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Distribution of human immunodeficiency virus (HIV) in the CNS of children with severe HIV encephalomyelopathy*

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Summary. The presence and distribution of human immunodeficiency virus (HIV) were examined in the CNS of two children with severe HIV encephalitis and myelitis. Using polymerase chain reaction-mediated DNA amplification and subsequent Southern analysis, proviral HIV gag sequences were identified in brain tissue of both patients. In situ hybridization using antisense oligonucleotide probes revealed abundant HIV gag and env/nef RNAs selectively in areas with histopathological evidence for HIV-induced tissue damage. The spinal cord of one patient exhibited a striking subpial accumulation of HIV RNAs strongly suggestive of a liquorigenic spread of the infection. HIV RNAs were typically associated with cells of the monocyte/macrophage lineage, as shown by a combined immunohistochemical and in situ hybridization procedure. The present study supports the view that the pattern and distribution of HIV-induced brain lesions is largely determined by the extent of focal HIV replication within the CNS.

Key words: HIV encephalitits – HIV myelitis – Human immunodeficiency virus – Polymerase chain reaction – In situ hybridization

The HIV epidemic involves an increasing number of children who usually acquire the virus from seropositive mothers. Common pathways of infection include transplacental or perinatal transmission during labor and delivery or postnatal infection through breast feeding [9, 28, 29]. In addition, infection by transfusion of contaminated blood products may occur [9]. When compared to adult AIDS patients, HIV-positive children show a higher incidence of HIV encephalopathies [3, 12, 15]. Neurological deficits may already develop during the 1st year of life [15]. Characteristic clinical manifestations include loss of developmental milestones and mental retardation, progressive deterioration of motor function and generalized weakness with pyramidal signs [1, 2, 14, 15]. In contrast, opportunistic infections such as toxoplasma and cytomegalovirus (CMV) encephalitis are less common in children with AIDS [1, 12, 24]. This low incidence of opportunistic infections probably reflects the fact that young children have not previously been exposed to these infectious agents [15].

Here we present the findings of a neuropathological and molecular biological analysis of the brains of two children with an unusual combination of AIDS-associated CNS lesions.

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Materials and methods

Histopathology and immunohistochemical reactions

Brain and spinal cord were fixed in 10% buffered formalin for 2 weeks. Tissue samples for microscopic examination were taken from the frontal, temporal, parietal and occipital cortex, the frontal white matter, basal ganglia, cerebellum, pons, medulla, cervical, thoracic and lumbar spinal cord and from all macroscopically conspicuous areas. Paraffin sections (4 μm) were stained with hematoxylin-eosin (H&E), Luxol-Nissl, Grocott and Ziehl-Neelsen. Immunohistochemical reactions were performed with antibodies to glial fibrillary acidic protein [GFAP, Dakopatts, Copenhagen, Denmark, dilution (dil.) 1:250], leucocyte common antigen (LCA, Dakopatts, dil. 1:100), CMV (Dakopatts, dil. 1:50), herpes simplex virus I+II (Dakopatts, dil. 1:200), Toxoplasma gondii (Virion, Rüschlikon, Switzerland, dil. 1:200), common capsid antigen of the polyomaviruses JC, SV40 and BK (kindly provided by Dr. D. L. Walker, University of Wisconsin, Madison, Wis.), the macrophage-specific antibody PG-M1 (provided by Dr. B. Falini, Istituto Clinica Media I, Perugia, Italy), a monoclonal CD20 antibody to B lymphocytes (clone L26, Dakopatts, dil. 1:150) and a monoclonal antibody to the CD45 Tcell antigen (Dakopatts, dil. 1:100). HIV gag proteins were detected employing monoclonal antibodies to the p17 and p24 antigens (Dupont de Nemours International, Geneva, Switzerland, C 274, C 289, dil. 1:150). An avidin-biotin-peroxidase or alkaline phosphatase/anti-alkaline phosphatase (APAAP) system were used to identify the reaction products.

In situ hybridization and double labeling

cRNA-probes for HIV-I were prepared by subcloning into the transcription vector pGEM1 (Promega Biotec, Heidelberg, FRG) of a *Bam*HI fragment and a *Hind*III fragment from the plasmid pBH10-R3 [21], which contains a HIV-1 provirus of the T cell line H9/HTLV-III. The *Bam*HI fragment (nucleotides 7831-8933) comprised the 3′-region of HIV-I, including the 3′-LTR, the nef and a portion of the env gene, whereas the *Hind*III fragment (nucleotides 408-1035) probe was specific for the HIV-I gag gene. After linearization of the pGEM1 constructs with appropriate restriction enzymes, antisense and sense (control) RNA probes were generated by run off-transcription with incorporation of ^{35}S-labeled nucleotides as described (average specific activity, 1.3×10^9 cpm/μg) [22, 30]. To increase tissue penetration, RNA transcripts were degraded to 100 to 150 base pairs (bp) length by controlled alkaline hydrolysis. Specificity and performance of the RNA probes were tested on cytospin preparations of the cell lines H9 (American Type Culture collection, Rockville, Md.), HIV-I-infected cell line H9/HTLV-III (donated by Dr. R. C. Gallo, Bethesda, Md.) and the HTLV-II-producing T lymphoid line Mo-T (supplied by Dr. I. Chen, Los Angeles, Calif.).

Paraffin sections (4 μm) were subjected to pre-hybridization using standard procedures [38]. Hybridization, post-hybridization washing (54 °C), RNase digestion of mismatched sequences as well as autoradiography were performed as described [31, 32]. Incubation of sections with RNase A or Micrococcus nuclease (both from Boehringer Mannheim, Mannheim, FRG) prior to in situ hybridization abolished the autoradiographic signal, confirming that RNA sequences were the targets of the hybridization procedure. Hybridization of HIV-infected brain tissue with RNA sense probes and hybridization of biopsy specimens obtained from seronegative individuals with RNA antisense probes were used as negative controls.

Immunohistochemistry was carried out prior to in situ hybridization using RNase free buffers and glassware. Tissue sections were deparaffinized in xylene for 2×20 min and rehydrated through graded ethanols. Sections were then rinsed in 0.85% saline solution and 1× PBS for 5 min, respectively. Following postfixation in 4% paraformaldehyde for 30 min at 4 °C, slides were rinsed in 1× PBS for 2×20 min and subsequently treated with proteinase K (Merck, Darmstadt, FRG; 0.5 mg/ml in 50 mM Tris-HCl/5 mM EDTA, pH 7.2) for 10 min at room temperature. After rinsing in 1× PBS for 5 min, slides were incubated with the primary monoclonal antibody (PG-M1, Dr. B. Falini, Perugia, Italy, dil. 1:8). All antibodies were diluted in freshly prepared RPMI 1640 medium, pH 7.5, containing 10 mg/ml bovine serum albumin, 1.0 mg/ml yeast tRNA, and 5000 U/ml heparin ammonium salt (Sigma) to inhibit RNase activity [23]. The incubations with the monoclonal antibody PG-M1, rabbit anti-mouse immunoglobulin (Dakopatts) and the APAAP complex (Dakopatts) were carried out for 30 min at room temperature. Secondary antibody and APAAP complex were diluted 1:20. The final two incubation steps were repeated once for 10 min each. Washes between the incubation steps were carried out with 1× TBS. The alkaline phosphatase was visualized as described [10, 42] and slides were immediately subjected to in situ hybridization [31, 32]. Autoradiographic exposure was 5 days. To estimate the RNA loss during immunostaining procedures, adjacent tissue sections were subjected to in situ hybridization without prior immunostaining.

Polymerase chain reaction

Unfixed tissue samples from frontal cortex and white matter were obtained within 24 h postmortem, snap frozen in liquid nitrogen and stored at −80 °C until further use. Aliquots (1 g) were homogenized in 5 ml of 1× lysis buffer (Applied Biosystems, Foster City, Calif.) and digested with RNase A and RNase T1 (1 h at 37 °C; 400 U/g tissue) and proteinase K (2 h at 37 °C; 85–170 U/g tissue). Lipid extraction was carried out with methanol/chloroform and DNA was purified by phenol/water/chloroform and ethanol extraction. Extracted DNA was then solubilized in water at 4 °C for 4 days. DNA aliquots (1 μg) were subjected to 50 cycles of polymerase chain reaction (PCR) on an automated thermal cycler (Perkin Elmer Cetus, Norwalk, Conn.). Oligomers flanking a 109-bp fragment of the HIV gag region were used as PCR primers (3′-ATCCACCTATCCCAGTAGGAGAAAT-5′, 3′-AGATGG-ATAATCCTGGGATTAAATA-5′, position 1091-1199 in HTLV III [34]). The reaction was carried out in a total volume of 100 μl with 3 units of Taq polymerase (Amplitaq, Perkin Elmer Cetus), 50 pmol of each primer and 300 mM of each deoxyribonucleoside triphosphate (dATP, dCTP, dGTP, dTTP) in PCR buffer (10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$). DNA samples from brain tissue of a HIV-seronegative individual and from HIV-infected H9 cells were used as controls. All samples were processed simultaneously. Cycling parameters were 2 min denaturing at 94 °C, 2 min annealing at 45 °C and 3 min polymerase reaction at 72 °C. The amplified 109-bp sequence was identified by agarose gel electrophoresis.

For Southern blot analysis, amplified and electrophoretically separated DNA was transferred to a nylon membrane and hybridized with a ^{32}P-labeled oligonucleotide (3′-GGTCCTTGT-CTTATGTCCAGAATGC-5′) corresponding to an internal fragment of the amplified 109-bp sequence. Hybridization products were visualized by autoradiography.

Patients

Patient 1

The first patient was a girl born a term, with an uneventful neonatal period. Her mother reported a 2-year history of intravenous drug abuse prior to pregnancy. By 6 months of age the child was able to sit. She vocalized the first words at 10 months but wasn't able to walk independently until the age of 22 months. Starting at the age of 18 months, the girl suffered from recurrent episodes of bronchitis and bilateral otitis. With 24 months she developed a persistent left-sided hemiparesis. At this time, no detailed neurological examination was carried out. Nine months later, i.e., at the age of 2 9/12 years, she was hospitalized with severe bronchitis, respiratory failure, convulsions and diarrhea. She now presented with signs of severe dystrophia, dehydration and significant developmental retardation. Diagnoses of interstitial pneumonia, oral and pharyngeal candidiasis and bilateral otitis were made. A screening test showed seropositivity for HIV. Neurological examination revealed spastic left-sided hemiparesis with hyperreflexia and spasticity of the lower limbs. The girl died from respiratory failure at the age of 2 10/12 years.

At general autopsy (A89/1168), severe *Pneumocystis carinii* pneumonia was diagnosed. Additional findings included oral candidiasis, esophagitis, fatty degeneration of the liver, thymic atrophy and lymphocytic depletion of tonsils and lymph nodes corresponding to AIDS lymphadenopathy grade III. Neoplasms were not detected.

Patient 2

The second patient, a 7-month-old boy, was born at term to a HIV-seropositive mother with a history of intravenous drug abuse. The child had an inconspicuous neonatal period. From the 3rd month, he showed failure to thrive and developed hepatomegaly, lymphadenopathy, anemia, oral candidiasis and hemorrhagic diarrhea. He was hospitalized at the age of 4 months with bronchopneumonia. At this time, antibodies to HIV and HIV-specific DNA sequences were detected in peripheral blood samples. The CSF showed positivity for HIV-1 antigen. The patient subsequently developed neurological symptoms including opisthotonus, tetraspasm and shrill crying. Computed tomography revealed a cortical

and subcortical atrophy. The further clinical course was characterized by recurrent intestinal hemorrhage of unknown origin. At 6 months of age, recurrent epileptic seizures were noted. Three weeks later, the boy developed a severe hepatic syndrome with hypalbuminemia and generalized edemas. He died at the age of 7 months due to respiratory failure.

General autopsy (A90/270) showed necrotizing interstitial pneumonia with occasional CMV inclusion bodies in pneumocytes. In the digestive tract, severe CMV esophagitis and enteritis were found. CMV-related inclusion bodies were also detected in the pancreas, thyroid gland, kidney and in the liver which showed necrotizing hepatitis. The spleen was atrophic and exhibited large infarcts. Atrophy was also prominent in the thymus and lymph nodes. This prompted the diagnosis of AIDS lymphadenopathy grade III.

Results

Neuropathological findings

Patient 1. The brain of the first patient showed a large necrotizing mass in the right parietal lobe (Fig. 1D).

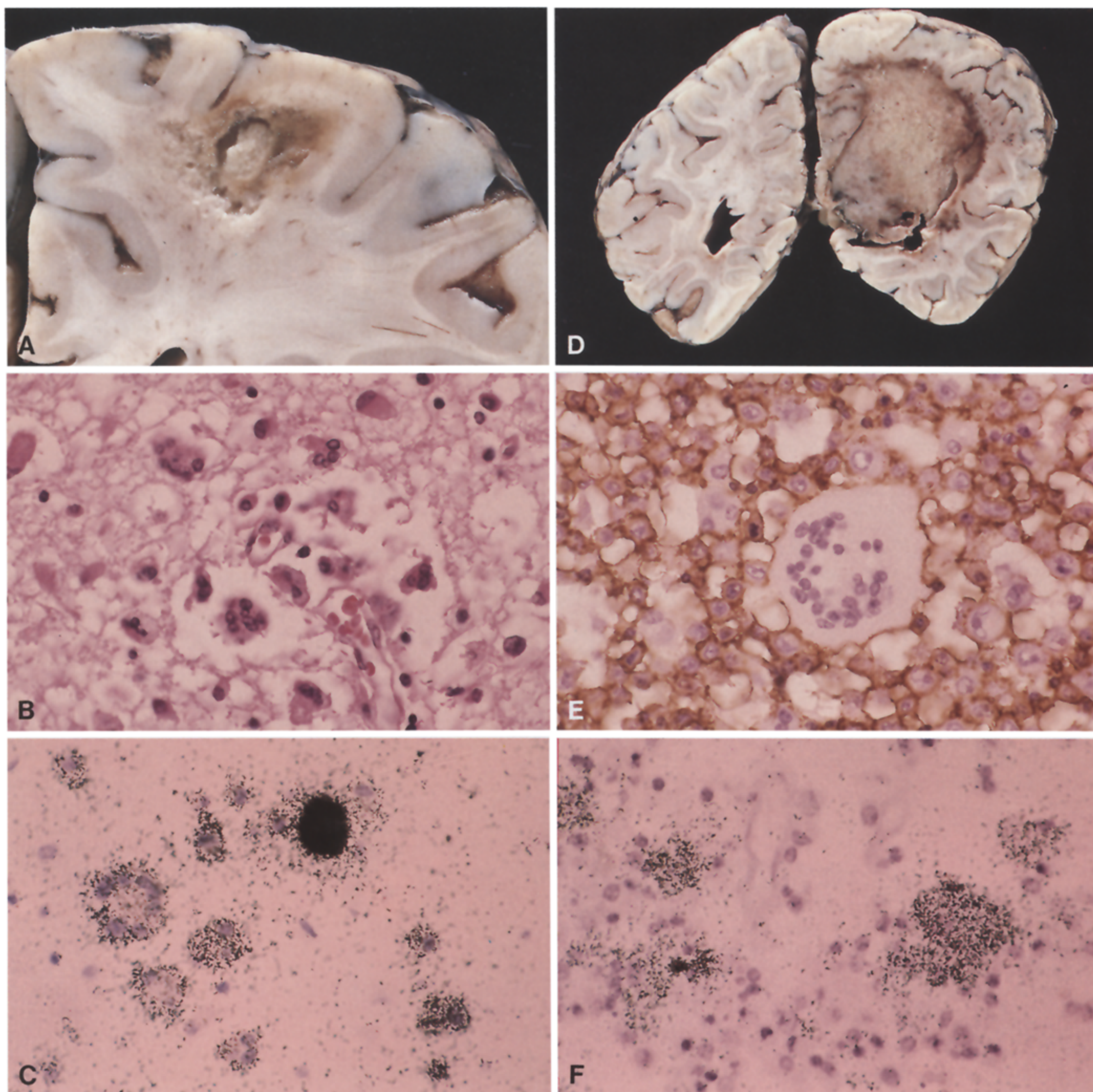

Fig. 1 A–F. Neuropathological findings in patient 1. **A–C** Necrotizing HIV encephalopathy in the right frontal lobe with numerous multinucleated giant cells (**B**) containing large amounts of HIV gag RNA (**C**; in situ hybridization). **D–F** Large cerebral lymphoma in the right parieto-occipital white matter. The tumor cell population is strongly immunoreactive with the B lymphocyte-specific monoclonal antibody L26 (**E**). Note the absence of immunoreactivity in the multinucleated giant cell. In situ hybridization reveals HIV RNA within the histiocytic giant cells whereas the neoplastic cell population does not contain HIV RNA (**F**). **B, C, E, F** × 330

Microscopically, a highly cellular tumor with the histopathological features of a malignant lymphoma was detected. The tumor cells contained large nuclei with prominent nucleoli and showed a small rim of cytoplasm. Immunohistochemical analyses revealed a strong immunoreactivity of the tumor with the monoclonal antibody L26 specific for the B cell antigen CD20 (Fig. 1E). In contrast, expression of CD45 antigen was restricted to a population of reactive T lymphocytes. These findings prompted the diagnosis of a primary cerebral non-Hodgkin lymphoma of B cell origin. The tumor exhibited an unusual component of multinucleated giant cells with as many as 40 nuclei. These cells showed a strong reaction with the antibody PG-M1 and were, therefore, regarded as histiocytic giant cells.

A second partially necrotic lesion involved both subcortical white matter and cortex of the right frontal lobe (Fig. 1A). Histopathological analysis revealed a large necrotizing focus with extensive demyelination, loss of neurons, reactive astrogliosis and a prominent population of macrophages and multinucleated giant cells (MGCs) indicating HIV encephalitis (Fig. 1B).

Microscopic foci of HIV encephalitis containing microglial cells, MGCs, and occasional lymphocytes were seen throughout the white matter of both cerebral hemispheres and in the brain stem. These foci generally occurred in a perivascular location and were associated with demyelination and reactive astrogliosis. Small perivascular calcifications were found in the basal ganglia. Few scattered MGCs and microglial nodules were also detected in the cerebral cortex.

The spinal cord showed a severe HIV myelitis. Numerous foci with demyelination, abundant reactive astrocytes, macrophages and MGCs were found in both gray and white matter. The inflammatory foci were preferentially located in the superficial areas of the cord. Few MGCs were detected in the leptomeninges. White matter vacuolation was absent.

Patient 2. Focal discoloration and softening of periventricular tissue was noticed in the brain of the second patient. The weight of this brain (670 g) was slightly reduced compared to the age standard (770 g). Microscopic analysis revealed a widespread HIV encephalitis. Numerous inflammatory foci containing MGCs were not only detectable in the white matter but also in the cerebral and cerebellar cortex and, again, showed a predominant perivascular location. The macroscopically conspicuous periventricular tissue was identified as necrotizing foci of HIV encephalitis. In the white matter, inflammatory foci were occasionally associated with microcalcifications. These calcifications were most prominent in the basal ganglia. Striking changes were observed in the brain stem with necrotizing foci of HIV encephalitis and patchy demyelination.

Infiltrates with microglial cells and MGCs were also detectable in the gray and white matter of the spinal cord and prompted the diagnosis of a HIV myelitis. A number of MGCs was found in close association with the central canal. No white matter vacuolation was observed.

Immunohistochemical studies

In the brain and spinal cord of both patients, HIV gag antigens p17 and p24 were identified immunohistochemically in macrophages and MGCs (Fig. 2B). The reaction product was confined to delicate cytoplasmatic structures which frequently showed a granular appearance. However, a large fraction of MGCs failed to exhibit immunoreactivity with the anti-gag antibodies. Within the inflammatory foci, few cells showed expression of LCA. A considerable proliferation of GFAP-positive reactive astrocytes was found adjacent to these lesions. Immunohistochemical reactions with antibodies to *Toxoplasma gondii,* CMV, herpes virus type I and II and papova virus failed to reveal opportunistic infectious agents. Fungal or mycobacterial infections were excluded by negative Grocott and Ziehl-Neelsen staining reactions.

In situ hybridization and double labeling

HIV env/nef and gag RNAs were identified in brain and spinal cord of both patients by in situ hybridization (Figs. 1, 2). Of the two antisense probes used, the env/nef construct yielded a consistently stronger hybridization signal. However, we could not detect significant differences in the distribution of viral env/nef and gag RNAs. Maximal levels of viral RNA were concentrated in macrophages and MGCs within the necrotizing lesions (Fig. 1C). HIV-specific RNA was also abundant in scattered inflammatory foci of the gray and white matter of brain and spinal cord (Fig. 2A, D). In contrast to the immunohistochemical findings, a hybridization signal was readily detectable in the majority of the monocytic and microglial cells and of multinucleated giant cells within affected areas. Double-labeling studies with the PG-M1 macrophage-specific antibody and subsequent in situ hybridization for HIV RNA confirmed the monocytic nature of the infected cells (Fig. 2C, E, F). We did not find evidence for HIV infection of neurons, astrocytes, oligodendrocytes, ependymal or endothelial cells.

The spinal cord of the first patient showed a remarkable distribution of HIV RNAs. The HIV-positive foci were predominantly localized in superficial, subpial areas of the cord (Fig. 3A) with few affected macrophages in the meninges and spinal nerve roots. In the spinal cord of the second patient, foci of HIV-infected cells were evenly distributed throughout the gray and white matter (Fig. 3B), but were also detectable in superficial structures (Fig. 2C). Occasionally, labeled cells with microglial morphology were observed in close association with spinal neurons (Fig. 2F).

The cerebral lymphoma (case 1) contained a distinct population of multinucleated histiocytic giant cells. We examined the tumor tissue for expression of HIV RNA. High levels of viral RNA were seen in the majority of these giant cells (Fig. 1F). Combined in situ hybridization and immunohistochemical analysis confirmed the histiocytic nature of the multinucleated cells. There was

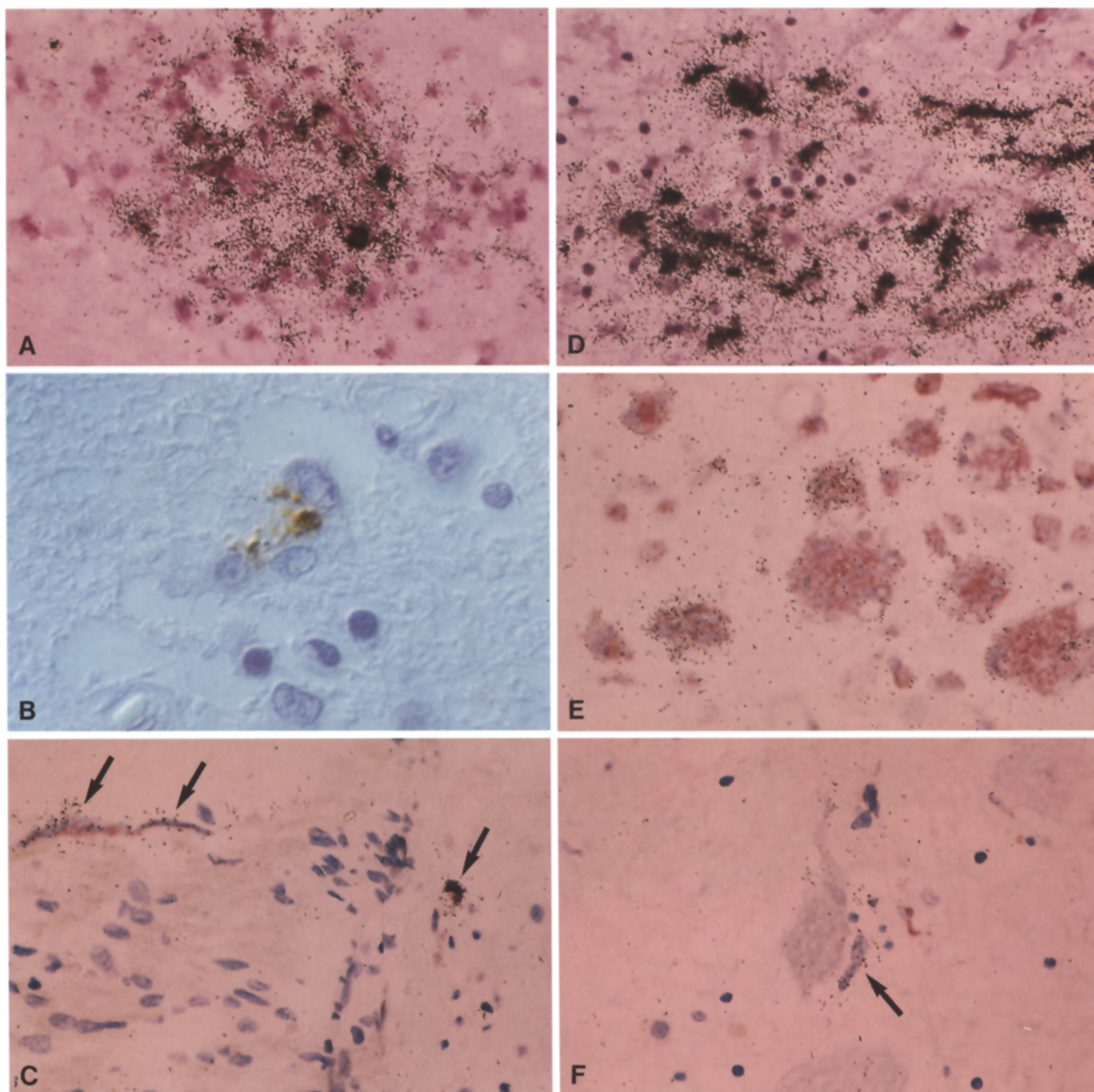

Fig. 2 A–F. In situ hybridization and immunohistochemical analysis. **A, D** Abundant HIV RNA within inflammatory foci of the cerebral cortex (**A**) and the spinal cord (**D**) of patient 2. **B** Occasional multinucleated giant cells show immunoreactivity with a monoclonal antibody to HIV gag p24 protein (patient 1). **C, E, F** Combined immunohistochemical and in situ hybridization reaction with the monocyte-specific antibody PG-M1 and a ribonucleotide probe complementary to a segment of the HIV env/nef genes. Double labeling is detectable in multinucleated giant cells (**E**; patient 1) and in cells with microglial morphology (**C**; patient 2). **C** shows double-labeled mononuclear cells in a spinal nerve root *(arrows)*. **F** HIV RNA in a satellite cell with microglial morphology *(arrow)*. Note the absence of viral RNA in the adjacent spinal neuron (patient 2) **A, C–F** × 330; **B** × 1000

no evidence for HIV infection of the neoplastic cell population.

No autoradiographic signal was detected after hybridization of biopsy specimens obtained from seronegative individuals or hybridization of HIV-infected brain tissue with RNA sense probes.

PCR-mediated amplification of proviral HIV sequences

A pair of conserved oligonucleotide primers was used to amplify a 109-bp fragment of the HIV-1 gag gene. An amplification product of the expected size was obtained

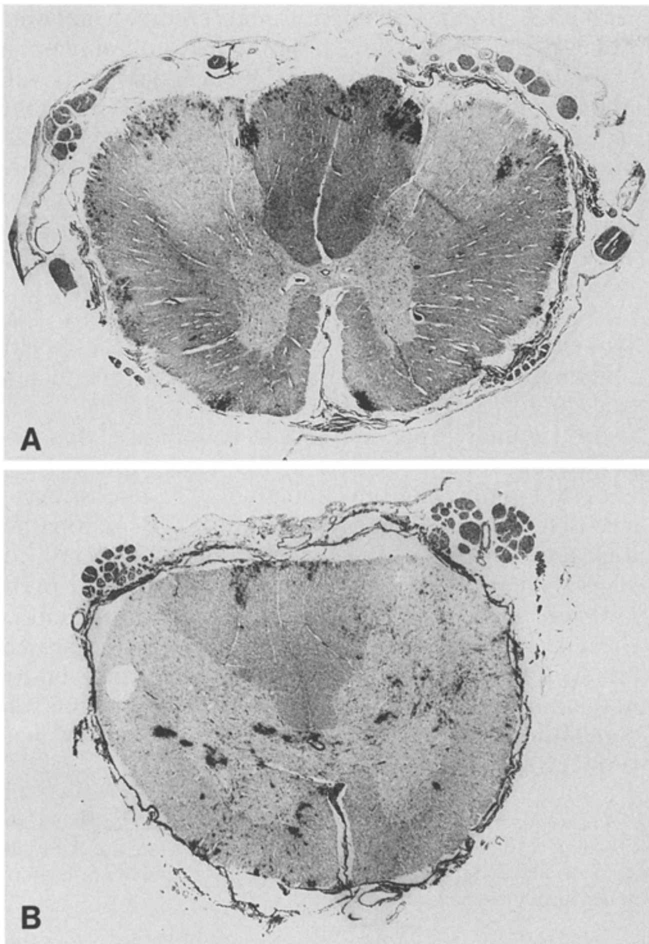

Fig. 3 A, B. In situ hybridization analysis of HIV env/nef RNA in the spinal cord. **A** Note the predominantly superficial localization of the foci in patient 1. **B** The spinal cord of patient 2 shows a more uniform distribution of HIV RNA in both gray and white matter. Occasional HIV transcripts which are not readily visible at this magnification were also detected in the spinal leptomeninges and spinal nerves of both patients. **A, B** × 7

with DNA extracted from frontal lobe tissue of the two patients and from HIV-1-infected H9 cells but not with cerebral DNA of a healthy individual. We confirmed the specificity of the PCR product by Southern transfer and hybridization with an oligonucleotide probe complementary to a segment of the amplified gag sequence (Fig. 4).

Discussion

The limited number of documented pediatric AIDS cases indicates that HIV-infected children are prone to develop HIV encephalopathies and myelopathies but rarely present with opportunistic CNS infections [1, 12, 24]. An important feature of the two cases described in this report is the unusual severity and distribution of HIV-associated CNS damage with the appearance of large necrotizing cortical and subcortical lesions. Such variants of HIV encephalopathies are not usually found in the brains of adult patients [26, 27]. However, Giangaspero et al. [16] have recently described a 4.5-year-old child who showed HIV encephalopathy with massive destruction of the cerebral cortex. These findings suggest that the developing brain exhibits a particular susceptibility to HIV encephalopathies. A similar observation was made in rhesus monkeys infected with the HIV-related lentivirus simian immunodeficiency virus (SIV) [40]. While adult animals are only mildly affected, juvenile monkeys develop encephalopathies which mimic important features of HIV encephalopathies in children.

In the brain and spinal cord of both patients HIV gag and env/nef RNAs were present in large amounts. This was surprising because the hybridization reactions had to be carried out on formalin-fixed postmortem tissue processed without prior inactivation of RNases. Although it remains to be determined if the in situ hybridization signals result from virus replication or from selective expression of HIV genes, the abundance of HIV RNAs in the CNS of our patients would support an active role of HIV gene products in the pathogenesis of HIV encephalopathies and myelopathies. Potential candidates for neurotoxic products of the HIV genome

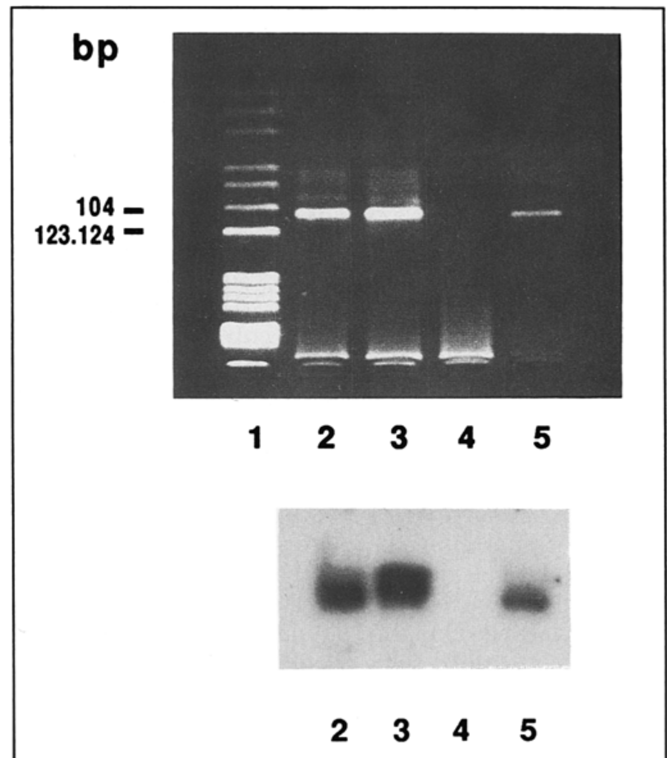

Fig. 4. Polymerase chain reaction-mediated amplification of HIV gag sequences. The *upper panel* shows an ethidium bromide-stained agarose gel after electrophoretic separation of marker DNA *(lane 1)* and amplification products with cerebral DNA of patients 1 and 2 *(lane 2* and *3),* of a HIV-seronegative individual *(lane 4)* and with DNA from HIV-infected H9 cells *(lane 5,* positive control). Note the 109-base pair gag amplification product in *lanes 2, 3* and *5.* The *lower panel* shows a Southern analysis of the same samples following capillary transfer onto nylon membrane and hybridization with an oligonucleotide probe specific for the amplified HIV gag sequence

include the gp 120 protein, which exerts calcium-mediated cytotoxicity on neuronal cultures [5, 13], and the tat protein, which is known to cause lethal neurotoxicity in mice upon intracerebral injection [18, 37].

Interestingly, the detection of HIV RNAs in the brains of these two children was virtually restricted to areas with histopathological evidence for HIV-associated tissue damage, suggesting that the development of HIV encephalopathies is closely associated with the presence and replication of HIV in the affected areas of the CNS. This finding extends the results of our recent study using PCR-mediated DNA amplification to identify proviral HIV gag sequences in postmortem brain tissue of adult AIDS patients (Leib SL et al., submitted for publication). In this study, HIV sequences were only detectable in the CNS of patients with histopathologically proven HIV encephalopathies but not in brain tissue of seropositive individuals withouth intracerebral lesions or of patients with opportunistic CNS infections.

The high number of HIV-infected monocytes and macrophages within the inflammatory foci and the absence of HIV RNA in neural and endothelial cells of both patients corroborates previous reports of the monocyte lineage as the major reservoir of HIV in the brain [6, 7, 25, 43]. A striking perivascular accumulation of HIV-infected mononuclear and multinucleated cells in both brains is compatible with the Trojan horse hypothesis of monocytes/macrophages carrying HIV into the brain. Secretory products of activated macrophages and MGCs have been implicated in the pathogenesis of HIV encephalopathies. Tumor necrosis factor (TNF), a cytokine secreted by activated monocytic cells, has a potential to destroy oligodendrocytes in vitro [39]. Preliminary attempts to identify TNF or TNF mRNA in children and adult patients with HIV encephalopathies have been unsuccessful (data not shown). Recently, Giulian et al. [17] have isolated a small neurotoxic molecule from HIV-1-infected mononuclear phagocytes. This factor was shown to activate excitatory *N*-methyl-D-aspartate (NMDA) receptors. It remains to be determined whether this molecule is also produced in affected brains in vivo.

The spinal cord of the first patient provided an interesting clue to potential pathways of HIV spread within the CNS. In situ hybridization revealed a striking superficial and subpial accumulation of HIV RNA in the cord. This pattern suggests that HIV or HIV-infected monocytic cells have reached the spinal cord via the cerebrospinal fluid. Although both children showed severe and widespread HIV myelitis, vacuolar degeneration of spinal fiber tracts, i.e., a hallmark of HIV myelopathy in adult AIDS patients, was absent. Several authors have pointed out that vacuolar myelopathy is very rare in pediatric AIDS [11, 24, 41]. This may reflect distinct pathogenetic mechanisms or a different susceptibility of the developing and the adult spinal cord to HIV-associated damage.

The unusual finding of HIV-infected MGCs within the CNS lymphoma of the first patient corroborates a similar observation by Gray et al. [19] in an adult patient with both HIV encephalopathy and cerebral lymphoma. Mizusawa et al. [33] have described a cerebral lymphoma immediately adjacent to HIV encephalopathy with MGCs in the brain of a patient with AIDS. Occasional reports of HIV-infected MGCs in extracerebral lymphomas [20] indicate that this phenomenon is not restricted to cerebral lymphomas coexisting with HIV encephalopathy. Epstein-Barr virus (EBV) has been associated with the pathogenesis of non-Hodgkin B cell lymphomas [4, 8, 35, 36], which represent the most common CNS mass lesion in pediatric AIDS patients [12]. However, we have not been able to identify EBV-specific sequences in the cerebral lymphoma of our first patient (data not shown).

In summary, our observations indicate that the developing human CNS is highly susceptible to HIV encephalopathies and myelopathies. The almost exclusive detection of HIV RNAs in areas with histopathological evidence for HIV encephalitis and HIV myelitis suggests that the presence and replication of HIV in the CNS are closely associated with the development of these lesions. Liquorigenic distribution may represent one pathway of viral spread in the CNS. The distribution and abundance of HIV RNAs in our patients are compatible with a neurotoxic potential of HIV gene products.

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