American Journal of Pathology, Vol. 139, No. 3, September 1991

Localization of Simian Immunodeficiency Virus in the Central Nervous System of Rhesus Monkeys

Andrew A. Lackner,*† Mary O. Smith,* Robert J. Munn,‡ Dale J. Martfeld,‖ Murray B. Gardner,‡ Preston A. Marx,* and Satya Dandekar‖

From the California Regional Primate Research Center, and the Departments of Veterinary Pathology,† Medical Pathology,‡ and Internal Medicine,‖ University of California, Davis, California*

Simian immunodeficiency virus (SIV), like the human immunodeficiency virus (HIV), is a lentivirus that is both immunosuppressive and neurovirulent. Rhesus macaques (*Macaca mulatta*) inoculated with SIV often develop a giant cell encephalitis similar to that seen in humans infected with HIV. The authors examined SIV expression by immunohistochemistry and RNA* in situ *hybridization in the cerebrum, cerebellum, choroid plexus, and spinal cord from five macaques with and two macaques without giant cell encephalitis. Selected portions of the central nervous system (CNS) also were examined by electron microscopy. Simian immunodeficiency virus was detected in the CNS of all seven monkeys whether or not they had giant cell encephalitis. Both SIV antigen and RNA were present in all levels of the CNS examined. Macrophage/giant cell lesions always contained viral RNA and antigen and were the only sites where viral particles were detected by electron microscopy. However, SIV antigen and RNA also were commonly associated with small vessels, the choroid plexus, and meninges; these were the only locations where virus was detected in animals without giant cell encephalitis. Immunophenotyping showed that the cellular infiltrates consisted primarily of monocyte/macrophages and occasional CD8-positive T cells. Macrophages and T cells also were present in the stroma of the choroid plexus and were intimately associated with vessels in the CNS of SIV-infected but not uninfected macaques. Simian immunodeficiency virus infection of the macaque CNS provides an excellent model for studying the pathogenesis, treatment, and prevention of HIV-1-encephalitis. (Am J Pathol 1991, 139:609–621)

Infection with human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS), often leads to neurologic disease.[1,2] At autopsy, about 50% of AIDS patients have subacute encephalitis (also called HIV-1-encephalitis or AIDS encephalopathy), which is the most common form of neurologic disease in AIDS patients.[1,3–5] Histologically this encephalitis is characterized by microglial nodules, multinucleated giant cells, white matter pallor, and gliosis.[5–7] In the spinal cord, a distinct pathologic process termed vacuolar myelopathy is present in about 25% of AIDS autopsies.[8] Human immunodeficiency virus type 1 has been demonstrated in the brain and spinal cord of AIDS patients using a variety of techniques,[9–17] and some authors[18] report a direct correlation between the amount of HIV-1 expression and extent of clinical and pathologic disease, although this is controversial.[6] Viral expression in central nervous system (CNS) tissues has been localized primarily to macrophages,[11–13,16,17,19] with less convincing evidence of infrequent infection of astrocytes, oligodendrogliocytes, endothelial cells, and possibly neurons.[13,19] The pathogenesis of AIDS-associated neurologic disease, however, is not well understood. A relevant animal model would help in elucidating the pathogenesis of HIV-1–associated neurologic disease.

The simian immunodeficiency viruses (SIV) are the closest known relatives of HIV-1 and HIV-2. They closely parallel their human counterparts in genetic organization and biologic properties.[20–24] Similar to HIV, SIV has lentiviral morphology,[25,26] extraregulatory genes,[21–24,27] uses the CD4 molecule as its cellular receptor,[28] infects CD4-positive monocyte/macrophages and lympho-

Supported by Public Health Service grants NF27338 from the National Institute of Neurologic Disorders and Stroke, RR00169 from the National Center for Research Resources, National Institutes of Health, AI62559 from the National Institute of Allergy and Infectious Diseases, and contract FOD-0629 from the National Cancer Institute. A. Lackner was supported by Public Health Service Special Emphasis Research Career Award in Laboratory Animal Science from the National Center for Research Resources, RR00039.

Accepted for publication May 15, 1991.

Address reprint requests to Dr Andrew A. Lackner, New Mexico Regional Primate Research Lab, Building 1264, Holloman AFB, Holloman, New Mexico 88330-1027.

cytes,[28–30] and causes an acquired immune deficiency syndrome in virtually all rhesus monkeys (*Macaca mulatta*) infected.[25,26,31–33] Neurologic disease is a common feature of simian AIDS in macaques infected with SIV, as in humans infected with HIV-1.[25,31,32,34,35] At necropsy, approximately 50% of rhesus macaques inoculated with SIV from either rhesus macaques (SIVmac) or sooty mangabeys (*Cercocebus atys*) (SIVsm) have a giant cell encephalitis characterized by multifocal, perivascular aggregates of macrophages and multinucleated giant cells that occur at all levels of the CNS. These aggregates often extend into the adjacent parenchyma, causing tissue destruction. The most common portion of the CNS to be involved is the cerebral white matter. The more diffuse involvement of the CNS in SIV infection, including involvement of the cerebral cortex, and a lower incidence of opportunistic infections, more closely resembles HIV infection in the nervous system of infants and children than in adult AIDS patients.[35] The overall genetic and biologic similarities between SIV and HIV, however, both *in vivo* and *in vitro,* suggest that the SIV-macaque model is suitable for studies of the mechanisms of HIV-induced neurologic disease in humans.

To investigate the expression of SIV in the CNS, we used immunohistochemistry and *in situ* hybridization to localize viral antigen and RNA, respectively, in the brain and spinal cord from five rhesus macaques with and two without giant cell encephalitis. Selected portions of the CNS from two animals also were examined by electron microscopy (EM). The immunophenotype of the cellular infiltrates in the CNS of these animals also was determined. We found that, in all animals, SIV was present in macrophage/giant cell lesions. Virus also was commonly associated with capillaries in the CNS of animals both with and without neurologic lesions. The immunophenotype of the associated cellular infiltrate was predominantly monocyte/macrophage, with lesser numbers of CD8-positive T cells.

Materials and Methods

Animals and Tissues

Seven rhesus macaques that had been inoculated with SIV were selected for this study based on histologic evaluation of their central nervous systems (Table 1). Five (16417, 21866, 21891, 22704, 23612) of the seven animals had a characteristic SIV-associated giant cell encephalitis,[31,34,35] with involvement of the spinal cord in two animals (16417, 21866). This lesion was characterized by multifocal, perivascular infiltrates of mononuclear cells and multinucleated giant cells, primarily in the white matter. Two of the animals (16417, 21866) had clinical neurologic signs and severe lesions with large areas of tissue destruction. Four of the seven animals (16417, 21866, 22657, 22704) were inoculated with SIVsm obtained from a captive sooty mangabey,[36] and three (21891, 21943, 23612) were inoculated with SIVmac provided by Dr. Ronald Desrosiers of the New England Regional Primate Research Center.[25,26] One animal in each group (22657 and 21943) lacked histologic evidence of giant cell encephalitis (Table 1). Only one of the seven animals (22704) had a persistent humoral immune response to SIV as determined by immunoblot.

All animals were killed when moribund with simian AIDS, and sections of cerebrum (parietal cortex), cerebellum, choroid plexus, spinal cord (segments C4–5 and L5–6) and spleen were collected and immediately snap frozen in liquid nitrogen-cooled freon and stored at −70°C. An adjacent block of tissue was fixed in 10% neutral buffered formalin. Adjacent cryostat sections, 6 μ thick, were examined by immunohistochemistry for viral antigen and immunophenotype of cellular infiltrates. Formalin-fixed tissues were embedded in paraffin, sectioned at 6 μ, and processed for *in situ* hybridization to detect SIV RNA. Formalin-fixed tissues were not used for immunohistochemistry because of poor antigen preservation. Matched tissues from two rhesus monkeys from the type D retrovirus-free and SIV-free colony at the California Regional Primate Research Center (CRPRC) were collected as uninfected controls. An additional control consisted of matched tissues from an SIV-negative rhesus macaque that died of immunosuppression subsequent to infection with type D retrovirus serotype 1 (formerly SRV-1).[37,38]

Viral Inocula

Stocks of SIVsm and SIVmac for inoculation were prepared as previously described.[39,40] All animals were inoculated intravenously with 10^3 to 10^4 intravenous 100% animal infectious doses, except for 21943, which was inoculated via the urethral mucosa with 10^4 intravenous animal infectious doses, three times over a 3-week period.[39] The disease resulting from intravenous or mucosal inoculation with SIVmac or SIVsm is indistinguishable, except that mucosal inoculation requires a higher dose and generally results in a more prolonged disease course.[39] In this group of animals, the time from inoculation to death ranged from 96 to 384 days, with a mean of 165 days.

Isolation of SIV

Four of the seven animals were viremic within 10 days, and all were viremic within 31 days after inoculation and remained persistently viremic. Infectious SIV was isolated

Table 1. *Pathologic and Viral Status of SIV-infected Macaques Grouped by Inoculum*

Animal	Age* (months)	First SIV isolation† (days)	Survival‡ (days)	Necropsy findings	Neuropathologic findings
SIVsm					
16417	145	9	127	Giant cell pneumonia and lymphadenitis; Intestinal amyloidosis	Severe‖ giant cell encephalitis and leukomyelitis; focal CMV meningitis
21866	49	5	144	Giant cell pneumonia and gastritis; Glomerulonephropathy;	Severe giant cell encephalitis and mild giant cell myelitis
22657	21	8	102	Gastroenterocolitis; CMV pericholecystitis	None
22704	19	30	384	Giant cell pneumonia; adenoviral pancreatitis; gastric trichomoniasis	Moderate giant cell encephalitis
SIVmac					
21891	43	29	127	Giant cell pneumonia; Glomerulonephropathy	Moderate giant cell encephalitis, CMV Peripheral neuritis
21943	41	31	176	Giant cell pneumonia, enteritis and lymphadenitis; glomerulonephropathy; disseminated CMV	CMV meningitis, mild
23612	11	5	96	Oral candidiasis; cryptosporidiosis, bile ducts and intestine; glomerulonephropathy; CMV interstitial pneumonia	Mild giant cell encephalitis
Controls					
3382	NA	NA	NA	None	None
20448	NA	NA	NA	None	None
19276	NA	NA	NA	Enterocolitis, chronic with villous atrophy; pulmonary acariasis	None

* Age at time of inoculation.

† Days after inoculation for initial isolation of SIV. Each virus-positive animal was persistently infected, as determined by multiple SIV isolations.

‡ Time from inoculation to euthanasia due to moribund condition.

‖ Mild, moderate, and severe were defined as follows: mild, 1 to 10 giant cell lesions/section; moderate, 10 to 20 giant cell lesions/section; Severe, more than 20 giant cell lesions/section and extensive tissue destruction. Both animals with severe encephalitis had clinical neurologic signs before euthanasia.

¶ Spontaneously infected with type D retrovirus serotype 1. Time from initial viral isolation to euthanasia was 24 months.

CMV, cytomegalovirus; NA, not applicable.

from rhesus peripheral blood mononuclear cells as previously described.[36,39]

Electron Microscopy

Samples of cerebrum (parietal cortex) and spinal cord collected at necropsy were fixed in Karnovsky fixative[41] for examination by electron microscopy. Tissues were postfixed in 1% osmium tetroxide, serially dehydrated for infiltration, embedded in epoxy, and sectioned at 60 to 90 nm. Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM-400 electron microscope.

Localization of Viral Antigen by Immunohistochemistry

A monoclonal antibody (MAb) (IgG2a isotype) (HE3) specific for the major core protein (p27) of SIVmac[42] was used in an avidin-biotinylated peroxidase complex technique with either 3-amino-9-ethylcarbazole or diaminobenzidine as the chromogen.[43,44] This MAb cross-reacts with p27 of SIVsm.[42] Control procedures included omission of the primary antibody and substitution of an isotype-matched MAb of irrelevant specificity. Additional controls included matched tissues from two normal uninfected rhesus monkeys and one type D retrovirus-infected rhesus monkey.

Immunophenotype of Cellular Infiltrates in the CNS

The immunophenotype of the cellular infiltrates in the CNS was determined by immunohistochemistry using cell-specific monoclonal antibodies. T cells were identified using the pan T (CD2) MAb T11 (Coulter Corporation, Hialeah, FL) and subsets identified with the CD8-specific MAb *Leu2*a (Becton Dickinson, Mountain View, CA) and the CD4-specific MAb 19Thy5D7.[45] B cells and

tissue macrophages were identified with B1 (CD20, Coulter Corporation, Hialeah, FL) and EBM-11 (CD68, Dako Corporation, Carpinteria, CA), respectively. These cell-specific antibodies have previously been shown to cross-react with rhesus determinants.[46–48] In addition, expression of major histocompatability complex (MHC) class II antigens was examined using an MAb specific for rhesus MHC II, provided by Dr. Peter Moore.[49]

In Situ *Hybridization*

In situ hybridization was performed using a 35S-labeled SIV DNA probe on formalin-fixed, paraffin-embedded tissue sections by a modification of the procedure described by Haase and co-workers.[50] Radioactive probes were produced with a specific activity of 1×10^8 cpm/μg or greater by nick-translation of a 9-kb fragment of the SIVmac genome (obtained from P. Sonigo, Institute Pasteur), consisting of the entire gag, pol, and env regions.[22] This probe cross-reacts very well with the closely related SIVsm.[21] Controls included: 1) SIV-infected and uninfected cultured T-cells (HUT78); 2) matched tissues from two uninfected rhesus monkeys and one type D retrovirus-infected rhesus monkey; 3) pretreatment of sections with RNase to demonstrate that hybridization was to RNA; and 4) hybridization with a nick-translated probe containing only the pSP64 plasmid vector.

Paraffin-embedded tissues were cut at 6-μ thickness and placed on Histostik (Accurate Chemical and Scientific Corp, Westbury, NY) coated glass slides. Slides were deparaffinized, hydrated through graded alcohols, and treated for 15 minutes at 37°C with proteinase K (1 μg/ml in 50 mmol/l [millimolar] ethylenediaminetetra-acetic acid [EDTA] and 100 mmol/l TRIS-HCl, pH 7.4), rinsed in phosphate-buffered saline, placed in 0.2 mol/l (molar) TRIS/0.1 mol/l glycine for 15 minutes, dehydrated, and air dried. The hybridization solution contained 50% deionized formamide, 10% dextran sulfate, 50 mmol/l NaH_2PO_4, 0.6 mol/l NaCl, 0.5 mmol/l EDTA, 1 × Denhardt's solution, 75 μg/ml *Escherichia coli* tRNA, 100 μg/ml salmon sperm DNA, and 20 mmol/l dithiothreitol (DTT). ^{35}S-labeled DNA probe (containing the 9-kb fragment of SIV DNA in the pSP64 plasmid vector, or pSP64 alone, nick-translated to a specific activity of 1×10^8 cpm/μg or greater) was added at 1×10^6 cpm/20 ml of hybridization solution. Before the addition of hybridization salts and DTT, the mixture was heated at 95°C for 10 minutes, chilled on ice, and dispensed onto each specimen, which then was placed in a humidification chamber to hybridize overnight at 42°C. After removal of coverslips and washing with 50% deionized formamide/2 × standard saline citrate at 42°C, followed by three washes with 2 × standard saline citrate at room temperature, the slides were dehydrated in 70% and 95% ethanols containing 0.3 mol/l ammonium acetate and air dried. Autoradiography was performed with NTB2 emulsion (Eastman Kodak, Rochester, NY) diluted 1:1 with 0.6 mol/l ammonium acetate. The slides were exposed at 4°C for 3 to 14 days, developed with Kodak D-19, and counterstained with hematoxylin.

Results

Localization of Viral Antigen

All seven of the SIV-infected animals had viral antigen detected by immunohistochemistry in two or more levels of the CNS, regardless of the presence or absence of giant cell encephalitis (Table 2). Within the CNS, infiltrates of macrophages and multinucleated giant cells were consistently positive for SIV-p27 (Figure 1a); however, only approximately half of the giant cells in these lesions stained for viral antigen. Such antigen-positive lesions were present primarily in the white matter of the cerebral hemispheres in all five animals with giant cell encephalitis as well as in the cerebellum of four animals (16417,

Table 2. ***Tissue Localization of SIV p27 and SIV RNA in Rhesus Monkeys Infected with SIV***

	Result of immunohistochemistry for SIV p27					Result of *in situ* hybridization for SIV RNA			
Rhesus monkey	Cerebrum	Cerebellum	Choroid plexus	Spinal cord	Spleen	Cerebrum	Cerebellum	Choroid plexus	Spinal cord
With SIV encephalitis									
16417	+	−	+	+	+	+	+	NA	+
21866	+	+	−	+	+	+	+	+	+
21891	+	−	+	NA	+	+	+	NA	NA
22704	+	+	−	NA	+	+	+	+	NA
23612	+	+	+	+	+	+	+	+	+
Without SIV encephalitis									
21943	+	+	NA	NA	+	+	NA	+	NA
22657	+	+	+	NA	+	−	+	−	+

NA, not available.

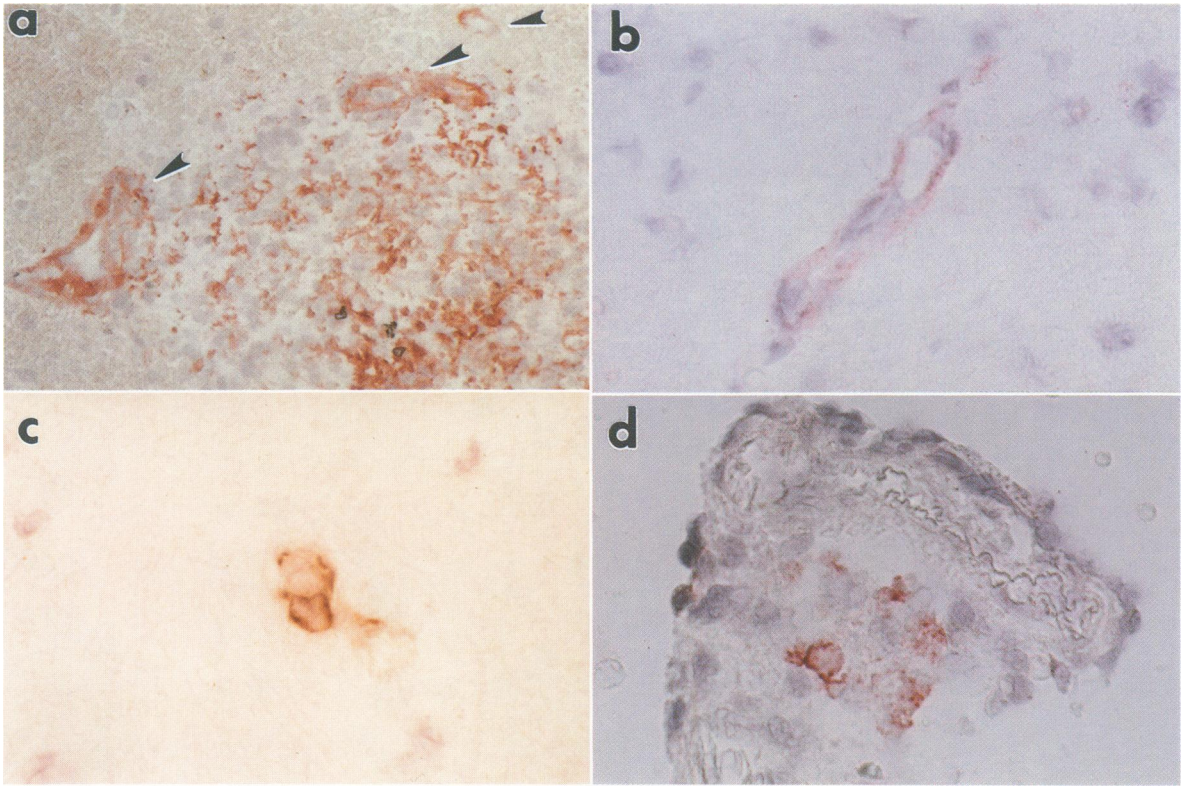

Figure 1. *Immunohistochemical localization of SIV-p27 in the central nervous systems of rhesus monkeys infected with simian immunodeficiency virus (SIV). Abundant viral antigen was present in cellular infiltrates of mononuclear cells and multinucleated giant cells* **(a)**. *Vessels associated with these lesions were often labeled for SIV-p27 (arrowheads). Other vessels not associated with cellular infiltrates were also positive for SIV-p27* **(b)**. *In addition, some SIV-p27–positive cells were identified in the brain parenchyma in areas without any demonstrable tissue damage* **(c)**. *In the choroid plexus scattered mononuclear cells were found that were positive for SIV* **(d)**. *Magnification:* **a**, ×*125;* **b**, ×*300;* **c**, ×*500;* **d**, ×*300.*

21866, 22704, 23612), and spinal cord of one animal (16417). No differences in the distribution of viral antigen in the CNS were noted between animals infected with SIVmac and SIVsm.

Although the distribution of viral antigen paralleled that of the infiltrates of mononuclear cells and multinucleated giant cells, viral antigen was not limited to these infiltrates. Viral antigen was commonly found associated with scattered capillaries and small vessels in the brain, spinal cord, meninges, and choroid plexus (Figure 1a, b). Less commonly, viral antigen was associated with individual cells or clusters of cells in these same tissues without any apparent association with vessels (Figure 1c). No staining was associated with any cell that morphologically appeared to be of neural origin.

In animals with giant cell encephalitis, vessels in the macrophage/giant cell lesions generally had staining adjacent to or within vessel walls (Figure 1a). In addition, occasional vessels not associated with lesions were also positive for viral antigen (Figure 1b). In animals without giant cell encephalitis (21943, 22657), viral antigen was limited to individual cells or clusters of cells associated with capillaries and small vessels in the parenchyma (primarily white matter) and meninges. Morphologically it appeared that viral antigen was associated with both endothelial cells and mononuclear cells in and around vessel walls and possibly perivascular microglia.

In the choroid plexus, viral antigen was detected in mononuclear cells in the plexus stroma (Figure 1d), but not in choroid plexus epithelial cells. The four animals with antigen in the choroid plexus (Table 2) included animals with and without giant cell encephalitis. No multinucleated giant cells were present in the frozen sections of choroid plexus. In adjacent blocks of tissue used for routine histology and *in situ* hybridization, however, two animals that were negative for viral antigen in the choroid plexus (21866, 22704) had individual multinucleated giant cells in the lateral ventricles, between fronds of the choroid plexus (Figure 2b, see below).

Although the presence of macrophage/giant cell lesions was directly correlated with the presence of viral antigen, the severity of the lesions was not predictive of widespread viral antigen. Thus the one animal with viral antigen detected in all sections of CNS examined (23612) had the least extensive lesions of the five animals with giant cell encephalitis.

Spleen from each of the animals was examined as a positive control. All animals had viral antigen localized to

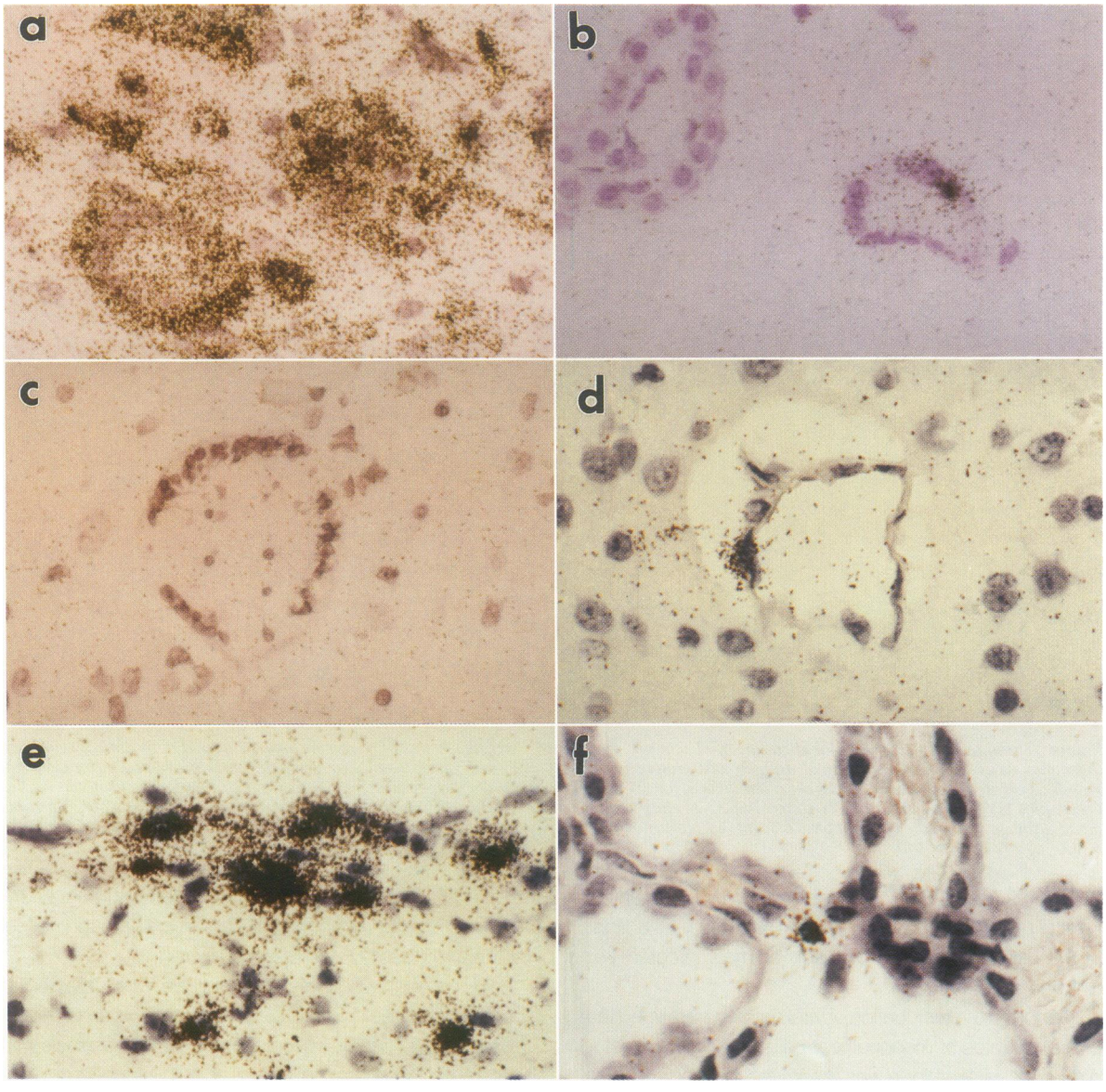

Figure 2. *Localization of SIV RNA by* in situ *hybridization in the CNS of SIV-infected rhesus monkeys. The macrophage/giant cell lesions in the CNS were consistently positive for SIV RNA, as they were for SIV-p27* **(a)**. *Scattered multinucleated giant cells had hybridization limited to a single nucleus* **(b)**. *This giant cell is present in the lateral ventricle of the brain between fronds of the choroid plexus. While most multinucleated giant cells hybridized for SIV RNA, many of them did not, as demonstrated by this giant cell in the brain of an animal with severe giant cell encephalitis* **(c)**. *Viral RNA was also commonly associated with vessels* **(d)**, *the meninges* **(e)**, *and the stroma of the choroid plexus* **(f)**. *The localization of SIV to vessels suggests that endothelial cells were infected or that virus-positive cells, such as macrophages or lymphocytes, were adherent to the endothelium or passing through the vessel wall. Magnification for all figures is ×300, except for* **f**, *which is ×450.*

germinal centers in a dendritic staining pattern (data not shown) consistent with the presence of viral antigen in or on follicular dendritic cells.[30] No staining was detected in matched tissues from two uninfected rhesus monkeys or a type D retrovirus-infected rhesus monkey.

Localization of Viral Nucleic Acid

All seven animals had SIV nucleic acid identified in two or more CNS tissues, including the two animals without neurologic lesions (Table 2). The distribution of SIV RNA-positive cells in the CNS detected by *in situ* hybridization correlated extremely well with the results of immunohistochemistry, but many more infected cells were identified by *in situ* hybridization (Table 2 and Figure 2). The macrophage/giant cell lesions in the CNS were consistently positive by *in situ* hybridization (Figure 2a), with occasional giant cells showing hybridization predominantly over one or two nuclei (Figure 2b). In addition to the macrophage/giant cell lesions in the brain and spinal cord, SIV RNA was commonly associated with scattered small

vessels throughout the CNS (Figure 2d), the meninges (Figure 2e), and choroid plexus (Figure 2b, f). In vessels, hybridization was present over mononuclear cells in the vessel walls, in the perivascular space, and over cells with the morphologic appearance of endothelial cells (Figure 2d). In the choroid plexus, SIV RNA was detected in individual mononuclear cells in the plexus stroma (Figure 2f) and in multinucleated giant cells (Figure 2b) trapped between fronds of the choroid plexus. Unique to *in situ* hybridization was localization of SIV to scattered rod-shaped cells in the parenchyma that were morphologically consistent with microglia, and to one perineuronal cell consistent with a satellite cell (data not shown). No labeling of neurons was detected in any animal. Matched tissues from uninfected and type D retrovirus-infected rhesus monkeys were uniformly negative. Pretreatment of SIV RNA-positive sections with RNase abolished hybridization. The results of *in situ* hybridization confirmed the localization of viral antigen and further demonstrated the wide distribution of virus in the CNS of macaques infected with SIV.

Ultrastructure

Electron microscopy was performed to determine whether the localization of SIV antigen and RNA correlated with productive infection. Morphologically, the cellular infiltrates in brain and spinal cord consisted primarily of vacuolated macrophages and multinucleated giant cells with small numbers of lymphocytes. The cellular infiltrate was intimately associated with the basal lamina of vessels, and many giant cells contained myelin inclusions. Viral particles were only identified within or near multinucleated giant cells, where they were generally present in large numbers (Figure 3). Not all multinucleated giant cells contained viral particles. When viral particles were present, they were within vesicles of smooth endoplasmic reticulum and small vacuoles. The particles had conical-shaped cores with eccentric nucleoids characteristic of lentiviruses.[51] In addition, many immature lentiviral particles and occasional budding particles were present (Figure 3). Thus the ultrastructural localization of viral particles was more limited than the distribution of viral antigen and RNA.

Immunophenotype of Cellular Infiltrates in the CNS

Immunohistochemistry with cell-specific monoclonal antibodies was performed to determine the phenotype of the cellular infiltrates in the CNS of the five animals with giant cell encephalitis. Immunophenotypic characterization of the infiltrates showed strong expression of rhesus MHC II (Figure 4a); approximately 90% to 95% of the cells (including the multinucleated cells) were monocyte/macrophages expressing the CD68 antigen identified by the MAb EBM-11 (Figure 4b). The remainder of the cells were T cells expressing the CD2 (T11) and CD8 (Leu 2a) antigens (Figure 4c, d). No cells expressing CD4 or CD20 (B1) were detected within cellular infiltrates in the brain.

Examination of the brain, spinal cord, and choroid plexus with these cellular markers demonstrated that the histologically discrete lesions were actually part of a larger, more diffuse infiltrate of CD68-positive macrophages extending into the adjacent neuropil (Figure 4b). In addition, numerous macrophages and lesser numbers of CD2 and CD8-positive lymphocytes were intimately associated with vessels (Figure 4d, e). Similar infiltrates were present in the meninges and stroma of the choroid plexus (Figure 4f). Rare B cells expressing B1 were identified in the stroma of the choroid plexus in two animals. Examination of matched CNS tissues from the two uninfected control monkeys disclosed no detectable B cells or T cells and only occasional perivascular macrophages.

Discussion

We have demonstrated SIV infection of the CNS in seven of seven rhesus macaques infected with SIV whether or not the animals had giant cell encephalitis. In animals with giant cell encephalitis, virus was always present in the discrete cellular infiltrates of macrophages and multinucleated giant cells, similar to earlier reports.[52] Thus there was a correlation between increasing numbers of macrophage/giant cell lesions and increasing amounts of viral antigen and nucleic acid. In addition to these discrete lesions, however, SIV RNA and antigen were also associated with the choroid plexus, meninges, and vessels. The age and sex of the animals, viral inoculum (SIVmac or SIVsm), antibody response in the peripheral blood, and length of survival had no apparent effect on the the distribution or level of SIV expression in the CNS. In animals without giant cell encephalitis, however, virus was limited to the meninges and small vessels. No evidence of infection of neural cells was detected in any animal.

In vessels, viral RNA and antigen were present in the vessel wall and in individual cells in the perivascular space. These virus-positive vessels were scattered throughout the CNS, in areas both with and without macrophage/giant cell lesions. The localization of lentiviruses to vessels in the CNS has previously been described in HIV-1–infected humans and in goats infected with caprine arthritis encephalitis virus (CAEV).[13,14,53] In both CAEV-infected goats and SIV-infected rhesus monkeys,

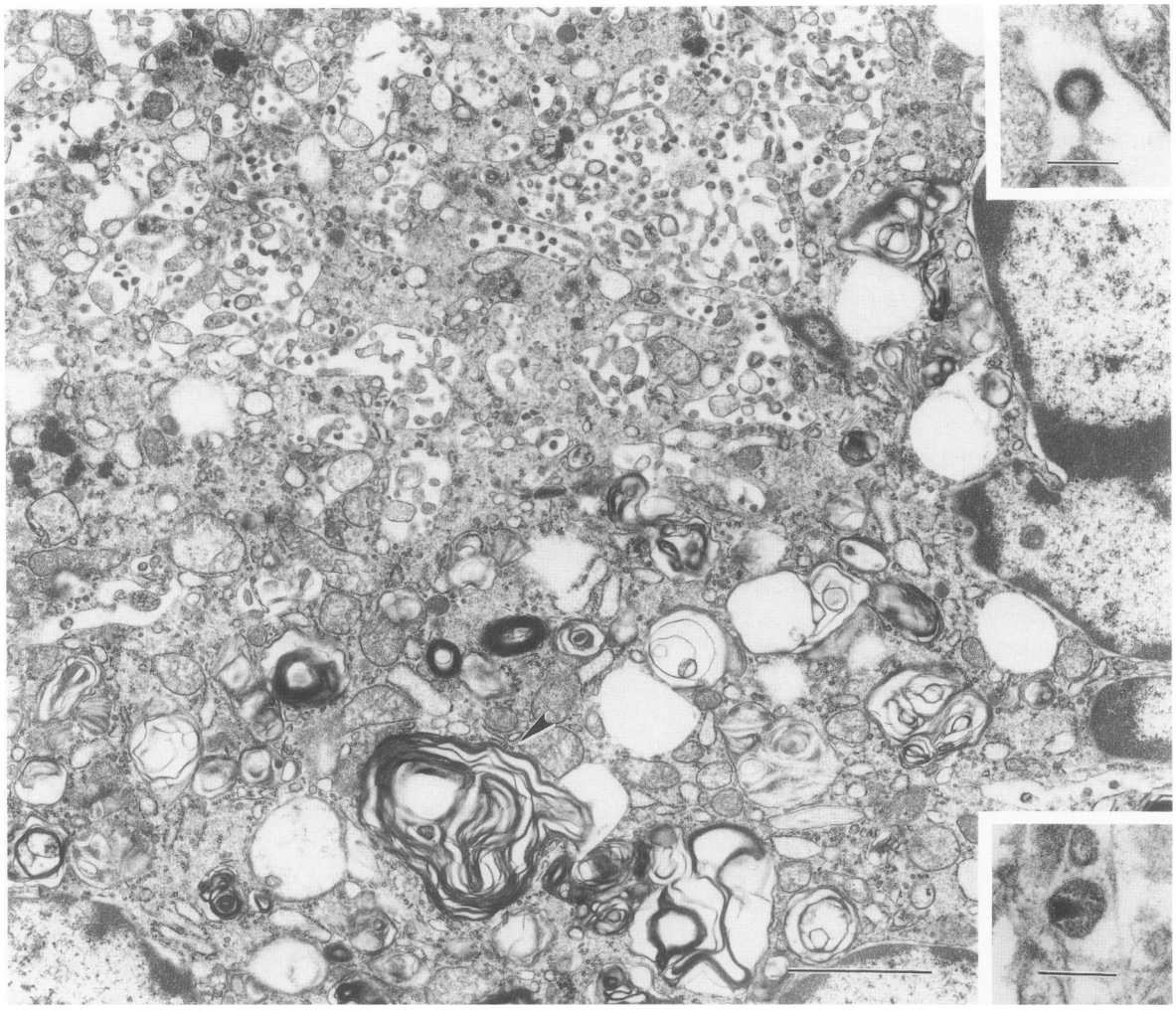

Figure 3. *Electron micrograph of a portion of a multinucleated giant cell in the CNS. Portions of several nuclei are present around the margins of the micrograph. The giant cell contains many viral particles within dilated endoplasmic reticulum and small vacuoles in the upper half of the micrograph. Myelin inclusions (arrowhead) are also present. The insets show a lentiviral bud (upper right) and a typical lentiviral particle with a conical core and eccentric nucleoid (lower right). All three electron micrographs are from the same giant cell. Bar = 2μm; in insets, bar = 0.2μm.*

lentiviral transcripts were associated with vessels in non-inflamed CNS. This suggests that lentiviral infection of vessels plays a key role in the pathogenesis of lentiviral-induced neurologic disease, as has been proposed in HIV-1-encephalitis.[13,14] The identity of the infected cells is, however, uncertain. In the SIV-infected macaques, it appeared that both endothelial cells and mononuclear cells in the vessel wall and perivascular space were positive. Immunohistochemical studies of the cell types associated with the vessels disclosed numerous CD68-positive monocyte/macrophages, with lesser numbers of CD8-positive T cells. Many of these cells within the vessel wall had flattened profiles that could easily be confused with endothelial cells. The intimate association of mononuclear cells with the basal lamina of vessels was seen also by electron microscopy. Double-label immunoelectron microscopy studies are needed to determine if both mononuclear cells and endothelial cells are infected or if what appears to be endothelial cell infection is actually infected mononuclear cells in transit through the vessel wall or possibly perivascular microglia.

The immunophenotype of the cells in the lesions was identical to that described in HIV-infected humans[54] and consisted primarily of macrophages and macrophage-derived multinucleated giant cells with lesser numbers of CD8-positive T cells. No CD4-positive T cells or B cells were identified in the lesions. The predominance of T cells of the CD8 phenotype raises the possibility that they may be mediating the tissue destruction, as has been proposed in HIV-infected humans[55] and sheep with visna.[56] In SIV-infected macaques with CNS lesions, we also found substantial numbers of monocyte/macrophages and CD8-positive T cells associated with vessels in the CNS and in the stroma of the choroid

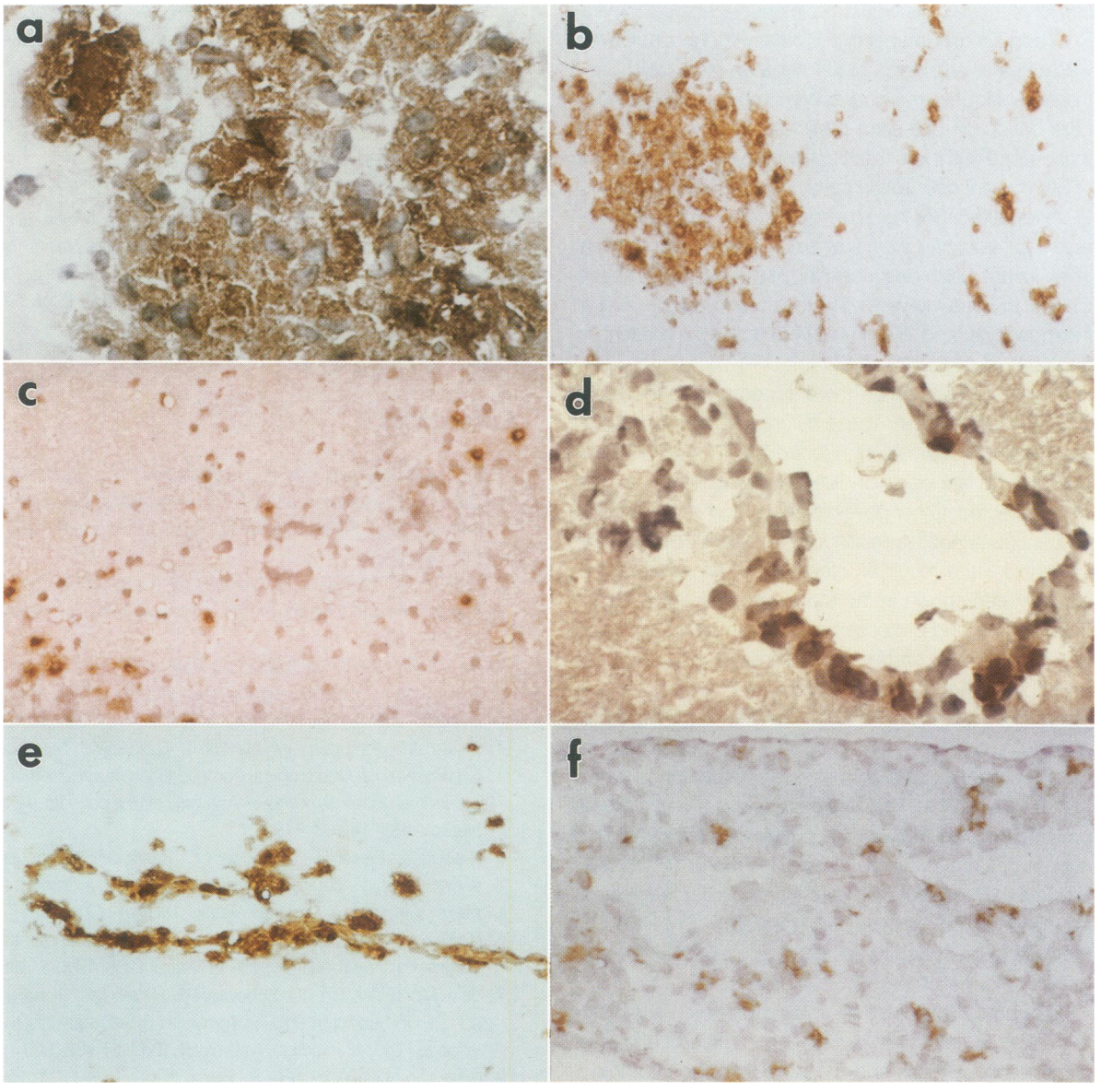

Figure 4. *Immunohistochemistry for rhesus MHC-II, macrophage, and lymphocyte markers revealed that the lesions in the CNS expressed abundant class II antigen* (a) *and were composed predominantly of CD68-positive monocyte/macrophages* (b) *and multinucleated giant cells. The latter photomicrograph* (b) *also shows that many macrophages were present in the parenchyma around the discrete cellular infiltrate. Lesser numbers of CD2-positive* (c) *and CD8-positive T cells* (d) *were present in lesions and around vessels. Many CD68-positive monocyte/macrophages were intimately associated with vessels* (e) *in the CNS and were also present in the stroma of the choroid plexus* (f). *Magnification:* a *and* d, *×300;* b, c, e, *and* f, *×125.*

plexus, locations where viral antigen and RNA were detected. These data suggest that the primary routes of CNS invasion by SIV are by vessels in the parenchyma and choroid plexus, with parenchymal vessels playing the major role because the majority of lesions in the CNS are perivascular. Although the meninges also contained many SIV-positive cells, it is less likely to be a major route of entry into the CNS because the adjacent cortical gray matter is rarely involved compared with cortical white matter.

Although multinucleated giant cells are considered the hallmark of HIV- and SIV-associated encephalitis, immunohistochemistry and *in situ* hybridization make it clear that viral infection and replication is much more extensive, and can take place without induction of giant cell syncytia. The converse also appears to be true, in that only a portion of the multinucleated giant cells contain SIV RNA, antigen, and particles. Considering that multinucleated giant cells are the only cells in the CNS in which lentiviral particles have been identified by electron microscopy, we assumed that, with the increased sensitivity of immunohistochemistry and *in situ* hybridization, virtu-

ally all of these cells would be intensely labeled for viral RNA and antigen, respectively. What we found, however, was a similar proportion of SIV negative giant cells by all three techniques, and increasing numbers of SIV-positive cells based on detecting particles, antigen, or RNA, in that order. Thus it is difficult to attribute the apparent lack of SIV in giant cells to the sensitivity of the techniques.

Alternatively SIV-negative giant cells may be associated with restricted viral expression at the transcriptional level, as has been described for visna virus in the sheep CNS.[57] In visna, however, the restriction is at the level of the monocyte, and little viral RNA or antigen is present. In contrast, in the CNS of SIV-infected macaques, and HIV-1 infected humans,[12] there appears to be abundant viral RNA and protein. These differences may be due to the immune suppression induced by SIV and HIV-1 or possibly the late stage of disease at which the samples were collected. Other possible explanations for SIV RNA and antigen-negative multinucleated giant cells include: 1) they are the result of infection by defective virions, as has been shown in the monocytic cell line U-937[58]; 2) nucleases and proteases may cause the degradation of viral RNA and protein; or 3) some of the giant cells reflect a host response to SIV-induced tissue damage rather than a direct effect of viral infection of macrophages.

Interestingly scattered multinucleated giant cells had hybridization for SIV RNA limited to one or two nuclei. The presence of only a few nuclei containing SIV RNA within a giant cell has previously been described.[59] This may indicate CD4-gp120–mediated fusion of infected and uninfected cells[60,61] with limited diffusion of SIV RNA into the cytoplasm. It also raises the question of the effect of giant cell formation on SIV RNA processing.

The pathogenesis of neurologic disease associated with HIV-1 is thought to involve the combined effects of immune suppression and monocyte/macrophage infection in the brain with local activation of these cells and release of cytokines.[1] In addition, the production and accumulation of potentially toxic substances such as unintegrated HIV-1 DNA, HIV-1-gp120, HIV-1 *tat*, and quinolinic acid in the CNS also may play a role.[62–66] This is also likely to be true in SIV-infected macaques.

This study reaffirms the relevance of the SIV-macaque model for studying AIDS in humans. The neurologic lesions, immunophenotype of the cells involved and distribution of SIV in the CNS are very similar to those described for HIV-1-encephalitis, particularly as it occurs in infants and children.[35] The clinical signs associated with the infection are, however, poorly described. Future work will have to include careful neurobehavioral studies if this deficiency of the model is to be addressed. Despite this problem, the model holds great potential for performing careful time-course studies, and the availability of pathogenic and nonpathogenic molecular clones of SIV[67,68] will enable careful study of the viral determinants of neuropathogenesis.

Acknowledgments

The authors thank Judy Walls, Ibsen Chen, Linda Antipa, and Steve Joye for expert technical assistance; Barbara Weiser for helpful discussions; Linda Lowenstine and Myra Jennings for SIVsm; and Ronald Desrosiers of the New England Regional Primate Research Center for SIVmac.

References

1. Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P: The brain in AIDS: Central nervous system HIV-1 infection and AIDS dementia complex. Science 1988, 239:586–592
2. Johnson RT, McArthur JC, Narayan O: The neurobiology of human immunodeficiency virus infections. FASEB J 1988, 2:2970–2981
3. Ho DD, Pomerantz RJ, Kaplan JC: Pathogenesis of infection with human immunodeficiency virus. N Engl J Med 1987, 317:278–286
4. Snider WD, Simpson DM, Nielson S, Gold JWM, Metroka CE, Posner JB: Neurological complications of acquired immune deficiency syndrome: Analysis of 50 patients. Ann Neurol 1983, 14:403–418
5. Nielson SL, Petito CK, Urmacher CD, Posner JB: Subacute encephalitis in acquired immune deficiency syndrome: A postmortem study. Am J Clin Pathol 1984, 82:678–682
6. Navia BA, Cho ES, Petito CK, Price RW: The AIDS dementia complex: II. Neuropathology. Ann Neurol 1986, 19:525–535
7. de la Monte SM, Ho DD, Schooley RT, Hirsch MS, Richardson EP Jr: Subacute encephalomyelitis of AIDS and its relation to HTLV-III infection. Neurology 1987, 37:562–569
8. Petito CK, Navia BA, Cho E-S, Jordan BD, George DC, Price RW: Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immunodeficiency syndrome. N Engl J Med 1985, 312:874–879
9. Ho DD, Rota TR, Schooley RT, Kaplan JC, Allan JD, Groopman JE, Resnick L, Felsenstein D, Andrews CA, Hirsch MS: Isolation of HTLV-III from cerebrospinal fluid and neural tissues of patients with neurologic syndromes related to the acquired immunodeficiency syndrome. N Engl J Med 1985, 313:1493–1497
10. Shaw GM, Harper ME, Hahn BH, Epstein LG, Gajdusek DC, Price RW, Navia BA, Petito CK, O'Hara CJ, Cho E-S, Oleske JM, Wong-Staal F, Gallo RC: HTLV-III infection in brains of children and adults with AIDS encephalopathy. Science 1985, 227:177–182
11. Koenig S, Gendelman HE, Orenstein JM, Dal Canto MC, Pezeshkpour GH, Yungbluth P, Janotta F, Aksamit A, Martin M, Fauci AS: Detection of AIDS virus in macrophages in

brain tissue from AIDS patients with encephalopathy. Science 1986, 233:1089–1093

12. Vazeux R, Brousse N, Jarry A, Henin D, Marche C, Vedrenne C, Mikol J, Wolff M, Michon C, Rozenbaum W, Bureau JF, Montagnier L, Brahic M: AIDS subacute encephalitis: Identification of HIV-infected cells. Am J Pathol 1987, 126:403–410
13. Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA: Cellular localization of human immunodeficiency virus infection within the brains of acquired immunodeficiency syndrome patients. Proc Natl Acad Sci USA 1986, 83:7089–7093
14. Gabuzda DH, Ho DD, de la Monte S, Hirsch MS, Rota TR, Sobel RA: Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. Ann Neurol 1986, 20:289–295
15. Orenstein JM, Janotta F: Human immunodeficiency virus and papovavirus infections in acquired immunodeficiency syndrome: An ultrastructural study of three cases. Hum Pathol 1988, 19:350–361
16. Eilbott DJ, Peress N, Burger H, LaNeve D, Orenstein J, Gendelman HE, Seidman R, Weiser B: Human immunodeficiency virus type 1 in spinal cords of acquired immunodeficiency syndrome patients with myelopathy: Expression and replication in macrophages. Proc Natl Acad Sci USA 1989, 86:3337–3341
17. Maier H, Budka H, Lassmann H, Pohl P: Vacuolar myelopathy with multinucleated giant cells in the acquired immune deficiency syndrome (AIDS). Acta Neuropathol 1989, 78:497–503
18. Weiser B, Peress N, LaNeve D, Eilbott DJ, Seidman R, Burger H: Human immunodeficiency virus type 1 expression in the central nervous system correlates directly with extent of disease. Proc Natl Acad Sci USA 1990, 87:3997–4001
19. Stoler MH, Eskin TA, Benn S, Angerer RC, Angerer LM: Human T-cell lymphotropic virus type III infection of the central nervous system: A preliminary in situ analysis. J Am Med Assoc 1986, 256:2360–2364
20. Gardner MB, Luciw PA: Simian immunodeficiency viruses and their relationship to the human immunodeficiency viruses. AIDS 1988, 2 (suppl):S3–S10
21. Hirsch VM, Olmsted RA, Murphey-Corb M, Purcell RH, Johnson PR: An African primate lentivirus (SIV_{sm}) closely related to HIV-2. Nature 1989, 339:389–392
22. Chakrabarti L, Guyader M, Alizon M, Daniel MD, Desrosiers RC, Tiollais P, Sonigo P: Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature 1987, 328:543–547
23. Kestler HW, Li Y, Naidu YM, Butler CV, Ochs MF, Jaenel G, King NW, Daniel MD, Desrosiers RC: Comparison of simian immunodeficiency virus isolates. Nature 1988, 331:619–622
24. Franchini G, Gurgo C, Guo HG, Gallo RC, Collalti E, Fargnoli KA, Hall LF, Wong-Staal F, Reitz MSJ: Sequence of simian immunodeficiency virus and its relationship to the human immunodeficiency viruses. Nature 1987, 328:539–543
25. Letvin NL, Daniel MD, Sehgal PK, Desrosiers RC, Hunt RD, Waldron LM, MacKey JJ, Schmidt DK, Chalifoux LV, King NW: Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III. Science 1985, 230:71–73
26. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, Kanki PJ, Essex M, Desrosiers RC: Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. Science 1985, 228:1201–1204
27. Fukasawa M, Miura T, Hasegawa A, Morikawa S, Tsujimoto H, Miki K, Kitamura T, Hayama M: Sequence of simian immunodeficiency virus from African green monkey, a new member of the HIV/SIV group. Nature 1988, 333:457–461
28. Kannagi M, Yetz JM, Letvin NL: In vitro growth characteristics of simian T-lymphotropic virus type III. Proc Natl Acad Sci USA 1985, 82:7053–7057
29. Ringler DJ, Wyand WS, Walsh DG, MacKey JJ, Sehgal PK, Daniel MD, Desrosiers RC, King NW: The productive infection of alveolar macrophages by simian immunodeficiency virus. J Med Primatol 1989, 18:217–226
30. Ringler DJ, Wyand MS, Walsh DG, MacKey JJ, Chalifoux LV, Popovic M, Minassian AA, Sehgal PK, Daniel MD, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid tissues: I. Immunohistochemistry and electron microscopy. Am J Pathol 1989, 134(2):373–383
31. Baskin GB, Murphey-Corb M, Watson EA, Martin LN: Necropsy findings in rhesus monkeys experimentally infected with cultured simian immunodeficiency virus (SIV)/delta. Vet Pathol 1988, 25:456–467
32. Benveniste RE, Morton WR, Clark EA, Tsai C-C, Ochs HD, Ward JM, Kuller L, Knott WB, Hill RW, Gale MJ, Thouless ME: Inoculation of baboons and macaques with simian immunodeficiency virus/Mne, a primate lentivirus closely related to human immunodeficiency virus type 2. J Virol 1988, 62:2091–2101
33. Murphey-Corb M, Martin LN, Rangan SRS, Baskin GB, Gormus BJ, Wolf RH, Andes WA, West M, Montelaro RC: Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys. Nature 1986, 321:435–437
34. Ringler DJ, Hunt RD, Desrosiers RC, Daniel MD, Chalifoux LV, King NW: Simian immunodeficiency virus-induced meningoencephalitis: Natural history and retrospective study. Ann Neurol 1988, 23(supp):S101–S107
35. Sharer LR, Baskin GB, Cho E-S, Murphey-Corb M, Blumberg BM, Epstein LG: Comparison of simian immunodeficiency virus and human immunodeficiency virus encephalitides in the immature host. Ann Neurol 1988, 23(supp):S108–S112
36. Lowenstine LJ, Pedersen NC, Higgins J, Pallis KC, Uyeda A, Marx PA, Lerche NW, Munn RJ, Gardner MB: Seroepidemiologic survey of captive old-world primates for antibodies to human and simian retroviruses and isolation of a lentivirus from sooty mangabeys (*Cercocebus atys*). Int J Cancer 1986, 38:563–574
37. Marx PA, Maul DH, Osborn KG, Lerche NW, Moody P, Lo-

wenstine LJ, Henrickson RV, Arthur LO, Gravell M, London WT, Sever JL, Levy JA, Munn RJ, Gardner MB: Simian AIDS: Isolation of a type D retrovirus and disease transmission. Science 1984, 223:1083–1086

38. Heidecker G, Lerche NW, Lowenstine LJ, Lackner AA, Osborn KG, Gardner MB, Marx PA: Induction of simian acquired immune deficiency syndrome (SAIDS) with a molecular clone of a type D retrovirus. J Virol 1987, 61:3066–3071
39. Miller CJ, Alexander NJ, Sutjipto S, Lackner AA, Hendrickx AG, Gettie A, Lowenstine LJ, Jennings M, Marx PA: Genital mucosal transmission of simian immunodeficiency virus: Animal model for heterosexual transmission of human immunodeficiency virus. J Virol 1989, 63:4277–4284
40. Sutjipto S, Pedersen NC, Miller CJ, Gardner MB, Hanson CV, Gettie A, Jennings M, Higgins J, Marx PA: Inactivated simian immunodeficiency virus vaccine failed to protect rhesus macaques from intravenous or genital mucosal infection but delayed disease in intravenously exposed animals. J Virol 1990, 64:2290–2297
41. Karnovsky M: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol 1973, 27:137A
42. Sutjipto S, Kodama T, Yee J, Gettie A, Jennings M, Desrosiers RC, Marx PA: Characterization of monoclonal antibodies that distinguish simian immunodeficiency virus isolates from each other and from human immunodeficiency virus types 1 and 2. J Gen Virol 1990, 71:247–249
43. Lackner AA, Marx PA, Lerche NW, Gardner MB, Kluge JD, Spinner A, Kwang H-S, Lowenstine LJ: Asymptomatic infection of the central nervous system by the macaque immunosuppressive type D retrovirus, SRV-1. J Gen Virol 1989, 70:1641–1651
44. Lackner AA, Rodriguez MH, Bush CE, Munn RJ, Kwang HS, Moore PF, Osborn KG, Lowenstine LJ, Marx PA, Gardner MB: Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid and salivary tissues. J Virol 1988, 62:2134–2142
45. Letvin NL, King NW, Reinherz EL, Hunt RD, Lane H, Schlossman SF: T lymphocyte surface antigens in primates. Eur J Immunol 1983, 13:345–347
46. Maul DH, Zaiss CP, MacKenzie MR, Shiigi SM, Marx PA, Gardner MB: Simian retrovirus D serogroup 1 has a broad cellular tropism for lymphoid and nonlymphoid cells. J Virol 1988, 62:1768–1773
47. Ringler DJ, Hancock WW, King NW, Letvin NL, Daniel MD, Desrosiers RC, Murphy GF: Immunophenotypic characterization of the cutaneous exanthem of SIV-infected rhesus monkeys: Apposition of degenerative Langerhans cells and cytotoxic lymphocytes during the development of acquired immunodeficiency syndrome. Am J Pathol 1987, 126:199–207
48. Ringler DJ, Walsh DG, MacKey JJ, Hunt RD, King NW: Immunophenotypic characterization of mononuclear phagocytes and dendritic cells in lymphoid organs of the rhesus monkey. Clin Immunol Immunopath 1988, 49:349–364
49. Moore PF, Ellingsworth LR, Toedter GP: Development and characterization of a monoclonal antibody to class II MHC antigens in rhesus macaques. Tissue Antigens 1986, 28:88–99
50. Haase AT, Brahic M, Stowring L, Blum H: Detection of viral nucleic acids by in situ hybridization, Methods in Virology. San Diego, Academic Press, 1984: pp 189–226
51. Munn RJ, Marx PA, Yamamoto JK, Gardner MB: Ultrastructural comparison of the retroviruses associated with human and simian acquired immunodeficiency syndromes. Lab Invest 1985, 53:194–199
52. Ward JM, O'Leary TJ, Baskin GB, Benveniste R, Harris CA, Nara PL, Rhodes RH: Immunohistochemical localization of human and simian immunodeficiency viral antigens in fixed tissues sections. Am J Pathol 1987, 127:199–205
53. Zink MC, Yager JA, Myers JD: Pathogenesis of caprine arthritis encephalitis virus: cellular localization of viral transcripts in tissues of infected goats. Am J Pathol 1990, 136:843–854
54. Pumarola-Sune T, Navia BA, Cordon-Cardo C, Eun-Sook C, Price RW: HIV antigen in the Brains of Patients with the AIDS dementia complex. Ann Neurol 1987, 21:490–496
55. Sethi KK, Naher H, Stroehmann I: Phenotypic heterogeneity of cerebrospinal fluid-derived HIV-specific and HLA-restricted cytotoxic T-cell clones. Nature 1988, 335:178–181
56. Narayan O, Kennedy-Stoskopf S, Zink MC: Lentivirus-host interactions: Lessons from visna and caprine arthritis-encephalitis viruses. Ann Neurol 1988, 23(suppl):S95–S100
57. Haase AT: Pathogenesis of lentivirus infections. Nature 1986, 322:130–136
58. Boulerice F, Bour S, Geleziunas R, Lvovich A, Wainberg MA: High frequency of isolation of defective human immunodeficiency virus type 1 and heterogeneity of viral gene expression in clones of infected U-937 cells. J Virol 1990, 64(4):1745–1755
59. Wyand MS, Ringler DJ, Naidu YM, Mattmuller M, Chalifoux LV, Sehgal PK, Daniel MD, Desrosiers RC, King NW: Cellular localization of simian immunodeficiency virus in lymphoid tissues: II. In situ hybridization. Am J Pathol 1989, 134(2):385–393
60. Sodroski J, Goh WC, Rosen C, Campbell K, Haseltine WA: Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature 1986, 322:470–474
61. Lifson JD, Reyes GR, McGrath MS, Stein BS, Engleman EG: AIDS retrovirus induced cytopathology: giant cell formation and involvement of CD4 antigen. Science 1986, 232:1123–1127
62. Dreyer EB, Kaiser PK, Offerman JT, Lipton SA: HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. Science 1990, 248:364–367
63. Pang S, Koyanagi Y, Miles S, Wiley C, Vinters HV, Chen ISY: High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. Nature 1990, 343:85–89
64. Brenneman DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, Pert CB: Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. Nature 1988, 335:639–642
65. Heyes MP, Rubinow D, Lane C, Markey SP: Cerebrospinal

fluid quinolinic acid concentrations are increased in acquired immune deficiency syndrome. Ann Neurol 1989, 26:275–277

66. Sabatier J-M, Vives E, Mabrouk K, Benjouad A, Rochat H, Duval A, Hue B, Bahraoui E: Evidence for neurotoxic activity of tat from human immunodeficiency virus type 1. J Virol 1991, 65:961–967
67. Kestler H, Kodama T, Ringler D, Marthas M, Pedersen N, Lackner A, Regier D, Sehgal P, Daniel M, King N, Desrosiers R: Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 1990, 248:1109–1112
68. Marthas ML, Banapour B, Sutjipto S, Siegel ME, Marx PA, Gardner MB, Pedersen NC, Luciw PA: Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. J Med Primatol 1989, 18:311–319