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Cerebral CD8+ lymphocytosis in HIV-1 infected patients with immune restoration induced by HAART

Abstract In HIV infected persons, highly active antiretroviral therapy (HAART) has reduced both the morbidity and incidence of several disorders. Its effects on direct HIV-induced damage to the CNS remain controversial. In addition, HAART may provoke an "immune reconstitution inflammatory syndrome" (IRIS). Herein we report two patients who, despite HAART, developed a diffuse encephalopathy. Their clinical, radiological and neuropathological features are described. Immunohistochemical and PCR analyses were used to detect HIV and to exclude other viruses in brain tissue. The unusual inflammatory reaction in the brain tissue was defined by immunohistochemistry. Both patients had advanced HIV disease with low CD4 counts and high HIV "viral loads" before starting HAART. In both, HAART induced an increase in CD4 count and a marked reduction in HIV viral load, which was accompanied, in patient one, by worsening of pre-existing, and, in patient two, by development of, acute encephalopathy. At post-mortem examination, the brain of patient one showed HIV encephalitis. In addition, the brains of both patients revealed HIV–DNA by PCR, diffuse microglial hyperplasia and massive and diffuse perivascular and intraparenchymal infiltration by CD8+/CD4– lymphocytes. We suggest that the rapid immune reconstitution induced by HAART in these two patients led to a redistribution of lymphocytes into peripheral blood. This was followed by recruitment of CD8+ lymphocytes into the brain, which resulted in the diffuse infiltration described. The appearances in patient two further suggest that HIV brain infection, even without encephalitis, is sufficient to trigger this response.

Keywords HAART · HIV · Immune reconstitution inflammatory syndrome

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Introduction

Combination anti-retroviral treatment, also known as highly active anti-retroviral therapy (HAART), results in reduction of HIV replication which is mirrored in peripheral blood by reduction of HIV "viral load" and increased CD4+ lymphocyte counts in HIV positive patients [5, 29]. As consequence of HAART, mortality and morbidity have declined [6], and the spectrum of diseases observed has changed considerably. In the context of the central nervous system (CNS), cerebral toxoplasmosis [18], cryptococcal meningitis [26] and most opportunistic infections [32] have decreased in frequency and survival of patients with CNS non-Hodgkin's lymphoma [16] and progressive multifocal leukoencephalopathy (PML) [1, 10] has increased.

In a minority of patients, partial restoration of specific immunity, induced by HAART, may unmask or worsen a pre-existing disease. This complication, named immune reconstitution syndrome, immune restoration disease or immune reconstitution inflammatory syndrome (IRIS), is defined as a "paradoxical deterioration in clinical status attributable to the recovery of the immune system during HAART" [37]. Mycobacterial infection [11, 13], cyto-megalovirus retinitis [17], and cryptococcal meningitis [41, 21] related to IRIS have been reported. In some patients with PML, who are receiving HAART, contrast enhancement seen on MR imaging suggests the existence of a florid inflammatory reaction, usually discrete in untreated individuals, which is confirmed by cerebral biopsy [27, 39].

The effect of HAART on the late complication of HIV infection, HIV encephalitis, associated, in a number of patients, with a form of subcortical dementia, is still a controversial issue. It has been reported that HAART is associated with improvements in cognitive function [34] and a decreased incidence of HIV-associated dementia [6, 33]. More pertinent to the present study is the report by Langford et al. [24] of seven patients who had received HAART, five of whom in life had HIV-associated dementia. The salient neuropathological changes (myelin loss, lympho-monocytic exudate, axonal injury and astrocytic gliosis) were considered to be more severe than those observed in the pre-HAART era. The most characteristic feature in these cases was the presence of perivascular inflammatory cells, which were interpreted as resulting from immune restoration [24].

We recently examined the brains of two patients with AIDS who had received HAART and were impressed not only by the density of the lymphoid infiltration, but also by its location which was not limited to the perivascular spaces, as reported by Langford et al. [24], but diffusely involved the white and, to some extent, the grey matter.

Patients and methods

As controls, the following brains were examined: (1) from 6 patients with advanced HIV infection, who, in life, had low CD4 lymphocyte counts and clinically apparent HIV-associated dementia; none had received HAART; (2) from 6 patients who had died while in the asymptomatic stage of HIV infection; and (3) from 6 patients who had received HAART, with documented improvement in CD4 counts and reduction in HIV VL. Patients in this group had the following clinico-pathological conditions: (a) chronic burnt-out VZV encephalitis (1 patient); (b) chronic burnt-out HIV encephalitis (2 patients); and (c) neither clinically apparent neurological disorders nor neuropathological abnormalities (3 patients).

Patient 1

A 45-year-old black African man had lived in the UK for the previous 3 years and had been heterosexually active in Africa for many years. He presented with an 8-week history of progressive cognitive decline, a confusional state and a 3-week history of right-sided weakness/neglect. A cranial MR scan showed generalised atrophy and high signal in the right hippocampus, amygdala, isthmus and left inferior temporal lobe. At lumbar puncture, cerebrospinal fluid (CSF) analysis showed WBC<1 and protein=0.9 g/l. Staining and culture for bacteria, mycobacteria and fungi were negative, and CSF toxoplasma, syphilis and cryptococcal serology were negative. The polymerase chain reaction (PCR) in CSF for detection of JC virus and human herpes viruses 1–8 was negative. An HIV-1 antibody test was positive. The CD4+ T lymphocyte count was 50 cells/μl and the HIV viral load (VL) detected in blood by PCR (Bayer Quantiplex HIV RNA assay; used also in case 2) was 838,100 copies/ml.

The patient was treated empirically for tuberculous meningitis, cytomegalovirus encephalitis and toxoplasmosis. One month after admission, his condition had not improved. A second cranial MR scan showed worsened appearances. At this time, the anti-infectious therapy was stopped and HAART (zidovudine, lamivudine and efavirenz) was started.

After 10 weeks, the patient's condition was unchanged. At this time the CD4+ T lymphocyte count was 320 cells/μl and the HIV VL=4000 copies/ml. An additional cranial MR scan showed severe generalised cerebral atrophy and extensive diffuse white matter signal abnormality, particularly in the periventricular area (Fig. 1a), with multiple foci of increased signal in the grey matter of the right

Fig. 1a, b Patient 1. Cranial MR. **a** Axial section, T2-weighted image shows diffuse periventricular white matter signal abnormality. **b** Coronal section. Fluid-attenuated inversion recovery (FLARE) sequence shows increased signal abnormality in the right hippocampus and bilaterally in cortical grey matter
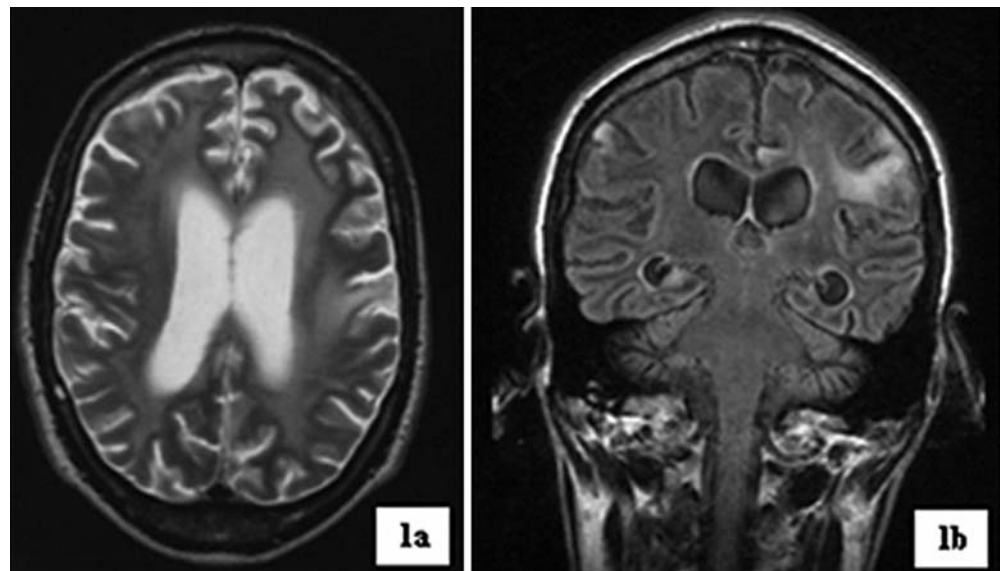
hippocampus, and bilaterally in cortical grey matter (Fig. 1b). At lumbar puncture, the opening pressure was 10.5 cm water and CSF analysis showed 23 lymphocytes and protein=1.15 g/l. Tests for pathogens (as above) were negative. One week later, the patient's condition deteriorated acutely with both focal and generalised seizures and he died.

Patient 2

A 32-year-old black African woman, resident in the UK for 14 years, was admitted to hospital following a sudden collapse. The patient had been diagnosed HIV-1 positive 7 months earlier, when she had presented with cutaneous Kaposi sarcoma (KS). At this time the CD4+ T lymphocyte count was 2 cells/µl and the HIV VL was >500,000 copies/ml. The patient began HAART (zidovudine, lamivudine and ritonavir/saquinavir) and the KS was treated with chemotherapy. Four months later, the CD4+ T lymphocyte count had increased to 122 cells/µl and the HIV VL had fallen to 1193 copies/ml.

On admission to hospital the patient gave a 17-day history of headache. General and neurological examination was normal. She had an abnormal affect and was poorly communicative. A lumbar puncture gave a bloody tap; the opening pressure was 13 cm water. A CSF analysis showed protein=0.4 g/l and WBC=12; staining and culture and serological tests on CSF (as for patient one) were negative. A cranial MR scan and an EEG suggested a diffuse encephalopathy. A CD4+ T lymphocyte count was 95 cells/µl and the HIV VL=291 copies/ml. During her stay in hospital the patient became increasingly withdrawn and uncooperative. She died on day 18 of admission.

Post-mortem examination was limited to the brain in patient 1; full post-mortem examination was carried out in patient 2. In both cases the brains were immersed in 10% buffered formalin and fixed for 3 weeks. Blocks were taken from a number of regions of the cerebral and cerebellar hemispheres, including the deep grey nuclei, midbrain and brain stem, and processed for embedding in paraffin wax. Sections were stained with routine and immunohistochemical (IHC) methods; the latter included the use of the following antibodies: anti-GFAP (Dako, 1:1500); anti-p24 (NEN, 1:300); anti-β-APP (Roche, 1:400); anti-Ki67 (Dako, 1:200); anti-CD68 (Dako, 1:100); anti-CD3 (Dako, 1:100); anti-CD20 (Dako, 1:500); anti-CD4 (Novocastra, undiluted); anti-CD5 (Dako, 1:25); anti-CD8 (Dako, 1:50); anti-TIA-1 (Abcam, 1:50); and anti-LN-3 (BioGenex, undiluted). Presence of the following organisms was excluded: Herpes simplex type 1 and 2, measles, EBV and *Toxoplasma* (by immunohistochemistry); CMV and JC (by in situ hybridisation); *Mycobacteria* and *Cryptococcus* (by histochemistry). DNA was extracted from formalin-fixed, paraffin-embedded tissue sections and nested PCR was used to amplify a fragment of the HIV *pol* gene, as described previously [2].

Results

Macroscopically, both brains were symmetric and did not show any focal lesions. In patient 1 the brain appeared atrophic (weight 930 g) with shrinkage of the cerebral gyri, widening of the sulci and ventricular dilatation. Mild grey discolouration of the white matter of the centrum semiovale was seen in both cases.

In patient 1 histological examination showed HIV encephalitis (HIVE), characterised by diffuse myelin pallor, astrocytic gliosis, confirmed by GFAP antibody, presence of large numbers of macrophages and activated microglial cells (Fig. 2a) and a small number of MGC, both in the white matter and surrounding blood vessels. Use of p24 antibody revealed moderate amounts of HIV protein in the cytoplasm of some MGC (Fig. 2a, inset) and macro-

phages. The encephalitis was circumscribed to the cerebral hemispheres and produced moderate axonal damage, as revealed by β-APP antibody. In addition, the basis pontis showed multiple, small and relatively well-circumscribed areas of myelin degeneration with presence of large macrophages and some preservation of axons and nerve cells, which are features of focal pontine leukoencephalopathy. In patient 2 the white matter was hypercellular, showed no myelin pallor, a smaller number of reactive astrocytes and fewer activated microglia and macrophages (Fig. 2b) than in patient 1; moreover, it did not show evidence of HIVE and IHC did not reveal presence of replicating HIV. Axonal damage was minimal.

An unusual feature in the white, and to lesser extent in the grey, matter and meninges of both patients was the large number of CD3+ T lymphocytes, seen either around blood vessels (Fig. 3a), where they were variably intermingled with macrophages, or scattered in the white matter (Fig. 3b) among the other types of cells or, less frequently, forming loose aggregates (Fig. 3c). The IHC confirmed that the lymphocytes were CD5+, CD8+ (Fig. 4), and CD4– (data not shown) T cells. In patient 2 (Fig. 4b), in whom lymphocytes were more numerous than in patient 1 (Fig. 4a), T cells contained cytotoxic granules as shown by positive staining with TIA-1 antibody. In patient 1 the cells were TIA-1 negative. In both patients there was a very low proliferation fraction shown by immunostaining with Ki67. LN3 antibody revealed an obvious up-regulation of histocompatibility type-II antigens in both brains; this was higher in patient 1 (Fig. 5a) than in 2 (Fig. 5b). The PCR revealed the presence of HIV DNA in both cases. All the infectious agents mentioned in "Materials and methods" were excluded.

Examination of the brain tissue of the patients from groups 1 and 2 showed occasional to few CD3+ lymphocytes; these were predominantly located around the vessels, with only a small proportion scattered in the white matter. The lymphocytes were CD8+ and CD4–. In all 12, the tissue was HIV DNA positive with PCR. Similar cell density, localisation and immunophenotype of lymphocytes were seen in the brains forming group 3. In patient 2 general post-mortem examination revealed a small focus of KS in an otherwise atrophic hilar lymph node.

Discussion

Despite HAART-induced improvements in CD4+ T-lymphocyte counts and HIV VL, the neurological condition of these two patients with AIDS (HIV-associated dementia and KS, respectively) deteriorated, and led to death. Neuropathological findings in both patients included the diffuse presence of activated microglia and infiltration of the white matter, leptomeninges and grey matter, in decreasing order of severity, by macrophages and CD3+/CD5+/CD8+/CD4– lymphocytes. Both types of cells were present also around blood vessels. In both brains there was up-regulation of histocompatibility class-II antigens and PCR evidence of HIV DNA. Differences between the
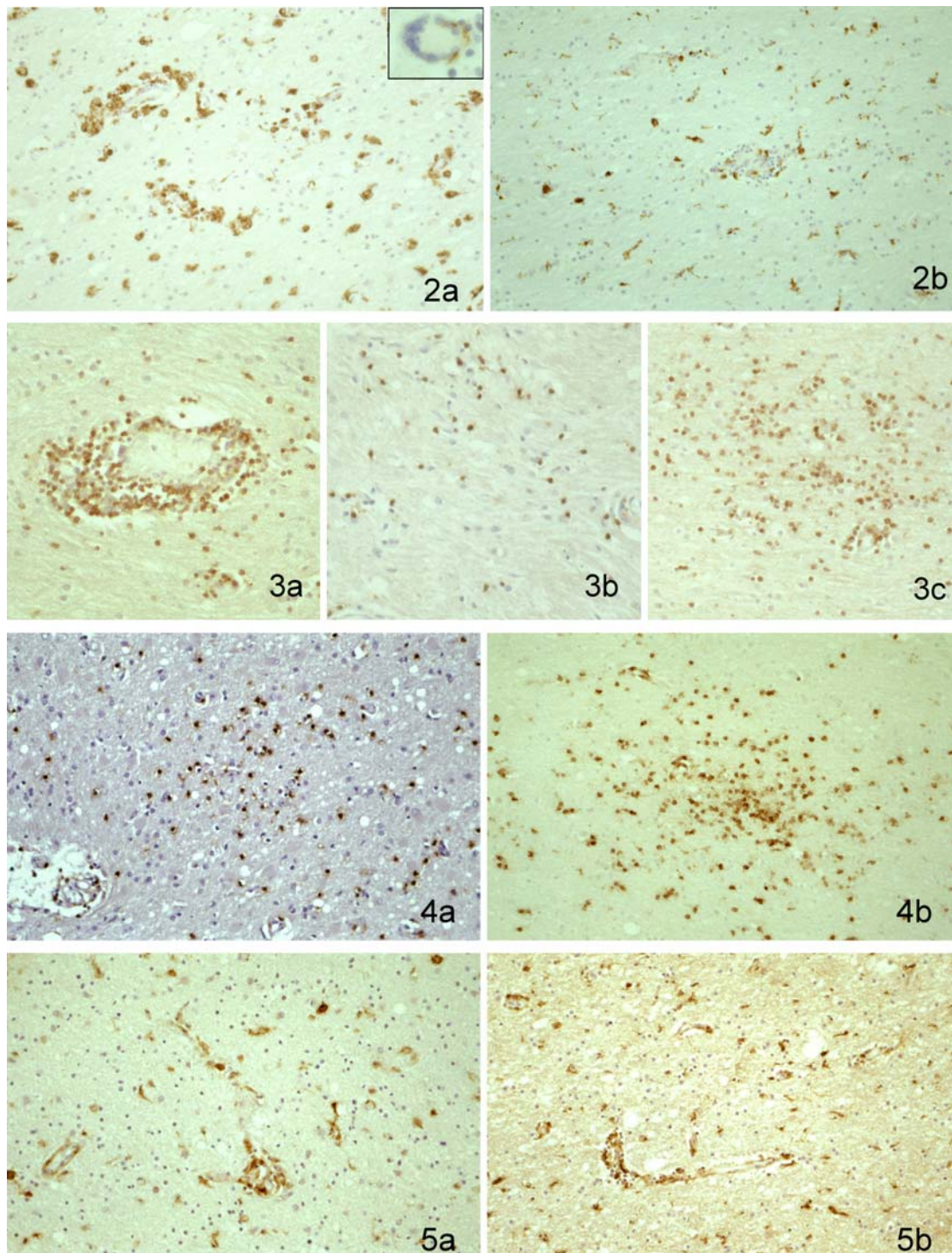
Fig. 2a, b CD68 antibody stains (a). A large number of activated microglial cells and macrophages in the white matter. *Inset:* in a multinucleated giant cell in the white matter of the same patient p24 antibody reveals productive infection by HIV (p24 antibody, ×633). b The density of CD68 positive macrophages and activated microglia in the white matter of patient 2 appears lower than that in patient 1. Magnification ×170

Fig. 3a–c T-lymphocytes, immunostained with CD3 antibody, form perivascular cuffs (a), or are diffusely infiltrating the white matter (b), where they may form loose aggregates (c). a, c Patient 2. b Patient 1. Magnification ×400

Fig. 4a, b Among the inflammatory cells most T lymphocytes are CD8+. a Patient 1. b Patient 2. Magnification ×170

Fig. 5a, b LN3 antibody reveals a diffuse upregulation of histocompatibility type-II antigens, which is higher in patient 1 (a) than in patient 2 (b). Magnification ×160

two brains included myelin pallor, HIVE and focal pontine myelinolysis in the former and larger lymphocytic perivascular cuffings and cytotoxic granules in the cytoplasm of brain lymphocytes in the latter.

The morphological and IHC features described in this study are quite unusual. They are unlike those previously reported either in patients with AIDS or those with asymptomatic HIV infection. The difference was confirmed by examination of the brains from patients in groups 1 and 2, in which the lymphocytic infiltration was discrete and located in the meninges and perivascular spaces with only minimal extension to brain parenchyma. The immunotype of these cells was identical to that of the two patients reported here. The only previous study of subsets of T cells in patients with HIVE not treated with HAART described a mixed population of CD4+ and CD8+ cells, localised to the perivascular spaces [30]. In addition, the changes we describe do not appear to be normally associated with HAART, as in the 6 patients in group 3, all of whom had undergone HAART-induced partial immune reconstitution, only occasional lymphocytes were seen in the brain parenchyma, with immunophenotype similar to that in the other groups.

The findings in the seven patients with AIDS and leukoencephalopathy, who received HAART reported by Langford et al. [24], bear some similarities with ours in that those patients had perivascular infiltration by macrophages and lymphocytes. In contrast to our cases, lymphocytes did not infiltrate the white matter and their immunophenotype was not described. The two cases described herein also contrast with the recent description of three patients with an "active" CSF, a raised CSF opening pressure and neuroimaging suggestive of meningo-encephalitis, all of which resolved on HAART [40]. In our 2 patients CSF opening pressure was not raised and their condition worsened once HAART was commenced.

The organisms implicated in HAART-induced IRIS are already present in the affected regions before the onset of the treatment, where they elicit a process which may be sub-clinical [37]. Our case 1 fulfils these criteria as, on admission, the patient was already suffering from HIV-associated dementia. The findings in patient 2 are more difficult to explain, as, at post-mortem examination, her brain showed no underlying lesion, except for the severe infiltration by lymphocytes and monocytes. On the other hand, she appeared confused before HAART was commenced. A hypothesis to explain the findings in patient 2 is that HIV is present in the brain from early in the course of HIV infection (even when patients are neurologically asymptomatic), where it creates a state of immune activation with production of toxic substances, including pro-inflammatory cytokines [3]. It is therefore possible that in the second patient, in whom HIV-DNA was demonstrated in brain by PCR, an already compromised situation, with likely endothelial damage [4], was exacerbated by HAART.

The HAART may exert a deleterious effect through different mechanisms: directly via the constituent drugs, via mitochondrial damage and abnormal lipid metabolism [9]; promoting additional phagocytosis of myelin by monocytes–macrophages [15]; or by relocation into peripheral blood of CD4+ and CD8+ T-lymphocytes [12], including CD8+ memory cells, previously sequestered in lymphoid tissue [23]. Support for the role of T-cell restoration comes from the study of two profoundly immunosuppressed HIV infected patients with PML [35], in whom a paradoxical worsening of their clinical and radiological condition occurred in the context of HAART-induced rapid improvement of CD4+ count and HIV VL. Histological examination of the brains showed a prominent inflammatory infiltration and evidence of JC virus by PCR. It was postulated that the immune restoration from HAART allowed an influx of memory T-cells that recognised JC viral antigens. The contribution of CD8+ cells cannot be assessed, as immunophenotyping of the lymphocytes was not performed.

CD8+ lymphocytes form the majority of cytotoxic cells and are present only in small numbers within the normal CNS [28]. In some circumstances, a strong immune-mediated response may result in their increase to the point that they outnumber CD4+ lymphocytes, as has been shown in multiple sclerosis [14] and in the SCID mouse model of HIV infection [31]. This response may be beneficial, when it leads to elimination of a pathogen, but in other instances, including in HIV-positive patients treated with HAART, the effect may be harmful. In both our patients rapid immune reconstitution took place, with a ≥sixfold rise in CD4+ count and a >2 $\log_{10}$ fall in VL. In this respect, our findings are at variance with those by Langford et al. [24], who described low CD4+ cell counts and poorly controlled HIV replication in their patients. The improvements in cell counts in our patients was detrimental, as it included also recruitment of CD8+ memory cells into the brain, in the absence of CD4+ cells. Similar results were shown in brain biopsies of five patients with PML, four of whom had received HAART [27]. Similar to our cases, most of the inflammatory cells seen in these patients were CD8+ suppressor, with absence of CD4+, cells.

The deleterious role of CD8+ cells in the syndrome of immune reconstitution is supported by the following reports. Following HAART, there is an increase in CD8+ T lymphocytes in peripheral blood [8, 20]; the CD8+ T lymphocyte count was the only risk factor in patients developing herpes zoster [25]; clinical hepatitis caused by hepatitis B and C virus appears to result from an increase in CD8+ T lymphocyte count [7, 19] and suppression of HIV viraemia correlates with a decrease in CD8+ T lymphocyte responses [22]. In contrast, in children [36] a low circulating CD8+ lymphocyte count predicts development of HIV-1 encephalitis. CD8+ cells exert their function in two main ways: either by ligating TNF receptor-like molecules by their corresponding ligands, triggering the apoptosis pathway, or by secreting the content of cytoplasmic vesicles (cytotoxic granules) [38]. The latter mechanism is suggested in our second patient, in whom toxic granules were detected in lymphocytes within the brain.

Conclusion

In conclusion, it is postulated that redistribution of lymphocytes from lymphoid tissue into peripheral blood induced by HAART permitted recruitment of CD8+ T lymphocytes into the brain. The diffuse cerebral infiltration with CD8+ T lymphocytes seen in these two cases contrasts with that previously described in the context of HAART-induced immune restoration, where infiltration of the brain by macrophages/monocytes and lymphocytes was confined to the perivascular spaces. In addition, our case 2 further suggests that HIV infection of the brain per se, without overt HIV encephalitis, is sufficient to trigger this response.

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