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Damage and Repair of DNA in HIV Encephalitis

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Abstract. Neuronal damage and dementia are common sequelae of HIV encephalitis. The mechanism by which HIV infection of CNS macrophages results in neuronal damage is not known. We examined the brains from 15 AIDS autopsies (8 with HIV encephalitis and 7 without) and 4 non-infected control autopsies for the presence of DNA strand breaks, for associated changes in the expression of the DNA repair enzymes KU80 and Poly (ADP-ribose) polymerase (PARP), and for accumulation of amyloid precursor protein (APP). Abundant DNA damage was observed with terminal transferase-mediated dUTP nick end-labeling (TUNEL), however, there was no morphologic evidence of significant neuroglial apoptosis. The DNA repair enzyme KU80 was immunocytochemically detectable in neuronal and glial cells in autopsy brains from patients with and without HIV encephalitis; however, in cases with HIV encephalitis the staining was more prominent than in the infected or non-infected controls without encephalitis. There was no difference in KU80 immunostaining in oligodendroglia from autopsies with and without encephalitis. Immunostaining for PARP was more intense in gray and white matter of cases with HIV encephalitis. No clear spatial relationship existed between expression of DNA repair enzymes and the spatial proximity of microglial nodules or HIV-infected macrophages. The cytoplasm of cortical and subcortical neurons immunostained for APP. Stronger cortical neuronal APP staining was observed in cases without HIV encephalitis. Staining of deep gray matter neurons was similar, irrespective of the presence or absence of encephalitis. While foci of intense APP staining were noted in white matter not related to HIV infection, they were associated with foci of opportunistic infections (e.g. due to CMV or PML). We conclude that damaged DNA and altered patterns of expression of DNA repair proteins and APP are common findings in the brains of AIDS patients at autopsy, but do not have a spatial relationship to HIV-infected macrophages.

Key Words: AIDS; Amyloid precursor protein; DNA damage; DNA repair; Encephalitis HIV; Poly (ADP-ribose) polymerase (PARP).

INTRODUCTION

Worldwide the prevalence of HIV infection now exceeds 30 million. In 1998 AIDS became the 4th leading cause of death, behind ischemic heart disease, cerebrovascular disease, and pneumonia. Approximately one quarter of individuals infected with HIV develop a dementing disorder originally termed AIDS dementia complex (ADC) (1) and more recently HIV-1 Associated Dementia Motor Complex (2). Clinically ADC appears as a subcortical dementia (3) consistent with the predominance of subcortical pathology observed on neuropathologic examination (4, 5). While the relationship between neuropathologic and clinical findings has not reached universal agreement, better agreement has been reached between the relationship of HIV and neuropathologic findings (6).

Various degrees of neuronal loss and synaptic damage have been observed in AIDS autopsies (7–10). There is some disagreement about the extent of neuronal damage in early stages of HIV infection (8, 10–13), however, most studies have shown pronounced neurodegeneration in late stages, particularly when HIV encephalitis is present. Unfortunately the pathogenesis of this neurodegeneration remains obscure. Several groups have looked for evidence of axonal damage or neuronal apoptosis in AIDS autopsies (14–18). Both axonal damage and neuronal apoptosis have been observed, but without a clear relationship to the presence of HIV-infected macrophages. Assessing mechanisms of neuronal damage in a chronic disease like ADC is complicated by the slow clinical time course. If HIV encephalitis runs a subacute course measured in months, there would be little morphologic evidence of apoptosis in tissue based autopsy studies even if apoptosis were responsible for degeneration of half of the nervous system.

As proposed for other neurodegenerative diseases, dementia may result from the summation of acute events (e.g. multi-infarct dementia) or the summation of chronic events (e.g. oxidative stress). The discovery of mutations in superoxide dismutase genes in familial amyotrophic lateral sclerosis (19) and the abundance of oxidative products in Alzheimer and Parkinson disease (20–23) support the role of oxidative stress in chronic neurodegeneration. Because the brain is completely dependent upon oxidative metabolism that produces a large amount of superoxides, CNS nucleic acids, proteins, and lipids are particularly susceptible to oxidative stress (defined as free radicals and their products in excess of anti-oxidant defenses). Classically, oxidative stress is thought to lead to apoptosis or necrosis, although alternate pathways to neuronal damage will surely be discovered.

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There has been considerable interest in the potential role of oxidative stress in mediating the neurodegeneration associated with HIV encephalitis. Unfortunately, given the short half-life of reactive oxygen and nitrogen species, it is difficult to document their presence in human autopsy tissues. Instead investigators have relied on identifying the footprints of oxidative damage by measuring lipid peroxidation, inactivation of enzymes, glyco-oxidation products, and DNA strand breakage. Several groups have utilized the end labeling of free 3′ nucleic acids as a means of detecting DNA strand breakage and apoptosis in the brains of AIDS autopsies (14–17).

Damage of the nervous system, by whatever mechanism, elicits reparative processes. Recently Love and coworkers have shown that neuronal damage resulting from insults as diverse as acute ischemia (cardiac arrest) (24) to chronic neurodegeneration (Alzheimer Disease) (25) elicit detectable changes in DNA repair enzymes. In the current study we examined brains obtained at autopsy of patients with and without HIV encephalitis and found abundant evidence of DNA damage and repair, although we were unable to identify a spatial relationship between these processes and local presence of HIV encephalitis.

MATERIALS AND METHODS

A total of 15 AIDS autopsies were selected based on the following criteria: postmortem time less than 24 h; absence of significant opportunistic neuropathology; and, absence of clinical and neuropathologic evidence of acute cerebral ischemia (without clinical history of lengthy resuscitation and without pathologic findings of ischemic change in Sommer's sector of the hippocampus). We limited our study population to those with postmortem times under 24 h, because while some have not observed a relationship between DNA nicking and postmortem time (16, 26), others have observed increased DNA nicking with extended postmortem times (27). Each case was subjected to standard neuropathologic examination, as previously described (28). Microscopic foci of opportunistic lesions (e.g. solitary focus of PML or CMV infection) that were not present in the original autopsy investigations were found in the research sections studied in 2 of the autopsies. Four (4) non-HIV-infected autopsies with postmortem times less than 24 h and without significant neuropathology were used as controls. The average age at death of the non-infected controls (53 yr) was higher than in the AIDS cases with or without HIV encephalitis (40 yr and 41 yr, respectively). Paraffin-embedded blocks of pontine tissue from autopsies with ponto-subicular necrosis were used as controls for apoptosis staining (26).

Assessment of HIV encephalitis was performed as previously described. In brief, immunocytochemistry for gp41 was quantitated in paraffin-embedded sections from cerebral cortex, deep white and gray matter. An arithmetic sum of the 3 regions was determined (scale 0–6). In some cases frozen CNS tissues and CSF were available for quantitation of HIV RNA. This was performed as previously described using the branched DNA technique (29).

TABLE 1

Antibody Specificity	Company	Dilution in Immunohistochemistry
APP	Boehringer Mannheim, Indianapolis, IN	1:10
GFAP	DAKO, Carpinteria, CA	1:100
HIV gp41	ALAV-41.1, Genetic Systems, Seattle, WA	1:750
HIV p24	DAKO	1:10
HLA-DR	DAKO	1:50
KU80	Serotec, Raleigh, NC	1:8,000
MAP-2	Sternberger, Lutherville, MD	1:1,000
PARP	Serotec	1:200

Table 1 lists the specificity, source, and dilution of commercial antibodies used in this study. GFAP = glial fibrillary acidic protein, HLA-DR = human histocompatibility locus antigen DR, HIV gp41 = HIV envelope glycoprotein molecular weight 41,000 Dalton, HIV p24 = HIV group associated antigen molecular weight 24,000 Dalton, APP = beta amyloid precursor protein, KU80 = DNA polymerase associated protein molecular weight 80,000 daltons. MAP-2 = Microtubule associated protein-2, PARP = Poly (ADP-ribose) polymerase.

Immunocytochemistry

Immunocytochemistry for multiple viral and cellular proteins was performed using either fluorescent or horseradish peroxidase-labeled antibodies. In the latter case, the reaction was developed with amino-ethyl carbazole followed by hematoxylin counterstaining. Counterstaining of immunofluorescence was accomplished with propidium iodide. The source of commercial antibodies and the dilutions used in immunocytochemistry are listed in Table 1. Assessment of intensity of immunocytochemical staining was done by 2 independent observers (CAW and GW). For the 3 different areas assessed (neocortical gray matter, basal ganglia, and white matter), nuclear and cytoplasmic staining was evaluated qualitatively on a 3 point scale. A consensus was defined by joint review of individual regions. Average staining intensity for the cells in each region were compared between the 3 groups. As HIV-infected non-encephalitis controls showed staining patterns similar to non-HIV-infected non-encephalitis controls, Table 3 summarizes the comparison of the immunocytochemical staining between encephalitis and non-encephalitis groups.

Double label immunofluorescence was performed according to the previously published general protocols (30). For some of the antibodies, direct tyramide-signal-amplification was first employed. Subsequently, the sections were incubated at RT for 2 h with the second primary antibody. After rinsing in PBS, sections were incubated at RT for 1 h with the appropriate secondary antibody (i.e. Cy5-labeled goat anti-mouse IgG serum, or Cy5-labeled streptavidin, 1:100, Jackson ImmunoResearch, West Grove, PA). For negative reagent controls, primary antibodies were replaced with normal (rabbit or mouse) serum at equivalent protein concentrations. Double immunofluorescence sections were analyzed on a laser-scanning inverted confocal microscope (Molecular Dynamics, Sunnyvale, CA), as previously described (30).

TABLE 2

Case#	gp41 Sum	CSF HIV RNA	Cortical Gray Matter HIV RNA	Cerebral WhiteMatter HIV RNA	Deep GrayMatter HIV RNA	TUNEL Tagged Neuroglial Nuclei
1	6	NA	38360	1000	—	0
2	4	12698000	174400	—	—	0
3	5	—	5036000	NA	—	0
4	5	22000	37360	NA	28114667	0
5	4	—	609600	NA	9670693	0
6	3	6460000	1044400	—	—	2
7	5	NA	35560	43720	46400	0
8	4	NA	NA	NA	NA	0
9	1	NA	—	—	329444	1
10	0	NA	—	—	—	0
11	0	NA	—	—	—	1
12	0	NA	—	—	—	1
13	0	NA	—	—	56000	0
14	0	—	—	NA	97980	2
15	0	NA	—	—	25000	2
16	NA	NA	—	—	NA	0
17	NA	NA	—	—	NA	0
18	NA	NA	—	—	NA	2
19	NA	NA	—	—	NA	0

Table 2 lists general findings for the 15 AIDS cases and 4 non-HIV-infected controls studied. *Only case 4 had a clinical history of dementia. gp41 Sum = the abundance of gp41 detected immunocytochemically in cortical gray matter, deep white and gray matter scored on a scale of 0-6 (33). HIV RNA is reported in copies per ml for CSF or copies per gram wet weight tissue (29). The symbol ↑ denotes the concentration of HIV RNA exceeded the assay limit of 10^8 copies/gram wet weight tissue. The symbol ↓ denotes the concentration was below the assay detection limit of 10^3 copies/gram wet weight tissue. NA = specimen not available. Semi-quantitative assessment of TUNEL labeling was rated according on a scale of 0-2: rare labeled nuclei (<5 per paraffin section) were rated 0, sections with occasional labeled nuclei (> = 5 but <15 per paraffin section) were rated 1, and sections with more labeled nuclei were rated 2.

TUNEL

Tissue sections were deparaffinized and then rehydrated. Tissue was post-fixed by immersing the slides in 4% methanol-free formaldehyde solution in PBS for 15 min at RT. Slides were washed twice in PBS. 300 μl of the 20 μg/ml freshly prepared Proteinase K solution was added to each slide for 15 min at RT. Slides were washed twice in PBS for 5 min. Tissue sections were fixed again in 4% methanol-free formaldehyde solution, washed, and covered with 100 μl of Equilibration Buffer. 50 μl of Nucleotide Mix was added to the tissue. Slides were incubated at 37°C for 60 min inside a humidified chamber to allow the tailing reaction to occur. Immersing the slides in 2×SSC at RT terminated the reaction. Slides were washed and RNA hydrolyzed by incubating in 1 μg/ml RNase for 15 min at RT. Sections were counterstained by immersing the slides in propidium iodide solution, freshly diluted to 1 μg/ml in PBS for 15 min at RT in the dark. After washing the slides were coverslipped with Gelvetol.

HIV RNA Quantitation

Regional quantitation of HIV RNA was performed as originally described (29). All human tissues were homogenized in TRIzol™ Reagent (Gibco-BRL, Life Technologies, Gaithersburg, MD). Sterile glass beads and frozen brain tissue were added to 2 ml tubes along with 1.0 ml of TRIzol™ Reagent per 100 mg of tissue. The mixture was then shaken in a mini-bead beater for 10 s, stored on ice briefly, and homogenized again for 10 s. This was followed by phase separation with the addition of 0.2 ml of chloroform per mL of TRIzol™ Reagent. The mixture was shaken well and centrifuged at 12,000 ×*g* for 15 min. The upper aqueous layer was then transferred to a new tube. To the tube containing the aqueous layer, 0.5 ml of isopropanol was added per ml of TRIzol™ Reagent. The mixture was then split by transferring one half to a fresh tube. One half (corresponding to 50 mg of tissue) was used for HIV RNA determination in the QUANTIPLEX™ bDNA SIGNAL AMPLIFICATION SYSTEM (Chiron Corporation Emeryville, CA). Following an overnight precipitation at −80°C, the mixture was centrifuged at 12,000 ×g for 10 min. The supernatant was discarded and the pellet was washed in 0.5 mL of 75% ethanol, followed by another brief spin for 5 min. The ethanol was decanted and pellet air-dried for 5 min.

For QUANTIPLEX™ quantitation of HIV RNA, 0.44 ml of HIV sample working reagent was added to this pellet. The suspension was vortexed vigorously and incubated at 53°C for 20 min, followed by an additional 10 s of vortexing at the end of incubation to ensure complete solubilization of the RNA pellet. 0.2 mL of the solubilized RNA was placed in duplicate HIV capture wells in a standard QUANTIPLEX™ HIV RNA assay including standards and controls. These were incubated overnight at 53°C before being measured by the luminometer for calculation of HIV equivalents/gram of wet weight tissue as per

TABLE 3
Summary Comparison of Markers of DNA Damage in HIV Encephalitis versus Non-Encephalitis Controls

	Cortex		Basal Ganglia		White Matter	
	Neuronal Nuclei	Neuronal Cytoplasm	Neuronal Nuclei	Neuronal Cytoplasm	Oligodendroglial Nuclei	Oligodendroglial Cytoplasm
KU80	↑	↑	↔	↔	↔	↔
PARP	↑	↑	↑	↑	↑	↔

Table 3 summarizes the immunocytochemical staining results for KU80 and PARP in cases with and without HIV encephalitis. The symbol ↑ indicates increased immunostaining in the indicated cellular compartments in encephalitis cases compared with non-encephalitis cases. The symbol ↓ indicates decreased immunostaining in the indicated cellular compartments, while the symbol ↔ indicates no consistent difference in immunostaining between encephalitis and non-encephalitis cases.

the manufacturer's specifications. If the duplicate QUANTIPLEX™ samples varied by greater than 35%, the test was considered to be invalid.

RESULTS

Eight of the 19 cases studied had HIV encephalitis as defined by the presence of abundant immunocytochemically detectable HIV envelope protein gp41 (Table 2). The remaining 7 HIV-infected cases had little to no detectable gp41. When frozen CSF and brain tissues were available in the cases with HIV encephalitis, HIV RNA was quantitated. Regional variations in HIV RNA loads were similar to those described in previous studies (e.g. high levels in the striatum and low levels in the midfrontal cortical gray matter or cerebellum) (29, 31).

TUNEL

TUNEL staining of free 3′ ends of DNA labeled apoptotic nuclei in the brainstem of a control pediatric case of ponto-subicular necrosis (Fig. 1). TUNEL staining of CNS tissues from the AIDS and control autopsies was substantially less intense, less common, and not clearly related to the presence of HIV encephalitis. To compare the frequency of labeled neuroglial nuclei in the 19 cases, a semi-quantitative scale was employed. Sections including neocortex and deep white and gray matter were blindly rated on a scale of 0–2 for density of TUNEL-positive nuclei. Sections with rare labeled nuclei (< 5 per paraffin section) were rated ''0,'' sections with occasional labeled nuclei ($\geq = 5$ but <15 per paraffin section) were rated ''1'' and sections with more labeled nuclei were rated ''2.'' While there was some difference in TUNEL staining between the groups, the total number of cells was small and intense labeling of condensed nuclear profiles consistent with apoptotic bodies was only rarely observed (Table 2). Microglial cells in many of the cases showed intense labeling of cytoplasm (Fig. 2). As activated microglial cells were more common in cases with HIV encephalitis, more labeled cytoplasm was identified in these cases, however, labeled microglia were also present in cases without HIV encephalitis.

KU80 Immunostaining

Immunocytochemistry for the DNA polymerase subunit KU80 stained gray matter glial and neuronal nuclei in cases with HIV encephalitis more intensely than in HIV-infected and non-infected cases without encephalitis (Fig. 3). In the white matter, approximately half of the glial nuclei stained intensely with only rare cytoplasmic staining (data not shown). Additionally, in cases with encephalitis, more cells showed cytoplasmic staining than in cases without encephalitis (Fig. 3C, inset). White matter staining was independent of the presence or absence of HIV encephalitis (Table 3). There was no relationship between deep white and gray matter nuclear staining. Double-label immunocytochemistry for KU80 and GFAP confirmed that while nuclei of oligodendroglia and astrocytes stained for KU80 (particularly in white matter), cytoplasmic staining was most prominent in cells with neuronal morphology (Fig. 4).

Double label immunofluorescence was performed to assess the relationship between KU80 expression and focal HIV infection. The nuclei of HIV-infected mono- and multinucleated macrophages labeled for KU80 while the cytoplasm did not (Fig. 5). Macrophages within microglial nodules showed moderate KU80 staining of nuclei, but adjacent neuroglial elements did not show a different distribution of staining compared to that in areas without microglial nodules (Fig. 6). As HIV encephalitis is accompanied by microglial activation in excess of HIV-infected cells, we assessed the relationship between KU80 staining and a marker of activated macrophages (HLA-DR) (Fig. 7). We observed no spatial relationship between activated macrophages and Ku80 stained neuroglial nuclei.

Poly (ADP-Ribose) Polymerase (PARP) Immunostaining

Immunocytochemistry for PARP in white matter and cortical and subcortical gray matter was more intense in HIV encephalitis then HIV-infected patients without HIV encephalitis and the control non-infected cases. In white matter the staining was restricted to oligodendroglial nuclei, while in gray matter staining was most intense in neuronal nucleoli, less intense in the surrounding nucleoplasm, and weakest in neuronal cytoplasm. As with KU80 staining, no spatial association was noted between PARP staining and HIV-infected macrophages (Fig. 8).

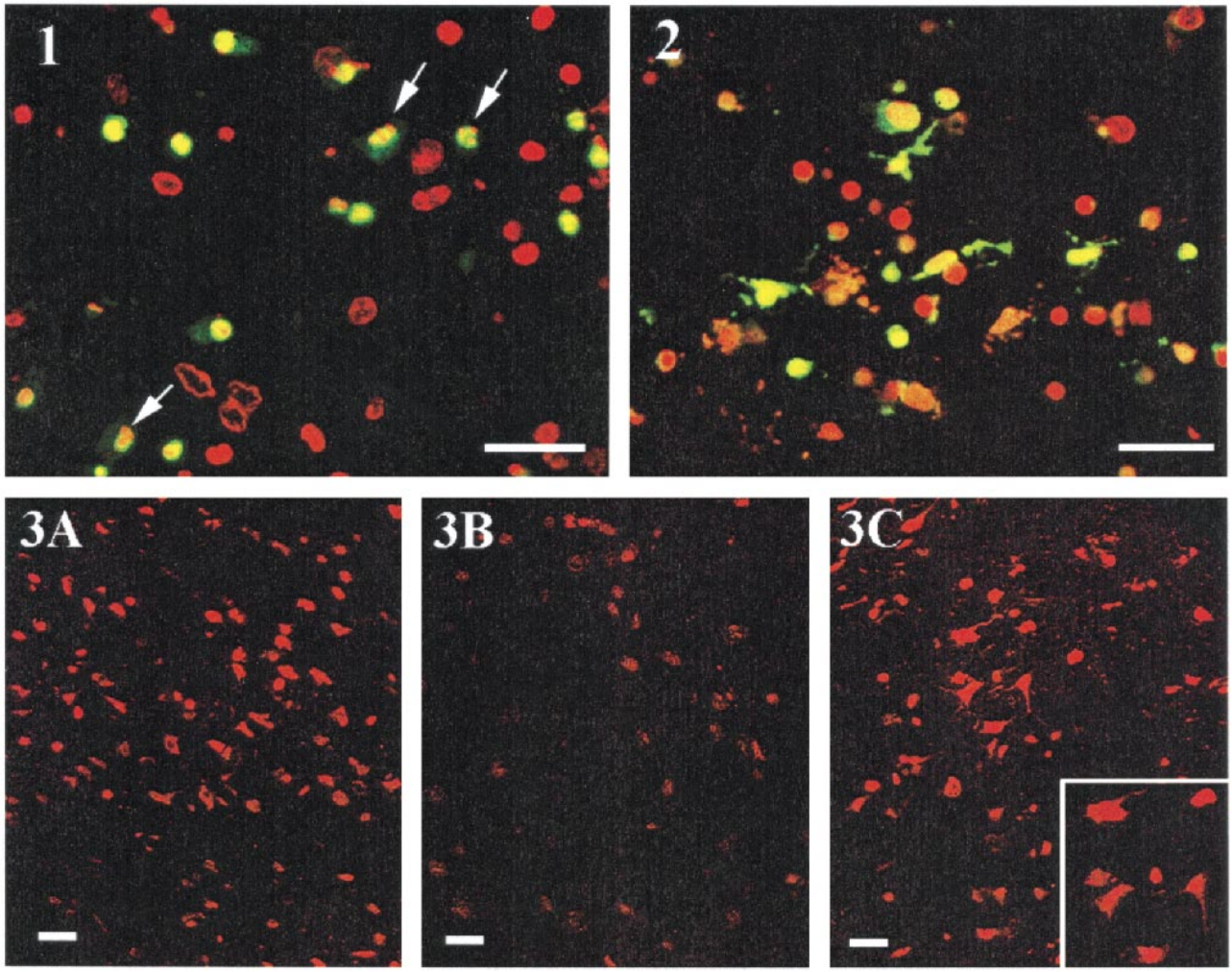


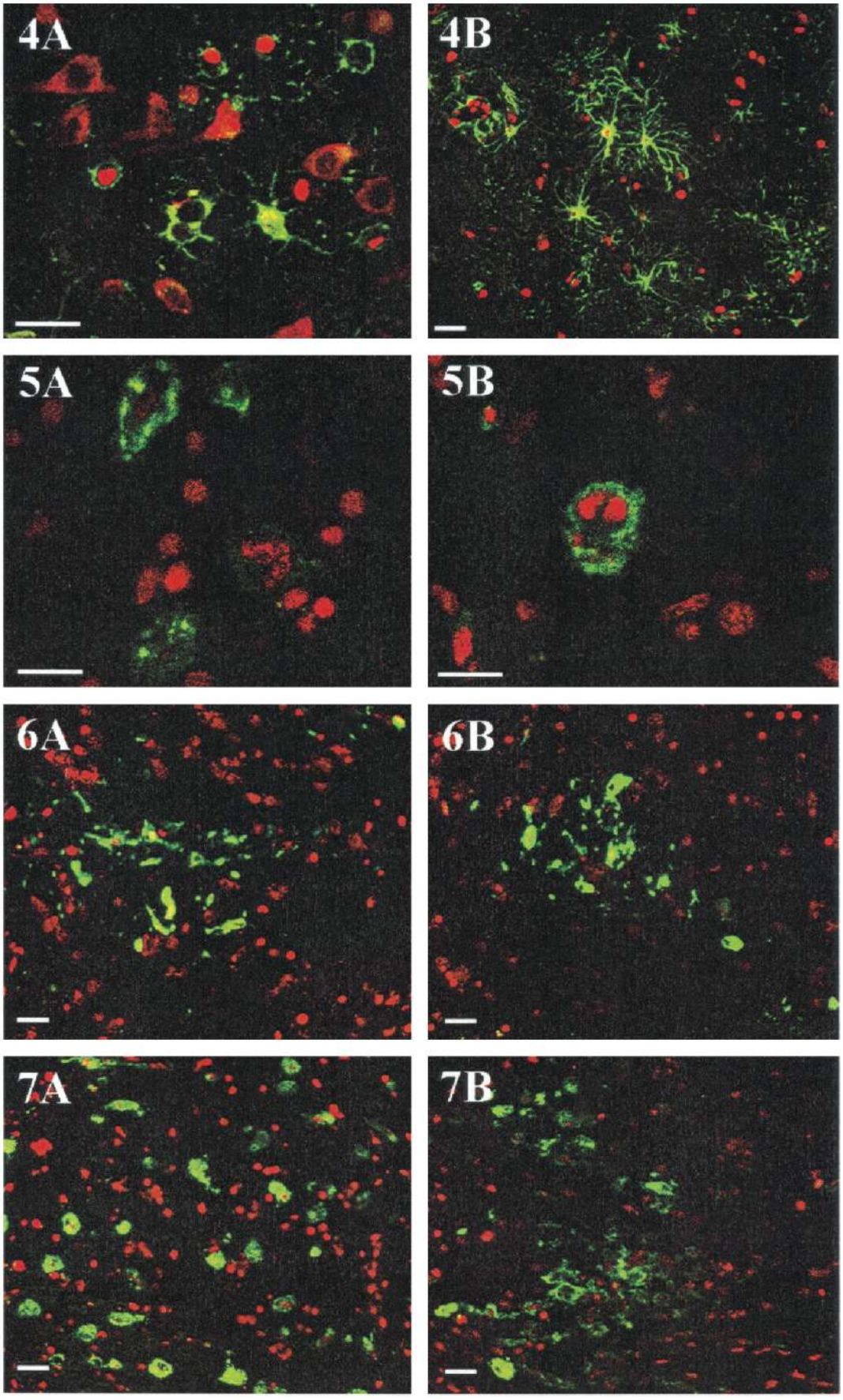
Fig. 1. As a positive control for apoptotic cells, TUNEL labeling (green) was performed on a paraffin section from the pons of a pediatric autopsy with ponto-subicular necrosis. The section was counterstained with propidium iodide (red). The presence of numerous TUNEL-positive cells demonstrates significant free 3′DNA. Several of the cells (arrows) also show nuclear profiles confirming apoptosis. Scale bar = 20 μm.

Fig. 2. Paraffin section from the brain of a patient with HIV encephalitis stained with the TUNEL (green) technique and counterstained with propidium iodide (red). A central microglial nodule shows both nuclear and cytoplasmic TUNEL staining (green). In autopsies with and without encephalitis, microglia stained with the TUNEL technique. Scale bar = 20 μm, case #2.

Fig. 3. Paraffin sections of the neocortex from AIDS autopsies with (A and C) and without (B) HIV encephalitis immunostained for KU80 (red). Overall KU80 staining is more intense in cases with HIV encephalitis. Additionally while most KU80 staining was restricted to the nucleus, in cases with HIVE cytoplasmic staining was also observed (C and inset). Scale bar = 20 μm, A and C from case #4, B from case #12.

APP Immunostaining

Immunocytochemical staining for the amyloid precursor protein (APP) diffusely labeled neuronal cytoplasm. Neuronal staining was seldom intense and there was a tendency for more neurons to be labeled in cases without encephalitis than in cases with encephalitis. Double-label immunofluorescent staining for KU80 and APP showed neuronal nuclear staining in regions of somal labeling for APP, but there was no apparent relationship between intensity of staining for these 2 proteins in any of the 3 groups. Occasionally, intensely stained white matter tracts were observed in some of the HIV-infected cases, but not in the non-infected controls. These tended to be multi-focal and confined to dystrophic-looking axonal processes showing beading. Double immunofluorescent labeling for KU80 and APP did not show higher or lower expression of KU80 around tracts expressing high levels of APP (Fig. 9). While presence of CNS opportunistic infections documented in the initial neuropathologic exam was use to exclude cases form study, 2 of the cases showed microscopic foci of opportunistic infections in deep-level sections of the blocks studied. In both instances (a microglial nodule related to CMV infection and a microscopic PML lesion), dilated axonal processes



stained with APP and were tightly associated with the lesions.

DISCUSSION

Several studies have looked for evidence of apoptosis in specific cell types in CNS tissue from AIDS autopsies. In the first of these, Petito and colleagues studied 15 AIDS autopsies (8 with HIV encephalitis and 7 without) and 8 control non-HIV-infected autopsies (16). These investigators found end-labeling of DNA in neurons and astrocytes and occasional multinucleated giant cells. In 5/8 autopsies with HIV encephalitis this labeling ''roughly correlated'' with location and severity of HIV encephalitis. Histologic features of apoptosis were only seen in 4 cases with HIV encephalitis. Similar findings were observed by Shi et al (17) and Adle-Biasette et al (14), however, Shi additionally found labeling of endothelial nuclei. Neither group found an association of end-labeling with regions of productive HIV infection, however there was an association with atrophy (14, 32). In contrast, Gelbard et al who studied 16 pediatric AIDS autopsies (7 with HIV encephalitis) reported an association between end-labeled neurons and chronic inflammation and HIV-infected macrophages. No fragmented DNA was observed in either astrocytes or oligodendrocytes. It is possible that this difference reflects a difference between pediatric and adult AIDS. AIDS can run a more rapid course in the pediatric population and the nervous system has been particularly severely affected in pediatric cases. In particular, immature neurons may be a more susceptible to apoptosis.

Our findings are in agreement with those of most previous investigations. We observed evidence of DNA strand breaks but could not discern a spatial relationship between the damaged DNA and HIV infection. Of the 3 groups studied, the HIV-infected but non-encephalitic cases showed more TUNEL labeling than the other 2 groups, however, this labeling was so infrequent in all of the 3 groups that it is difficult to draw conclusions regarding its significance. If observations are restricted to cells with neuronal morphology, this problem is exacerbated. While other groups have noted ''at least some neurons'' (16) undergoing apoptosis, the relationship between neuronal apoptosis and HIV encephalitis is not clear. In previous studies cortical neuron apoptosis showed no relationship to local HIV, while basal ganglia neuronal apoptosis did appear to be associated with cells that immunostained for p24 (29, 33). This latter finding may reflect the increased frequency of HIV-infected cells in the basal ganglia. Additionally, if the word ''apoptosis'' is restricted to cells with nicked DNA accompanied by nuclear morphologic changes, then others and we have not observed evidence to support apoptosis as an important path to neurodegeneration in HIV encephalitis. This may, however, simply reflect the rapid kinetics of apoptosis. Since HIV encephalitis runs a course of months, the probability of identifying a cell in apoptosis in histological sections at a single point in time would be relatively small.

Despite the lack of histological evidence of apoptosis, we and others have documented an increased amount of free 3′ ends of DNA in the brains of AIDS patients, consistent with oxidative damage. In the present study, the DNA repair enzymes KU80 and PARP were both detected at higher levels within neuronal and oligodendroglial elements of patients with HIV encephalitis than in brains from patients without encephalitis. However, we were unable to demonstrate a spatial link between expression of the reparative proteins and presence of local HIV infection. Even if HIV infection was directly associated with DNA damage, it is possible that such a link would be lost amongst the noise associated with upstream and downstream neuronal degeneration. Distinguishing proximal from remote effects might be better dissected using an animal model such as SIV encephalitis, where the abnormalities can be assessed at specific time points.

After oxidative stress a cell must ''decide'' whether to commit its residual energy stores to repairing damaged molecules and organelles or to initiation of apoptosis. The processes appear to be mutually exclusive. One of

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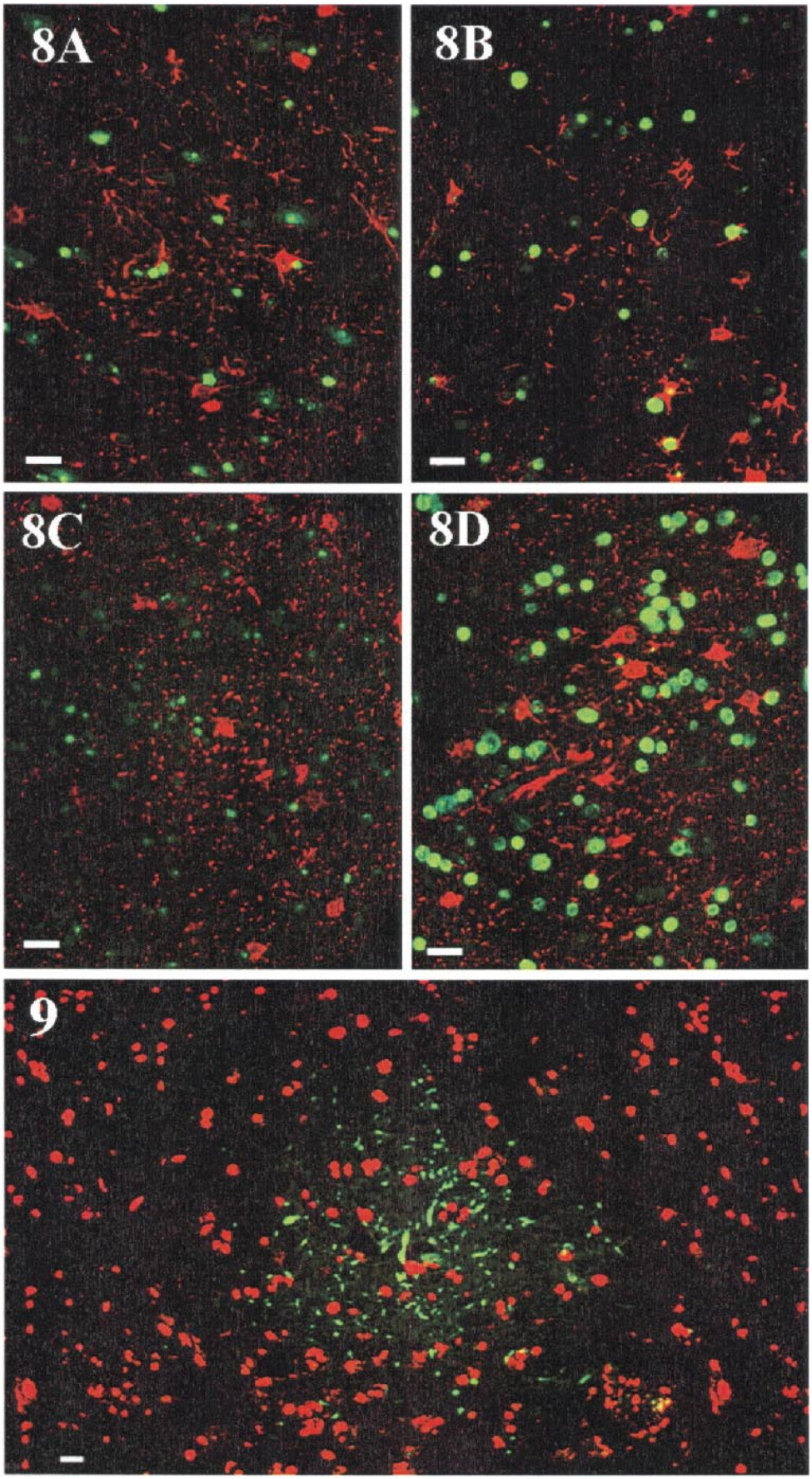
Fig. 4. Paraffin sections of the neocortical gray matter (A) and deep white matter (B) from AIDS autopsies with HIV encephalitis immunostained for GFAP (green) and KU80 (red). In both neocortical gray and white matter, KU80 immunostaining was most intense in non-astrocytic elements. Scale bar = 20 μm, case #4.

Fig. 5. A, B: Paraffin sections of the neocortex from an AIDS autopsies with HIV encephalitis immunostained for KU80 (red) and HIV p24 (green). In HIV-infected macrophages and multinucleated giant cells KU80 staining was restricted to the nucleus. Scale bar = 20 μm, case #4.

Fig. 6. A, B: Paraffin sections of the neocortex from an AIDS autopsies with HIV encephalitis immunostained for KU80 (red) and HIV p24 (green). While KU80 staining is more intense in cases with HIV encephalitis no clear spatial relationship could be discerned between KU80 staining and HIV-infected macrophages. Scale bar = 20 μm, case #2.

Fig. 7. A, B: Paraffin sections of the neocortex from an AIDS autopsies with HIV encephalitis immunostained for KU80 (red) and HLA-DR (green). While KU80 staining is more intense in cases with HIV encephalitis, no clear spatial relationship could be discerned between KU80 staining and HLA-DR-positive cells (activated macrophages). Scale bar = 20 μm, case #4.



the first steps in a cell's commitment to apoptosis is mediated by caspase 3 cleavage of reparative enzymes like PARP. PARP is a nuclear enzyme that responds to DNA nicking by adding chains of poly (ADP-ribose) to nuclear proteins. This presumed reparative process is conducted at the expense of significant consumption of energy through metabolism of NAD (34). After cerebral infarction, energy expenditure from high PARP activity is associated with additional cell death and expansion of the penumbra (35). Several PARP inhibitors have been shown to protect against this delayed effect (36–38). Recently this was elegantly demonstrated in PARP knockout mice which, compared to wild type mice, are subject to less neuronal destruction following experimental cerebral infarction (39, 40). PARP enzyme activity in the nucleus is well described. Recently PARP has been localized to the cytoplasm of some types of neuron suggesting that it may have additional activities in other subcellular organelles (41).

While acute oxidative stress clearly causes neuronal damage and death, a potential role of chronic oxidative stress has been proposed for neurodegenerative disease. Love and colleagues have shown that PARP and ADP-ribose tagged proteins are more readily detected in Alzheimer disease than in age-matched controls (25). Large projection neurons in layers 3 and 5 of the neocortex contain abundant PARP and its enzymatic products. Curiously, neurons with tangles did not show more intense immunocytochemical staining for PARP. To clarify the immunocytochemical observations, future Western-blot studies in AD and AIDS can examine the presence or absence of cleaved PARP to confirm cellular commitment to apoptosis.

Several investigators have looked for evidence of axonal damage in AIDS by examining the distribution of the beta-amyloid precursor protein, APP. APP is a rapidly anterogradely transported protein of unclear function, potentially involved in cell adhesion, neuronal growth, and response to injury. It is a sensitive and early marker of diffuse axonal injury (42–45). Within 2 h of injury, APP accumulates within axons and may be demonstrable for periods of up to several months (46). Scaravilli and coworkers examined APP distribution in several dozen AIDS (7 with HIV encephalitis, 29 without neurologic symptoms) and non-AIDS (other neurologic disease) autopsies (47, 48). They found no correlation between the pattern of APP reactivity and pre-mortem clinical evidence of dementia. Intense APP staining was associated with degenerating white matter tracts and regions of myelin pallor. Raja et al (49) studied APP distribution in 55 AIDS autopsies. They found no variation in APP distribution between different cortical regions and no co-localization with HIV. However, they did appreciate a vascular distribution of APP reactivity and an association with CMV lesions, consistent with our more limited observations. In the present study we have extended previous observations in documenting the absence of a relationship between APP staining and DNA damage or repair.

The mechanism of neuronal damage in HIV encephalitis continues to be an enigma. Data from this and previous studies suggest that DNA damage is present, presumably resulting from oxidative stress. What could be inducing oxidative stress in HIV encephalitis? Activated macrophages are one likely source. Macrophages are capable of producing significant quantities of reactive oxygen and nitrogen species (50, 51). Additionally, we have shown that neurotrophic factor expression is aberrant in HIV encephalitis, as others have shown in neurodegenerative diseases (52). In the developing nervous system, the withdrawal of neurotrophic factors leads to increased production of reactive oxygen and nitrogen species and programmed cell death. Whether or not the mature nervous system responds similarly is the subject of much current research.

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Fig. 8. Paraffin sections of the neocortical gray matter (A, B) and deep white matter (C, D) from AIDS autopsies without (A, C) and with (B, D) HIV encephalitis immunostained for PARP (green) and counterstained for GFAP (red). Overall PARP staining is more intense in cases with HIV encephalitis. In both neocortical gray and white matter, astrocytes did not stain intensely for PARP; rather PARP staining was most intense in cells with profiles most compatible with neurons or oligodendrocytes. Scale bar = 20 μm, A and C from case #11, B and D from case #1.

Fig. 9. Paraffin sections of the neocortical white matter from an AIDS autopsy immunostained for KU80 (red) and APP (green). Occasional bundles of axons intensely stained for APP. These AAPP-positive bundles showed no spatial relationship to KU80 staining or presence of HIV-infected macrophages. Scale bar = 20 μm, case #4.

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