemistry

Paraffin wax embedded postmortem material was available from patient 6 two years after he presented with rapid onset of dementia. Brain biopsies from the frontal cortex and adjacent DWM were performed in patients 4 and 5 (table 1). All three specimens were collected, paraffin wax embedded, and evaluated at the New England Medical Center (Boston, USA) between 1996 and 1998. Histopathological evaluation revealed no inclusion bodies and was not suggestive of cytomegalovirus infection or progressive multifocal leucoencephalopathy (PML). Serial 7 μm sections were cut and mounted on precleaned Superfrosted (Fisher, Scientific, Pittsburgh, Pennsylvania, USA) slides. The sections were dewaxed and rehydrated using xylene and graded changes of ethanol. Antigen retrieval was achieved by autoclaving the slides in buffer (0.01M EDTA) for six minutes.[31] Antibodies to the following antigens were used: gp41 (1/100 dilution; monoclonal; Genetic Systems, Redmond, Washington, USA), TNFα (1/500 dilution; polyclonal; Sigma, St Louis, Missouri, USA), TNFα (1/1000; monoclonal; R&D, Minneapolis, Minnesota, USA), HLA-DR (1/1000 dilution; monoclonal; ENZO, Farmingdale, New York, USA), and glial fibrillary acidic protein (1/30 dilution; Dako, Carpinteria, California,
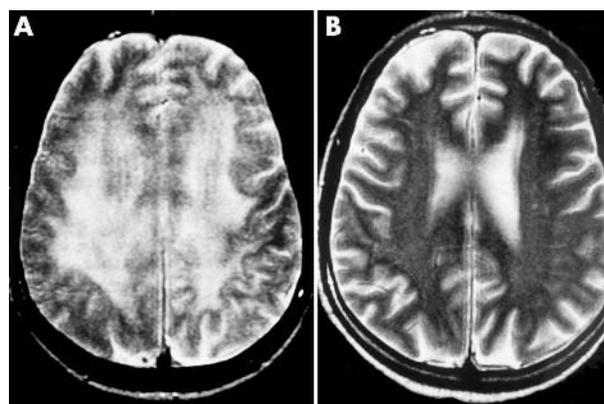

Figure 1 T_2 weighted axial magnetic resonance images of patient 1 (A) before and (B) after antiretroviral treatment showing substantial improvement of the deep white matter abnormalities.

USA). All antibodies were diluted in 5% bovine serum albumin. ABC kits (Vector, Burlington, California, USA) were used for the secondary antibody and the streptavidin–peroxidase label. Slides were then developed in 3,3'-diaminobenzidine-H_2O_2 (Sigma) solution and counterstained with Harris's haematoxylin. A lymph node from an HIV positive patient was used as a positive control. The primary antibody was omitted as an internal control. Tissue from the normal brain of a 76 year old man, who died of heart failure, was used as a negative control. Peptide blocking studies using recombinant TNFα protein (Boehringer Mannheim, Germany) were carried out to confirm the specificity of the polyclonal and monoclonal anti-TNFα antibodies. TNFα peptide (1/100 dilution; R&D Systems) was preincubated with the polyclonal or monoclonal TNFα antibody for four hours and then applied to sections from the postmortem case (patient 6). All sections were stained with Luxol-Fast-Blue and haematoxylin and eosin to assess myelin pallor, multinucleated giant cells, and microglial nodule formation. The degree of staining was rated semiquantatively as absent, mild (up to five positive cells/high power field), moderate (five to 20 positive cells), or prominent (more than 20 positive cells).

RESULTS

From 1993 to 1998, six patients were identified who presented with rapid progressive cognitive and motor decline over a period of four to 12 weeks, consistent with ADC stage 2 or greater, as their initial AIDS defining illness (table 1). Cranial MRI studies showed extensive, bilateral, confluent

Table 1 Clinical features, antiretroviral therapy, and time interval between the first and second MRI study of the brain in six patients with HIV leucoencephalopathy

Patient	Age/sex	CD4 count ($\times 10^6$/l)	Clinical presentation	Drug treatment	Time interval to 2nd MRI and CD4 count	Biopsy results
1	31/M	105	Depression for 6 months, followed by confusion, falls, leg weakness for 3 months	AZT 600 mg/day	5 weeks/375	–
2*	42/M	80	Failing memory, frequent falls for 3 months	AZT 800 mg/day	12 weeks/567	–
3	41/M	160	Memory problems for 4 months	AZT 800 mg/day	4 weeks/NK	–
4	40/M	98	Mental decline for 10 weeks	AZT 1000 mg/day	5 months/439	Extensive TNFα, HLA-DR staining
5†	46/M	110	Personality changes, memory difficulties for 2 months	AZT 600 mg/day Saquinavir	–	Extensive TNFα, HLA-DR staining
6	33/M	<50	Confusional state for 4 weeks	AZT 600 mg/day D4T 60 mg/day	5 weeks/170	Necropsy: gp41, TNFα, HLA-DR staining

*Only patient 2 had neuropsychological testing at the time of clinical presentation and after the symptoms had improved; †only patient 5 had received AZT 600 mg/day for a period of 3 months before the onset of cognitive decline.
AZT, AZT, 3'-azido-3'-deoxythymidine; HIV, human immunodeficiency virus; MRI, magnetic resonance imaging; NK, not known; TNFα, tumour necrosis factor α.

abnormal T_2 signals in the DWM of the centrum semiovale in the anterior and posterior regions in all six patients (fig 1).

All six patients were started on antiretroviral treatment or had previous treatment regimens modified (table 1). AZT was included in all regimens and was given in a range of 600 mg/day to 1000 mg/day. In four patients, it was the only drug administered. All patients showed pronounced clinical improvement over the following weeks. Subsequent MRI studies showed improvement, with reduced white matter signal abnormalities in five of the six patients. Patient 5 had no follow up study. The time interval between the MRI study before antiretroviral treatment and the subsequent scan was four to five weeks in cases 1, 3, and 6 and three and five months in cases 2 and 5, respectively (table 1). The case histories of two patients are described below.

Case 1 (patient 1)

A 31 year old man presented with a six month history of abdominal pain, weight loss of 9 kg, and mood changes. Gastritis and depression were diagnosed and antidepressants were commenced with good response. However, three months later, he developed a precipitous decline in his cognitive abilities. Subsequently, he was readmitted with frequent falls, confusion, and double incontinence. His neurological examination revealed that he was orientated to his name but not time and place. He was only able to follow a one step command. He showed generalised weakness and symmetrical hyper-reflexia, with extensor bilateral plantar responses. The history and neurological findings were consistent with a diagnosis of ADC stage 3, based on the MSKCC classification.[32] Cerebrospinal fluid (CSF) studies were remarkable for a lymphocytic pleocytosis (9×10^6 white blood cells/litre, 96% lymphocytes, CSF protein 660 mg/litre, CSF glucose 690 mg/litre, serum glucose 1030 mg/litre); Gram stain, cultures, cryptococcal antigen, VDRL, cytology, and toxicology screen were normal. His chest x ray and Mantoux test were normal. His HIV screen, previously negative, was positive, and his CD4 count was 105×10^6/litre. His cranial MRI scan showed diffuse bilateral

increased T_2 signals in the cerebral white matter, in addition to increased signal in the internal capsule and brainstem (fig 1A). The patient was started on AZT 600 mg/day. Six weeks later, the patient was alert, oriented, able to walk, and performed all activities of daily living. He continued to have mild difficulties on tasks of sustained attention and showed mild distal weakness of his upper extremities, accompanied by brisk reflexes and extensor plantar responses, consistent with ADC stage 1. His AZT dose was increased to 1000 mg/day. A subsequent cranial MRI scan five weeks after onset showed only minimal DWM T_2 signal hyperintensities (fig 1B).

Case 2 (patient 4)

A 40 year old man presented to the neurological clinic (New England Medical Center, Boston, USA) with a 10 week history of mental decline. His relatives noted problems with attention and forgetfulness. Apart from a history of drug abuse, he had no medical problems. His neurological examination revealed no focal neurological signs, apart from a mild truncal ataxia. However, his attention span, short term memory, and finger tapping skills were greatly reduced. His HIV screen was positive and his CD4 count was 98×10^6/litre. The history and neurological findings were consistent with a diagnosis of ADC stage 2. CSF studies were unremarkable (no pleocytosis, CSF glucose 590 mg/litre/serum glucose 990 mg/litre, Gram stain and cultures were normal). Chest x ray revealed no signs of tuberculosis. He was started on antiretroviral treatment with AZT 600 mg/daily. A week later, his condition worsened with increasing difficulties in walking and cognition. In view of the cranial MRI scan, which showed diffuse bilateral increased T_2 signals in the cerebral white matter, a brain biopsy of the frontal cortex and adjacent white matter was obtained primarily to exclude PML. The daily dose of AZT was increased to 1000 mg. Over the following six weeks the patient showed steady improvement. The MRI scan of the brain was repeated after five months and showed a pronounced improvement of the DWM abnormalities.
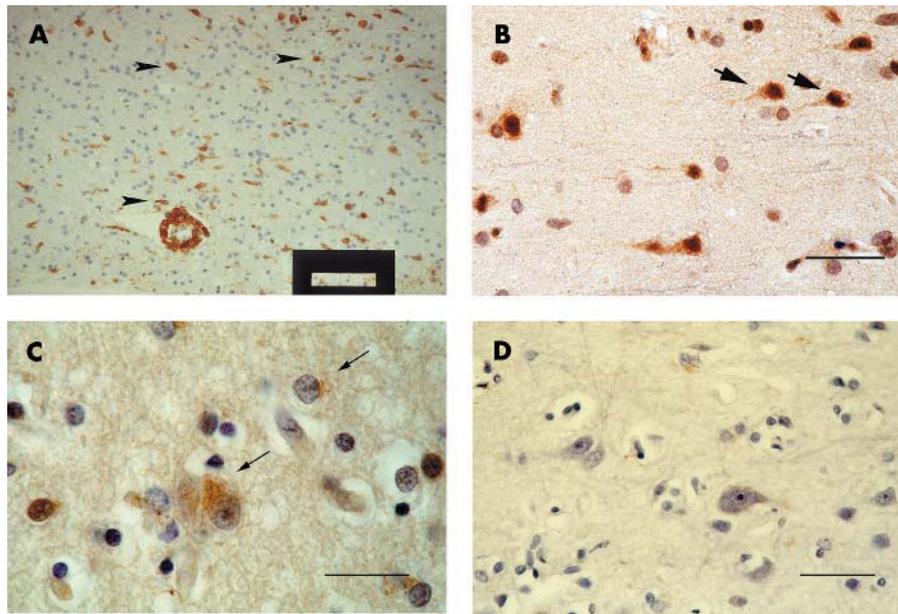

Figure 2 (A) Immunohistochemical staining for HLA-DR shows prominent activation of microglia/macrophages (arrows) in the biopsy of patient 4 (original magnification, ×20). (B) Immunohistochemical staining with a rabbit polyclonal antibody against tumour necrosis factor α (TNFα) in the biopsy of patient 4 shows staining of the cytoplasm of cortical neurones (arrows). (C) Staining with a monoclonal antibody to TNFα seen in the cytoplasm of neuronal cells (arrows) in the frontal cortex of patient 6 with severe AIDS dementia complex and human immunodeficiency virus encephalitis. (D) Preabsorption studies performed in adjacent sections from the frontal cortex of patient 6. After preabsorption with TNFα peptide, staining with a monoclonal antibody against TNFα was negative (diaminobenzidine was used as the chromagen in all slides; all scale bars, 20 μm).

Immunohistochemistry

Biopsies from the frontal brain region were obtained from patients 4 and 5. Patient 6 came to necropsy two years after his initial presentation. At the time of death, he was extremely undernourished and had acquired a fulminant pneumonia, but had no clinical evidence of a central nervous system infection. He was not orientated to his name, time, or place. He showed generalised weakness and was unable to walk. History and neurological findings were consistent with a diagnosis of ADC stage 4. Histopathological evaluation of all three specimens revealed no signs of cytomegalovirus infection or PML.

Immunohistochemical staining showed prominent immunoactivation in the DWM, as demonstrated by the number of HLA-DR positive macrophages/microglia in all three cases (fig 2A; patient 4). Only a few microglia/macrophages were positive for gp41 staining in the biopsy cases. Prominent expression of TNFα in macrophages/microglia was seen in the biopsy cases with a polyclonal antirabbit antibody. In addition, in both subjects, the cell body and axons of multiple neurones stained positive for TNFα with either the monoclonal or polyclonal antibody (fig 2B; patient 4). In the frontal cortex, hippocampus, and basal ganglia of patient 6, TNFα was detected in microglia/macrophages, and to a lesser degree in astrocytes. TNFα staining was also found in neurones of the frontal cortex and basal ganglia (fig 2C). In addition, the expression of gp41 and inducible nitrous oxide synthetase was prominent in all brain regions studied, accompanied by myelin pallor and astrocytosis in the DWM.

To assess the specificity of the neuronal expression of TNFα, we examined the cellular and regional expression of TNFα in postmortem brain sections from 16 other HIV infected patients previously reported with various stages of cognitive impairment and without a clinical history of a focal central nervous system process. ADC staging was as follows: four subjects were stage 0, five subjects were ADC stage 1, three subjects were stage 2, and four subjects were stage 3/4.[23] None of these patients showed the radiographic pattern of white matter abnormalities described above. No further neuronal staining for TNFα was found in the subjects.

Peptide blocking studies were performed on serial brain sections from patient 6. Staining for TNFα using either antibody was abolished by the blocking peptide in macrophages/microglia, astrocytes, and neurones (fig 2D).

DISCUSSION

Our study examines the clinical presentation of six patients with acute/subacute and reversible cognitive motor decline as their main AIDS defining illness, all of whom had similar findings on cranial MRI, characterised by diffuse, bilateral T_2 hyperintense signal changes in the DWM. The striking association of white matter abnormalities, the distinct clinical course, and the rapid response to antiretroviral drug treatment suggest that these patients may have a unique form of ADC.

In a previous study, Bouwman et al described the clinical characteristics of rapid progressors and non-progressors with HIV dementia.[33] Rapid progressors showed a decline in neurocognitive function over months compared with the non-progressors, who had more stable disease. However, several aspects distinguish these rapid progressors from our group of patients. Patients of the above mentioned study developed dementia towards the end of their illness and did not present with dementia as their AIDS defining illness. Furthermore, radiological features such as atrophy and white matter abnormalities did not differ between the rapid progressors and the non-progressors. Third, no differences were noted in antiretroviral treatment response between rapid progressors and non-progressors.

The term HIVL has been used previously to describe the pathological findings in the white matter of patients with AIDS, ranging from patchy isolated lesions to extensive bilateral and confluent white matter changes involving the midbrain and pons. Histological features include myelin pallor, astrogliosis with evidence of immune activation, and viral infection predominantly localised to microglia and macrophages.[34]

The pathophysiological basis of the reversible DWM changes in these patients is not well understood. Three studies implicate altered BBB function as an important underlying mechanism. Smith et al noted striking changes in the microvasculature, including mural thickening, increased cellularity, and enlargement of endothelial cells in patients with features of HIV encephalitis and leucoencephalitis.[35] Two groups detected a significant accumulation of serum proteins in the cerebral white matter, consistent with breakdown of the BBB.[36 37]

All patients in our present report showed rapid cognitive improvement soon after the introduction of antiretroviral treatment, accompanied by a reversal of DWM lesions. In contrast, in patients with the more slowly progressive form of ADC treated with antiretroviral drugs, restitution of cognitive functions tends to occur slowly and MRI changes do not improve substantially.[38] One possible explanation might be that the prompt institution of antiretroviral treatment caused a decline in peripheral viral load and circulating inflammatory cytokine concentrations, leading to a reversal of BBB abnormalities and improvement in cognitive function.

Immunohistochemical studies of the two biopsies showed prominent HLA-DR expression but only mild viral protein (gp41) expression, in contrast to the findings in the postmortem case. This observation raises the possibility that HIV proteins in the brain might not be an important factor in the acute or early stages of ADC compared with its later stages. Furthermore, in all three cases, prominent TNFα staining was seen in astrocytes and perivascular microglia/macrophages of the DWM, consistent with previous reports.[25 26 39] TNFα positive cells such as microglia/macrophages and astrocytes have a crucial role in the induction of astrocytosis, and may also play a role in alteration of the BBB.[30 40]

The surprising finding of our study was the detection of TNFα staining in neurones in all three subjects. Interestingly, Cowan et al showed that NT2 cells, which share morphological and functional features of mature primary neurones, were able to produce TNFα upon stimulation with human T cell leukaemia virus 1 Tax1.[41] Several other groups have reported that certain HIV virions and HIV proteins (gp120 and Tat) can stimulate the cellular production of TNFα in macrophages, astrocytes, and neurones.[42 43] Further support for the notion that human neuronal cells can produce TNFα comes from a recent immunohistochemical study, in which prominent neuronal staining with antibodies to TNFα was found in cortical areas adjacent to the DWM lesions in preterm infants with periventricular leucomalacia.[44] The reason why neurones produce TNFα particularly under pathological conditions is at present unclear. One hypothesis is that TNFα is produced in an autocrine fashion, in an attempt to promote cell survival via activation of nuclear factor κB and the upregulation of survival proteins.[26 45]

The results of our study suggest that certain HIV infected patients may be predisposed to develop a primary cytokine induced encephalopathy. Quasney et al found that the presence of the A allele at the TNFα −308 site in the promoter region was over-represented among adults with HIV dementia compared with those without dementia and healthy controls, suggesting that this locus may play a role in the pathogenesis of HIV dementia.[46] In vitro studies have

shown that the TNF2 allele increases the transcription of TNFα, consistent with the finding that homozygotes for the TNF2 allele have significantly raised concentrations of TNFα.[47] This raises the possibility that polymorphisms in the TNFα gene may predispose a subgroup of patients with HIV to a more rapid cognitive decline.

CONCLUSION

HIVL is a unique form of HIV associated brain injury, which is characterised by a more rapid onset of clinical symptoms and prominent, reversible white matter changes. Furthermore, we show evidence of TNFα expression in neurones in three patients, suggesting that the process underlying this rapidly progressive form of ADC may reflect indirect mechanisms mediated by host factors, particularly TNFα.

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