Infection of *Macaca nemestrina* brain with human immunodeficiency virus type 1

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We previously reported that pigtailed macaques (*Macaca nemestrina*) became infected after intravenous inoculation with the LAI strain of human immunodeficiency virus type 1 (HIV-1), an isolate that replicates in both *M. nemestrina* lymphocytes and blood-derived monocyte/macrophages. In the current study we investigated the presence of HIV-1 and pathology in the postmortem brains of four of these macaques. Histopathological findings revealed focal lesions in white matter in the frontal and occipital lobes of one macaque, with myelin loss, nerve fibre loss, and gliosis within these lesions. Semi-quantitative, solution-based PCR revealed HIV-1 DNA in the brains of two of the other macaques. Using slide-based PCR-driven *in situ* hybridization, we studied these two macaques further and detected intranuclear, circular HIV-1 DNA in vascular endothelia and other non-neuronal brain cells. These findings indicate that *M. nemestrina* brain can be infected with HIV-1 *in vivo* and may provide a useful animal model for understanding early HIV-1 brain infection in humans.

Introduction

Human immunodeficiency virus type 1 (HIV-1) invades the central nervous system (CNS) of many asymptomatic, seropositive individuals soon after infection. Evidence of early neuroinvasiveness includes the findings of intrathecal anti-HIV antibody synthesis (Resnick et al., 1988), detection of viral nucleic acid and infectious virus in cerebrospinal fluid (CSF) by PCR and culture (Chiodi et al., 1992; Sönerborg et al., 1991), and changes in electrophysiological indices (Koralnik et al., 1990) and metabolic patterns (Pascal et al., 1991) of brain function in seropositive individuals without clinically overt systemic or neurological disease. Early invasion of the CNS is also suggested by the development of aseptic meningitis in association with seroconversion to HIV (Gabuzda & Hirsch, 1987) and silent CNS infection of a patient who died of non-neurological causes 15 days after a mistaken transfusion with HIV-infected white blood cells (Davis et al., 1992). Early invasion of the CNS can progress to severe HIV-1-related encephalopathy (Spencer & Price, 1992).

Ultrastructural and *in situ* hybridization studies of HIV-1-infected brain have shown that microglia, macrophages and, rarely, vascular endothelial cells and astrocytes, but not neurons, can be infected by HIV-1 (Epstein & Gendelman, 1993; Spencer & Price, 1992). How HIV-1 invades the CNS during clinically silent periods, and the molecular characteristics of HIV-1 that contribute to neurotropism for non-neuronal brain cells, remain unclear (see Levy, 1993).

Development of an animal model in which HIV-1 infects the brain would be useful for investigating both the neuroinvasiveness and neurotropism of HIV-1 infection of human brain.

Chimpanzees, gibbons, and pigtailed macaques (*Macaca nemestrina*) are the only non-human primates known to be susceptible to infection with HIV-1 (Agy et al., 1992; Fultz et al., 1986; Lusso et al., 1988). There are no published reports of virological or pathological analyses of brain from HIV-1-infected chimpanzees or gibbons, partly owing to the endangered status and limited supply of these two species. In a previous study we described six *M. nemestrina* that showed infection by virus recovery, seroconversion, and DNA PCR using peripheral blood mononuclear cells (PBMCs) after being intravenously inoculated with HIV-1$_{LAI}$ (Agy et al., 1992; Frumkin et al., 1993). In the current study we investigated for the presence of HIV-1 and pathology in the postmortem brains of four of these macaques 60 weeks after inoculation with HIV-1$_{LAI}$.
Methods

In vitro infection of M. nemestrina cultured monocytes with HIV-1LAI. Previous experiments showed that HIV-1LAI replicated in M. nemestrina PBMCs and purified CD4+ T cells (Agy et al., 1992; Frumkin et al., 1993). Because of the relation between neurotropism and macrophage-tropism of HIV-1 isolates (see O’Brien, 1994), we performed a series of experiments to assess whether M. nemestrina primary monocyte-derived macrophages (cultured monocytes) were susceptible to in vitro infection with HIV-1LAI. Peripheral blood-derived monocytes were isolated by seeding 4 x 10^6 PBMCs from an HIV-1 antibody-negative macaque or human donor into 25 cm^2 tissue culture flasks at a concentration of 5 x 10^6 cells/ml macrophage medium (Macrophage-SFM; Gibco). Monocytes were allowed to adhere for 24 h in a 5% carbon dioxide atmosphere at 37°C; non-adherent cells were then eliminated by multiple washings with PBS free of calcium and magnesium. Adherent cells were detached by incubation (30 min at 4°C) in PBS supplemented with 5 mM-EDTA. At this time, non-specific esterase and monoclonal antibody (CD14) staining showed that adherence purification yielded a monocyte population of > 95% purity. Viable cells were then replated in triplicate at 10^6 cells/well (24-well tissue culture plates) in 1 ml macrophage medium supplemented with macrophage-colony stimulating factor (1000 U; Cetus Corporation); streptomycin (50 μg); penicillin (50 U); and glucose (0.3 mg) and incubated for 7 days prior to inoculation, as differentiation after 1 week in culture can increase the susceptibility of blood monocytes to in vitro HIV-1 infection (Rich et al., 1992). The stocks of HIV-1JR.FL, a monocyte-tropic strain of HIV-1 (Koyanagi et al., 1987), and the T-cell tropic HIV-1LAI (Wain-Hobson et al., 1991) were grown and titrated on human PBMCs with a TCID_50 of 10^8 (HIV-1JR.FL) and 10^6 (HIV-LAI). We inoculated human and M. nemestrina monocytes with (a) HIV-1LAI, (b) HIV-1JR.FL, or (c) heat-inactivated (60°C for 30 min) HIV-1LAI (negative control) at an m.o.i. of 0.01/cell. Following a 2 h incubation at 37°C, the wells were washed four times with PBS to remove unattached virus and 1 ml of macrophage medium was placed in each well. A 50% volume replacement with macrophage medium was performed every 3 days with all wells, with volume replacement of sampled culture supernatant at each time point. Cultured supernatants were sampled for the presence of HIV-1 antigen by whole-virus antigen capture (Frumkin et al., 1993) on days 0, 7, 14, 21 and 28 post-infection.

Table 1. HIV-1 specific oligodeoxynucleotides used as PCR primers and probes in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' → 3')</th>
<th>Region</th>
<th>Location*</th>
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<tr>
<td>KK1</td>
<td>AAATATGAGCGTTCCTATG</td>
<td>env</td>
<td>6546–6570</td>
</tr>
<tr>
<td>KK2</td>
<td>TTTTCCAGTGTGTTTCACAAGGCCTC</td>
<td>env</td>
<td>6575–6599</td>
</tr>
<tr>
<td>KK3</td>
<td>GAGCTTTCCTGCTGCTCCCCAGACC</td>
<td>env</td>
<td>7785–7809</td>
</tr>
<tr>
<td>KK4</td>
<td>TCTAGATCTGCTTCTTTCTCTCTTCT</td>
<td>env</td>
<td>7735–7759</td>
</tr>
<tr>
<td>KK5</td>
<td>AATTCCCTCTACATTTCAACTG</td>
<td>env</td>
<td>7344–7368</td>
</tr>
<tr>
<td>KK6</td>
<td>AGCAGTACATGGTCATACAGTG</td>
<td>env</td>
<td>6944–6963</td>
</tr>
<tr>
<td>SK32</td>
<td>AAATATGAGCGTTCCTATG</td>
<td>gag</td>
<td>1551–1578</td>
</tr>
<tr>
<td>SK33</td>
<td>TTTATGAGCGTTCCTTTTCTCTCT</td>
<td>gag</td>
<td>1665–1638</td>
</tr>
<tr>
<td>LTR R</td>
<td>GAGGCTAAGCAGTGGTTC</td>
<td>LTR</td>
<td>9591–9610</td>
</tr>
<tr>
<td>LTR US</td>
<td>GTCTGTAGTGGTCTGCTG</td>
<td>LTR</td>
<td>9650–9679</td>
</tr>
<tr>
<td>Probe</td>
<td>GGGAGGCTCCTGCGCTAA</td>
<td>LTR</td>
<td>9568–9587</td>
</tr>
<tr>
<td>SK19</td>
<td>ATCCCTGGAATATAGTAAGAGAATATAGGCTCATC</td>
<td>gag</td>
<td>1595–1635</td>
</tr>
</tbody>
</table>

* The positions for primers SK 38/39 and probe SK 19 were numbered relative to the genome of HIV-1 strain SF2 (Kellogg & Kwok, 1990; GenBank accession number K03551). The positions for the remaining primers and probe were numbered relative to the HIV-1 strain HXB2 (Bukrinsky et al., 1993; GenBank accession number K03455).
studies were performed blind on specimens without the examiner having knowledge of the HIV-1 infection status of the animals.

Detection of HIV-1 in macaque brain by solution-based PCR and PCR-driven in situ hybridization. With no focal lesions seen on gross examination of the brains, we used the presence of histopathology to determine areas to be evaluated for the presence of HIV-1. We selected the frontal cortex with adjacent subcortical white matter for virological determination areas to be evaluated for the presence of HIV-1. We selected examination of the brains, we used the presence of histopathology to having knowledge of the HIV-1 infection status of the animals. Studies were performed blind on specimens without the examiner knowledge of one macaque, and reports of HIV-1 proviral DNA (Sinclair et al., 1994; Sinclair & Scaravilli, 1992) in frontal cortex and subcortical areas in brain tissue of infected humans.

We extracted DNA from fixed, paraffin-embedded tissue from the frontal lobe of each inoculated and control macaque as previously described (Korber et al., 1994). Brain tissue was dispersed in 4 M-guanidium isothiocyanate. Total nucleic acids were extracted with phenol–chloroform, ethanol precipitated, and resuspended in deionized water. Total DNA from the extracted brain tissue was then used in a semi-quantitative, solution-based PCR assay using the Gen-Probe Chemiluminescence Detection System (Gen-Probe) as described (Whetsell et al., 1992; Jackson et al., 1993). This assay uses a chemiluminescent acridinium ester-labelled probe designed to hybridize with the HIV-1 gag sequences generated by the primer pair SK 38/39 (Table 1; Ou et al., 1988). The quantity of HIV-1 DNA was estimated by comparing the luminometer reading from each 500 ng sample of macaque brain DNA with a standard curve of 0, 5, 10, 20 and 50 HIV-1 genome copy numbers that were co-processed. The cutoff value for a positive result was > 10000 relative light units (Jackson et al., 1993).

Four separate repeat assays were done for the macaque brain specimens initially demonstrated to be PCR positive in the solution-based assay. All four repeats for each macaque were positive with light units ranging from 11000 to 50000; variability in the light reading appeared to be related to sampling in the original tissue block, as repeat assays of specimen from each block gave a similar reading.

To confirm the presence of HIV-1 and to identify the infected cells, PCR-driven in situ hybridization was performed as described previously (Korber et al., 1994). This assay has been previously used to locate HIV-1 DNA in non-neuronal brain cells from postmortem tissue of patients with advanced HIV disease (Korber et al., 1994). We obtained tissue for PCR-driven in situ hybridization from (a) areas of macaque brain positive by solution-based PCR, (b) homologous brain areas from control macaques, and (c) deltoid muscle from HIV-1-inoculated macaques, as a negative control of potentially infected tissue. In situ hybridization was performed using HIV-1 gag primers SK 38/39 (Ou et al., 1988) and probed with either specific (SK 19) (Kellogg & Kwok, 1990) or non-specific (cytomegalovirus IE-3) biotinylated probes as previously described (Dirks et al., 1990).

Assay for HIV-1 circular DNA and mRNA in macaque brain. The state of the viral genome was evaluated from HIV-1-infected brain tissue of two macaques (numbers 2 and 6) using PCR followed by Southern transfer and hybridization. DNA was extracted from brain tissue as above and amplified using primer sets LTR R/US (Table 1) that identified 2-1.5-TR-containing circular forms of HIV-1 DNA found in the nuclei of differentiated, non-dividing cells (Bukrinsky et al., 1993). PCR products were resolved on a 1% agarose gel and transferred using the Turboblotter System (Schleicher & Schuell). The amplified product was then detected using the internally conserved 32P-labelled oligonucleotide probe R (see Table 1) (Bukrinsky et al., 1993). HIV-1 transcriptional activity was assessed by PCR for reverse-transcribed tat mRNA as previously described (Furtado et al., 1991). Preliminary experiments indicated that the sensitivity of the assay was approximately 25 copies of tat mRNA when synthesized material.

Determination of amino acid sequence of HIV-1 third variable (V3) region in macaque brain tissue and PBMCs. To provide further evidence of HIV-1 infection, the envelope gene (first through fifth variable regions) of the PBMC isolate from week 8 after inoculation and brain tissue from macaque 6 was cloned as described (Furtado et al., 1991). Total DNA was prepared from fresh and fixed brain tissue and PBMCs of the HIV-1-infected macaque as described above. HIV-1 envelope gene was amplified using nested primer sets (Table 1; outer set, KK1/3; inner set, KK2/4) and cloned into pAMp (Gibco). Automated sequencing was performed using primer set KK8/40 (Table 1) with a 373A Laser Sequencer (Applied BioSystems) as described (Furtado et al., 1991).

Results

In vitro infection of M. nemestrina cultured monocytes with HIV-1

HIV-1 LA1 and HIV-1 JR.FL replicated in M. nemestrina and human cultured monocytes (Fig. 1). M. nemestrina cultures infected by HIV-1 LA1 showed an increase in

![Graph](image-url)
Fig. 2. For legend see opposite.
HIV-1 antigen from 81 pg/ml on day 7 to 879 pg/ml on day 28. Values for concomitantly infected cultured human monocytes were 274 pg/ml and 2809 pg/ml for the same period. Peak HIV-1 antigen levels for M. nemestrina and human cultured monocytes infected by HIV-1JRFL averaged 1 to 1.2 log_10 higher than that achieved for comparable cultures infected by HIV-1LAI, despite a similar amount of input virus. Values for HIV-1JRFL were 63 and 1123 pg/ml for M. nemestrina monocyte cultures on days 7 and 28 respectively, and 340 and 46000 pg/ml for human monocyte cultures for the same period. M. nemestrina and human cultures treated with heat-inactivated HIV-1LAI had < 30 pg/ml HIV-1 antigen during the 28 day culture period.

**Neuropathology of M. nemestrina brain**

No gross abnormalities were seen in the brains of any of the inoculated or control macaques. Macaque number 1, inoculated with cell-associated HIV-1, showed focal lesions in the subcortical white matter of the frontal and occipital lobes (Fig. 2, frontal lobe). Special stains demonstrated loss of myelin, gliosis, underlying nerve fibre loss, and small localized haemosiderin deposits within these lesions (Fig. 2a-c). Microscopic examination of brain tissue from the other inoculated macaques (numbers 2, 5, and 6) and controls revealed no abnormalities.

**Detection of infected cell type in M. nemestrina brain**

Using solution-based, semi-quantitative chemiluminescent PCR, we readily detected HIV-1-specific DNA in multiple brain tissue sections from the frontal lobes of two HIV-1-inoculated macaques (numbers 2 and 6). Between 10 and 100 copies of HIV-1-specific DNA per 500 ng of total DNA were detected in each preparation. Adequacy of DNA preparation was verified using HLA-DQα DNA primers (Patterson et al., 1993). DNA from brain tissue of macaques 1 and 5 and the two controls showed no HIV-1-specific sequences. Using in situ PCR, we confirmed the presence of HIV-1 DNA in all of six sections that were randomly selected from the same block of frontal lobe for macaques 2 and 6 (macaque 6, Fig. 3). HIV-1 DNA sequences were present in situ in non-neuronal (vascular, perivascular, and intraparenchymal) brain cells (Fig. 3c, d), identified morphologically on specimens as vascular endothelia, microglia, astrocytes, and possibly oligodendrocytes based on correlation of counterstained PCR slides with consecutive paraffin sections (Fig. 3e). No HIV-1 DNA was detected in situ from homologous regions of the frontal cortex and subcortical white matter of the control macaques (Fig. 3b) or deltoid muscle of any HIV-1 inoculated macaque.

**Detection of circular HIV-1 DNA in M. nemestrina brain**

DNA was identified in circular forms in brain tissue from macaques 2 and 6 in all three DNA extractions tested from the same tissue blocks (Fig. 4). Tat mRNA was not detected in the frontal lobe of any of the HIV-1 inoculated macaques.

**Comparisons of amino acid sequence of HIV-1 V3 region in macaque brain tissue, PBMCs and inoculum virus**

We compared the consensus V3 amino acid sequence (Wain-Hobson et al., 1991) of the inoculum strain, HIV-LAI, with V3 amino acid sequences from the brain and PBMC clones isolated from macaque 6. The consensus V3 region of HIV-1LAI, the brain clones, and the clones sequenced from the PBMCs obtained 8 weeks after inoculation showed 100% identity in their amino acid sequence (Fig. 5).

**Discussion**

We have demonstrated HIV-1 DNA in the postmortem brains of two M. nemestrina 60 weeks after intravenous inoculation. Infection was diffuse, with a high number of brain cells containing HIV-1 DNA that included circular forms in tissue sections from the frontal lobes of these macaques (numbers 2 and 6). Based on morphology, HIV-1 DNA was localized to vascular endothelia and other non-neuronal brain cells (e.g., microglia). An additional HIV-inoculated macaque (number 1) showed neuropathology consisting of loss of myelin, underlying nerve fibre loss, gliosis, and haemosiderin deposits in subcortical white matter of the frontal and occipital lobe.

In humans, macrophages and related microglia are the predominant cells infected by HIV-1 in the brain (Epstein

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Fig. 2. (a) Luxol fast blue staining. A white matter lesion (arrows) was seen in the frontal lobe of brain obtained from a M. nemestrina (number 1) inoculated intravenously with cell-associated HIV-1LAI, 60 weeks earlier (Frumkin et al., 1993). Bar marker represents 800 μm. (b) Holzer stain demonstrated gliosis (arrow) in the white matter lesion. Bar marker represents 800 μm. (c) Bodian stain demonstrated underlying nerve fibre loss (arrow at margin of loss) in the white matter lesion. Dark structures in the lower half of the figure are glial nuclei. Bar marker represents 100 μm.
Fig. 3. Detection of HIV-1 DNA by tissue-based PCR-driven in situ hybridization in brain tissue obtained from a *M. nemestrina* (number 6) inoculated intravenously with cell-free HIV-1 \textsc{La} 60 weeks earlier (Frumkin et al., 1993). (a) Low-power photomicrograph of cerebral cortex shows non-neuronal cells (dark staining) containing HIV-1 DNA. Bar represents 200 μm. (b) Cortical brain tissue from an uninoculated *M. nemestrina* was negative for both HIV-1 gag and a biotinylated cytomegalovirus (CMV)-specific probe. Bar represents 200 μm. A contiguous section from frontal cortex shown in (a) amplified with HIV-1 gag primers SK 38/39 (Ou et al., 1988) and probed with CMV-specific probes was also used as a negative control and did not stain for HIV-1 (not shown). (c) Higher power of (a) shows non-neuronal cell (arrow) containing HIV-1 DNA and surrounding neuron identified by a prominent nucleolus (arrowhead). Bar represents 15 μm. (d) Enlargement of a region (boxed) shown in (a) demonstrates vascular endothelial cell (arrow) and perivascular non-neuronal cells containing HIV-1 DNA. Bar represents 15 μm. (e) Consecutive H & E-stained section from the same macaque and cortical layer shown in (c) demonstrates normal histology and frequent perineuronal astrocytes (arrowheads). Bar represents 15 μm. All in situ PCR sections were counterstained with nuclear fast red.
Infection of *M. nemestrina* brain with HIV-1

Fig. 4. Autoradiogram showing HIV-1 circular DNA in brain tissue of the *M. nemestrina* described in legend of Fig. 3, using PCR followed by Southern transfer and hybridization. Lane 1, amplified circular HIV-1 DNA from the persistently HIV-1LaA-infected WE17 T cell line (Willard-Gallo et al., 1990). This T cell line is derived from a human T cell lymphoma and contains high copy numbers of both 2-LTR and 1-LTR HIV-1 DNA; lane 2, amplified circular HIV-1 DNA from brain tissue of the *M. nemestrina* described above; lane 3, absence of circular HIV-1 DNA from uninfected control CEM cells.

& Gendelman, 1993; Gartner et al., 1986). Occasional infection of oligodendrocytes (Esiri et al., 1991), astrocytes (Saito et al., 1994), and capillary endothelial cells (Wiley et al., 1986) has also been noted. We have not yet developed a technique for double-labelling of *in situ* PCR sections with immunohistochemistry. We thus identified several types of infected non-neuronal cells (microglia, astrocytes, vascular endothelia, and possible oligodendrocytes) in the brains of macaques 2 and 6 based on nuclear fast red counterstaining of *in situ* PCR slides and correlation with consecutive sections stained with H & E (Fig. 3e).

Circular DNA forms were found in the brains of macaques 2 and 6. While macaques 2 and 6 also lacked evidence of neuropathology, minimal pathology with large amounts of proviral and unintegrated HIV-1 DNA can also occur in HIV-1-infected human brain (Dickson et al., 1993; Vazeux et al., 1992).

Neuropathology was observed in only one macaque (number 1) and was not accompanied by any evidence of HIV-1 in the frontal lobe of this animal. Similar neuropathological changes have been reported in early human HIV-1 infection (Gray et al., 1993; Jones et al., 1988). We did not find evidence, in any of the four macaques, of the multinucleated giant cell encephalitis that is characteristic of more advanced and productive human HIV-1 infection (Price, 1994).

The mechanism of the loss of myelin, gliosis, and nerve fibre loss in macaque 1 is unclear. It is possible that local responses to infected or uninfected, activated cells at other, adjacent brain regions not assayed for HIV-1 may have contributed to these neuropathological changes. Viral coat products such as gp120 can bind to a glycolipid

<table>
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<tr>
<th>Consensus HIV-1LaA</th>
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<tr>
<td>Sequence 292 QLNQ5VEINCTRPNNTKRSIRGPGRAVFTIGKIGNWROAHCNISRAKWNATLQIASK 362</td>
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*Fig. 5. Amino acid sequences of the V3 region (underlined) of gp120 of HIV-1LaA consensus sequence (Wain-Hobson et al., 1991) compared with that of brain and PBMC clones from a *M. nemestrina* intravenously inoculated with HIV-1LaA and described in legends of Figs 3 and 4. PBMCs were obtained 8 weeks after inoculation; brain was obtained 60 weeks after inoculation. Numbers above the amino acid sequences refer to relative positions of the amino acids of each clone. All sequences are in single-letter code and dashes represent identical amino acids. The V3 loop region is identical for all clones. Amino acid sequences from regions flanking the V3 loop show a single amino acid substitution (position 296) for one brain-derived clone, BP552.18.*
expressed on oligodendrocytes (Bhat et al., 1991), and cytokines (e.g., tumour necrosis factor-α and interleukin-1) secreted by locally infected or uninfected cells have been implicated in HIV-1 neuropathogenesis, including myelin damage (Gendelman et al., 1994; Tyor et al., 1992). Alternately, cytokines or other neurotoxins derived from an immune response to systemic HIV-1 infection may have played a role in the neuropathology of this macaque (Price, 1994; Vitkovic et al., 1994). The neuropathology of macaque 1 may also be unrelated to HIV-1 infection and instead be caused by a reaction during (or more often after) infection with another virus (Murray et al., 1992; Sharova et al., 1987).

How HIV-1 enters the macaque CNS is unknown. Although there are probably several mechanisms of viral entry, a favoured hypothesis for human infection is that monocytes infected by HIV-1 in the peripheral blood carry the virus into the brain (see Epstein & Gendelman, 1993 for review). In addition, neurotropic isolates of HIV-1 have tropism for cells of monocyte/macrophage lineage (O'Brien, 1994). We therefore studied the in vitro susceptibility of M. nemestrina cultured monocytes to our inoculum strain (HIV-1La1). While HIV-1La1 replicated to a limited extent in M. nemestrina (and human) cultured monocytes infected in vitro, several factors strongly indicate that residual lymphocytes were unlikely to have caused the persistent HIV-1 antigen production. These factors include (a) the use of multiple washes to remove non-adherent cells, (b) the presence of non-specific esterase stain positivity, (c) a characteristic cell morphology, (d) no use of exogenous interleukin-2, phytohaemagglutinin-P, or donor PBMCs during the 28 day culture period, (e) peak production of HIV-1 antigen at around 28 days, and (f) significantly greater HIV-1 antigen production with the monocyte-tropic HIV-1JR-FL compared with HIV-1La1# despite similar amounts of input virus.

In vitro susceptibility of M. nemestrina cultured monocytes to HIV-1 does not necessarily imply in vivo susceptibility. This is particularly important given the failure to detect in vivo infection by culture or PCR of blood monocytes from HIV-infected chimpanzees (Schuitemaker et al., 1993) despite successful in vitro infection (Eibl et al., 1992). However, we have found HIV-1 nucleic acid by PCR in non-cultured primary monocytes obtained from two HIV-1La1-infected M. nemestrina that were not included in the current study (L. Frumkin et al., unpublished results).

We have mainly used HIV-1La1 in our in vivo studies, as viral stocks of this T cell tropic strain are well characterized and grow better than other HIV-1 strains in M. nemestrina lymphocytes infected in vitro (Agy et al., 1992; Frumkin et al., 1993). Infection of M. nemestrina with highly macrophage-tropic strains of HIV-1 would be of interest, especially considering the association between HIV-1 macrophage-tropism and neurotropism in humans. Whether the state and site of viral infection and replication in M. nemestrina differ with highly macrophage-tropic strains versus that of HIV-1La1 requires further study.

Our in situ hybridization studies clearly demonstrate the widespread neuroinvasiveness of HIV-1 in the brains of two M. nemestrina. Semi-quantitative techniques consistently detected 10 to 100 HIV-1 DNA copies/500 ng sample of M. nemestrina brain DNA. The nuclear localization of both HIV-1 DNA and circular forms by in situ PCR suggests that HIV-1 entered and effectively reverse transcribed in M. nemestrina cells in vivo. At the time of examination, there was no evidence of transcriptionally active HIV-1 as demonstrated by the inability to isolate virus from brain tissue and the lack of tat mRNA by PCR. The lack of intrathecal HIV-specific immunoglobulin and HIV-1 antigen production in CSF from all macaques at several points between 1 month post-inoculation and necropsy is also consistent with our failure to detect productive infection within the CNS. However, all macaques maintained stable levels of antibodies to HIV-1 envelope and gag proteins until time of death, suggesting continued antigen production.

The cause of apparently restricted long-term viral replication in the brains of these macaques is not known, but data from our laboratory suggest that containment of replication may occur in persistently HIV-1-infected macaques. We have shown that both CD8-mediated cytotoxic and CD4-mediated proliferative T cell responses to HIV proteins are present early and persist for 100 weeks in HIV-1-infected M. nemestrina (Kent et al., 1995). The large amounts of HIV-1 DNA seen in our macaques may represent a reservoir of latent infection, particularly in non-dividing cells such as microglia.

We undertook the sequencing of the V3 loop region as a means to confirm the HIV-1 PCR results from brain tissue. The reasons behind the identical amino acid sequence of the V3 loop from blood and brain clones obtained from macaque 6 are unclear; perhaps it is related to minimal rates of replication of HIV-1 in the CNS of the macaques that we studied. A better understanding of the molecular determinants of HIV-1 neurotropism in our macaques awaits additional experiments with a larger sample size, different HIV-1 strains, examination of other tissues such as lymph node and spleen by in situ PCR, and assessment of isolates from blood and brain at additional times, especially early after inoculation.

In summary, the finding of HIV-1 infection in M. nemestrina brain is the first demonstration of HIV-1 in the brain of a non-human primate. We did not observe the multinucleated giant cell encephalitis, productive
infection, or behavioural changes often seen in humans with symptomatic and advanced HIV-1 brain disease (Price, 1994). However, the ability of HIV-1 to infect *Macaca nemestrina* brain and the finding of persistent, circular forms of HIV-1 DNA in non-neuronal brain cells are similarities between human and *M. nemestrina* infection. Further development of the *M. nemestrina* model may help define factors involved in HIV-1 neuroinvasiveness and neurotropism in human brain.

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