American Journal of Pathology, Vol. 146, No. 5, May 1995

Evidence of Apoptotic Cell Death in HIV Encephalitis

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The mechanism of cell death in the brains of patients with acquired immune deficiency syndrome was examined in 15 cases, 8 of whom had human immunodeficiency virus (HIV) encephalitis, and in 8 control cases. Postmortem formalin-fixed, paraffin-embedded sections were prepared for routine histology and immunohistochemistry to detect cell-specific antigens. Apoptosis was detected by its morphology and by* in situ *end labeling of its characteristic oligonucleosomal fragments. Combined* in situ *end labeling and immunohistochemistry identified specific cell types. Six acquired immune deficiency syndrome brains, 5 of which had HIV encephalitis, contained positive nuclei by* in situ *end labeling. Colabeling studies identified the cells as neurons, reactive astrocytes, and, rarely, the multinucleated giant cells of HIV encephalitis. The only control with nuclei positive by* in situ *end labeling had hepatic encephalopathy and Alzheimer type II astrocytes; the location and absence of cell-specific markers suggested a glial origin for the labeled cells. These results demonstrate that at least some neuronal and astrocytic death in HIV infection occurs by apoptosis. Its stimuli are unknown, but likely candidates include tumor necrosis factor or HIV viral products. Additionally, we hypothesize that apoptotic death of reactive astrocytes may be a normal mechanism whereby the brain removes an excess number of astrocytes that have proliferated after certain types of brain injury. (Am J Pathol 1995, 146:1121–1130)

Brain atrophy is common in patients with human immunodeficiency virus (HIV) infection and often develops in the absence of acquired immune deficiency syndrome (AIDS), dementia, or HIV encephalitis (HIVE).[1–3] Quantitative analysis of regional changes indicate loss of both white matter and gray matter structures in the cerebral hemispheres, although atrophy of subcortical nuclei correlates best with AIDS dementia.[4–6] Histological studies clearly document white matter pathology[7] as well as neuronal and synaptic losses,[8–12] the severity of which correlates with regional concentrations of HIV antigen.[13] Astrocytosis and microgliosis are prominent in AIDS brains,[14–16] and the severity of the latter also correlates with brain atrophy.[14]

The mechanisms of and the stimuli for brain atrophy are unknown.[17] Current hypotheses include defective viral infection of glial cells, toxic factors released from activated or infected monocytes or macrophages, and hematogenously derived neurotoxins. Tumor necrosis factor and HIV envelope glycoprotein gp120 are likely candidates for the local or systemically derived neurotoxins, as reviewed below. Both kill neuroectodermal cells by the process known as programmed cell death or apoptosis.[18,19] In contrast to necrosis, apoptosis is a gene-directed[20,21] process initiated by the induction or activation of a Ca^{2+}/Mg^{2+}-dependent endonuclease that cleaves nuclear DNA into 180 to 200-kb oligonucleosomal fragments.[22,23] After compaction and fragmentation of chromatin and cytoplasmic bleb formation, the cell breaks up into small round cytoplasmic bodies (apoptotic bodies), many of which contain fragments of condensed chromatin. Plasma membranes and organelles are intact and functional. Apoptosis is an important mechanism of T lymphocyte loss during HIV infection (see Refs. 24 and 25 for review). Its specific mediators may be multiple but include HIV gp120 envelope protein, intercellular signals between infected and noninfected T cells, HIV-related superantigens, and autoimmune mechanisms.

We occasionally have encountered structures that appear to be apoptotic bodies on routine light mi-

Supported by a grant from the National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health (RO1-NS27416 to CKP).

Accepted for publication January 3, 1995.

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croscopy of brain tissue from patients with severe HIVE (personal observations). As apoptosis can be prevented by various inhibitors, its identification in AIDS brains may have potential therapeutic as well as mechanistic importance. Consequently, we examined postmortem brains of patients with AIDS and non-AIDS controls for the presence of apoptotic bodies. In addition, we used an *in situ* end label (ISEL) technique to identify the nuclear oligonucleosomal fragments of apoptotic cells. This recently developed method greatly facilitates the detection of apoptosis as the apoptotic bodies themselves are present for only short periods of time.[26] Combined immunohistochemistry/ISEL allowed identification of the cell type undergoing apoptosis. The results were correlated with associated pathological changes in the specimens examined. A preliminary report has been published.[27]

Materials and Methods

Both AIDS and non-AIDS cases were selected from the 1991 to 1994 autopsy files of Jackson Memorial Hospital. Cases with focal brain lesions or anoxic/ischemic encephalopathy were excluded. We initially included only those cases with postmortem intervals of 26 hours or less. When it appeared that this did not affect the ISEL, we later added AIDS cases with longer postmortem intervals. All brains were fixed in 10% buffered formalin for 10 to 14 days before gross examination, sectioning, and paraffin embedding as previously described.[8]

Apoptosis was investigated in basal ganglia in 21 cases, in thalamus in 1 AIDS case, and cerebellum and midbrain in 2 control cases. We specifically examined the sections for the presence of HIVE, using the criteria outlined by Budka et al,[28] and for reactive astrocytosis, microglial cell proliferation, apoptosis, and ischemic neuronal necrosis. Immunohistochemistry was used to detect antigens of HIV, glial fibrillary acidic protein (GFAP), leukocyte common antigen, T lymphocytes (CD45), macrophages (CD68), and nonphosphorylated neurofilaments in neuronal cell bodies (SMI 33). Deparaffinized sections were incubated overnight with the following monoclonal antibodies: HIV gp41, 1:750 (Genetics System, Seattle, WA), GFAP 1:600 (Dako Corp., Carpinteria, CA), CD45, 1:400 (Dako Corp.), and CD68; 1:200 after trypsin digestion (Dako Corp.), and SMI 33, 1:16,000 (Sternberger Monoclonals Inc, Baltimore, MD). This was followed by sequential incubation with biotinylated secondary antibody, the avidin-biotin complex with horseradish peroxidase, 3,3′-diaminobenzidine (DAB), and hydrogen peroxidase (H_2O_2). Positive controls included a case of HIVE (for HIV gp41), intrinsic immunoreactivity of subpial astrocytes and neurons (for GFAP and SMI-33), and a lung carcinoma and a central nervous system CNS lymphoma (for apoptosis and for inflammatory cell markers). Omission of the primary antibody was used as a negative control.

We identified oligonucleosomal fragments by ISEL, using a slight modification of the method of Wijsman et al.[29] Deparaffinized sections were pretreated with 2X standard saline citrate and digested with pepsin. They were then incubated for 1 to 2 hours at 37°C with 100 to 200 U of DNA polymerase and a mixture of dATP, dGTP, dCTP, and biotinylated dUTP. After blocking intrinsic peroxidase and thorough washing, sections were incubated with HRP-conjugated avidin (Vector Laboratories, Burlingame, CA) and then exposed to 3,3′-diaminobenzidine and H_2O_2 for 10 minutes. A light methyl green counterstain was used in some of the cases. To ensure reliability of this procedure, parallel sections of all cases were initially exposed to DNAse, a procedure that fragments DNA and thus labels all nuclei. Parallel sections also were treated as above with the omission of the DNA polymerase, a step that abolishes labeling. Known positive controls (lung carcinoma and brain lymphoma) were prepared in a similar fashion. A commercially available end-labeling kit was also tried but not used as it caused some diffuse nuclear and cytoplasmic staining and because Wijsman's method was more cost-efficient. Combined ISEL/immunohistochemistry was used to identify the cell type undergoing apoptosis. The ISEL procedure with DAB (brown reaction product) was done first and was followed by immunohistochemistry with the cell-specific markers and substitution of vasoactive intestinal peptide (purple reaction product) for DAB.

All microscopic evaluations were completed without knowledge of the experimental conditions.

Results

Altogether, 15 AIDS cases and 8 non-AIDS controls were selected for this study. The average postmortem interval of the controls was 16.9 ± 7.6 (range, 5 to 26) hours and their mean age was 57.8 ± 15.4 (range, 45 to 86) years. Systemic diseases in controls included 4 with neoplastic disease and 1 each with myocardial infarction, cirrhosis, end stage renal disease, and Alzheimer's disease. There were two groups of AIDS patients. Group 1 had postmortem intervals similar to the controls and included 9 cases with an average

postmortem interval of 15.8 ± 7.9 (range, 7 to 26) hours and an average age of 33 ± 17.4 (range, 0.5 to 63) years. Group 2 had 6 AIDS patients with prolonged postmortem intervals that averaged 52 ± 17.6 (range, 29 to 73) hours and an average age of 38 ± 7.4 (range, 26 to 47) years. The average postmortem interval of the entire group of AIDS cases was 30 ± 22 (range, 7 to 73) hours. All had systemic manifestations of AIDS including opportunistic infections, lymphoma, or Karposi's sarcoma. Focal brain diseases, including infections, neoplasms, strokes, or hemorrhages, were absent.

Neuropathological diseases in control brains included Alzheimer type II astrocytosis secondary to hepatic encephalopathy, Alzheimer's disease, and neoplastic meningitis. The remaining five were normal. Local brain pathology in the sections studied for apoptosis included Alzheimer's type II astrocytosis in one case and a focus of postmortem autolysis in a second case. Eosinophilic neuronal necrosis, apoptotic bodies, reactive astrocytosis, and HIV gp41 immunoreactivity were absent in all.

Of the 15 AIDS patients, 8 had HIVE, and this infection was also present in the sections examined for apoptosis. All were immunoreactive for HIV gp41 (Figure 1A). The remaining 7 AIDS cases were immunonegative for HIV gp41, 5 of which had diffuse or focal gemistocytic or fibrillary astrocytosis in the section examined for this study and 2 of which were normal (Table 1). Apoptotic bodies were found in 4 of the HIVE cases on hematoxylin and eosin (H&E)-stained sections. Most were round and measured less than 5 μ in diameter (Figure 1B, C). Some were situated in the perineuronal or perivascular regions of grey matter or were scattered throughout white matter. By location, therefore, these cells were more consistent with glial or inflammatory cells than with neurons. Other apoptotic bodies were present in larger triangular shaped cell bodies resembling neurons.

Pretreatment with DNAse produced ISEL-positive staining of all nuclei in all cases (Figures 2A and 3A) except case 16 in which ISEL was consistently negative. This case was not further evaluated for ISEL. Postmortem intervals did not appear to increase the ISEL of the sections with or without DNAse pretreatment (Figure 3A, B) so the two AIDS groups were grouped together. Apoptotic nuclei in the positive tumor controls showed prominent ISEL, and occasional leptomeningeal tumor cells in case 7 also contained labeled nuclei, although the brain parenchyma was negative. Combined ISEL/immunohistochemistry on slides pretreated with DNAse showed successful double labeling with GFAP, CD45 for T cells, and CD68 for macrophages. The pepsin digestion required for the ISEL procedure greatly diminished the immunoreactivity with leukocyte common antigen and altered the immunostaining pattern of SMI-33, resulting in prominent axonal staining and diminished perikaryal staining.

Seven of the eight control basal ganglia sections were negative for ISEL. The one case with hepatic encephalopathy and Alzheimer type II astrocytes (case 3) had scattered ISEL-labeled nuclei in the basal ganglia (Figure 2B). There were no T lymphocytes or monocyte/macrophages in the brain parenchyma although occasional cells within the vascular

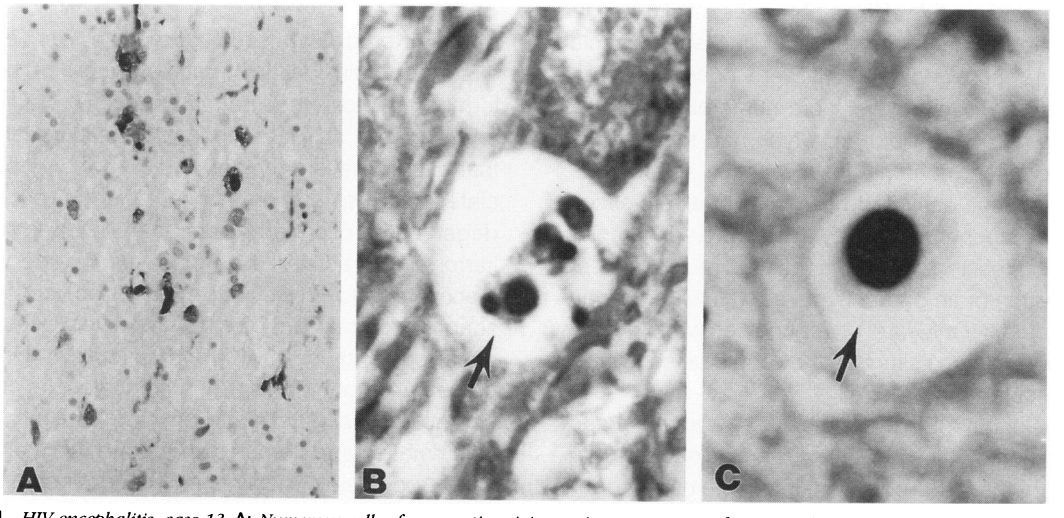

Figure 1. *HIV encephalitis, case 13.* **A:** *Numerous cells of monocytic origin are immunoreactive for HIV gp41. Hematoxylin counterstain; original magnification, ×400.* **B:** *A collection of small, round, hyperdense nuclear fragments surrounded by a thin rim of eosinophilic cytoplasm, consistent with apoptotic bodies (arrows). H&E; original magnification, ×1000.* **C:** *The center of the field shows a vacuole with a probable apoptotic body containing hyperdense chromatin material. H&E; original magnification, ×1000.*

Table 1. *Summary of Pathology and Apoptosis in Control and AIDS Brains*

Case	Age (years)	PMI (hours)	Dx	Basal ganglia pathology*	GFAP	APO bodies	ISEL	ISEL/IH
Controls								
1	45	26	MI, PE	Focal autolysis	0	0	0	
2	53	25	Cancer	0	0	0	0	
3	42	10	Cirrhosis, PE	Alz II glia	0	0	1–2+	None
4	49	21	ESRD	0	0	0	0	No
5	65	16	Cancer	0	0	0	0	
6	50	21	Cancer	0	0	0	0	
7	73	11	Cancer	0	0	0	0(tumor +)	
8	86	5	AD	AD	0	0	0	
AIDS								
9	0.5	3	HIVE	HIVE, 1+	0	+(periv)	+(periv)	None
10	45	7	Normal	Vascular calcification	Focal	0	+(periv)	
11	46	9	HIVE	HIVE, 3+	Diffuse	0	0	
12	27	16	Put Gliosis	Gliosis	Diffuse	±	0	
13	23	18	HIVE	HIVE, 3+	Diffuse	+	+	Neuron, astrocyte
14	33	19	HIVE	HIVE, 1+	Focal	0	0	
15	31	20	HIVE	HIVE, 3+	Diffuse	0	+(periv)	None
16	63	24	Normal	0	0	0	NA	
17	29	26	HIVE	HIVE, 2+	Diffuse	+(periv)	0	
18	43	29	Normal	0	0	0	0	
19	40	36	Normal	0	Focal	+	0	
20	26	51	Normal	0	Focal	0	0	
21	47	53	HIVE	HIVE, 3+	Focal	±	+	Neuron, astrocyte, multinucleated cell
22	34	70	Normal	0	0	±	0	
23	38	73	HIVE	HIVE, 3+	Diffuse	0	+(periv)	None

PMI, postmortem interval; Dx, major diagnosis at autopsy; APO, apoptotic; IH, immunohistochemistry; MI, myocardial infarct; PE, pulmonary emboli; ESRD, end stage renal disease; AD, Alzheimer's disease; Alz II glia, Alzheimer type II astrocytes; periv, perivascular; put, putamen; NA, not available.

*Cerebellum in case 2, midbrain in case 7, and thalamus in case 15. HIVE was graded as mild (1+), moderate (2+), or severe (3+).

lumina or around blood vessels were immunoreactive for their respective antigens. Combined ISEL/immunohistochemistry was negative for GFAP, SMI-33, and T cell and macrophage markers.

Six of the fifteen AIDS brains contained ISEL-positive nuclei (Figures 3B and 4). Five of the six had HIVE in the section prepared for ISEL and the sixth showed only astrocytosis and vascular mineralization. Conversely, five of the eight cases with HIVE contained labeled nuclei. In four cases, the labeled nuclei were infrequent and generally were found in perivascular regions. They were more numerous in the remaining two cases, both of which had severe HIVE and prominent neuronal loss, reactive astrocytosis, and numerous activated microglia. In all six cases, immunohistochemistry showed increased numbers of monocytes and macrophages (and multinucleated cells) within brain parenchyma as well as in perivascular spaces. Only rare T lymphocytes were found in brain parenchyma although they could be seen in the intravascular spaces.

Some of the ISEL-positive cells were immunoreactive for SMI-33, thus identifying them as neurons (Figure 5A). Other ISEL-positive nuclei were hypertrophic astrocytes that had numerous, enlarged cell processes immunoreactive for GFAP (Figure 5B). Labeled nuclei never co-labeled with T cell markers although occasional multinucleated cells were labeled for both fragmented DNA and monocyte/macrophage markers (Figure 5C). In the two cases with numerous ISEL nuclei, most of the labeled nuclei failed to co-localize with cell markers.

Discussion

In 1965, Kerr distinguished two types of liver necrosis after portal vein ligation[30]: necrosis and what he later termed apoptosis (see Ref. 22 for review). Necrosis is an accidental cellular response to an external injury that often is severe. Its onset is abrupt and is associated with loss of membrane integrity, swelling and degeneration of cell organelles, cell lysis, and nuclear karrolysis. Reactive tissue changes include inflammation, phagocytosis, and conversion to collagen scars. In contrast, apoptosis is an active process generally requiring both mRNA and protein synthesis and preservation of high energy phosphates. Its onset is characteristically delayed from 3 to 20 hours[31] and inflammation and fibrous scarring are minimal or absent. Apoptosis not only is an important component of normal development and differentiation but also is the mechanism of cell death in tumors and after such injuries as are caused by radiation, toxic chemicals,

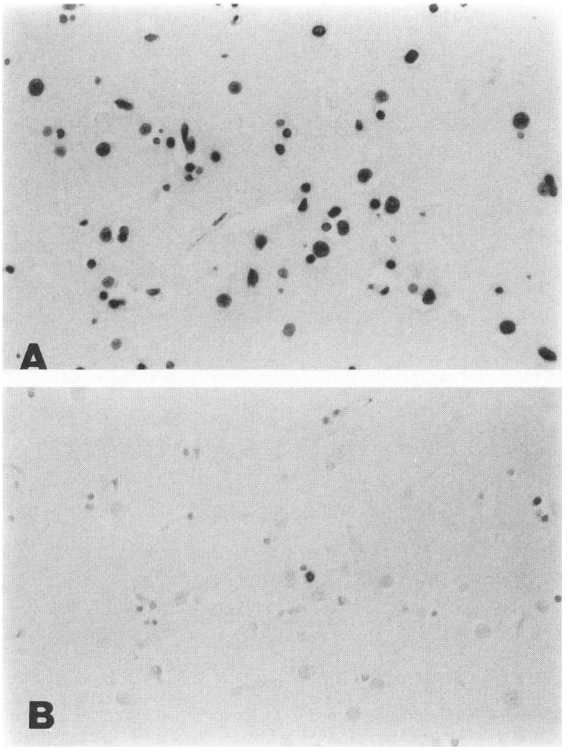

Figure 2. *Non-AIDS control case 3 (ISEL with no counterstain). The ISEL-positive nuclei are indicated with a brown chromogen that is depicted here as black.* **A:** *DNAse pretreatment, which produces diffuse chromosomal breaks, causes ISEL positivity in all nuclei. Original magnification, ×400.* **B:** *With no DNAse, only scattered nuclei are ISEL positive. Original magnification, ×400.*

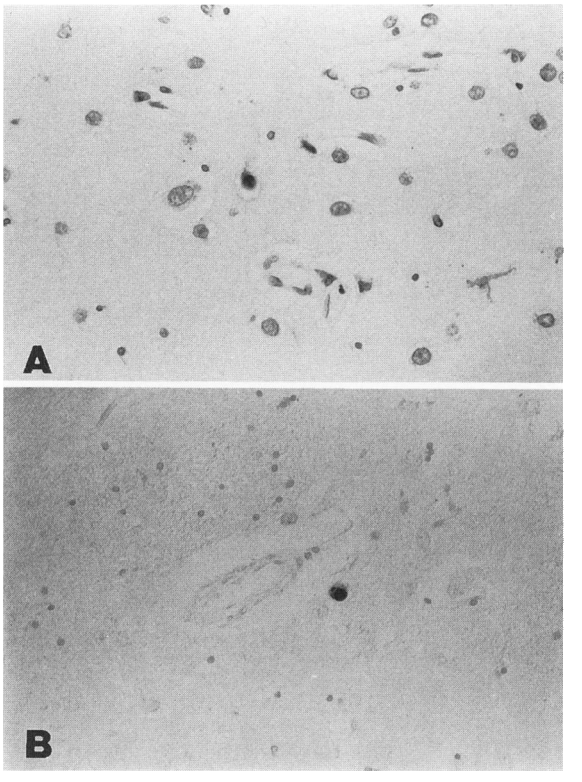

Figure 3. *HIV encephalitis, case B. A prolonged postmortem interval of 73 hours did not increase the number or intensity of ISEL with* (**A**) *or without* (**B**) *DNAse pretreatment. Only rare nuclei showed ISEL as depicted in* **B**.

and hormones. In certain circumstances, mild injury induces apoptosis whereas a more severe form of the same insult leads to cell necrosis.[32] Some authors distinguish between programmed cell death and apoptosis,[33] restricting the former term to describe the apoptotic cell death that occurs during normal development or metamorphosis.

In the nervous system, stimuli for programmed cell death during normal development and differentiation include the presence or absence of trophic factors or hormones and signals from adjacent neurons or glia (see Raff et al for review[34]). External stimuli that induce or enhance apoptosis in neuroectodermal cells *in vitro* include low potassium[35] and retinoic acid.[36] Apoptosis in the nervous system *in vivo* occurs after cerebral ischemia according to some[37,38] but not all[39] investigators. It is also seen in experimental allergic encephalomyelitis[40,41] although most of those apoptotic cells are lymphocytes rather than primary neuroectodermal cells. Apoptosis of infiltrating T lymphocytes recently has been demonstrated in human T lymphotropic virus type I-associated myelopathy.[42]

Histology is considered to be a reliable method for determining apoptosis in tissue sections and, indeed, was the original method used by Kerr.[30] However, apoptotic bodies may be difficult to find as they may be infrequent and because they are so rapidly taken up by adjacent cells. Electron microscopy is the best way to specifically identify apoptosis,[22] but this technique is not useful when apoptotic cells are infrequent or when post-reaction autolysis prevents adequate tissue preservation for ultrastructural analysis. Recently, the oligonucleosome fragments of DNA have been identified by ISEL in formalin-fixed, paraffin-embedded sections. This technique enables the observer to identify apoptotic cells before their rapid engulfment by neighboring cells. In addition, it helps distinguish between necrotic cells with pyknotic nuclei and apoptotic cells with condensed chromatin, a distinction that can be difficult, especially in the CNS where the presence of apoptosis in injury has not been well established. Finally, it is a practical technique for examining large regions of tissue, such as brain, when apoptotic cells may be absent or rare.

The technique of ISEL works well on formalin-fixed, paraffin-embedded materials, but possible artifacts may interfere with the interpretation of the results. First, ISEL will label pyknotic nuclei of necrotic cells. This can be distinguished from ISEL in apoptosis as

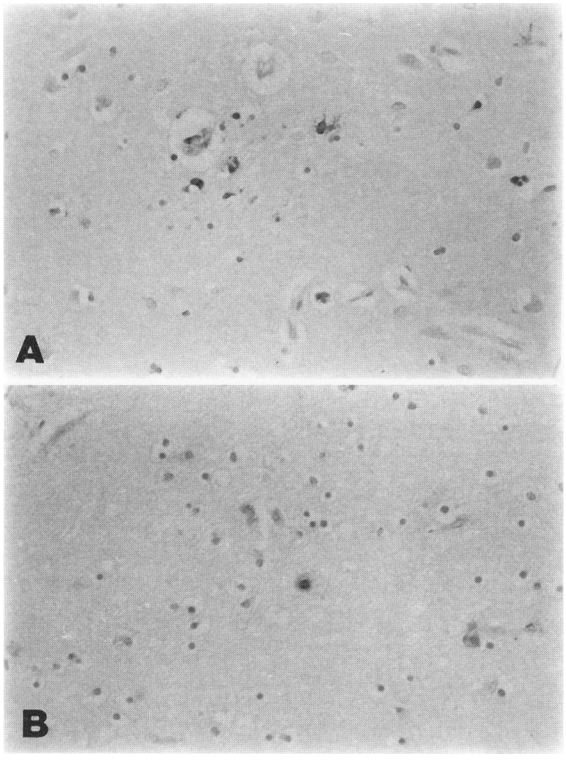

Figure 4. *HIV encephalitis, case 10. ISEL-positive nuclei are present as focal collections in perivascular parenchyma* (**A**) *or are scattered throughout the neuropil* (**B**). *Original magnification,* ×*400.*

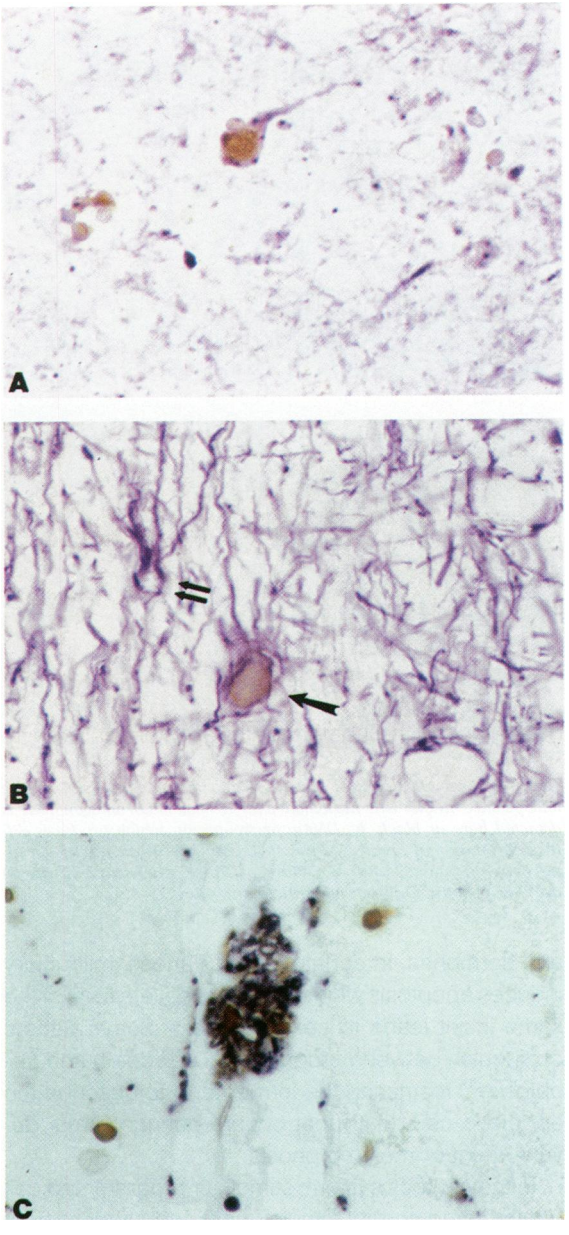

Figure 5. *HIV encephalitis. Combined ISEL/immunohistochemistry. The ISEL-positive nuclei are expressed as a brown color and the immunohistochemistry for cell-specific markers is expressed as a purple color. Original magnifications, all* ×*1000.* **A:** *Neuron with combined ISEL/SM1–33 immunoreactivity (case 21).* **B:** *Hypertrophied astrocytes with GFAP immunoreactivity, one of which has an ISEL-positive nucleus (arrow). The other astrocyte has an ISEL-negative nucleus (arrowhead) and thus shows no evidence of DNA fragmentation (case 13).* **C:** *Multinucleated giant cell with its nuclei showing ISEL positivity and its cytoplasm immunoreactive for CD68, a monocyte/macrophage marker (case 21).*

the DNA changes in necrosis follow, rather than precede, disintegration of the plasma membrane and cell organelles. For example, with kannic acid-induced excitatory necrosis[43] or brain infarction,[37] ISEL follows obvious cell necrosis by 18 and 48 hours, respectively. In the present study, we ruled out the nonspecific ISEL of cell necrosis as the cells with ISEL nuclei had morphologically intact cytoplasm. Another cause of false positive ISEL may arise from the procedure itself.[29] Both acid-induced DNA breaks occurring during the pepsin digestion, or overexposure to the DNA polymerase, can cause diffuse ISEL. We controlled for this by establishing optimal conditions for our particular tissue that ensured uniform ISEL of the DNAse-treated slides and an absence of ISEL tissue without apoptotic cells. Lastly, postmortem delay in fixation could cause nonspecific ISEL. We excluded this unavoidable complication as much as possible by using control brains with postmortem intervals that were similar to the first group of AIDS cases (26 hours or less). Furthermore, in the second group of six AIDS cases with prolonged postmortem intervals, four of six had no evidence of ISEL. Interestingly, two recent autopsy studies of apoptosis in postmortem brain did not encounter nonspecific ISEL in their cases[42,44]; unfortunately, neither study identified the postmortem intervals of the cases examined.

The present study is the first *in vivo* demonstration of apoptosis in brains of patients with AIDS. It was

designed to determine whether or not HIV infection was associated with apoptotic cell death in the nervous system, and specific studies examining frequency, distribution, and anatomic relationship of apoptosis to HIV encephalitis are in progress. However, the present study does suggest that apoptosis is associated with HIVE as it was detected by light microscopy and ISEL in six of the eight patients with this brain infection. Furthermore, the number and localization of ISEL-positive nuclei roughly correlated with location and severity of the HIV encephalitis. In all cases, the number of ISEL-positive nuclei outnumbered the number of apoptotic bodies. It is controversial whether or not ISEL simply enhances the detection of apoptotic cells[29] or increases the number of apoptotic cells by identifying those with early DNA fragmentation before morphological evidence of apoptosis occurs.[44] The recent study does not address this point although our results suggest that ISEL enhanced rather than increased detection of apoptotic cells.

Co-labeling studies clearly identified some of the ISEL-positive cells as neurons or reactive astrocytes. The majority of the labeled nuclei failed to localize with cell-specific markers for neurons, reactive astrocytes, and monocyte/macrophages. Although their origin was thus uncertain, we can rule out a lymphocytic origin as these cells were rare or absent. Some may be oligodendroglia on the basis of their localization in white matter. Alternatively, they may represent severely degenerated astrocytes or neurons.

Apoptosis is likely to be an important mechanism of brain damage in patients with AIDS. This study shows that at least some neuronal loss is clearly mediated via apoptosis and suggests a direct correlation between its frequency and the severity of HIVE. Apoptotic death of astrocytes could indirectly contribute to brain atrophy. Astrocytes are important in maintaining normal levels of extracellular potassium, ammonium, and glutamate and thus serve a neuroprotective function.[45,46] Their loss could lead to a secondary degeneration of neurons or their processes. The direct contribution of apoptosis to white matter lesions in AIDS is only theoretical as we were unable to directly identify this process in oligodendrocytes. If present, however, it could be responsible for some of the white matter changes in brain and spinal cord (see Ref. 47 for review).

The restriction of the ISEL-positive nuclei to the one control case that had Alzheimer type II raises the hypothesis that apoptosis of reactive astrocytes represents a protective mechanism whereby the brain removes the excess numbers of astrocytes that proliferate after certain types of brain injury.[48] Although combined ISEL/immunohistochemistry for cell markers in that case was negative, it is likely that these cells were astrocytes. They were relatively concentrated in gray matter, they frequently were perineuronal, and marker studies ruled out an inflammatory cell origin. Absence of GFAP immunoreactivity does not exclude an astrocyte origin for the apoptotic cells. It often is not found in gray matter astrocytes from formalin-fixed postmortem material. This may be especially true in cases of hepatic encephalopathy in which GFAP immunoreactivity is diminished. Thus, the detection of apoptosis in the hypertrophied astrocytes in AIDS brains and its possible occurrence in astrocytes in the patient with hepatic encephalopathy could therefore be more related to the presence of reactive astrocyte hyperplasia rather than being a specific sign of HIV-related astrocyte injury.

Apoptotic signals in the CNS that may be particularly pertinent to the pathogenesis of HIV-associated brain damage include tumor necrosis factor and HIV gp120. Both are elevated in serum, cerebrospinal fluid, and brains of AIDS patients.[49–52] Enhanced vascular permeability, common in AIDS brains even in the absence of apparent CNS pathology,[53,54] indicates the potential for a serum-derived source of these two neurotoxins in those AIDS patients without HIVE but with brain atrophy. Both are toxic to neurons or glia *in vitro*[18,55–59] and induce apoptosis in neuroectodermal cells.[18,19] The present study did not distinguish between the relative importance of these two neurotoxins. Although the severity of the apoptosis correlated with HIVE, the abundance of monocytes and macrophages, as well as the abundance of HIV antigens suggests that both tumor necrosis factor as well as HIV gp120 were present in large amounts.

Interestingly, HIV gp120-induced neuronal death is inhibited by a number of factors including coincubation with memantine, an *N*-methyl-D-aspartate receptor blocker,[19] anti-HIV gp120 antibodies,[55] vasoactive intestinal peptide,[56] and calcium channel antagonists.[57] Some of these antagonists may work by inhibiting the process of apoptosis. In other model systems, neuronal apoptosis also is abolished by certain agents. For example, the proto-oncogene *bcl-2* prevents the apoptosis in neurons that is caused by the withdrawal of neurotrophic factor[60] or glutathione deprivation [61] (cyclic AMP inhibits apoptosis due to serum deprivation[62] or low potassium[35]), and inhibition of protein and mRNA synthesis inhibits neuronal apoptosis due to nerve growth factor deprivation.[63] Such inhibitors may prove applicable to HIV-related apoptosis of neurons and glia as well.

Acknowledgments

The authors appreciate the secretarial assistance of Lee Ann Moffett.

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