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Simian immunodeficiency virus encephalitis in the white matter and degeneration of the cerebral cortex occur independently in simian immunodeficiency virus–infected monkey

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Highly active antiretroviral therapy (HAART) has been successful to reduce progression of acquired immunodeficiency syndrome (AIDS). Nevertheless, recent autopsy analysis of the brain from patients with human immunodeficiency virus (HIV)-1 infection reported same or even increasing numbers of AIDS encephalopathy. This insufficient effect of HAART for central nervous system (CNS) complication might be explained by independent pathogenetic processes in lymph node and CNS. We inoculated macaques with three Simian immunodeficiency virus (SIV) strains and investigated relationship between degree of the lymph node pathology and that of AIDS-related brain pathology. Animals infected with T-cell-tropic viruses SIVmac239 and SHIV-RT developed typical AIDS pathology in the lymph node 46 to 156 weeks after infection. The cerebral cortex of these animals showed focal or diffuse gliosis, and electron microscopic analysis demonstrated degenerative changes, such as accumulation of dense lamellar bodies in the dendrites and swelling of astrocytic processes. However, there was no evidence of microglial nodules or multinucleated giant cells in the white mater. The animals infected with macrophage-tropic SIV239env/MERT did not develop lymph node pathology of AIDS in the same or longer period of infection. The white mater of the animal, however, showed microglial nodules with multinucleated giant cells, a pathological hallmark of AIDS encephalopathy. SIV immunoreactivity was demonstrated in these giant cells as well as macrophage/microglia cells. On the other hand, there was no abnormality in the cerebral cortex. These findings suggest that there are two independent pathogenetic processes in AIDS encephalopathy: immune response against virus infected macrophage/microglial cells in the white mater without immunodeficiency and cortical degeneration caused in the late stage of AIDS. *Journal of NeuroVirology* (2003) **9,** 508–518.

Keywords: AIDS dementia complex; animal model; cerebral cortex; leukoencephalopathy; lymph node; neuropathology

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Introduction

With the use of highly active antiretroviral therapies (HAARTs), the number of patients with acquired immunodeficiency syndrome (AIDS) is reduced and human immunodeficiency virus type 1 (HIV-1)–infected individuals become asymptomatic for long time. HIV-1 infection is frequently associated with a specific neurological symptom known as AIDS dementia complex or AIDS encephalopathy, and its incidence is also presumed to reduce accordingly. Nevertheless, recent autopsy analysis of the brain from patients with HIV-1 infection reported same or even increasing numbers of HIV encephalitis, another name of neuropathological diagnosis for AIDS dementia complex (Dore *et al*, 1999; Masliah *et al*, 2000; Jellinger *et al*, 2000), and this has become an important subject to study.

HIV encephalitis is histopathologically characterized by diffuse and nodular microgliosis with formation of multinucleated giant cells (MNGCs) in the white mater of the brain (Budka *et al*, 1991; Dickson *et al*, 1993). Myelin pallor (Budka *et al*, 1987) and axonal damage (Raja *et al*, 1997; An *et al*, 1997; Giometto *et al*, 1997) with abundant HIV-infected macrophages and microglial cells were demonstrated in the white mater (Budka, 1991; Wiley *et al*, 1999). On the other hand, it has been repeatedly reported that there were a variety of neuronal damages, such as neuronal loss in the cortex or basal ganglia (Everall *et al*, 1991; Aylward *et al*, 1993; Gonzalez *et al*, 2000), apoptosis of neurons (Kaul *et al*, 2001), synaptic and dendritic simplification (Wiley *et al*, 1991; Masliah *et al*, 1992; 1997), and atrophy of the cerebral cortex (Budka, 1991). However, it is unclear how each of these findings contributes to the pathogenesis of HIV-1–associated brain damages.

To understand the pathogenesis and specificity of HIV-1–associated brain pathology, it would be important to know when these alterations begin and how they relate to each other, and the relationship between the degree of lymph node pathology of AIDS and the HIV-1–associated brain pathology. In order to analyze these points, we used a simian immunodeficiency virus (SIV)-macaque model because there are striking similarities between SIV-induced disease in macaque monkeys and HIV-1–induced disease in humans. We inoculated macaques with three different SIV strains and analyzed the relationship between characteristics and degrees of the CNS pathology and those of the lymph node pathology, and compared them between each groups of animals infected with different SIV strains.

Results

Clinical manifestation

Table 1 summarized clinical data, including viral RNA loads and CD4+ cell counts in the peripheral blood at the time of autopsy, from the seven SIV-infected rhesus macaques. Three macaques, 532, 627, and 682, were infected with T-cell-tropic virus SIVmac239. Macaque 627 showed rapid decreases of CD4+ cell counts and became morbid within 46 weeks after infection. Macaque 532 had prolonged clinical courses, showed vary high viral loads, and decreased CD4+ cell counts 133 weeks after infection, and became morbid and was diagnosed as AIDS because of very low CD4 + CD29high T cells (less than 1% of peripheral blood mononuclear cells [PBMCs]). Macaque 682 had prolonged clinical courses too, and showed vary high viral loads and decreased CD4+ cell counts. Because of self-bite behavior, it was sacrificed for autopsy 115 weeks after infection. Two macaques, 631 and 677, were infected with the T-cell-tropic virus SHIV-RT. They showed decreased CD4+ cell counts and diagnosed as AIDS at the time of autopsy. Macaque 631 developed B-cell lymphoma. Two macaques, 531 and 626, were infected with the macrophage-tropic virus SIV239env/MERT, and showed very slow progression of clinical course. CD4+ cell counts remained moderate decrease even long after infection. They were sacrificed for autopsy 154 and 218 weeks after infection, respectively. None of the infected animals showed apparent neurological manifestation, except self-bite behavior observed in macaque 682.

Table 1 Clinical data of macaques examined

Animal number	*Sex*	*Age at virus inoculation (years)*	*Duration of infection (weeks)*	*Viral inoculums*	*Viral RNA load in plasma at autopsy (copies/ml)*	*CD4+ cell count in PBMCs at autopsy (per μl)*	*Clinical information*
532	M	5	133	SIVmac239	214,300	380	Body weight loss and morbid
627	F	7	46	SIVmac239	25,000	90	Body weight loss and morbid
682	F	3	115	SIVmac239	480,000	140	Body weight loss and self-bite
531	M	4	154	239/envMERT	277,800	270	Poor appetite
626	M	3	218	239/envMERT	1000	220	Poor appetite
631	M	2	108	SHIV-RT	2500,000	200	Body weight loss and inactive, inguinal B cell lymphoma
677	M	2	156	SHIV-RT	6900	100	Body weight loss and morbid
630					Control		
671					Control		

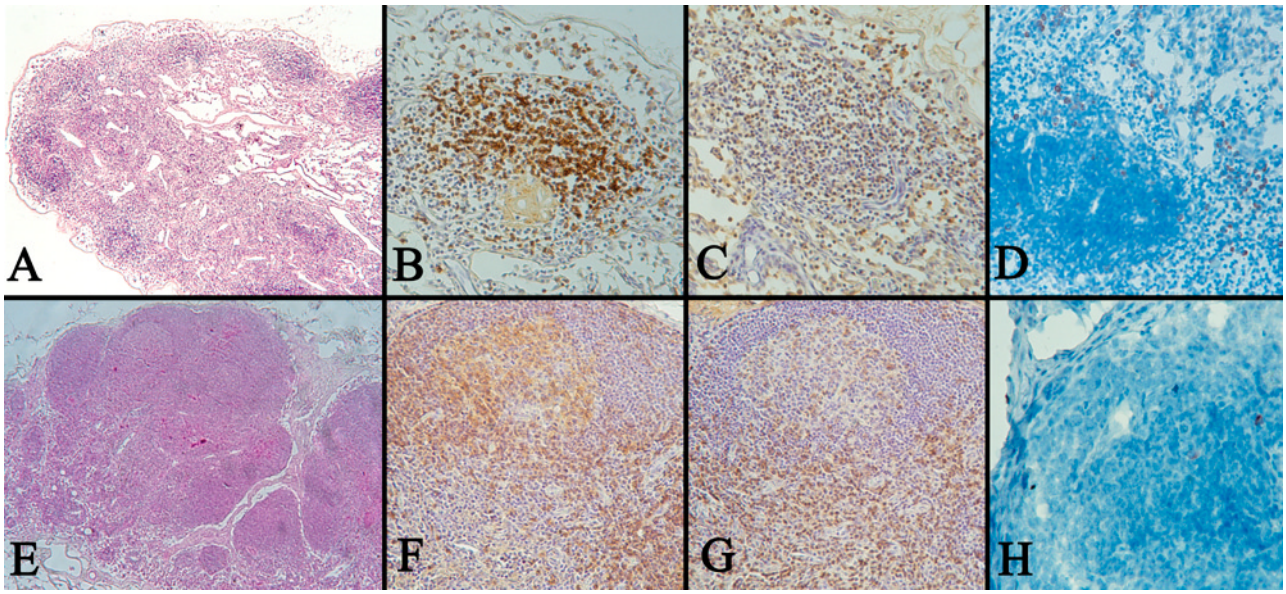

Figure 1 Lymph node pathology of SIVmac239-infected macaque 682 (**A**, **B**, **C**, **D**) and SIV239env/MERT-infected macaque 531 (**E**, **F**, **G**, **H**). Lymph node of macaque 682 showed collapse of follicles, marked decrease of T cells (**B**, **C**), and many virus-infected cells (**D**). Lymph node of macaque 531 showed hyperplasia of follicles (**E**, **F**, **G**) and a few virus-infected cells (**H**). (**A**, **E**) H&E stain; (**B**, **F**) anti-CD20; (**C**, **G**) anti-CD3; (**D**, **H**) anti-SIVenvgp160/gp32.

Lymphatic tissue pathology

All animals infected with SIVmac239 showed marked depletion of follicles with T-cell decrease in the spleen. Many lymph nodes became collapsed (Figure 1**A**, **B**, **C**, **D**). Lymphatic tissues of animals infected with SHIV-RT showed follicular atrophy and degeneration. Most of remaining germinal centers were disrupted. Moderate decrease of T cells was seen in the paracortex. The animals infected with the macrophage-tropic SIV239env/MERT showed hyperplasia of the lymph follicles, germinal centers of which showed irregular shapes (Figure 1**E**, **F**, **G**, **H**). Immunostaining by anti-SIVenv gp160/gp32 demonstrated many virus-infected cells, mainly in the paracortex of the lymph nodes of all SIVmac239- (Figure 1**D**) and SHIV-RT–infected animals. On the other hand, a few virus-infected cells were detected in the lymph nodes of SIV239env/MERT–infected animals (Figure 1**H**).

Brain pathology

Cerebral white matter: Macaque 531 infected with macrophage-tropic SIV239env/MERT showed some typical microglial nodules with MNGCs in the cerebral white matter (Figure 2**A**), a pathological hallmark of AIDS encephalopathy. Microglial nodules were mainly composed with CD68-positive (CD68+) macrophages/microglias (Figure 2**B**), and CD3-positive (CD3+) T cells were scattered in the surrounding areas (Figure 2**C**). Immunostaining by anti-SIVenvgp160/gp32 was positive in macrophages/microglias and MNGCs of microglial nodules (Figure 2**D**), and a few perivascular macrophages of noninflamed areas in the white mater. Astrocytic gliosis was not accentuated in the areas of microglia nodules. Another macaque infected with SIV239env/MERT, 626, showed scattered CD3+ cells and CD68+ cells in the perivascular regions of the white matter (Figure 2**E**, **F**) and the meninges. The viral burden assessed by anti-SIVenvgp160/gp32 immunoreactivity was demonstrated in a few perivascular macrophages in the white mater (Figure 2**G**, **H**). However, there was no microglial nodule with MNGCs.

On the other hand, in SIVmac239-infected macaques, 532, 627, and 682, as well as SHIV-RT–infected macaques, 631 and 677, there was no evidence of microglial nodules nor MNGCs in the white matter. CD3+ cells were rarely seen even in the perivascular areas and the meninges. A few CD68+ cells were noted around blood vessels, but a similar degree of CD68+ cells was also seen in the control animals.

Cerebral cortex: With routine histopathologic examination, apparent neuronal degeneration and loss were not detected in the cerebral cortex of all macaques examined. However, focal or diffuse gliosis of the neuropil was readily identified by the immunohistochemical staining for glial fibrillary acidic protein (GFAP) in the frontal cortex as well as hippocampus of macaques infected with SIVmac239 (Figure 3**A**, **C**, **D**). In order to determine the severity of neuropil changes, semiquantification of GFAP staining was performed in the frontal cortex of all macaques. Diffuse and the greatest fold increase

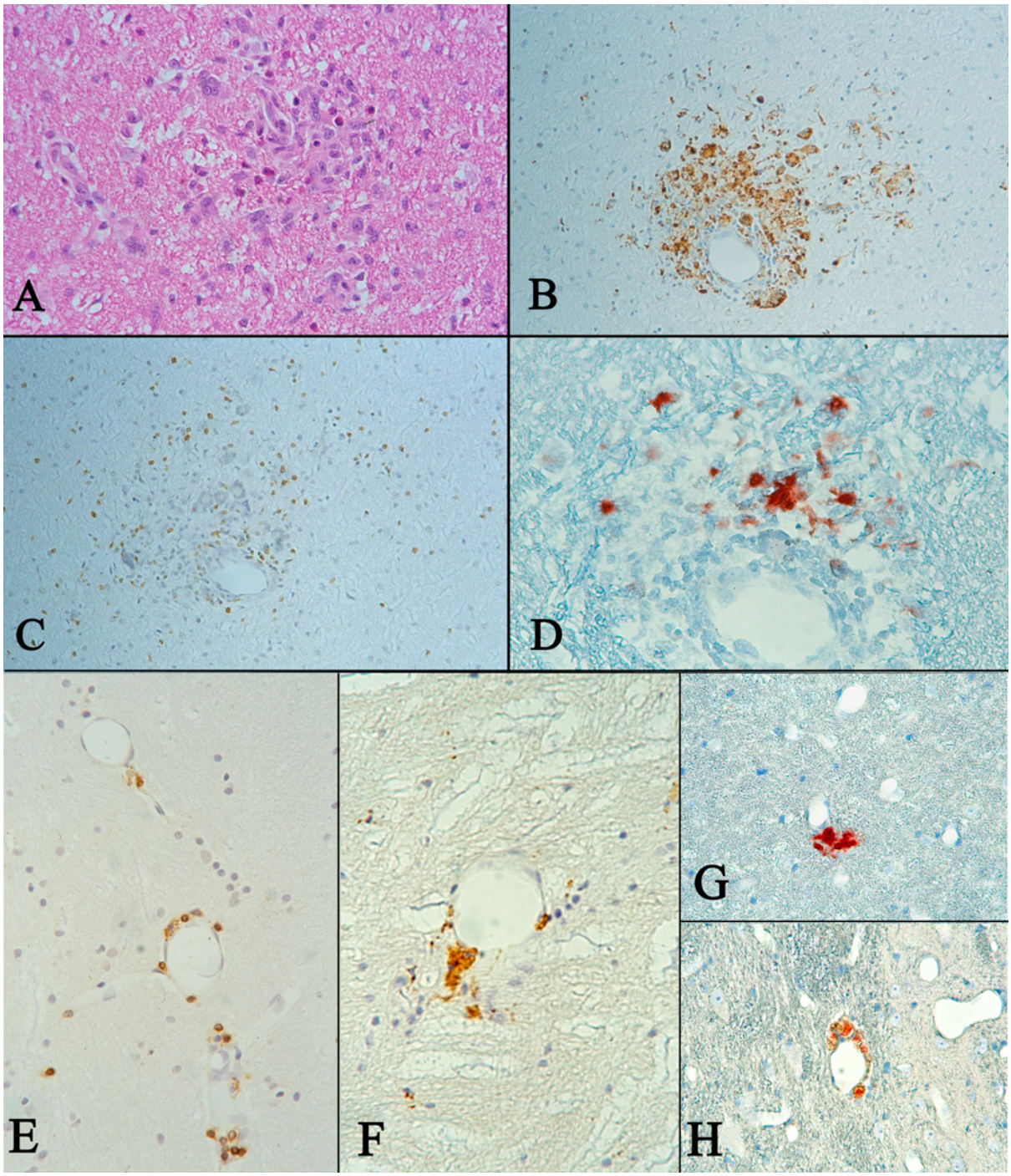

Figure 2 Brain pathology in the frontal white mater of SIV239env/MERT-infected macaques 531 (**A**, **B**, **C**, **D**) and 626 (**E**, **F**, **G**, **H**). A microglial nodule is mainly composed with CD68-positive macrophage/microglial cells and MNGCs (**A**, **B**), and T cells are diffusely infiltrated in and around the lesion (**C**). Expression of a SIV envelope protein is demonstrated in some MNGCs as well as macrophage/microglial cells (**D**). Another macaque 626 showed scattered CD3+ cells (**E**) and CD68+ cells (**F**) in the perivascular regions. Expression of a SIV envelope protein is demonstrated in a few perivascular macrophages (**G**, **H**). (**A**) H&E; (**B**, **F**) anti-CD68; (**C**, **E**) anti-CD3; (**D**, **G**, **H**) anti-SIVenvgp160/gp32.

in GFAP staining was observed in SIVmac239-infected macaque 682 (Figure 3**A**) and SHIV-RT–infected macaque 677. Focal staining of moderate degree was seen in SIVmac239-infected macaques 627 (Figure 3**C**) and 532, and SHIV-RT–infected macaque 631. Faint and focal staining was obtained in SIV239env/MERT-infected macaques 531 (Figure 3**B**) and 626. Noninfected controls showed only a few positive astrocytes in the frontal cortex. In all animals, there was no inflammatory cell

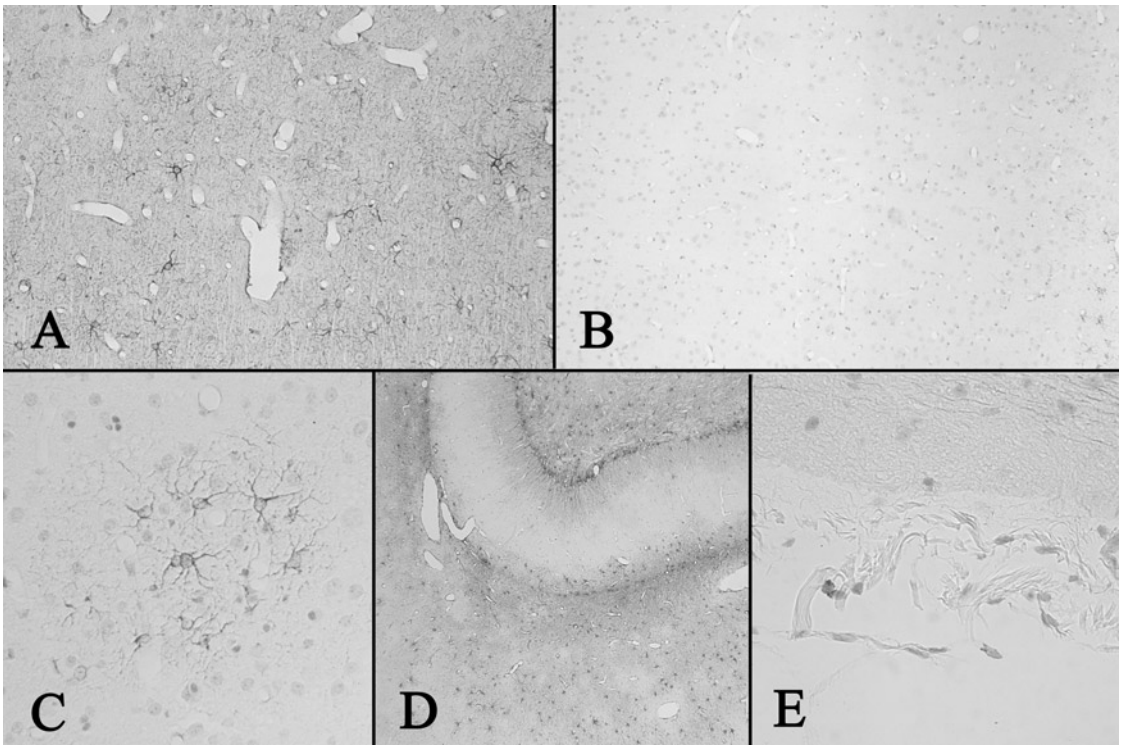

Figure 3 Pathologic changes in the cerebral cortex of SIV-infected macaque. Diffuse (**A**) or focal (**C**) astrocytic gliosis were observed in the frontal cortex of macaques infected with SIVmac239 (**A**, 682; **C**, 627). The hippocampus (**D**, 627) also showed gliosis. Macaque 531 infected with SIV239env/MERT showed no abnormity in the frontal cortex (**B**). A few SIV-infected cells are detected only in the meninges of the frontal cortex of macaque 682 infected with SIVmac239 (**E**). (**A**, **B**, **C**, **D**) Anti-GFAP; (**E**) anti-SIVenvgp160/gp32.

infiltration in the cerebral cortex. SIVenvgp160/32-positive SIV-infected cells could not be detected in the cerebral cortex. Only a few mononuclear cells were positive for anti-SIVenvgp160/32 immunostaining in the meninges of the macaque 682 infected with SIVmac239 (Figure 3**E**).

For better understanding of the nature of these neuropil gliosis, we performed immunohistochemistry using antibodies against various neuronal components, such as synaptophysin (SYN), a 38-kDa calcium-binding protein associated with membranes of neuronal presynaptic vesicles and involved in neurotransmitter release; microtubule associated protein (MAP-2), a protein in neuronal cell bodies and dendrites and involved in the polymerization of tubulin into microtubules (Bissel *et al*, 2002); and calbindin, a 28-kDa calcium-binding protein that function as an intraneuronal calcium buffer system, preventing toxic accumulation of cytosolic free calcium (Mattson *et al*, 1991; Masliah *et al*, 1995). We also performed electron microscopy for the neuropil changes.

Immunohistochemistry In the staining with anti-SYN and –MAP-2 antibodies, there was an uneven staining pattern in each section. However, we could not detect any difference in pattern or intensity of the immunostaining among animals infected with each virus as well as controls (Figure 4**A**, **B**). Such an uneven staining pattern did not correlate with focal or diffuse distribution of GFAP-positive astrocytes by the double-label immunohistochemistry (Figure 4**C**, **D**). In the staining with anti-calbindin, some small neurons of the second and third layers of the frontal cortex and a few of the fifth to sixth layers were positively stained and some of them showed positive staining of their dendritic processes in control animals (Figure 5**A**). Similar patterns but much weaker staining were observed in SIVmac239-infected macaques (Figure 5**B**). In SIV239env/MERT-infected macaques, macaque 626 showed similar numbers and patterns of positive neurons compared with control animals (Figure 5**C**), and only a few neurons could be stained in macaque 531.

Electron microscopy The frontal cortex of SIVmac239-infected macaques 627 and 682, in which focal or diffuse gliosis was noticed in GFAP immunostaining, showed apparent degenerative changes in the neuropil, such as accumulation of dense bodies (Figure 6**A**), deposition of glycogen-like granules, and increase of lamella bodies in the dendrites (Figure 6**B**). There were scattered swollen astrocytic processes, as an early reaction of astrocytes (Figure 6**C**) and some astrocytic processes were filled with dense bundles of glial fibrils (Figure 6**B**). Occasional dendrites had decreased synapse formation. Such a degenerative change could not be detected in the animals infected with macrophage-tropic SIV239env/MERT (Figure 6**D**). Table 2 summarized the pathologic changes observed

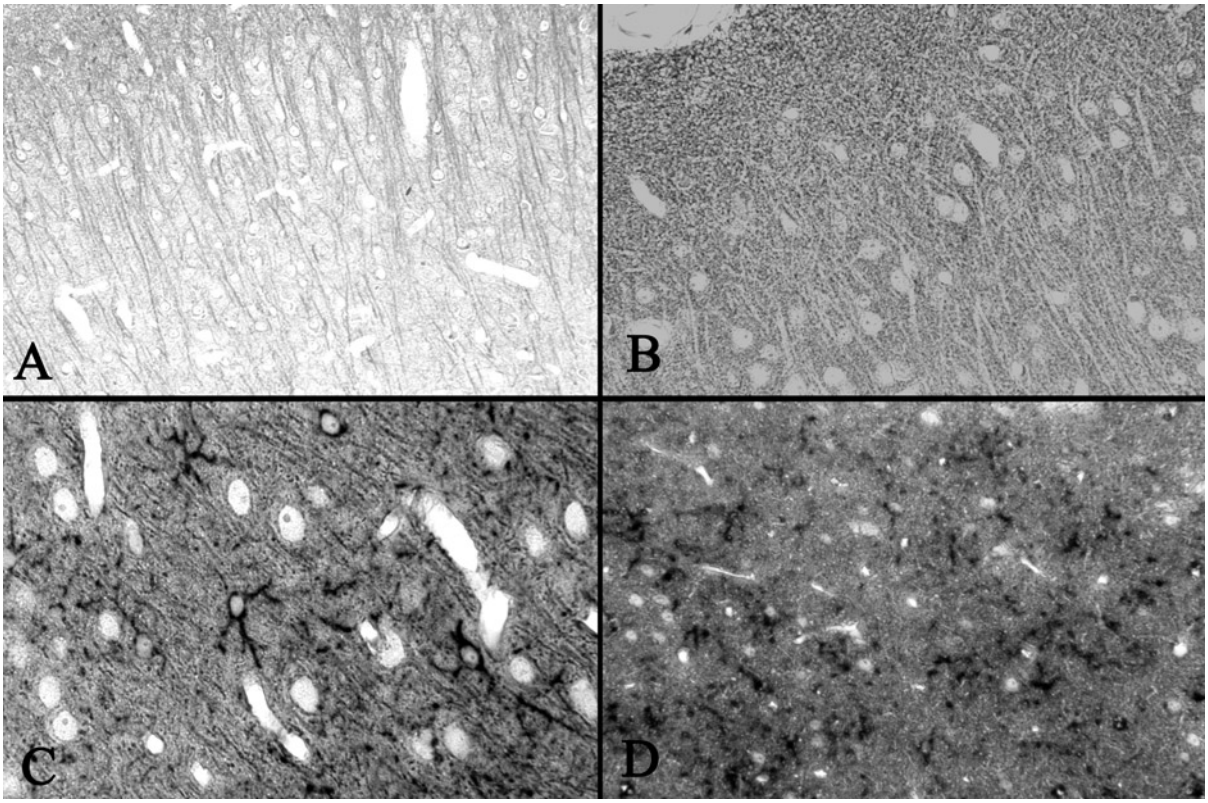

Figure 4 MAP-2 and SYN immunohistochemistry of the frontal cortex of macaque 682 infected with SIVmac239. Apparent decreases of MAP-2 (**A**, **C**) and SYN (**B**, **D**) are not observed even in the areas with gliosis. (**A**) Anti-MAP-2; (**B**) anti-SYN; (**C**) double-labeling of anti-GFAP and MAP-2; (**D**) double-labeling of anti-GFAP and SYN.

in the seven SIV-infected and two noninfected rhesus macaques.

Discussion

Microglial nodules with the MNGCs and diffuse myelin pallor in the white matter are well recognized to be a specific pathologic hallmark for AIDS dementia complex. On the other hand, a variety of neuronal damages have been repeatedly reported in the cerebral cortex. These two pathologic changes have been suggested to occur as different pathogenetic pathways for HIV damage to CNS tissue (Budka, 1991). However, the relative contribution of each of these processes to the pathogenesis of HIV-1–associated

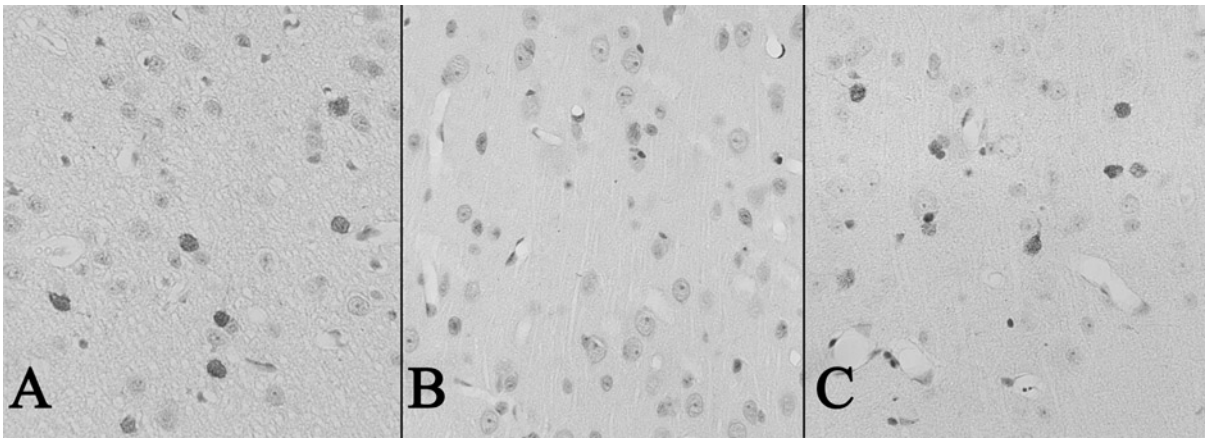

Figure 5 Calbindin immunohistochemistry of the frontal cortex of macaques. An uninfected control animal, 630, showed normal patterns of calbindin immunoreactive interneurons and pyramidal neurons (**A**), weaker staining was observed in SIVmac239-infected macaque 682 (**B**), and similar numbers and patterns of positive neurons were observed in SIV239env/MERT-infected macaque 626 (**C**).

Table 2 Pathologic changes from the SIV-infected and noninfected macaques

Animal number	*Sex*	*Viral inoculums*	*Lymph node pathology*	*Pathology of the cerebral white matter*	*Pathology of the frontal cortex*
532	M	SIVmac239	Collapses	None	Focal gliosis and neuropil degeneration
627	F	SIVmac239	Collapses	None	Focal gliosis and neuropil degeneration
682	F	SIVmac239	Collapses	None	Diffuse gliosis and neuropil degeneration
531	M	239/envMERT	Hyperplasia of follicles	Microglial nodules with $MNGC_S$	Faint and focal staining for GFAP
626	M	239/envMERT	Hyperplasia of follicles	Perivascular infiltration of T cells and macrophages	Faint and focal staining for GFAP
631	M	SHIV-RT	Follicular atrophy and degeneration	None	Focal gliosis and neuropil degeneration
677	M	SHIV-RT	Follicular atrophy and degeneration	None	Diffuse gliosis and neuropil degeneration
630		Control	None	None	A few astrocytes for GFAP
671		Control	None	None	A few astrocytes for GFAP

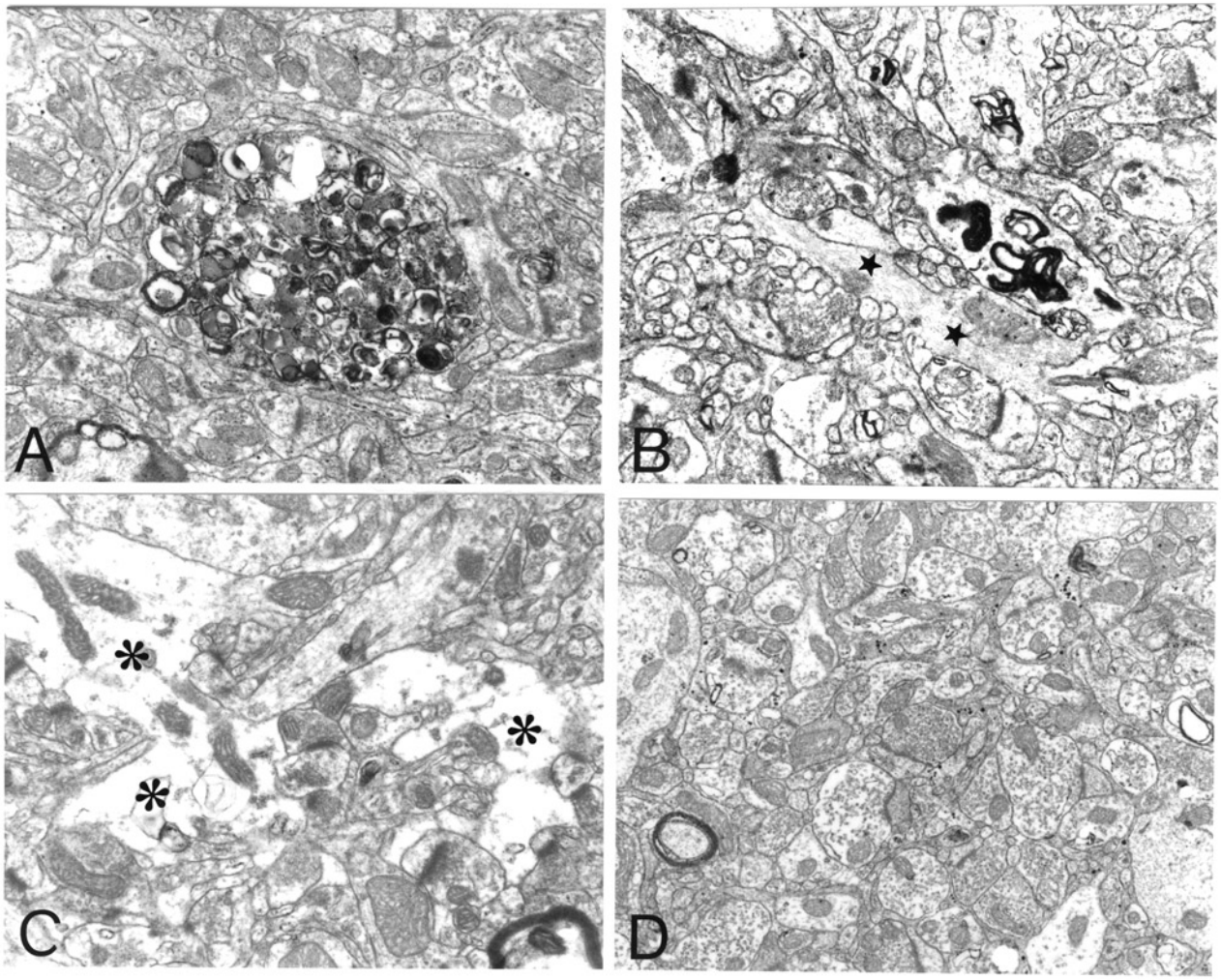

Figure 6 EM pictures of the frontal cortex of macaques 627 (**A**, **B**) and 682 (**C**) infected with SIVmac 239. Accumulation of dense bodies (**A**) and lamella bodies (**B**) are seen in the dendrites. Bundles of glial fibrils in the astrocytic processes (⋆) are frequently observed (**B**). Swollen astrocytic processes as an early reaction of astrocytes (∗) are seen (**C**). Macaque 626 infected with SIV239env/MERT does not show any degenerative change (**D**).

brain damages is unknown, and further, poor correlations between clinical manifestations and pathologic findings have been repeatedly pointed out. Glass *et al* (1993) reported that routine histopathologic examination of the brain failed to detect MNGCs and diffuse myelin pallor in 50% of patients dying with the clinical diagnosis of AIDS dementia complex. On the other hand, examination of the brain of asymptomatic HIV-1–positive individuals who died accidentally revealed HIV-1 infection and inflammatory response in the cerebral white matter (Gray *et al*, 1996).

The viruses isolated in the early phase of HIV-1 infection are predominantly macrophage-tropic and virus-harboring cells in the brain of patients with AIDS dementia complex are perivascular and parenchymal macrophages, MNGCs, as well as microglial cells (Pumarola-Sune *et al*, 1987). It seems reasonable that the viruses isolated from the brain of the patients with AIDS dementia complex are invariably macrophage-tropic in nature (Cheng-Mayer *et al*, 1989). On the other hand, AIDS dementia complex is frequently observed clinically in the late stage of AIDS and T-cell-tropic viruses are generally predominant in such advanced stages. Therefore such difference of virus tropism might contribute to the difference of pathologic processes for HIV damage to the CNS tissue.

Concerning the neurovirulence of SIV, Westmoreland *et al* (1998) performed a retrospective analysis of necropsies on 124 macaques with SIV-induced AIDS and reported that animals infected with uncloned SIVmac251 had a higher incidence of SIV encephalitis than animals infected with SIVmac239; however, the difference was not statistically significant. On the other hand, it was reported that the viruses found in the CNS lesions have been changed to macrophage-tropic mutants in the animals infected with SIVmac239 and having CNS involvements (Desrosiers *et al*, 1991; Zhu *et al*, 1995; Kodama *et al*, 1993). It has been also reported that macrophage-tropism alone was not sufficient for neurovirulence (Mori *et al*, 1992; Mankowski *et al*, 1997). These reports, however, were not concerned about neuronal changes of the cerebral cortex as observed in the present study.

In this study, we used SIV strains with different cell tropism and compared lymph node pathology and brain pathology. SIVmac239 is a cloned virus that causes AIDS (Kestler *et al*, 1990) and replicates very poorly in macrophages *in vitro* (Desrosiers *et al*, 1991). SHIV-RT is a T-cell-tropic chimeric virus that consists of a SIVmac239 virus backbone and the reverse transcriptase (RT) gene is replaced by the HIV-1 HxB2 RT gene (Uberla *et al*, 1995; Mori *et al*, 2000). This SHIV-RT has been shown to induce AIDS in experimentally infected rhesus macaques (Uberla *et al*, 1995; Ten Haaft *et al*, 1998). SIV239env/MERT is a virus with four amino acid substitutions in the *env* gene, V67M, K176E, G382R, and K573T, that are responsible primarily for high replication capability of the variant virus in macrophages (Mori *et al*, 1992; Dhar *et al*, 2000).

Macaques infected with SIV239env/MERT developed typical microglial nodules with MNGCs or mononuclear cell infiltration in the cerebral white matter. Immunohistochemistry for the viral antigen could demonstrate positive cells around blood vessels of noninflamed areas in addition to typical microglial nodules in these animals, though they were not clinically AIDS yet and the lymphatic tissue damage was not prominent. Similar findings have been reported by Mankowski *et al* (1997). These results suggest that microglial nodules with MNGCs in the cerebral white matter were formed independently with progression of AIDS. On the other hand, Willams *et al* (2001) reported that productive SIV infection of the brain was detectable only in early infection and in terminal AIDS. Further studies with a large number of macaques infected with SIV239env/MERT will answer the question whether SIVE can occur before development of AIDS. Contrary to SIV239env/MERT infection, macaques with SIVmac239 and SHIV-RT showed lymphatic tissue pathology of advanced AIDS, and in the brain, gliosis and neuropil degeneration were observed in the cerebral cortex. This cortical degeneration appeared to be parallel with the evolution of AIDS. However, microglial nodule with MNGCs in the cerebral white matter seen in macaques with SIV239env/MERT was hardly observed.

From these results of our SIV-infected animals, we can speculate that there are two independent pathogenetic processes in AIDS dementia complex. One is an immune response to infected macrophage/microglial cells in the cerebral white mater, which may occur from the early phase of HIV-1 infection, and the other is neuronal degeneration in the cerebral cortex, which is dependent on progression of immunodeficiency in the later stage of AIDS. These two independent pathogenetic processes might well explain the poor clinicopathologic correlation in human AIDS dementia complex above mentioned. Probably because routine histopathologic examination is not sensitive enough for neuronal damage in the cerebral cortex, and, from the clinical viewpoint, it might be true that neurological examination is not sensitive enough to detect small number of microglial nodules with MNGCs in the cerebral white matter.

Concerning the cortical damages, both presynaptic and postsynaptic damages have been reported during HIV encephalopathy (Everall *et al*, 1991; Wiley *et al*, 1991; Masliah *et al*, 1992, 1997). SYN is widely used to mark presynaptic terminals and to approximate synaptic density. Similarly, MAP-2 is widely used to mark postsynaptic elements. Decreases of these proteins in the cortex have been reported in the patients with AIDS dementia complex. Secreted products of activated macrophages have been suggested to directly act on neurons or indirectly act

on supporting glial cells, initiating synaptic damage and neuronal death. In our study, however, the immunostaining with the MAP-2 and SYN were not different among infected animals as well as noninfected controls. The distribution of focal or diffuse gliosis of the neuropil did not show any correlation with pattern of immunostaining with the MAP-2 and SYN. It might be possible that the degree of neuropil degeneration in our animals was not severe enough to detect by immunostaining with MAP-2 and SYN. Instead, we could demonstrate apparent neuropil degeneration in the frontal cortex and hippocampus of the macaques infected with SIVmac239 using electron microscopy, where varying degrees of astrocytic gliosis were observed. Czub *et al* (2001) reported vacuolization of dendritic processes as an ultrastructural finding of SIV encephalopathy during early SIV infection treated with the dopaminergic drug selegiline, and such vacuolar changes were not observed in SIV-infected/selegiline-untreated animals. Similarly, we could not detect vacuolization of the dendrite, though we demonstrated mild degenerative changes in the dendrite.

Calbindin is a neuronal marker that is specific to gamma-amino butyric acidergic (GABAergic) neurons (Mattson *et al*, 1991; Masliah *et al*, 1995), and loss of calbindin staining has been reported to indicate neuronal death or cellular injury in HIV-1–infected individuals (Gonzalez *et al*, 2000). In our study, decrease of calbindin-immunoreactive neurons was observed in the frontal cortex of SIVmac239-infected animals. On the other hand, calbindin-immunoreactive neurons were rarely observed in the frontal cortex of a SIV239env/MERT-infected animal with typical microglial nodules and another SIV239env/MERT-infected animal without microglial nodules showed normal staining patterns as seen in controls. Decreased calbindin-immunoreactivity might indicate mild loss of interneurons in our SIVmac239-infected animals; however, marked decrease of calbindin-immunoreactivity seen in one of SIV239env/MERT-infected animals is unlikely to be due to loss of neurons because astrocytic gliosis, which in principal follows neuronal loss, was not observed in the cortex of this animal. Although a correlation between lesions of SIV encephalitis and diminished calbindin-immunoreactivity might be suggested, further studies using more animals are required to clarify this point.

Masliah *et al* (2000) analyzed 390 AIDS autopsy cases obtained from 1982 to 1998. Despite the beneficial effects of HAART, involvement of the brain by HIV continued to be the second most frequently autopsy finding, and HIV encephalitis continued to be detected in at least 25% of the cases. Another autopsy series analyzed by Jellinger *et al* (2000) reported that despite introduction of HAART, frequency of HIV-associated CNS lesions did not change and, interestingly among the HIV-associated CNS lesions, HIV encephalitis was increased and diffuse glial poliodystrophy was decreased after era of HAART. These observations indicate that HAART is not effective to reduce HIV-related CNS pathology, especially for HIV encephalitis and leukoencephalopathy. On the other hand, diffuse glial poliodystrophy and degeneration of the cerebral cortex, could be controlled by HAART. If degeneration of the cerebral cortex occurs in late stage of AIDS, the findings observed in these autopsy analysis support our proposal that two different pathogenetic processes may exist in AIDS dementia complex. If such a speculation is true, it is suggested that the clinical features of AIDS dementia complex will change from subacute progressive encephalopathy in the terminal stage of HIV-1 infection to slowly progressive neurological deficits occurring without immune deficiency under HAART, and development of treatments targeting macrophage-infected viruses in the brain at pre-AIDS stage is urgently required to control AIDS dementia complex under HAART. Our macaque model infected with SIV239env/MERT may be useful for the development of the therapy as well as analysis of its pathogenesis of henceforth emerging AIDS dementia complex.

Materials and methods

Virus

Molecularly cloned SIVmac239 is T-lymphocyte-tropic whose pathogenic properties have been previously described. This virus causes immunosuppression and develops AIDS in macaques. SIV239env/MERT comprises four amino acid substitutions in the *env* of SIVmac239 backbone, and replicates as efficiently as the highly macrophage-tropic virus SIVmac316 in the alveolar macrophages (Mori *et al*, 1992). A chimeric virus SHIV-RT consists of a SIVmac239 virus backbone in which the SIV RT gene was replaced by the HIV-1 HxB2 RT gene as previously described (Uberla *et al*, 1995). This SHIV-RT has been shown to induce AIDS in experimentally infected rhesus monkeys (Uberla *et al*, 1995; Ten Haaft *et al*, 1998).

Animals and inoculation of viruses

Nine rhesus monkeys were screened and found to be seronegative for SIV, STLV, B virus, and type D retroviruses. Three macaques, 532, 627, and 682, were infected intravenously with 100 TCID50 of SIVmac239; two macaques, 531 and 626, with SIV 239/envMERT; and the other two, 631 and 677, with SHIV-RT. Another two uninfected macaques, 630 and 671, were used as controls (Table 1). Each animal were housed in individual cages and maintained according to the rules and guidelines of the National Institute for Infectious Diseases (NIID) for experimental animal welfare. These animals were sacrificed at a range of times after infection when they became morbid as shown in Table 1.

CD4+ cell counts and viral RNA loads
To measure the level of virus replication in the periphery, viral RNA was quantified in plasma at autopsy. Viral RNA in the plasma of inoculated macaques was measured by real-time reverse transcriptase–polymerase chain reaction (RT-PCR). And CD4+ cell counts were performed in peripheral blood at the time of autopsy.

Autopsy and histopathological examination
Under deep anesthesia, these animals were perfused with heparinized physiological saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Visceral organs, including lymphatic tissues such as spleen and lymph nodes, and serial coronal sections of the brain were embedded in paraffin. For routine light microscopy, the paraffin sections were stained with hematoxylin and eosin (H&E), and Kluever-Barrera (K-B) stain for the brain. Small pieces of the frontal cortex and hippocampus from animals 531, 626, 627, 682, and 630 were postfixed in 1% osmium tetroxide, and embedded in epoxy resin. One-micron semithin sections of epon-embedded samples were stained with toluidine blue and safranine. For electron microscopy, sections were stained with uranium acetate and lead citrate and examined by a Hitachi H-7000 electron microscope.

Immunohistochemistry
We used EnVision system (DAKO) for immunohistochemistry. The following antibodies were used as the first antibodies: monoclonal antibody (mAb) anti-GFAP (1:100; Chemicon International), rabbit anti-GFAP (1:1; DAKO), rabbit anti-human CD3 (1:50; DAKO), mAb mouse anti-human macrophage CD68 (KP1; 1:50; DAKO), mAb mouse anti-human B cell CD20 (1:100; DAKO), mAb mouse anti–microtubule-associated protein (MAP-2) (1:500; Sigma), mAb mouse synaptophysin (1:500; Boehringer Mannheim Biochemica), mAb mouse anti–calbindin-D-28K (1:1000; Sigma), and mAb mouse anti–SIV envelope gp160/gp32, KK41 (1:50; NIBSC). Paraffin sections were deparraffinized, hydrated using decreasing concentration of ethanol, and washed in Tris-buffered saline (TBS). For blocking of endogenous peroxidase, the sections were incubated in 3% H_2O_2/methanol for 10 min. Sections were incubated with each antibody for overnight at 4°C. EnVision HRP anti-mouse or anti-rabbit was applied for 60 min at room temperature. Immunoreactivity was visualized using either diaminobenzidine/peroxidase or AEC substrate–chromogen system (DAKO). Light counterstaining was done with hematoxylin.

Double-label immunohistochemistry
We performed double-label immunohistochemistry for GFAP and either SYN or MAP-2. After paraffin sections were deparraffinized and incubated in 3% H_2O_2/methanol, and treated with rabbit anti-GFAP (DAKO) for 10 min. EnVision alkaline phosphatase was applied for 60 min at room temperature, immunoreactivity was visualized using NBT/BCIP. After washing in TBS, sections were treated with anti–MAP-2 or SYN antibodies for overnight at 4°C. EnVision HRP anti-mouse was then applied for 60 min at room temperature. Immunoreactivity was visualized using AEC substrate-chromogen system (DAKO).

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