Reduced Expression of Excitatory Amino Acid Transporter 2 and Diffuse Microglial Activation in the Cerebral Cortex in AIDS Cases With or Without HIV Encephalitis

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Abstract
To determine the relationship between the human immunodeficiency virus type 1 (HIV-1) encephalitis (HIVE) and diffuse polymyodystrophy in the acquired immunodeficiency syndrome dementia complex, we examined the neuropathologic features in brain autopsy tissue specimens of HIV-1−infected patients with (n = 11) or without HIVE (n = 9). The brains were free of opportunistic diseases and major cerebrovascular lesions. In both groups, there was diffuse microglial activation, astrocytic gliosis, and decreased excitatory amino acid transporter 2 (EAAT-2) immunoreactivity. These changes did not correlate either with the severity of encephalitis or local HIV-1 infection as detected by p24 immunostaining. Some activated microglia expressed EAAT-2; interleukin-1β and tumor necrosis factor were detected only in microglial nodules of HIVE cases but not in areas with diffusely activated microglia. There was a significant negative correlation between the areas of EAAT-2 expression and numbers of activated microglia (p < 0.01) in cases with decreased EAAT-2. These data indicate that diffuse cortical changes may occur independently of HIVE in acquired immunodeficiency syndrome patients. The expression of EAAT-2 by activated microglia suggests that they might exert a compensatory effect that protects neurons from glutamate neurotoxicity.

Key Words: Astrocytes, Cerebral cortex, Excitatory amino acid transporter2, HIV encephalopathy, Immunohistochemistry, Microglia, Neuroprotection.

INTRODUCTION
The acquired immunodeficiency syndrome (AIDS) dementia complex (ADC) is characterized by diffuse and nodular inflammatory infiltrates and multinucleated giant cells (MNGCs) with myelin pallor and axonal damage in brain white matter. Its pathological substrate is termed human immunodeficiency virus encephalitis (HIVE) (1–8), in which abundant HIV-infected macrophages and microglial cells can be demonstrated. However, poor correlations between the clinical manifestations of brain dysfunction and these pathological findings have been repeatedly noted (9, 10). On the other hand, neuronal damage, as indicated by atrophy of the cerebral cortex (7), neuronal loss (11–13), neuron apoptosis (14), and synaptic and dendritic simplification (15–17) in the cerebral cortex are additional histopathologic features of ADC that have been termed diffuse polymyodystrophy (DPD) (3, 7). The precise relationships between these histopathologic patterns (i.e., the inflammatory process in the white matter and degenerative process in the cortex) are unclear, in part, because of the complexity of histopathologic findings in human autopsy brains.

In the simian immunodeficiency virus (SIV)−infected macaque model, we previously demonstrated that an inflammatory process with virus-infected MNGCs in the white matter and degenerative changes of the cerebral cortex along with development of immunodeficiency can occur independently according to the cell tropism of inoculated viruses (18). Further analysis of the frontal cortex of macaques with advanced AIDS demonstrated astrocytic abnormalities such as apoptosis and decreased expression of excitatory amino acid transporter 2 (EAAT-2) and alternative expression of EAAT-2 by diffusely activated microglial cells; the latter finding suggests a neuroprotective role for activated microglia. Virus-infected cells and SIV encephalitis could not be found in and around such cortical lesions (19), suggesting that astrocytic degeneration and microglial activation might play a role in the pathogenesis of AIDS dementia independently of the development of HIVE.

To determine whether similar changes occur in HIV−1−infected brains, we examined autopsy specimens from HIV−1−infected individuals, particularly focusing on microglial activation, apoptosis, and EAAT-2 expression, as...
well as the localization of HIVE, using various immunohistochemical methods.

MATERIALS AND METHODS

Case Material
Among autopsy cases at the Institute of Neurology, Medical University of Vienna, since 1983, there have been 429 patients who died from HIV-1 infection. The records of these cases were screened, and those who had opportunistic infections or neoplasm in the brain and those with massive cerebrovascular lesions were excluded from this analysis. Twenty cases were selected (Table); 11 had HIVE and 9 had no apparent histopathologic changes in the brain. These patients had died between 1987 and 2002; only 5 patients had died before 1991, before highly active antiretroviral therapy became available. Brain sections of 2 autopsy cases from patients with amyotrophic lateral sclerosis were used as controls. These patients did not show any clinical and histopathologic brain abnormalities other than the pyramidal tract involvement. Paraffin sections of the frontal lobe and pons were processed for further histopathologic examination.

Histopathologic Examination
The histopathologic methods used in this study have been described elsewhere (18). We used various immunohistochemical stains for detecting activated microglia and astrocyte abnormalities and virus-infected cells. Brain tissue specimens were embedded in paraffin, sectioned, and mounted on glass slides. The EnVision system (DAKO, Carpinteria, CA) was used for the immunohistochemical staining, except for the guinea pig anti-gial glutamate transporter 1, EAAT-2 antibody, with which the avidin-biotin-peroxidase complex method (Vector, Burlingame, CA) was applied. Immunoreactivity was visualized using either diaminobenzidine peroxidase (brown) or the 3-amino-9-ethylcarbazole substrate-chromogen system (DAKO; red). Light counterstaining was done with hematoxylin.

Antibodies
To identify activated microglia, we used mouse monoclonal antibody to human macrophage CD68 (KP1, 1:50; DAKO, Glostrup, Denmark) and rabbit anti-ionized calcium-binding adaptor molecule-1 (Iba1) antibody (1:500; Wako Chemicals, Osaka, Japan). To characterize astrocyte abnormalities, we used guinea pig anti-gial glutamate transporter-1, EAAT-2 antibody (1:6000; Chemicon, Temecula, CA). For HIV-infected cells, we used mouse anti-HIV-1 p24 antibody (1:10, DakoCytomation, Kyoto, Japan).

Mouse anti-human Ki-67 antibody (1:300; DAKO), which detects cells in all active phases of the cell cycle (i.e. G1, S, G2, and M), was used to detect dividing cells; sections of tonsil were used as positive controls. Mouse anti-human tumor necrosis factor (TNF) antibody (1:400; Abcam, Cambridge, MA) was used for identifying microglia with activated morphology.

Paraffin sections of the frontal lobe and pons were processed for further histopathologic examination.

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<tr>
<th>Patient No.</th>
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ALS, amyotrophic lateral sclerosis control cases; EAAT-2, excitatory amino acid transporter 2; HIV, human immunodeficiency virus; HIVE, HIV encephalitis; No HIVE, HIV-1–positive cases without HIVE; nd, not determined.
Cambridge, MA) and rabbit polyclonal antibody to interleukin 1β (IL-1β, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) were used to detect the respective cytokines. A tonsil with chronic inflammation was used as a positive control. We also performed glial fibrillary acidic protein (GFAP), ubiquitin carboxyl-terminal esterase L1, and CD20 immunohistochemical staining for routine cell characterization.

**Double Label Immunohistochemistry**

To determine the phenotype of apoptotic cells, we performed double label immunohistochemistry for GFAP or CD68 using the EnVision system (DAKO) and for single-stranded DNA (ssDNA) using the avidin-biotin-alkaline phosphatase complex method (Vector); double labeling was performed with diaminobenzidine peroxidase followed by Vector blue alkaline phosphatase. To examine the phenotype of EAAT-2-positive cells, we performed double label immunohistochemistry for Iba1 and EAAT-2 using the same method.

To determine the phenotype of proliferating cells, double label immunohistochemistry was first performed for Iba1 or GFAP using the avidin-biotin-alkaline phosphatase complex method and then for Ki-67 using the EnVision system. Double labeling was performed using Vector blue alkaline phosphatase followed by the 3-amino-9-ethylcarbazole substrate-chromogen system.

**Apoptosis**

In situ terminal deoxynucleotidyl transferase 2′-deoxyuridine 5′-triphosphate–mediated nick end labeling (TUNEL) of fragmented DNA was done using an ApopTag in situ apoptosis detection kit (Chemicon). We also performed immunohistochemistry using affinity-purified polyclonal rabbit immunoglobulin G directed specifically against the active form of caspase 3 (1:1000; R&D Systems, Minneapolis, MN) and anti-ssDNA antibody (1:250; DakoCytomation) to identify apoptotic cells. Sections of tonsil were used as positive controls.

**Quantitative Analysis of EAAT-2 Expression and Iba1 Microglial Activation**

Five red-green-blue images of light microscopic pictures (original magnification: 100×) were randomly acquired from the second to the fifth layers of the frontal cortex stained with EAAT-2 using a Sony digital camera system. These red-green-blue images were converted to blue-subtracted images using Adobe Photoshop to obtain better contrast; EAAT-2-positive areas (pixels) were measured by the National Institutes of Health Image analysis program.

Iba1 antibody-positive cells were counted in 5 light microscopic fields (original magnification: 200×) of the second to the fifth cortical layers of the middle frontal gyrus. When more than 600 Iba1-positive cells were counted, this was considered evidence of an increase in the activated microglia. We also performed semiquantitative assessments for the immunohistochemical assessment of astrocytic gliosis, Ki-67 or CD68-positive cells, and TNF and IL-1β expression.
FIGURE 2. Increase and activation of microglia in the cerebral cortex and pons. (A-D) Anti-Iba1 immunostaining (original magnification: 200×). Patient 12 without human immunodeficiency virus 1 encephalitis (HIVE) shows diffuse microglial activation in the frontal cortex (A) and in the pons (B). On the other hand, Patient 7 with HIVE in the cortex (C) and pons (D) shows localized Iba1-positive cells in perivascular infiltrates but no diffuse microglial activation. (E) Ki-67-positive proliferating cells are randomly distributed in the frontal cortex of Patient 12 without HIVE (original magnification: 200×). (F) Ki-67-positive proliferating cells are localized in the microglial nodules of the frontal cortex of Patient 7 with HIVE (original magnification: 200×). (G) Double label immunohistochemistry with anti-Iba1 (blue) and anti–Ki-67 (red) of the frontal cortex of Patient 12 without HIVE. Proliferating cells were also Iba1-positive microglial cells (original magnification: 200×). (H) Double label immunohistochemistry of anti-glial fibrillary acidic protein ([GFAP] blue) and anti–Ki-67 of the frontal cortex of Patient 12 without HIVE. Proliferating cells were not GFAP-positive astrocytes (original magnification: 400×).
FIGURE 3. Apoptosis of glial cells and neurons in the frontal cortex. (A–D) Patient 10 with human immunodeficiency virus 1 encephalitis (HIVE). Scattered positive glial cells and neurons are demonstrated in the second layer ([A] original magnification: 400×) and the fifth layer ([B] original magnification: 400×) by ApopTag in situ. By anti–activated caspase 3 immunostaining, scattered positive cells are also detected in the second layer ([C] original magnification: 400×) and the fifth layer of the cortex ([D] original magnification: 800×). (E, F) Patient 16 without HIVE. Some positive glial cells are demonstrated in the second layer ([E] ApopTag in situ, original magnification: 400×) and the fifth layer ([F] anti–activated caspase 3, original magnification: 400×). (G, H) Patient 5 with HIVE. Scattered positive glial cells are demonstrated in the second layer ([G] anti–single-stranded DNA [ssDNA], original magnification: 200×), and some of ssDNA-positive cells were glial fibrillary acidic protein (GFAP) positive by double label with anti-GFAP (brown) and anti-ssDNA (blue) ([H] original magnification: 400×).
Statistical Analysis

Statistical significance was determined using commercial software (StatView for Windows; SAS Institute, Cary, NC). We analyzed the correlation between areas of EAAT-2 expression and numbers of Iba1-positive activated microglia in the frontal cortex.

RESULTS

Routine Histopathologic Examination

The pathological diagnosis of HIVE was suspected when either perivascular infiltration and MNGCs were present or it was present along with microglial nodules; the diagnosis was confirmed by positive HIV-1 p24 immunohistochemistry. Of the 20 brain tissue specimens from HIV-1-infected cases, 11 showed the features of HIVE including microglial nodules and perivascular infiltration with MNGCs in the frontal white matter (Fig. 1A) and pons (Fig. 1B). Microglial nodules were mainly composed of CD68-positive macrophages/microglia, and ubiquitin carboxyl-terminal esterase L1-positive T cells were scattered in the surrounding areas (not shown).

Immunohistochemical staining with anti-HIV-1 p24 antibody was positive in the macrophages/microglia and MNGCs of the inflammatory foci (Fig. 1B) and in a few perivascular macrophages of noninflamed areas in the white matter. Diffuse or focal astrocytic gliosis was detected in the frontal cortex of 5 cases with HIVE (Figs. 1C, E). In the 9 cases without HIVE or any other coexisting pathological lesion, we did not detect HIV-1–infected cells by HIV-1 p24 immunohistochemical staining; of these cases, diffuse or focal astrocytic gliosis was observed in the frontal cortex of 5 cases (Fig. 1D).

Activated Microglia in the Frontal Cortex and Pons

Iba1-positive microglial cells could be identified by their extended branches. Fewer than 300 immunopositive cells in 5 fields (original magnification: 200×) were counted in the 2 control amyotrophic lateral sclerosis cases. Of the 20 brain tissue specimens from the HIV-1–infected cases, 12 showed a diffuse increase of Iba1-positive microglia; that is, more than 600 positive cells in 5 fields in the frontal cortex (Fig. 2A). Of these 12 cases, 5 had HIVE (5/11, 45.5%), and 7 did not have HIVE (7/9, 77.8%); 4 of the 7 cases without HIVE also showed diffuse microglial activation in the pons (Fig. 2B). On the other hand, diffuse activation of microglia was not detected in the other 6 cases with HIVE or in 2 cases without HIVE; in these cases, Iba1-positive cells were restricted in their localization to the microglial nodules in the frontal cortex (Fig. 2C) and in perivascular infiltrates in the pons (Fig. 2D).

Ki-67-positive cells were randomly distributed in the frontal cortex in cases with diffuse microglial activation (Fig. 2E), whereas positively stained cells were localized in the microglial nodules in the cases with HIVE (Fig. 2F).
Some Iba1-positive microglia were also Ki-67 positive by double label immunohistochemistry (Fig. 2G). Ki-67-positive cells were not observed among the GFAP-positive astrocytes by double label immunohistochemistry with anti-GFAP and anti-Ki-67 antibodies (Fig. 2H).

Apoptosis of Glial Cells and Neurons in the Frontal Cortex in Cases With or Without HIVE

We used the in situ TUNEL method and immunohistochemical staining for the active form of caspase 3 and ssDNA to detect apoptosis. We found that 14 of the 20 HIV-1-infected cases, 10 with and 4 without HIVE, showed apoptosis of glial cells and/or neurons. In cases with HIVE, TUNEL-positive cells were mainly demonstrated in the second layer of the cortex and seemed to be both cell types (Fig. 3A). In the fifth layer of the cortex, positive labeling was also demonstrated in some large pyramidal neurons (Fig. 3B). In the activated caspase 3 staining, positive cells showed intracytoplasmic and nuclear labeling, and most of them seemed to be glial cells (Figs. 3C, D). Similar patterns of TUNEL-positive cells (Fig. 3E) and caspase 3 staining (Fig. 3F) were also observed in the cases without HIVE. The ssDNA-positive cells seemed to be small neuronal and glial cells (Fig. 3G). Some of the ssDNA-positive cells were also positive for GFAP by double label immunohistochemistry (Fig. 3H, arrows).

Reduction of EAAT-2 Expression in the Neuropil of the Frontal Cortex

The EAAT-2 was predominantly detected in the neuropil of the cerebral cortex; this is consistent with astrocytic expression of EAAT-2. The EAAT-2-positive areas were 70% to 76% (169,765–183,612 pixels) of the total area (240,975 pixels) in the control brain samples. In the HIV-1-infected cases, 8 cases showed more than 70% EAAT-2-positive areas (Fig. 4A). The other 12 cases showed less than 70% EAAT-2-positive areas; for example, 30% of the area was stained in Patient 15 (Fig. 4B), and 6% was stained in Patient 12 (Fig. 4C). Of the cases with reduced EAAT-2 staining, 7 had HIVE and 5 did not. In addition,
findings in the frontal cortex are summarized in the Table.

...Y in any of the 12 cases with diffuse microglial activation (Figs. 7E-G). Semiquantitative assessments of the histopathologic findings in the frontal cortex are summarized in the Table.

**Correlation Between Activation of Microglia and Reduction of EAAT-2 Expression by Astrocytes in the Cerebral Cortex**

In the 12 cases with decreased EAAT-2 expression, we assessed the correlation between the EAAT-2-positive areas and numbers of Iba1-positive microglia by Spearman rank correlation test and regression analysis (Fig. 5). A significant negative correlation was demonstrated between the areas of EAAT-2 expression and numbers of Iba1-positive microglia (p < 0.01, R = -0.598) among cases with decreased EAAT-2 expression.

There was strong immunopositivity for EAAT-2 in microglial cells, which was demonstrated in the double label immunohistochemical staining with anti-Iba1 and anti-EAAT-2 antibodies (Fig. 4D, arrows).

**IL-1β and TNF Immunostaining in Inflammatory Lesions but Not in Diffusely Activated Microglia**

Interleukin-1β was detected only in the cells of the inflammatory lesions in all 11 cases with HIV (Figs. 7A, C); TNF was also detected in the inflammatory lesions of 7 cases with HIV (Figs. 7B, D). In the cases without HIV, IL-1β and TNF were detected in very few perivascular cells. Diffusely activated microglia in the frontal cortex did not show immunopositivity for these proinflammatory cytokines in any of the 12 cases with diffuse microglial activation (Figs. 7E-G). Semiquantitative assessments of the histopathologic findings in the frontal cortex are summarized in the Table.

**DISCUSSION**

We previously demonstrated that inflammation and cortical damage occur independently according to viral tropism in an SIV-infected macaque model of AIDS dementia (18); animals infected with T-cell tropic SIV developed advanced AIDS but no inflammatory changes in the brains (19). There was, however, primary injury to the astrocytes, including apoptosis and decreased EAAT-2 expression in the neuropil, diffuse activation of microglia, and limited neuronal damage. An apparent decrease in the EAAT-2 expression was observed in the animals with prolonged SIV infection. Apoptosis of the astrocytes and decreased EAAT-2 expression in the neuropil suggested that astrocytes are primarily involved in the cortical degeneration of AIDS encephalopathy. Some activated microglia also expressed EAAT-2 but not TNF and IL-1β, and SIV-infected cells were not detected in or around cortical lesions. These results suggested that astrocytic abnormalities and compensatory activation of microglia might provide a protective effect against neuronal degeneration in SIV-infected macaques without SIV encephalitis (19).

The results obtained in the present study are very similar to those of the SIV-infected macaque model. We observed various histopathologic changes in the frontal cortex of both HIVE and non-HIV groups of HIV-1-infected patients, including diffuse microglial activation, apoptosis of astrocytes and neurons, and decreased EAAT-2 immunostaining. These cortical abnormalities were independent of the presence or absence of HIVE, as well as of its severity. It would be of great interest to identify a relationship between these pathological findings and the severity of the cognitive disorder in these cases, but this clinical information was not available. In this regard, an autopsy case of ADC has been reported in which prominent cortical atrophy and severe neuronal loss were observed, with minimal inflammatory changes in the white matter and basal ganglia (20). These observations suggest that HIVE and cortical degeneration may occur independently in HIV-1-infected individuals.

Petito and Roberts (21) reported evidence of apoptosis in neurons and astrocytes in HIV encephalitis and hypothesized that apoptosis of astrocytes may be a normal mechanism, whereby the brain removes excessive astrocytes that have proliferated after certain types of brain injury. In the present study, we examined proliferating cells in the frontal cortex by Ki-67 immunostaining and found that most of the positive cells were microglia rather than astrocytes by double label immunohistochemistry. This suggests that astrocytes may not proliferate in this condition, but astrocyte apoptosis supports the idea that they are primarily injured in HIV-infected individuals.

Another astrocytic change we observed was a remarkable decrease in the expression of EAAT-2 in the neuropil. Astrocytes are a major cellular component of the brain and have neuroprotective roles mediated by the expression of glutamate transporters (EAAT-1 and EAAT-2). Glutamate transporters maintain a low extracellular glutamate concentration in the brain and prevent excitotoxic neuronal cell death. Increase of extracellular glutamate is believed to be an important factor in the pathogenesis of many CNS disorders, including Huntington disease, Alzheimer disease, and multiple sclerosis (22–26). We believe, therefore, that in AIDS patients, injury to astrocytes results in a decrease of glutamate transporters in the cortex that enhances neuronal damage via excitotoxicity of glutamate.
To determine how the decrease of EAAT-2 may occur, Wang et al (27) demonstrated that HIV-1 or surface glycoprotein 120 induces transcriptional downmodulation of the EAAT-2 transporter gene in human astrocytes and attenuates glutamate transport by the cells in vitro. Although we did not find a correlation between the decrease of EAAT-2 and HIV-1, apoptosis seemed to be more frequent in cases with HIV-1. Many studies have reported that viral factors such as HIV-1/glycoprotein 120 and HIV transactivator (Tat) protein may induce neuronal damage and apoptosis through indirect and/or direct pathways in vitro as well as in vivo (28–34). Because high viral loads and increase of the soluble form of viral proteins in the blood as well as in the cerebrospinal fluid are common features in the late stage of AIDS, it might be possible that an increase in soluble viral antigens in the frontal cortex through the blood-brain barrier results in the astrocytic abnormality/injury, thus permitting subsequent neuronal damage. On the other hand, Boycott et al (35) recently reported that the decrease of EAAT-2 in cultured astrocytes is mediated by TNF in a hypoxic condition. Although TNF was not detected in astrocytes and diffusely activated microglia in the area showing decrease of EAAT-2 in our study, an increase of TNF in the blood has been reported in the late stage of AIDS, and this might explain the decrease of EAAT-2 expression in astrocytes in the present study.

We also observed diffuse activation of microglia in the cortex; the numbers of activated microglia correlated with a

![Images of microglia and cytokines](http://jnen.oxfordjournals.org/)

**FIGURE 7.** Interleukin-1β (IL-1β) and tumor necrosis factor (TNF) immunopositivity are restricted to the inflammatory lesions. (A, B) Frontal cortex white matter of Patient 6. Some IL-1β-positive cells are detected in the perivascular infiltration and multinucleated giant cells (MNGCs) ([A] original magnification: 100×). The TNF-positive cells are detected in some perivascular infiltrates and surrounding microglia ([B] original magnification: 200×). (C, D) Frontal cortex of Patient 7. The IL-1β-positive cells are detected in some MNGCs ([C] original magnification: 200×). The TNF-positive cells are detected in the perivascular infiltrate ([D] original magnification: 100×). (E–G) Frontal cortex of Patient 13. The IL-1β ([E] original magnification: 100×) and TNF ([F] original magnification: 100×) are not detected in an area with numerous diffuse Iba1-positive microglia ([G] anti-Iba1, original magnification: 40×).
decrease of EAAT-2. Some of the activated microglia were immunopositive for EAAT-2, but their expressions of TNF and IL-1β were not detected by immunohistochemistry. In general, microglial cells are distributed ubiquitously throughout the CNS and become activated in response to harmful stimuli (36). Activated microglia release proinflammatory cytokines such as IL-1β and TNF and thus have potential neurotoxic functions. In AIDS encephalopathy, HIV-infected macrophages and microglia may produce viral neurotoxins and neurotoxic cytokines that lead to neuronal dysfunction and death (37–40). Thus, activated microglia have been considered to be effectors of neuronal degeneration. On the other hand, activated microglia can also secrete some neurotrophic factors and serve neuroprotective functions (41). The expression of EAAT-2 by microglia has been reported in AIDS brains by others (41–42), and we confirm EAAT-2 expression by activated microglia in this study. These data suggest that similar to astrocytes, microglia might clear extracellular glutamate, thereby playing a neuroprotective role in AIDS brains.

Neuronal apoptosis is considered to be one of the major pathological changes in HIV infection (14, 43, 44). We observed apoptosis of some large pyramidal and small neurons in the frontal cortex of HIV-infected brains, whereas neuronal apoptosis was rarely noted in our previous study of SIV-infected animals. The difference might be explained by the difference in the stage of DPD. In human ADC, an autopsy is usually performed at the advanced stages of the disease, whereas the animals in our previous study were at a subclinical stage, and the findings observed might have been the early changes of DPD. We hypothesize that abnormalities of astrocytes and microglial activation might precede neuronal damage in early DPD and that when microglia fail to supply sufficient neuroprotection, neuronal damage becomes apparent later in DPD.

In summary, this is the first report of primary injuries to astrocytes (i.e. apoptosis and decreased EAAT-2 expression in the neuropil) in HIV-infected human autopsy brains. We also demonstrated an increase of activated microglia and apparently compensatory expression of EAAT-2 by microglia in the frontal cortex in these cases. These findings in the frontal cortex did not correlate with the severity of HIVE, which suggests that astrocytic degeneration and microglial activation may also play a role independent of HIVE in ADC.

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