Expression of Excitatory Amino Acid Transporter-1 in Brain Macrophages and Microglia of HIV-Infected Patients. A Neuroprotective Role for Activated Microglia?

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Abstract. Recent experimental studies showed that activated macrophages/microglia (AMM) express excitatory amino acid transporters (EAATs), suggesting that, in addition to their neurotoxic properties, they also have a neuroprotective role by clearing extracellular glutamate and producing antioxidant glutathione. To test this hypothesis in human, the brain of 12 HIV-positive patients and 3 controls were immunostained for EAAT-1. EAAT-1 was expressed by AMM in all HIV-infected cases but not in HIV-negative controls. Expression varied according to the disease stage. In 5 cases with active HIV-encephalitis (HIVE), AMM strongly expressed EAAT-1 in the white matter and basal ganglia, analogous to HLA-DR and CD68 expression. There was weaker expression in the cortex and perineuronal microglial cells were not involved. In a case with “burnt out” HIVE following highly active antiretroviral therapy (HAART), EAAT-1 expression was mild, identical to that of HLA-DR and CD68 in the white matter and cortex and involved perineuronal microglial cells. In 3 AIDS patients without HIVE and in 3 pre-AIDS cases, EAAT-1 expression in the white matter was weaker than HLA-DR and CD68 expression; there was stronger correlation in the gray matter where perineuronal microglial cells were stained predominantly. Our findings in humans tend to confirm that AMM, particularly perineuronal microglial cells, play a neuroprotective role in the early stages of HIV infection and, possibly, following treatment. This is in keeping with the early microglial activation seen in pre-AIDS cases, and the late occurrence of neuronal loss. It may also explain the reversible cognitive disorders following treatment in some cases.

Key Words: Central nervous system; Excitatory amino acid transporter; Glutamate; HIV dementia; Human immunodefiiciency virus; Macrophage; Microglia.

INTRODUCTION

Nearly 20 years after the first autopsy cases of AIDS patients were reported, and despite an enormous amount of research, the precise causes and mechanisms of neuronal dysfunction underlying the cognitive disorders in AIDS patients (HIV dementia) are still unclear (1). Neurotoxic factors related to HIV infection, either locally released or blood-borne, probably play a leading role (2). The neurotoxicity of HIV proteins and substances produced by activated glial cells has already been shown (3–5). The final irreversible stage of neuronal damage in HIV encephalopathy is neuronal death, the mechanisms of which are only partly understood, but appear to involve apoptosis (6–11), oxidative stress (12–14), and glutamate-receptor-mediated toxicity (15–21) (for review, see [3]).

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Na+-dependent glutamate transporters, also called excitatory amino acid transporters (EAATs), were primarily identified in astrocytes (EAAT-1 and EAAT-2) and neurons (EAAT-3) (22, 23). EAATs keep extracellular glutamate concentration low in the brain and prevent excitotoxicity (24). In addition, it has been shown that glutamate entry into the cell via EAATs activates the entry of cystine through a mechanism of trans-stimulation and leads to increased glutathione synthesis, a critical activity in antioxidant response (25, 26). Several in vitro studies show that function and expression of astrocyte EAATs are depressed by HIV, probably due to the effects of inflammatory mediators and viral proteins (27–31). However, in vitro studies of cultured primary microglia from different species and human adult monocyte-derived macrophages (32–37), and in vivo studies of microglial cells activated following mechanical injury in rats (37, 38), show that these cells also express EAATs. A recent study by our group in simian immunodeficiency virus (SIV)-infected macaques also showed that activated microglia and brain macrophages (AMM) express EAAT-2 (39).

The latter study suggests that in chronic brain infection, particularly retroviral infection, besides their largely documented neurotoxic properties, AMM may have neuroprotective functions both against oxidative stress and glutamate mediated excitotoxicity, probably the 2 main mechanisms of neuronal dysfunction in HIV dementia (2, 3). The present study was undertaken to test this hypothesis in human HIV infection.
HIV (AIDS, HIVE) ("Burnt out" HIVE) 6 M 69 bronchopneumonia

Viral load was extremely high (J Neuropathol Exp Neurol, Vol 62, May, 2003). Slowing and gait disturbances. CD4 count was 313/mm³. The case with "burnt out" HIVE was diagnosed HIV-positive in 1997. In January 2000, he presented with intellectual decline and was found to have a CD4 count of 455/mm³. His laboratory parameters had improved (CD4: 455/mm³), and p24 was negative in cerebrospinal fluid (CSF). Magnetic resonance imaging (MRI) showed cerebral atrophy and multifocal high signal intensity in the deep white matter, suggestive of HIVE. The 3 AIDS patients without HIVE had no pathological or associated pathology. The 3 HIV-positive, non-AIDS cases were drug addicts who died from heroin overdose and were diagnosed HIV-positive at postmortem examination. None was previously known to be HIV-positive and none had clinical symptoms of AIDS-associated illness or cognitive disorder. Postmortem examination did not show any systemic or CNS lesions of AIDS-associated illness. Neuropathological examination showed mild diffuse myelin pallor and perivascular mononuclear cuffs in the leptomeninges and deep white matter. Epidemiological and clinical data of the 15 cases, including controls, are summarized in Table 1.

For each case, samples of formalin-fixed, paraffin-embedded frontal and temporal cortex with the underlying white matter, basal ganglia, and brainstem were examined. In addition to the different stains used in our series (6), we used immunohistochemistry to identify productive HIV infection, astrocytosis, and microglial activation. We also used immunohistochemistry to look for expression of EAAT-1, the main transporter implicated in glutamate uptake by human monocyte derived macrophages in vitro (35). Apoptotic cells were identified by immunohistochemistry for activated caspase 3, and in situ end labeling (ISEL). ISEL was performed using the Apoptag kit (Oncor, Gaithersburg, MD), as previously described (7).

### MATERIAL AND METHODS

#### Patients

Brains from 12 cases of HIV-infected patients at different stages of the disease were studied. They included 5 cases with full-blown AIDS and active HIVE, 1 case with "burnt out" HIVE, 3 AIDS patients without HIVE, and 3 HIV-positive, asymptomatic non-AIDS cases. Three HIV-negative, "normal" controls were also studied according to the same protocol.

Active HIVE was defined as diffuse microglial activation with microglial nodules and multinucleated giant cells and marked expression of HIV protein p24 in microglial cells. The 5 selected cases did not have any other AIDS-related neuropathology. All 5 had a history of cognitive disorders, of which 3 fulfilled the criteria for HIV dementia.

The case with "burnt out" HIVE was diagnosed HIV-positive in 1997. In January 2000, he presented with intellectual slowing and gait disturbances. CD4 count was 313/mm³ but his viral load was extremely high (>500,000 copies/ml), and p24 was positive in cerebrospinal fluid (CSF). Magnetic resonance imaging (MRI) showed cerebral atrophy and multifocal high signal intensity in the deep white matter, suggestive of HIVE. He received highly active antiretroviral therapy (HAART). In April 2000, his laboratory parameters had improved (CD4: 455/mm³, viral load was negative, p24 was negative in CSF), and on MRI the deep white matter showed a diffuse low signal intensity, more like HIV leukoencephalopathy than HIVE. Despite mild improvement of his gait, his intellectual status progressively deteriorated and he died from bronchopneumonia in June 2000. Neuropathological examination confirmed marked cerebral atrophy and myelin pallor of the deep white matter. The histology showed diffuse poliodystrophy with neuronal loss, frequent apoptotic neurons, astrocytosis, and mild microglial activation. There was myelin pallor of the deep white matter with marked astrocytosis at the cortico-subcortical junction and a few perivascular AMM. There were no microglial nodules, no multinucleated giant cells, no inflammation, and p24 immunostains were negative. There was no other AIDS-related neuropathology. Thus, HIV, the presumed diagnosis on clinical, radiological, and biological grounds in January 2000 appeared to have been cured by HAART.

The 3 AIDS patients without HIVE had no pathological or immunohistochemical evidence of HIVE, opportunistic, or malignant central nervous system (CNS) pathology and died from a systemic complication of AIDS (hepatitis B, hepatitis C-related cirrhosis, Kaposi sarcoma). Only 1 had a history of terminal cognitive disorder, which did not fulfill the criteria for HIV dementia.

The 3 HIV-positive, non-AIDS cases were drug addicts who died from heroin overdose and were diagnosed HIV-positive at postmortem examination. None was previously known to be HIV-positive and none had clinical symptoms of AIDS-associated illness or cognitive disorder. Postmortem examination did not show any systemic or CNS lesions of AIDS-associated illness. Neuropathological examination showed mild diffuse myelin pallor and perivascular mononuclear cuffs in the leptomeninges and deep white matter.

#### Table 1

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Cognitive disorders</th>
<th>Cause of death or associated pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>25</td>
<td>HIVD</td>
<td>bronchopneumonia</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>33</td>
<td>mild, terminal</td>
<td>Kaposi sarcoma</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>40</td>
<td>HIVD</td>
<td>bronchopneumonia</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>38</td>
<td>HIVD</td>
<td>bronchopneumonia</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>34</td>
<td>severe, terminal</td>
<td>bronchopneumonia</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>69</td>
<td>HIVD</td>
<td>bronchopneumonia</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>31</td>
<td>0</td>
<td>Kaposi sarcoma, suicide</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>58</td>
<td>0</td>
<td>septic shock</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>40</td>
<td>mild, fluctuating</td>
<td>hepatitis C-related cirrhosis</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>42</td>
<td>0</td>
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</tr>
<tr>
<td>11</td>
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<td>34</td>
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</tr>
<tr>
<td>12</td>
<td>M</td>
<td>34</td>
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</tr>
<tr>
<td>13</td>
<td>M</td>
<td>20</td>
<td>0</td>
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<tr>
<td>14</td>
<td>M</td>
<td>56</td>
<td>0</td>
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</tr>
<tr>
<td>15</td>
<td>M</td>
<td>35</td>
<td>0</td>
<td>decapitation</td>
</tr>
</tbody>
</table>

Abbreviation: HIVD, HIV dementia.
Immunohistochemistry was performed using a peroxidase-based method with the following commercial antibodies: a polyclonal antibody (Ab) raised against the glial fibrillary acid protein (GFAP) (anti-GFAP, Dako, Glostrup, Denmark); a mouse monoclonal antibody (mAb) raised against major histocompatibility class II antigens (HLA-DR) (CR3/43, Dako) and a mouse mAb to CD68 (KP1, Dako) to identify AMM; a mAb against microglial cells (PNMC) are tabulated in the first 3 columns for the 5 groups. Microglial activation, EAAT-1 expression in astrocytes, astrocytosis, and neuronal apoptosis are tabulated in the other columns.

Production of Human EAAT-1-Specific Antiserum

The peptide (C)- K-E-N-M-H-R-E-G-K-I-V-R-V-T, corresponding to amino acids 152–165 of human EAAT-1 sequence, was synthesized in the laboratory using a Milligen 9050 (Waters, Milford, MA) with standard protocol, and purified by HPLC. The extra Cys residue allowed specific covalent coupling with both immunogenic carrier (maleimido-BSA) and enzyme label (maleimido-acetylcholinesterase, AChE) via its thiol function as previously described (41). Balb/c mice were immunized (40 μg/mouse in complete Freud’s adjuvant) in the footpad. Booster injections were given every 3 weeks. The presence of specific anti-peptide antibodies was checked by enzyme immunoassay (EIA) as previously described (42). Titers ranging from 1/10,000 to 1/200,000 were obtained in EIA competition assay. Pre- and postimmunization mouse sera were then screened for immunohistochemistry using both frozen and paraffin-embedded brain sections from a control and an infected animal. Negative controls performed on serial sections included (i) omission of the serum, (ii) incubation with the preimmune serum of each mouse, and (iii) incubation with the immune serum previously depleted by overnight incubation at 4°C with 100 μg/ml of the immunogen peptide to ensure specific signal extinction. These controls enabled confirmation of signal specificity for the peptide used in the immunization protocol. We selected a serum that exhibited the highest signal-to-noise ratio, without nonspecific binding, and used it throughout the study. Brain sections from 1 sample that exhibited high signal during the screening were used with each series as positive controls.

Semiquantitative Analysis

The intensity of EAAT-1, GFAP, HLA-DR, CD68, and p24 expression was evaluated semiquantitatively blindly by 3 neuropathologists (FG, AVD, FC), subsequently reviewed jointly, and scored as follows: 0 = absent; 1 = mild; 2 = marked; and 3 = intense. Evaluation of the density of apoptotic neurons by ISEL and Caspase 3 immunostaining gave concordant results. Caspase 3-immunopositive neurons were usually positively stained by ISEL on successive slides. Caspase 3-immunopositive neurons usually had a normal appearance. Neurons positively stained by the ISEL method were more numerous, and some had the characteristic shrunken cytoplasm and pyknotic nuclei of apoptotic cells.

The severity of neuronal apoptosis was also evaluated semiquantitatively and scored as follows: 0 = no apoptotic cells; 1 = occasional isolated apoptotic neurons; 2 = occasional groups of apoptotic neurons; and 3 = frequent apoptotic neurons. Endothelial cells, which have a rapid turnover and often undergo apoptosis, served as a positive internal control.
Fig. 1. Immunostaining of successive sections from the cortico-subcortical frontal area in case 4 using antibodies raised against EAAT-1, HLA-DR, and GFAP showing clear correlation of EAAT-1 expression with that of HLA-DR, whereas GFAP expression is distinct. This is particularly obvious in the cortico-subcortical area, which shows marked astrocytosis and no positivity on EAAT-1 or HLA-DR immunostains (×40).

**Statistical Analysis**

The association between EAAT-1 immunostaining in various sites (white matter, cerebral cortex, and perineuronal microglial cells) and a history of cognitive disorders was evaluated using the Yates chi-square. The association between EAAT-1 and neuronal apoptosis was similarly assessed. The intensity of EAAT-1 and neuronal apoptosis was categorized as low/weak (score = 0 or 1) or high/strong (score = 2 or 3). A p value of less than 0.05 was considered statistically significant.

**RESULTS**

Semiquantitative evaluation of the intensity of EAAT-1 expression in AMM and astrocytes in the white matter and cerebral cortex, the degree of astrocytosis (GFAP expression) and microglial activation (CD68 and HLA-DR expression) in the cerebral cortex and white matter, and that of neuronal apoptosis in the cerebral cortex (ISEL and expression of caspase 3) is summarized in Table 2.

EAAT-1 immunostains were clearly positive in all HIV-infected cases, and mild or negative in HIV-negative controls. In HIV-positive cases, EAAT-1 expression did not correlate topographically with GFAP expression but correlated closely with CD68 and HLA-DR expression. However, the intensity of EAAT-1 expression and its predominance in the gray or white matter varied according to the stage of the disease.

In the 5 cases with active HIVE, EAAT-1 expression was strong and correlated clearly with HLA-DR and CD68 expression but not with GFAP. This was particularly obvious in the cortico-subcortical areas, which showed marked astrocytosis but were negative on EAAT-1, CD68, or HLA-DR immunostains (Fig. 1). EAAT-1 expression predominated in the white matter particularly in microglial nodules (Fig. 2A) and multinucleated giant cells (Fig. 2B), although some very large, dystrophic giant cells were sometimes negative (Fig. 2C). Perivascular macrophages also strongly expressed EAAT-1 (Fig. 2D). In contrast, cells resembling activated astrocytes were usually negative. Rare protoplasmic astrocytes in the subcortical white matter showed pale cytoplasmic positivity very different from AMM expression (Figs. 2C, 3A) and similar to what we observed occasionally in controls (Fig. 6). In the basal ganglia, EAAT-1 expression also predominated in microglial nodules and multinucleated giant cells, whereas astrocytes were negative. In contrast, in the cerebral cortex, except in occasional microglial nodules (Fig. 3A), EAAT-1 expression was mild and mainly in perivascular macrophages. Perineuronal microglial cells were not stained (Fig. 3B).

In the case with “burnt out” HIVE, EAAT-1 expression in the white matter was analogous to that of HLA-DR and CD68 in diffuse scattered microglial cells and perivascular macrophages (Fig. 4A). In the gray matter, EAAT-1 expression was less than that of HLA-DR and CD68 and was seen in perivascular microglia and in occasional perineuronal microglial cells (Fig. 4B).

In AIDS patients without HIVE, EAAT-1 expression in the white matter was much weaker than HLA-DR and CD68 expression. There was a better correlation in the gray matter, mostly in perineuronal microglial cells (Fig. 5A, B).
Fig. 2. EAAT-1 immunostaining of the white matter of the cerebral hemispheres and brainstem and of the basal ganglia in HIVE. A, B: Case 2, temporal white matter (A) and basal ganglia (B): strong immunostaining of activated microglial cells within microglial nodules and multinucleated giant cells. Note that reactive astrocytes (arrows) are not stained (×200). C: Case 1, white matter of the pons, with marked immunopositivity of activated microglial cells some of which are multinucleated. Note that very large dystrophic multinucleated giant cells are not stained (×100). D: Case 4, frontal white matter, with strong immunopositivity of perivascular macrophages (×200).

The pattern of EAAT-1 immunostaining was comparable, but less intense, in pre-AIDS cases. It was almost negative in the white matter, whereas CD68 and HLA-DR clearly showed activated microglial cells and perivascular macrophages (Fig. 5C, D). In the gray matter, a few perivascular and perineuronal cells were EAAT-1-positive, comparable with CD68 and HLA-DR.

In the HIV-negative controls, only rare activated macrophages/microglia were identified by CD68 or HLA-DR immunostains and were not stained by EAAT-1. Occasional reactive astrocytes showed weak cytoplasmic EAAT-1 positivity (Fig. 6).

Correlation of EAAT-1 expression with the cognitive disorders showed that demented patients with HIVE had a significantly increased expression of EAAT-1 in the white matter (p = 0.004). Conversely, the intensity of EAAT-1 expression in the cerebral cortex did not correlate with the clinical outcome of the patients.
There was an apparent inverse association between EAAT-1 expression in perineuronal microglial cells and dementia in HIV-infected cases, but it did not reach statistical significance (p = 0.067), possibly due to the small study group. EAAT-1-positive perineuronal microglial cells were present only in HIV-positive patients without cognitive disorders and not in demented patients with HIVE. The 2 exceptions were the case with...
Fig. 5. AIDS patients without HIVE (A, B) and HIV-seropositive non-AIDS cases (C, D). A: Case 7, EAAT-1 immunostaining of the frontal cortex: marked immunopositivity involving almost exclusively perineuronal microglial cells (×200). B: Case 9, EAAT-1 immunostaining of the temporal cortex: strong immunopositivity of a perineuronal microglial cell (×1000). C: Case 10, CD68 immunostaining of the frontal white matter shows activated microglial cells and perivascular macrophages (×100). D: Same case, successive section immunostained for EAAT-1 showing no convincing positivity (×100).
FIG. 6. EAAT-1 immunostaining of the frontal white matter of a control (case 14) showing slight positivity in the cytoplasm of a rare protoplasmic astrocyte (×500).

“burnt out” HIVE (case 6) with only occasional EAAT-1-positive perineuronal microglial cells and 1 AIDS case without HIVE (case 9) who had a history of fluctuating cognitive disorders probably due to hepatic encephalopathy. There was no association between EAAT-1 expression in the white matter or the cerebral cortex and neuronal apoptosis.

DISCUSSION

Our study shows that in HIV-infected patients, activated microglial cells and brain macrophages (AMM) express the high affinity glutamate transporter EAAT-1, with little or no expression by reactive astrocytes. This substantiates the previous demonstration, in SIV-infected macaques, of EAAT-2 expression by AMM (39). It is also in keeping with in vivo studies in rodents that showed that activated microglia express high affinity glutamate transporter following nerve axotomy (37) or cortical injury (38), and with in vitro demonstration of EAAT expression and function in cultured rat microglia (32–34, 36, 37) and human adult monocyte-derived macrophages (35). Only minimal astrocyte expression was found in our 3 HIV-negative controls, clearly different from that of AMM and of doubtful significance. It may just reflect background staining, but it could also reflect the low sensitivity of our technique, which may only detect high levels of EAAT-1 and not physiological expression. Indeed, different morphological studies (22, 43–45) have shown that normal expression of the glial glutamate transporters EAAT-1 and EAAT-2 is located in astrocyte processes.

In HIV-infected patients, reactive astrocytes were mostly EAAT-1-negative. This is consistent with previous in vitro demonstration that glutamate uptake by human astrocytes is impaired by HIV-1 infection, or after addition of gp 120 (27, 30, 31) or TNF-α (28). A rodent study showed that EAAT expression in microglial cells occurred following decreased EAAT expression by astrocytes, probably in response to a rise in glutamate concentration detectable in CSF (38). In addition, TNF-α, which down-modulates glutamate uptake by astrocytes (28), induces EAAT function in differentiating monocytes in vitro (35). One can postulate that in HIV infection, impairment of glutamate uptake by astrocytes due to viral protein and inflammatory factors (27–31, 46) may induce expression of EAAT by AMM. These cells could temporarily have a compensatory action, counteracting glutamate excitotoxicity by clearing extra cellular glutamate and oxidative stress by producing antioxidant glutathione (25, 35). Indeed, glutathione has been shown to protect neurons from HIV gp41 lytic peptide-induced neurotoxicity (47).

If AMM, as well as having neurotoxic properties, also exert a neuroprotective role, this could explain the puzzling absence of correlation at various stages of HIV infection between the degree of microglial activation and cognitive impairment or neuronal apoptosis. Microglial activation was proposed as a better correlate for cognitive impairment than viral multiplication in full-blown AIDS (48), but no clear correlation between microglial activation, neuronal damage, and cognitive disorders could be found at that stage (6). This discrepancy is particularly striking in pre-AIDS cases that show conspicuous microglial activation (49, 50) but have no obvious cognitive disorder, no neuronal loss (51), and little or no neuronal apoptosis (6, 7, 49, 52).

In HIV infection, EAAT-1 was almost exclusively expressed by AMM, although not all AMM expressed EAAT-1, and expression varied according to the stage of the disease and the region examined.

Perineuronal microglial cells did not express EAAT-1 in HIV demented cases with many apoptotic neurons, whereas they were the primary EAAT-1-expressing cells in AIDS cases without HIVE and in pre-AIDS cases without specific cognitive disorders and with few or no apoptotic neurons. This is similar to the situation in SIV-infected macaques without SIV encephalitis, in which numerous EAAT-positive microglial cells were observed in a perineuronal satellite position (39). Comparable findings were also reported in the rat controlled cortical impact injury model (38), where numerous perineuronal EAAT-positive microglial cells were only observed at a distance from the traumatized area. The authors proposed that, in the traumatized region, blood-borne infiltrating macrophages were EAAT-negative. On another hand, transformation of microglial cells into phagocytes around
apoptotic cells might also suppress EAAT expression. The latter hypothesis, if verified, would account for our finding of EAAT-negative perineuronal microglial cells in HIVE demented patients. Although our findings did not reach statistical significance, and a larger series will be necessary to confirm these preliminary findings, there was a clear negative association between HIV dementia and the expression of glutamate transporter by perineuronal microglial cells. This suggests that perineuronal microglial cells may play a neuroprotective role in the early stages of HIV infection, in asymptomatic HIV carriers, and in AIDS patients without HIVE. We did not find any either topographical or quantitative association between EAAT-1 expression by AMM and neuronal apoptosis. This does not exclude a possible neuroprotective role for AMM. The causes of neuronal apoptosis are multifactorial and vary according to site and stage of HIV disease (6), so correlation is difficult.

All patients with active HIVE had cognitive disorders; EAAT-1 expression predominated in microglial nodules and multinucleated giant cells in the white matter and basal ganglia, the same sites where p24, proinflammatory cytokines, and enzymes involved in oxidative stress (superoxide-dismutase and inducible nitric oxide synthetase) (6) were expressed. This joint expression suggests a possible balance between the expression of neurotoxic and neuroprotective factors.

We observed only 1 case with probable HIVE that regressed following HAART. Recent clinical reports have described improved neuropsychological performance in patients with AIDS-dementia receiving HAART (53, 54), but to date there are no supporting neuropathological studies. In our case, it seems likely that treatment was too late to prevent secondary lesions, including diffuse poliodystrophy with neuronal loss and leukoencephalopathy. The secondary changes were sufficient to explain the clinical deterioration and death. In this case, EAAT-1 expression was quite different to that in HIVE. There were no microglial nodules or multinucleated giant cells in the white matter and basal ganglia, and EAAT-1 expression only involved diffuse scattered microglial cells, analogous to HLA-DR and CD68. Interestingly, in the cerebral cortex, occasional perineuronal microglial cells expressed EAAT-1. One could speculate that in patients treated before neuronal death occurs, whose cognitive disorders improved, there would be associated re-expression of glutamate transporter by perineuronal microglial cells.

Our findings support the view that, in HIV infection, activated microglial cells play a dual role. Besides their classical neurotoxic properties involving glutamate-mediated excitotoxicity and oxidative stress, they have a counterbalancing neuroprotective role by clearing extracellular glutamate and producing antioxidant glutathione (25, 35). In the early stages of infection, EAAT-1 expression, mainly in perineuronal microglial cells, is probably sufficient to counteract the impairment of glutamate control by astrocytes brought out by HIV infection, and prevent the neuronal dysfunction underlying HIV dementia. In contrast, in terminal HIVE with HIV dementia, there is no longer expression of EAAT-1 in perineuronal microglial cells and glutamate increases in CSF and plasma (55). In these cases, EAAT-1 is strongly expressed by AMM in the deep white matter and basal ganglia, where productive HIV infection and microglial activation are prominent. The sites of expression are identical to those of HIV proteins, proinflammatory cytokines, and enzymes involved in oxidative stress (6), suggesting a possible balance between the expression of neurotoxic and neuroprotective factors. The resulting disordered glutamatergic system may participate in the neuronal dysfunction underlying HIV dementia (56). In patients successfully treated with HAART in whom productive HIV encephalitis has disappeared, our observations, in a single case, suggest that regression of the cognitive disorders might be associated with re-expression of EAAT-1 by perineuronal microglial cells.

Further experimental studies are necessary to determine the kinetics of EAAT expression following infection and its relationship to neuronal death. The role of EAAT expression in reversing HIV-related cognitive disorders will require the study of more human cases in whom HIV dementia regressed following HAART and who died from other causes.

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