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HIV infection of choroid plexus in AIDS and asymptomatic HIV-infected patients suggests that the choroid plexus may be a reservoir of productive infection

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The choroid plexus (CPx) may be an important site of viral dissemination since monocytes and dendritic cells in its stroma are infected with HIV in AIDS patients and since the ratio of CPx to brain infection is more than 2 : 1. In order to see if CPx infection also develops in asymptomatic (ASY) HIV-infected patients, we examined archival formalin-fixed brain and CPx from 14 AIDS and seven ASY cases, using routine histology, immunohistochemistry for HIV gp41, and DNA extraction and gene amplification for HIV DNA. Eight of 14 AIDS (57%) had HIV-positive cells in the CPx and four (29%) had HIV encephalitis. Two of seven ASY cases (29%) had HIV-positive cells in the CPx but none had HIV encephalitis. Extracted DNA from brain, CPx and systemic organs of five ASY cases was amplified by nested PCR with or without Southern blotting for HIV *env* gene. It was positive in systemic organs in five cases; in CPx in four cases; and in brain in one case. This study shows that the CPx is a site of HIV infection in ASY patients and that the frequency of CPx infection is higher than seen in brain in both AIDS and ASY cases. The results are consistent with the hypothesis that the CPx may be a site for hematogeneous spread and a reservoir for HIV infection during the period of clinical latency.

Keywords: viral reservoir; brain; immunohistochemistry; PCR

Introduction

Human immunodeficiency virus (HIV) is found in the central nervous system (CNS) at all stages of infection. Approximately one quarter of HIV-infected asymptomatic patients (ASY) have HIV in their CSF (Ho *et al*, 1985; Goudsmit *et al*, 1986; Resnick *et al*, 1988; Chiodi *et al*, 1992; McArthur *et al*, 1998) and this incidence increases over time. During this period, mild brain atrophy and a T cell lymphocytic meningitis may occur (Gray *et al*, 1992; Bell *et al*, 1993; Kibayshi *et al*, 1996; An and Scarivelli, 1997). HIV DNA sequences have been amplified from brains of ASY patients (Sinclair *et al*, 1994; DiStefaro *et al*, 1996; An and Scarivelli, 1997) but HIV encephalitis (HIVE) is limited to patients with AIDS. The autopsy incidence of HIVE may be as high as 30% in the era prior to antiretroviral therapy (Petito *et al*, 1986; Sharer, 1992) and its associated brain atrophy increases with the duration of AIDS (Post *et al*, 1986; Stout *et al*, 1998).

Parallel studies with simian immunodeficiency virus (SIV) reveal a similar relationship between viral entry in the CSF and productive brain infection. Between one and 2 weeks after systemic infection, a time at which plasma viral load is high and anti-SIV antibodies have not yet developed, CNS inflammation and viral DNA are common (Smith *et al*, 1995; Tracey *et al*, 1997). Brain inflammation and viral load decrease in tandem with the production of anti-SIV antibodies and reduction in plasma virus. Like HIVE, SIV encephalitis is not seen until the later stages of infection and requires systemic or cerebral inoculation of macrotropic viral strains (Sharma *et al*, 1992).

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We previously showed that the choroid plexus (CPx) contains HIV-infected cells in approximately half of all AIDS cases studied (Falangola *et al*, 1995). Indeed, the frequency of CPx infection (52%) was higher than the frequency of brain infection (20%) in the same patients. The infected cell type is controversial. Harouse *et al* (1989) suggests that the infected cell is a CPx fibroblast since it is the cell type capable of infection in tissue culture. In contrast, Bagasra *et al* (1996) suggest that the CPx epithelial cells are infected since they found HIV DNA and RNA in epithelial cells in three of three AIDS autopsies. The methodologies used for *in situ* PCR in that study were quite sensitive since DNA was present in neurons, astrocytes, oligodendrocytes and endothelial cells in 50% or more of their AIDS cases. Our own studies indicate that monocytes and dendritic cells in the CPx stroma and CPx epiplexus are the infected cell on the basis of their immunoreactivity for viral proteins *in vivo* (Falangola *et al*, 1995, Hanly and Petito, 1998). Preliminary studies suggest that HIV viral sequences in the CPx are admixtures of brain and blood sequences (Petito *et al*, 1998). In two cases from which we isolated virus from CPx, replication and p24 antigen production was greater in macrophage/monocyte culture than in lymphocyte cultures (unpublished data).

We hypothesized that the CPx may be pivotal in the neuropathogenesis of HIVE by providing a site for hematogeneous dissemination of systemic virus and by providing a reservoir for HIV infection in ASY patients or in patients on anti-retroviral infection. We reasoned that identification of CPx HIV infection in ASY patients would support this hypothesis since HIVE is absent in these patients. Accordingly, we compared brain and CPx pathology in AIDS and ASY patients with specific attention directed toward the presence or absence of HIV infection. Formalin-fixed material was available from 14 AIDS patients and seven HIV-infected ASY cases which were part of a prior study comparing brain pathology in these two populations (Kibayashi *et al*, 1996). Routine immunohistochemistry and nested polymerase chain reaction (PCR) identified HIV protein and DNA sequences. A preliminary report has been published in abstract form (Petito *et al*, 1999).

Results

Table 1 summarizes the results. The average age of the 14 AIDS patients was 39.1 ± 9.9 years. All but one were male. Four (29%) had HIVE and HIV-immunoreactive cells in brain (Figure 1A). Three of these had HIV-infected cells in the CPx. Two additional cases (Nos 5 and 6) had scattered HIV-immunoreactive cells in basal ganglia without multinucleated cells; one of these had CPx HIV infection. Two AIDS cases had minute foci of organized or organizing necrosis, the etiology of which was undetermined; one had calcific vasculopathy and six had normal brains.

Table 1 HIV in brain and choroid plexus of AIDS and asymptomatic patients

Case No.	*Age*	*Sex*	*Clinical*	*HIVE*	*gp41B Brain*	*gp41 CPx*	*PCR SYS*	*PCR CPx*	*PCR Brain*	*Microscopic, other*
1	44	M	AIDS	X	X	X				o
2	31	F	AIDS	X	X	X				CMV ventriculitis
3	31	M	AIDS	X	X	X				Chronic meningitis, CaV
4	27	M	AIDS	X	X	0				Focal necrosis
5	35	M	AIDS	0	X	X				Focal scars, rare MGN
6	35	M	AIDS	0	X	0				o
7	35	M	AIDS	0	0	X				o
8	38	M	AIDS	0	0	X				o
9	47	M	AIDS	0	0	X				o
10	34	M	AIDS	0	0	X				Minute foci necrosis
11	48	M	AIDS	0	0	0				o
12	32	M	AIDS	0	0	0				Focal scars, Ca1 gliosis
13	45	M	AIDS	0	0	0				CaV
14	65	M	AIDS	0	0	0				o
15	38	M	ASY	0	0	X	X	X	0	o
16	30	F	ASY	0	0	X	X	X[a]	0	Chronic meningitis
17	47	M	ASY	0	0	0	X	X	0	Focal scars
18	30	M	ASY	0	0	0	X	X[a]	X[a]	o
19	32	M	ASY	0	0	0	X[a]	0	0	o
20	41	M	ASY	0	0	0				Autolysis
21	43	M	ASY	0	0	0				CPx infl

Abbreviations: gp41, HIV gp41 immunoreactivity; Cpx, choroid plexus; PCR, polymerase chain reaction for HIV *env* sequences; SYS, systemic organs; CaV, calcific vasculopathy; HIVE, HIV encephalitis; CMV, cytomegalovirus; MGN, microglial nodule; inf, inflammation. [a]Detected only by Southern blots of amplified PCR products.

Eight of the 14 AIDS cases (57%) (Nos 1–3, 5, 7–10) had HIV-immunoreactive cells in the CPx. Their distribution and appearance was similar to our prior reports (Falangola *et al*, 1995, Hanly and Petito, 1998). In six of the eight cases, the infected cells were located in the CPx stroma and had a dendritic cell-like appearance with cytoplasmic processes (Figure 1B) or monocyte-like appearance with round or oval-shaped perikarya (Figure 1C). They were distributed as single cells or in small clusters

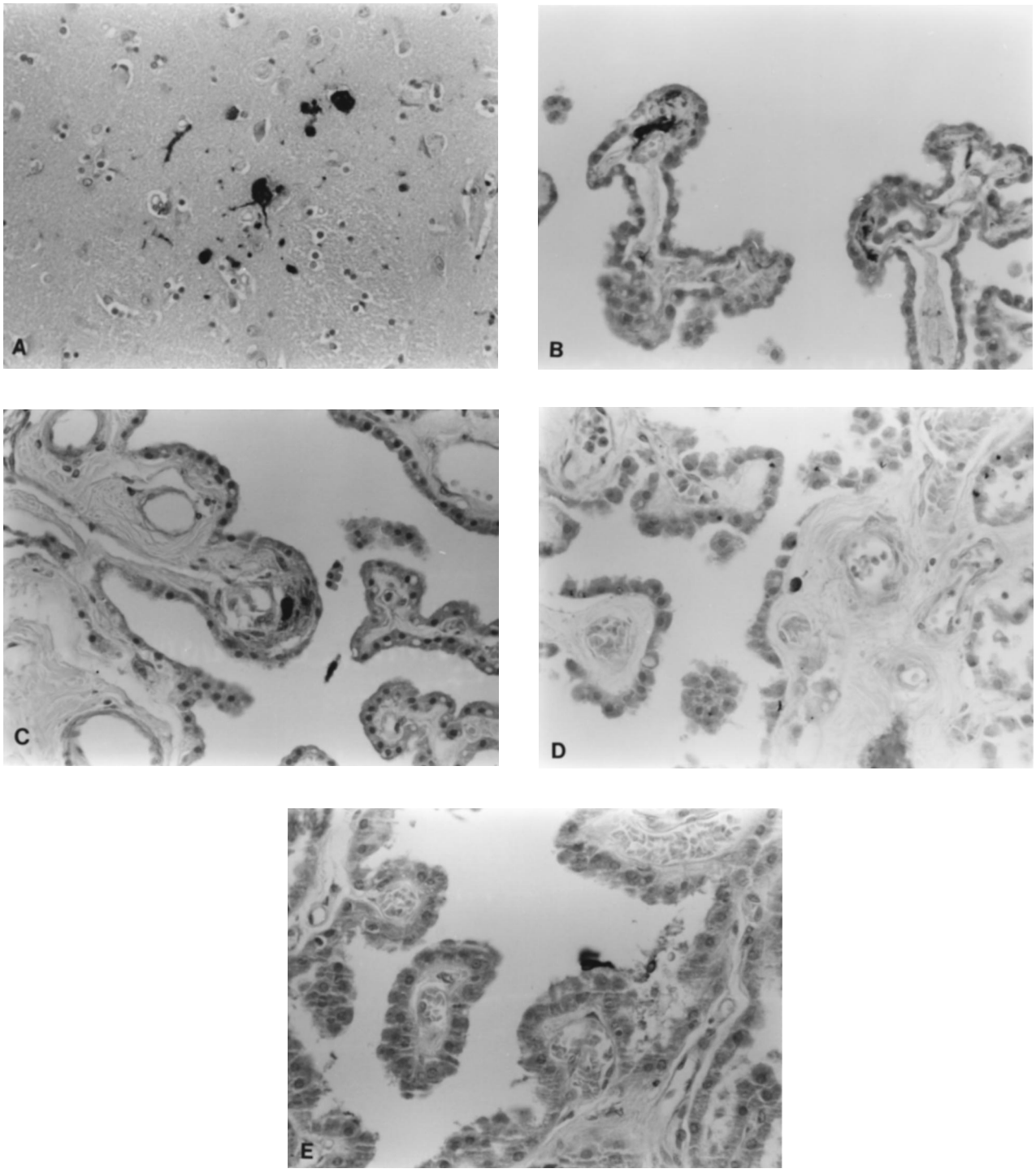

Figure 1 (**A–C**) Brain and choroid plexus (CPx) of AIDS cases: (**A**) HIV gp41 immunoreactivity is positive in brain microglial nodule (No. 2). (**B**) Dendritic-like cells in CPx stroma (No. 10). (**C**) Monocyte-like cells in CPx stroma (No. 2). (**D–E**) CPx of asymptomatic (ASY) cases. (**D**) HIV gp41 immunoreactivity in ASY CPx is positive in monocyte-like cell in CPx stroma (No. 15). (**E**) Dendritic-like cell on epithelial surface (No. 16). HIV gp41 immunohistochemistry, hematoxylin original magnification ×400.

of cells. The presence of nuclei in most HIV immunoreactive deposits identified these as intracellular virus although it was not possible to distinguish between intracellular versus extracellular virus in those deposits in which nuclei could not be identified. One of the six also had HIV-positive epiplexus cells. In two cases, HIV-immunoreactive cells were only seen on the epiplexus surface of the CPx epithelium. The infected cells ranged from 9 to >20 per slide and averaged 8.8 ± 7.9 per cm^2 CPx.

Brain pathology in the eight cases with HIV-infected CPx included HIVE in two, HIVE plus cytomegalovirus (CMV) encephalitis in one and HIV-immunoreactive cells in a fourth. Four of the eight had no evidence of HIV brain infection. One of these had minute foci of necrosis and three had normal brains.

The average age of the seven ASY patients was 38.5 ± 6.8 years. All but one were male. None of the ASY cases had HIVE in the prior neuropathology evaluation (Kibayashi *et al*, 1996) or in the five additional sections submitted for the present study. None of the brain sections contained HIV-immunoreactive cells in the brain. Brain pathology in the seven ASY patients included one case each with focal cortical scars; chronic meningitis; and chronic inflammation of the CPx.

Two of the seven ASY cases (29%) (Nos 15 and 16) had HIV-immunoreactive cells in the CPx. Case No. 15 only had rare HIV-positive monocytic-like cells (<1 cell per cm^2) in the CPx stroma (Figure 1D). Brain examination revealed chronic meningitis. Case No. 16 contained HIV-positive in the stroma and on its epithelial surface; they numbered 8 per cm^2 (Figure 1E). The brain was normal.

Gene amplification for HIV DNA was performed on CPx, basal ganglia and systemic organs of five of the seven ASY cases, including the two with HIV-positive CPx. Systemic organs included two spleens, two lungs and one kidney. The GAPDH gene was amplified in all specimens, thereby ensuring the integrity of the post-mortem DNA for the extraction and amplification procedures. HIV *env* sequences were detected by PCR or by PCR–Southern blotting in CPx, brain and systemic organs in one case (No. 18); in CPx and systemic organs, but not brain in three cases (Nos. 15–17); and in systemic organs only in case No. 19.

Discussion

Hematogeneous dissemination of HIV to the CNS develops shortly after initial systemic infection. If human disease is analogous to SIV, this initial dissemination may be lymphotropic, CXCR4-using virus with a later CNS dissemination by macrophage-tropic, CCR5-using viral strains, the variant required for productive brain viral infection. Alternatively, intra-CNS mutations from CXCR4 to CCR5 strains or initial infection with CCR5 virus, which remains dormant until the onset of immunosuppression, may provide the appropriate viral strain for productive viral infection.

Mechanisms of viral dissemination to the CNS may be multiple. Because most isolated CNS virus is M-tropic; and since macrophages and monocytes, including microglia, are the cell type harboring productive HIV infection, a leading hypothesis concerning viral entry suggests that brain becomes infected when HIV-infected macrophages or monocytes traverse an intact or damaged blood–brain barrier (BBB). The high incidence of co-infection by opportunistic organisms, especially CMV, also suggests a 'Trojan Horse' hypothesis, with entrance of HIV-infected monocytes in response to other brain infections. The potential for HIV-infected CD4 lymphocytes to enter and infect brain has not been explored, largely because isolated brain virus is predominately M-tropic. Direct infection of cerebral endothelium by circulating virus or virally infected cells is another potential mechanism for viral entry. It occurs in SIV infection *in vivo* (Mankowski *et al*, 1994; Flaherty *et al*, 1997; Edinger *et al*, 1997; Strelow *et al*, 1998); with HIV *in vitro* (Moses *et al*, 1993, Polland *et al*, 1995; Gilles *et al*, 1995) and possibly with HIV in some *in vivo* studies as well (Wiley *et al*, 1986; Gabzuda *et al*, 1986; Gyorkey *et al*, 1987, Bagasra *et al*, 1996).

The CPx is an alternative site for viral dissemination to the CNS since it has a high rate of HIV infection in AIDS patients. Hematogeneous spread to the CPx is not surprising since it lacks a BBB (Tennyson and Pappas, 1968) and contains immune cells, including trafficking lymphocytes, and tissue monocytes and dendritic cells in its stroma (Hicky *et al*, 1991; Maxwell *et al*, 1992; Matyszak *et al*, 1992; Serot *et al*, 1997 Hanly and Petito, 1998). In addition, the CPx may be the site of hematogeneous dissemination for other brain infections such as protozoa, fungi and bacteria (Bertrand *et al*, 1969; Netsky and Shuangshoti, 1975; Queiroz *et al*, 1979; 1991; Falangola and Petito, 1993). The CPx also is a site for SIV infection. Lackner *et al* (1991) detected SIV proteins and RNA in CPx of SIV-infected animals with or without SIV encephalitis and described the infected cells as monocytes and T lymphocytes. Similarly, Baskin *et al* (1992) found SIV-infected cells in 17 of 66 animals with SIV-induced brain inflammation. However, CPx infection with SIV does not take place during the initial few weeks following systemic venous infection (Chakrabarti *et al*, 1991; Smith *et al*, 1995).

The viral tropism of CPx infection may be both macrophage and T lymphocyte-tropic. The former is suggested by the localization of HIV to CPx monocytes and DCs; the localization of SIV to CPx

monocytes; and the macrophage tropism of isolated CPx virus in two of our cases. Lymphotropic strains are suggested by viral localization to T lymphocytes in SIV and by our preliminary findings of both brain and blood viral sequences in CPx of AIDS autopsies (Petito *et al*, 1998). Since the cases were end-stage AIDS patients, the blood-related CPx sequences might, in fact, be lymphotropic variants since this is the predominant blood-borne viral isolate at this stage of disease (Connor *et al*, 1997; Lu *et al*, 1997; Speck *et al*, 1997).

As reviewed in the Introduction, and as confirmed in the present study, HIV infection of the CPx is common in AIDS patients studied at post-mortem examination. The present study also found productive HIV infection in the CPx in ASY patients although the incidence and severity of infection was lower (two of seven cases) than that found in AIDS (eight of 14 cases). We also looked for the presence of HIV sequences in the CPx of five ASY cases by nested PCR. All had intact DNA in post-mortem paraffin blocks of brain, CPx and systemic organs, as determined by our ability to amplify the housekeeping gene GAPDH. We found HIV DNA in the CPx of four of five ASY cases but only in one of the five brain samples. The greater frequency of CPx HIV by PCR than by immunohistochemistry is likely to be related to the greater sensitivity of PCR.

The present study has certain methodological limitations. First, the incidence of HIVE in the AIDS cases may be underestimated since the number of sections submitted for microscopic examination was limited and lower than the usual examination in which ten or more sections are routinely submitted (Petito *et al*, 1986). However, the incidence of HIVE in the present study is similar to that found in other studies and the areas examined include basal ganglia and cerebral white matter, regions in which HIVE is most common. Similarly, the frequency of HIV-immunoreactive cells in the CPx may be underestimated since the number of positive cells is usually low. This may account for those cases with HIVE but negative CPx, as seen in case No. 6 herein as well as in several cases in our earlier report (Falangola *et al*, 1995). Second, cross-reactivity between tissues and anti-HIV antibodies is well known for gp41 and has also been described with other HIV proteins as well (Parmentier *et al*, 1992; Spehar and Strand, 1995; Dominguez *et al*, 1998; Chen and Dietrich, 1998). For that reason, detection of HIV DNA assists in establishing the presence of HIV although, by itself, does not distinguish between productive *versus* latent infection. Third, false-negative nested PCR for HIV may occur if the copy numbers are low or if post-mortem degradation of DNA resulted in some loss of HIV genomic material. While we cannot exclude these possibilities, the sensitivity of our PCR reaction was ten or more copies of HIV per reaction and we were able to amplify GAPDH in all samples. Fourth, contamination in PCR studies is always of concern but, as described below, our procedures avoided this as much as possible.

These results support the hypotheses that the CPx is a reservoir for HIV infection in the CNS. They also suggest that the CPx may be a site of hematogeneous dissemination to the CNS, since immunoreactive cells and HIV sequences were present in ASY cases without HIV encephalitis as well as in AIDS cases. Our data suggests that CPx infection is productive since HIV gp41 was identified by immunohistochemistry, although confirmation by detecting HIV RNA or by viral isolation has not yet been done. Immune competency is associated with brain protection and may also limit the productive infection in the CPx. With the advent of immune suppression and AIDS, increased viral load in the CPx could permit dissemination into the CSF and then into brain. The concentration of HIVE in basal ganglia and cerebral white matter is consistent with a CSF spread although, by itself, is insufficient to support this hypothesis.

The protective mechanisms in the CPx that allow persistent infection to continue during the ASY period are not known. Choroid plexus immune complexes, which we previously showed to be present in more than 75% of AIDS autopsies (Falangola *et al*, 1994), might be important and function in a manner similar to what has been proposed for systemic lymphoid organs by Tacchetti *et al* (1997). These authors found that viral particles are entrapped in immune complexes of lymph node germinal centers and retained in follicular dendritic cells as free cytoplasmic viruses without production of virus by membrane budding. These authors hypothesize that the immune complex entrapment of virus in the interdendritic spaces, and the inhibition of viral production by the dendritic cells once they have taken up HIV-containing immune complexes, protects the DCs from immune cell-mediated lysis and thus allows them to serve as HIV reservoirs. Studies examining the relationship between immune complexes and HIV infection in the CPx of AIDS and ASY patients are pending, but their close anatomical relationship in the CPx suggests that mechanisms similar to those hypothesized by Tacchetti *et al* (1997) for lymph nodes could be operative in this site as well.

Materials and methods

We obtained archival formalin-fixed brain and CPx from 14 AIDS patients and seven ASY cases that were autopsied in 1993–94 and included in a prior report (Kibayashi *et al*, 1996). At the time of the

initial post-mortem examination, the brains were fixed in 10% buffered formalin, sectioned and examined grossly. All cases had HIV antibodies in their post-mortem blood, documented by enzyme-linked immunoabsorbent assay and Western blot (Kibayashi *et al*, 1996).

Archival formalin pieces of basal ganglia, frontal cortex and white matter, hippocampus and CPx were embedded in paraffin, sectioned and stained with hematoxylin-eosin. A piece of spleen, or other systemic organ when spleen was not retained, was also embedded in paraffin to serve as a source of systemic virus. Deparaffinized sections were prepared for immunohistochemical analysis of HIV gp41 (Genetics Systems, Redmond, CA, USA, 1 : 750 dilution) using the avidin-biotin complex technique and 3,3′ diaminobenzidine and H_2O_2 for chromogen detection.

We extracted DNA from three 20 μm sections of the basal ganglia following removal of paraffin and tissue digestion, using ISOQUICK nucleic acid extraction kit (ORCA Research Inc., Bothell, WA, USA). HIV sequences were amplified in a DNA thermal cycler (Perkin-Elmer) using the 2.5 U Taq polymerase and the primer set: Henv-1 5′-GTA TGA ATT CAA CTG CTG TTA AAT GGC ACT-3′ and Henv-2 5′-ATG GAA TTC ACT TCT CCA ATT GTC CCT CAT-3′. 5 μL of the above first round PCR products were used as templates for nested PCR using the internal primers: Henv-3 5′-CGG AAT TCG CAG AAG AAG AGG TAG TAA TTA G-3′ and Henv-4 5′-TGT TCT AGA GTG TTA TTC CAT TGT G-3′. To avoid contamination between specimens, we cleaned the microtome knife with dilute acid between specimens, extracted the DNA in a room separate from the PCR reaction, and performed the biochemical procedures in a laminar flow hood. Samples with weakly amplified signals were further analyzed by southern blot for confirmation. This procedure detects at least ten copies of HIV per reaction. DNA was extracted from tissues from patients without HIV infection and run in parallel with each batch of DNA from the ASY case.

All histological sections were evaluated without knowledge of the patient group. HIV encephalitis was diagnosed as inflammation plus the characteristic multinucleated giant cell or HIV immunoreactivity for HIV protein (Budka *et al*, 1991) and graded as present or absent. In addition, the number of HIV-infected cells in the CPx was counted per cm^2 CPx. Nested PCR, including DNA extraction, also were done without knowledge of patient group.

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