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# Circular Forms of Unintegrated Human Immunodeficiency Virus Type 1 DNA and High Levels of Viral Protein Expression: Association with Dementia and Multinucleated Giant Cells in the Brains of Patients with AIDS

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**Thirty-one histologically abnormal brains from patients with AIDS were studied in order to establish the relationship between multinucleated giant cells, viral protein expression, the various forms of human immunodeficiency virus type 1 (HIV-1) DNA, and clinical evidence of dementia. Unintegrated HIV-1 DNA of 2 to 8 kb was found in 22 of the 31 brains. Multinucleated giant cells without any other pathology were found in 14 cases; unintegrated 1-long terminal repeat (1-LTR) circular forms of HIV-1 DNA and strongly positive immunohistochemistry for gp41 and p24 were found in most of these brains. Most of these patients had a clinical diagnosis of HIV-1-associated dementia and cerebral atrophy. In all the other brains studied, 1-LTR circles were absent and immunohistochemistry for gp41 and p24 was usually negative. Very few of these patients had a clinical diagnosis of dementia. Sequence comparison of the LTR region from integrated HIV-1 DNA with that from unintegrated 1-LTR circular forms of HIV-1 DNA in 12 cases showed no significant differences. A further comparison of these brain-derived LTR sequences with LTR sequences derived directly from lymphoid tissue also showed strong sequence conservation. The V3 loop of the virus from the brain was sequenced in 6 cases and had a non-syncytium inducing–macrophage-tropic genotype. Our results show that (i) although unintegrated HIV-1 DNA was present in most brains from patients with AIDS, molecular evidence of high levels of viral replication was associated with the presence of multinucleated giant cells and dementia, and that (ii) the HIV-1 LTR is not a determinant of neurotropism. These observations suggest that replication of HIV-1 and not just the presence of HIV-1 DNA within giant cells makes the important contribution to central nervous system damage.**

Neurological disease is a major complication of human immunodeficiency virus type 1 (HIV-1) infection, with large postmortem studies having demonstrated neuropathological abnormalities in 79 to 94% of brains from patients with AIDS (7, 15). Cells of monocyte/macrophage lineage constitute the majority of infected cells in the brain, with the formation of multinucleated giant cells reflecting one end of a spectrum of macrophage-microglial pathology. They are found in 18 to 20% of cases in large postmortem studies on brains from patients with AIDS (6, 7, 15, 39), and their presence is considered to be the histopathological hallmark of productive HIV-1 infection in the brain (6).

Several studies have also shown a large HIV-1 DNA load in the brains of some patients with AIDS (2, 5, 26). Most of it appears to be unintegrated (26), and although its presence has been correlated with dementia, its role in the pathogenesis of HIV-1-associated dementia has never been determined. Unintegrated HIV-1 DNA can exist as a linear form and as a circular form with either one or two long terminal repeats (LTRs). Linear forms are found in the cytoplasm, and circular forms are found in the nucleus, where 2-LTR circles represent preintegration complexes. Their presence is the molecular hallmark of active viral replication. When cells of monocyte/macrophage origin are infected by HIV-1 in vitro, this leads to the accumulation of unintegrated HIV-1 DNA but without cell death (27, 34). Recent in vitro studies have also shown that unintegrated HIV-1 DNA in macrophages can give rise to the synthesis and secretion of viral proteins in the absence of infectious-virus particle synthesis (8, 13, 36, 40); it is possible that these chronically infected cells are an important source of those viral proteins (e.g., gp120) which can cause neuronal cell injury (37).

The aims of this study were (i) to establish the relationship between multinucleated giant cells, viral protein expression, and the various forms of HIV-1 DNA in brains from patients with AIDS and (ii) to ascertain whether the tropism and expression of HIV-1 in the brain are determined by specific LTR sequences.

## MATERIALS AND METHODS

The histological studies were performed on formalin-fixed tissue, and the molecular studies were performed on frozen, frontal lobe tissue from 31 HIV-1-positive patients. All had been diagnosed with an AIDS-defining illness during life. Brain tissue was collected postmortem, coded, and stored at −70°C in the Medical Research Council's brain banks in London and Edinburgh. The remainder of each brain was fixed in 10% formol saline. Control brain tissue ($n = 6$) included cases with a diagnosis of motor neuron disease, Alzheimer's disease, and schizophrenia.

**Histological and immunohistochemical studies.** Detailed neuropathological examination was performed on formalin-fixed, paraffin wax-embedded sections from multiple areas. Large blocks, 2.0 to 4.0 by 1.5 to 3.0 cm in size, were taken

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from frontal, parietal, temporal, and occipital lobes; striatum; and thalamus. A large cerebellar hemisphere block and complete blocks of midbrain, pons, and medulla were also examined. Special stains for organisms, gliosis, demyelination, and axon loss were performed as appropriate. Cytomegalovirus (CMV) encephalitis was diagnosed when classical Cowdry A inclusions were present in enlarged glial cells. These were either in areas of focal inflammation or in association with necrotizing ependymitis. In those cases where CMV was suspected but inclusion bodies were not seen, immunohistochemistry (IH) for CMV antigens was performed. Primary cerebral non-Hodgkin's lymphoma was identified with an anti-CD20 antibody (i.e., B-cell origin; L26, Dako) and an anti-CD3 antibody (UCHT1, Dako) for T-cell infiltration. Brains with multiple pathology (e.g., giant cells and opportunistic infections and/or lymphoma) were not included in this study, because such cases serve to confuse rather than clarify the role of giant cells.

**IH for gp41 and p24 antigens.** The proteolytic cleavage of gp160 yields gp41 and gp120. As high-affinity antibodies are not available to perform gp120 IH on postmortem tissue, IH for gp41 was performed on sections with an average size of 3 by 1.5 cm, using an antibody from Genetic Systems, Seattle, Wash., and detected with the ABC system (Dako). IH was also performed for the core HIV-1 protein p24 with an antibody from Cambridge Biotech, Rochester, Mass., by methods previously described (2). The intensity of the signal for gp41 and that for p24 were scored blind. Using sections of approximately 4.5 $cm^2$, the grading system used was as follows: A, >100 positive cells/section; B, 11 to 99 positive cells/section; C, 1 to 10 positive cells/section; D, 0 positive cells/section.

**PCR analysis of integrated and unintegrated HIV-1 DNA.** Frontal cortex was used for the molecular studies, because giant cells were found predominantly in this area on detailed neuropathological examination and because Bell et al. (3) have shown that the presence of giant cells in the frontal lobe is associated with large amounts of HIV-1 DNA. Frozen brain tissue (0.5 to 1.0 g) from the frontal cortex was macerated and digested overnight at 37°C in 4 volumes of lysis buffer (50 mM Tris [pH 8.0], 100 mM EDTA, 150 mM NaCl, with 200 μg of proteinase K per ml and 0.1% sodium dodecyl sulfate). Sodium perchlorate (5 M) was added to a final concentration of 1 M, and 500 μl of the samples was extracted with chloroform-isoamyl alcohol. The DNA was precipitated with ethanol and redissolved in 50 μl of Tris-EDTA buffer, giving a mean DNA concentration of 0.47 ± 0.02 (standard deviation) mg/ml. Total brain DNA was screened by PCR and dot blot hybridization with the primer pair SK38-SK39 (25, 31). DNA from brains which gave a positive hybridization signal was then subjected to field inversion gel electrophoresis (FIGE).

Integrated and unintegrated forms of HIV-1 DNA resolved by FIGE were used for our PCR-based studies. Fragmentation of brain DNA due to either postmortem delay or the extraction of DNA was not seen. FIGE was performed on 1-μg samples of DNA resolved in 0.6% agarose-Tris-acetate-EDTA gels with a Bio-Rad FIGE Mapper. Running conditions were 50 V for 18 h, with an initial 0.3-s forward pulse and 0.1-s back pulse ramped to a final 3-s forward pulse and 1-s back pulse. Each gel was then photographed, and every gel lane was carefully sectioned with a gel cutter designed for the purpose. Nine 5-mm agarose cubes, which spanned the distance from the sample well to below the 4-kb marker, were obtained. A separate gel cutter was used for each lane. Gel cutters and the gel apparatus were cleaned before and after use by soaking them in 10% sodium hypochlorite (Chlorox; Sigma) for 10 min.

The primers used in all PCRs are shown in Fig. 1. Direct PCR amplification from each agarose cube eliminated any loss of DNA which could occur with phenol extraction. PCR amplifications were not affected by agarose, provided the amplified target size was less than 500 bp and the final agarose concentration did not exceed 0.12% in the PCR mix. PCR amplification was performed with the HIV-1 *gag* primer pair SK38-SK39 and the HLA DQα primer pair 895.1-895.2 (31). The cycling conditions were 95°C for 1 min for denaturation, 52°C for 1 min for annealing, and 72°C for 1 min for extension. Thirty-five cycles were performed. An HIV-1 *gag* probe was generated by $^{32}P$ labelling of a product which was generated by PCR amplification of the HIV-1 plasmid BH10 (16) (MRC AIDS repository, London, United Kingdom) with the primer pair ADP 816-ADP 817 (32). Each brain was tested on at least two separate occasions with appropriate negative controls, and precautions were taken to prevent carryover (23).

The presence of an HLA DQα signal in the high-molecular-weight DNA fraction was used to confirm that amplification had taken place, and its absence was used to show that the low-molecular-weight DNA was not contaminated with high-molecular-weight DNA. All hybridization signals were quantified with an LKB Ultrascan XL Enhanced Laser Densitometer linked to an IBM-compatible computer running Gelscan XL 1.21 and Fig P 6.0 software (Software Corp., Durham, N.Car.).

For PCR analysis of 1-LTR and 2-LTR circular forms of HIV-1 DNA, a *gag* primer and a *nef* primer (Fig. 1) were used. Two rounds of PCR of 35 cycles each were performed on both total DNA and size-fractionated DNA. The amplified products from the 1-LTR and 2-LTR circles of HIV-1 DNA were distinguished by their size. They were 1,017 bp for 1-LTR circles and 1,642 bp for 2-LTR circles. The presence of 1- and 2-LTR circle-amplified products was confirmed by Southern blotting and hybridization with a labelled LTR probe which was generated by amplification of pBH10 DNA with the primers SLP-1 and SLP-2. The presence of 2-LTR circles was also confirmed by a second round of nested PCR with the 2-LTR-specific primers M844 and M667 (26).

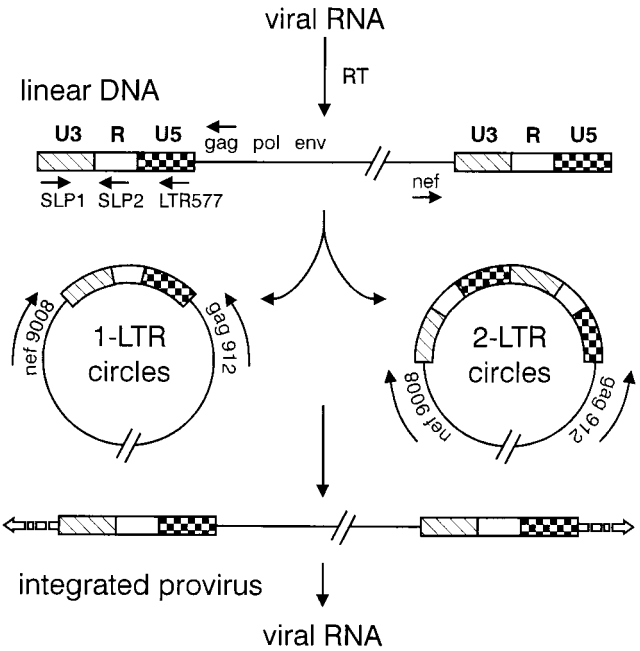


FIG. 1. Schematic representation of the various forms of integrated and unintegrated HIV-1 DNA and the sequences and positions of the primer pairs used for PCRs. The primer pairs used are as follows: SK38 (5′-TAAATCCAC CTATCCCAGTAGGAGAAA-3′) and SK39 (5′-TTTGGTCCTTGTCTTATG TCCAGAATG-3′) (Ou et al. [25]); 895.1 (5′-GTGCTGCAGGTGTAAACTT GTACCAG-3′) and 895.2 (5′-CACGGATCCGGTAGCAGCGGTAGAGTTG -3′) (Shaunak et al. [31]); ADP 816 (5′-GGTACATCAGGCCATATCACC-3′) and ADP 817 (5′-ACCGGTCTACATAGTCTC-3′) (Simmonds et al. [32]); M667 (5′-GGCTAACTAGGGAACCCACTG-3′) and M844 (5′-CTGATCCCT GGCCCTGGTGTG-3′) (Pang et al. [26]); *nef* primer (5′-GCAGCTNTAGAT CTTAGCCACTT-3′) and *gag* primer (5′-GATTAACTGCGAATCGTTC-3′) (Heinzinger et al. [17]); SLP-1 (5′-CACACACAAGGCTACTTCCCTGA-3′) and SLP-2 (5′-CCAGAGAGACCCAGTACAGGC-3′); and LTR577 (5′-GAG GGATCTCTAGTTACCAGAG-3′). RT, reverse transcriptase.

**DNA sequencing of the HIV-1 LTR region.** As transcription from unintegrated HIV-1 DNA in vitro has been reported (8), we sequenced the LTR from both integrated and unintegrated HIV-1 DNA in brain. Our aim was to establish whether unintegrated, 1-LTR circles have the correct control elements to allow transcription. We also compared the LTR sequences in brain with those in lymphoid tissue in order to determine whether a unique neurotropic LTR sequence exists.

A region from U3 of the LTR to just downstream of the TAR loop was sequenced in 12 brains and matched lymphoid tissues. FIGE-resolved gel slices containing the >20-kb integrated HIV-1 DNA were used for a seminested PCR with the SLP-1 and *gag* 912 primer pair (first round) and the SLP-1 and L577 primer pair (second round). For unintegrated 1-LTR forms of HIV-1 DNA, total genomic DNA was first amplified with the *gag-nef* primer pair. The amplified products were then resolved on 2.5% agarose gels, and the bands corresponding to the 1-LTR product were excised and reamplified with the primers SLP-1 and LTR577. In the case of matched lymphoid organs, 1 μg of total genomic DNA was amplified with the primer pair SLP-1 and LTR577. All PCR products were purified with Promega PCR Wizard kits. PCR products were then blunt ended with T4 polymerase (Boehringer) and ligated into *Sma*I-cut M13mp18 (Amersham) with a Boehringer Rapid Ligation kit. The presence and the orientation of LTR sequences in recombinant M13 plaques were established by amplification of phage in a PCR with an M13 (−20) sequencing primer and either primer SLP-1 or LTR577. The purified products of these PCRs were sequenced with an Applied Biosystems dye terminator sequencing kit and an Applied Biosystems 373A Automated DNA Sequencer. Alignments were performed against the HIV-1 isolates JR-CSF (cerebrospinal fluid) (22), JR-FL (frontal lobe) (for which the LTR sequence data is incomplete [see reference 24] (HIV sequence database [http://hiv-web.lanl.gov.], Los Alamos National Laboratory, 1996), and HXB-2 (29) with the Clustal program. At least three sequences from both DNA strands were obtained from each patient sample, either directly from amplification products or from PCR-amplified M13 clones.

A retrospective case note review was undertaken after completing the laboratory-based studies. All results are given as the mean ± standard error of the mean (SEM). The data in the Tables was analyzed with Fisher's exact test. All

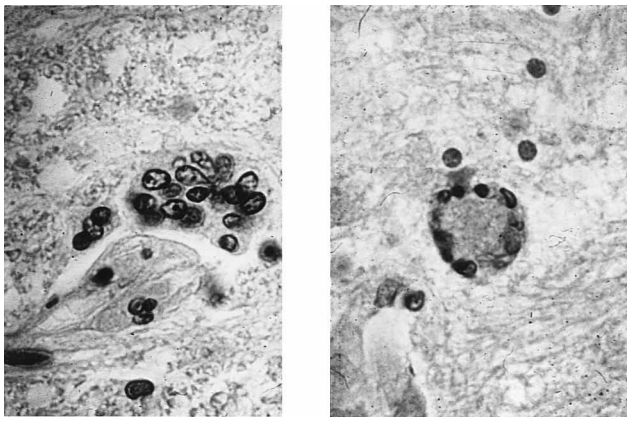

FIG. 2. Multinucleated giant cells in the brain of a patient with AIDS. Note the dense round or elongated nuclei. Hematoxylin and eosin stains were used.

other groups of data were analyzed with a Mann-Whitney U test (Graphpad; Instat).

## RESULTS

Multinucleated giant cells without any other pathology were seen in 14 brains (Fig. 2). In 13 of these brains, giant cells were seen in sections taken from multiple areas of the brain, and in 1 case they were confined to the midbrain. In another 17 brains, there was histological evidence of some other pathology, such as CMV encephalitis ($n = 8$), non-Hodgkin's lymphoma ($n = 4$), toxoplasmosis ($n = 2$), mycobacterial infection ($n = 2$), perivascular lymphocyte cuffing ($n = 2$), and perivascular macrophages ($n = 1$). Giant cells were not present in this group. Based upon the results from the histological studies, the brains were divided into two groups according to whether multinucleated giant cells were present (Table 1).

Unintegrated, circular forms of HIV-1 DNA were found only in brains with giant cells (group 1). The 1-LTR circles predominated, comprising 80 to 98% of the total hybridization signal for circular forms (Fig. 3). The 2-LTR circles were always the minority and were never found in isolation. Brains in which circular forms of HIV-1 DNA were found also had strongly positive IH for both gp41 and p24. In those cases in which 1-LTR circles were also amplified from size-fractionated DNA, the amplified signal was found in the 5- to 8-kb fractions. 2-LTR circles were not amplified from any of the size-fractionated DNA. In the single brain where the presence of giant cells was confined to the midbrain, IH of frontal lobe tissue was negative for gp41 and only weakly positive for p24 (<10 positive cells/4.5-cm$^2$ section); 1-LTR circles were not found in this case.

In brains with other pathology and without giant cells (group 2), circular forms of HIV-1 DNA were not found and IH for p24 was negative; in the four cases which were weakly positive for gp41, the histopathological diagnosis was CMV encephalitis.

Unintegrated linear and circular forms of HIV-1 DNA (2 to 8 kb) were present in 22 of the 31 brains. The mean ratio of unintegrated to integrated HIV-1 DNA in group 1 was 1.9 ± 0.6 (range, 0.3 to 6.7). In group 2, only those brains with macrophage-microglial nodules seen on histological examination had unintegrated HIV-1 DNA; the mean ratio was 1.1 ± 0.3 (range, 0.12 to 3.0; $n = 8$). The difference between the mean ratios for these two groups was not statistically significant ($P = 0.17$).

A retrospective case note review of the 31 patients was undertaken to determine if there was an association between the presence of giant cells and HIV-1-associated dementia (19). Of the 14 patients with giant cells in their brains, 12 had a clinical diagnosis of HIV-1-associated dementia. Cerebral atrophy was also demonstrated during life by computer tomography scanning of the head in 11 of 11 scans performed. In contrast, only 2 of the 17 patients with other pathology and without giant cells had a clinical diagnosis of dementia (Fisher's exact test, $P < 0.0001$). The histological diagnoses for these two patients were CMV encephalitis and cerebral toxoplasmosis, respectively.

The V3 loop of the virus from 6 of the brains with giant cells had a non-syncytium-inducing–macrophage-tropic genotype (11, 33a). We were unable to confirm that a conserved histidine at position 305 in the V3 loop is associated with HIV-1-associated dementia (28).

A DNA sequence analysis of the HIV-1 LTR was performed on nine brains in group 1 (integrated and 1-LTR circles) and on three brains in group 2 (integrated). There was no significant difference between the DNA sequences obtained from integrated HIV-1 DNA when they were compared with unintegrated 1-LTR circles obtained from the same brain (mean identity, 98.5% ± 0.3%; range, 96.8% to 99.4%) (Table 2). Although some differences were found when comparisons were made between brains in each group, there was no evidence of clustering. Comparison of these brain-derived LTR sequences with those from JR-CSF or JR-FL did not reveal an identity closer to the cerebrospinal fluid isolate (mean identity,

TABLE 1. Summary results from histological, PCR, and immunohistochemical studies ($P < 0.0001$, Fisher's exact test)

| Group no. and histological description | Result (no. of brains) for: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | PCR with HIV-1 DNA | | IH[a] | | | | | | |
| | | | gp41[b] | | | | p24[c] | | | |
| | Total (*gag*) | 1-LTR:2-LTR | A | B | C | D | A | B | C | D |
| 1 (multinucleated giant cells without any other pathology; $n = 14$) | 14 | 13:13[d] | 4 | 5 | 1 | 0 | 1 | 8 | 2 | 3 |
| 2 (other pathology without any multinucleated giant cells; $n = 17$)[e] | 17 | 0:0 | 0 | 1 | 3 | 13 | 0 | 0 | 0 | 17 |

[a] A, >100 positive cells/4.5-cm$^2$ section; B, 11 to 99 positive cells/4.5-cm$^2$ section; C, 1 to 10 positive cells/4.5-cm$^2$ section; D, no positive cells.

[b] For group 1, 10 brains showed positive cells (4 brains were not examined). For group 2, four brains showed positive cells, and all four brains had a histopathological diagnosis of CMV encephalitis.

[c] For group 1, 11 brains showed positive cells. For group 2, no brains showed positive cells.

[d] Circular forms of HIV-1 DNA were not found in frontal lobe tissue from the one brain in which multinucleated giant cells were confined to the midbrain. This patient did not have clinical evidence of dementia.

[e] Group 2 pathology: CMV encephalitis ($n = 8$), non-Hodgkin's lymphoma ($n = 4$), toxoplasmosis ($n = 2$), mycobacterial infection ($n = 2$), perivascular lymphocyte cuffing ($n = 2$), perivascular macrophages ($n = 1$).

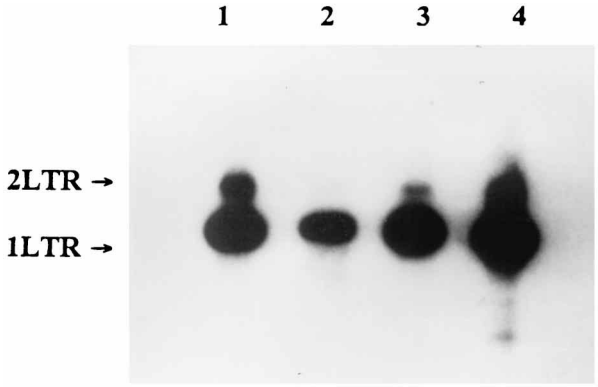


FIG. 3. PCR amplification of brain DNA from group 1 patients demonstrating the presence of 1- and 2-LTR circular forms of HIV-1 DNA. Shown are examples from brains with giant cells (group 1).

93.5% ± 0.4%) or to the frontal lobe isolate (mean identity, 93.4% ± 0.4%) or even to the blood-derived isolate HXB-2 (mean identity, 93.1% ± 0.6%) (Table 2). Furthermore, very few differences were found when the integrated HIV-1 LTR sequences from brains in group 2 were compared with the integrated HIV-1 LTR sequences from brains in group 1.

A comparison was also made between the integrated LTR sequences obtained from brain with those obtained from matched lymphoid tissue for both group 1 (two patients, five lymphoid tissues) and group 2 (three patients, five lymphoid tissues). For group 1, this showed a duplication of part of the T-cell factor-1α (TCF-1α) region in three lymphoid tissues. The length of the TCF-1α site duplication varied in the organs studied. In contrast, no duplication of the TCF-1α site was seen in HIV-1 LTR sequences derived from the lymphoid tissues of group 2 (Table 3).

## DISCUSSION

Our results show that unintegrated, circular forms of HIV-1 DNA and strongly positive IH for gp41 and p24 are associated with the presence of multinucleated giant cells in brains from patients with AIDS (Table 4). Most of these patients (group 1) also had a clinical diagnosis of HIV-1-associated dementia and cerebral atrophy. Histopathological evidence of any other pathology in the brain (in the absence of giant cells) was not associated either with molecular evidence of active viral replication (i.e., absence of 1- and 2-LTR circular forms of HIV-1 DNA) or with immunohistochemical evidence of p24 and gp41 viral protein production. Most of these patients (group 2) did not have a clinical diagnosis of dementia. The highly significant association (Table 1 and Materials and Methods; Fisher's exact test, $P < 0.0001$) between the presence of 1- and 2-LTR HIV-1 DNA circles, positive IH for viral proteins, the presence of giant cells, and HIV-1-associated dementia suggests that these observations are related.

There are some correlates for our in vivo observations in brains from patients with AIDS in experiments carried out in vitro with lymphocytes and macrophages. Acute infection of T-cell lines by HIV-1 is associated with the appearance of 1- and 2-LTR circles followed by syncytium formation and the production of infectious viral particles (20). The infection is cytopathic. This mirrors observations with the peripheral blood mononuclear cells of patients during seroconversion in whom there is an increase in the number of LTR circles and a fall in the CD4 count (20). In contrast, infection of monocyte-derived macrophages by laboratory-adapted isolates of HIV-1 results in the accumulation of unintegrated, circular forms of HIV-1 DNA (34) which can give rise to the synthesis of viral proteins in the absence of infectious-virus particle synthesis (8, 13, 36, 40). Furthermore, the persistence of unintegrated HIV-1 DNA

TABLE 2. Percentage identity of the HIV-1 LTR sequences from brains from groups 1 and 2 compared to those from isolates JR-CSF, JR-FL, and HXB2

| Isolate | % Identity with sequence from brain of patient[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group 1 | | | | | | | | | Group 2 | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| JR-CSF | 94.3 | 93.5 | 92.3 | 94.7 | 94.3 | 94.3 | 94.7 | 90.7 | 92.3 | 93.8 | 94.0 | 93.5 |
| JR-FL | 94.1 | 93.4 | 92.8 | 94.1 | 94.2 | 94.3 | 94.2 | 90.1 | 93.2 | 93.7 | 94.6 | 94.7 |
| HXB2 | 95.0 | 93.3 | 92.3 | 93.5 | 94.3 | 95.0 | 93.8 | 89.0 | 91.6 | 95.2 | 94.7 | 95.2 |

[a] The mean degree of identity between integrated and unintegrated forms of HIV-1 LTR DNA was 98.5% ± 0.3% (range, 96.8% to 99.4%). Mean ± SEM for JR-CSF, JR-FL, and HXB2: 93.5% ± 0.4%, 93.4% ± 0.4%, and 93.1% ± 0.6%, respectively.

TABLE 3. Percentage identity of the HIV-1 LTR sequences from lymphoid organs (lymph node and/or spleen) from groups 1 and 2 compared to those from isolates JR-CSF, JR-FL, and HXB2

| Isolate | % Identity with sequence from lymphoid tissue of patient[a]: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 1 | 1 | 2 | 2 | 10 | 11 | 11 | 12 | 13 |
| JR-CSF | 89.5 | 89.6 | 93.5 | 91.2 | 90.2 | 90.6 | 91.6 | 91.6 | 93.5 | 90.7 |
| JR-FL[b] | 90.9 | 90.5 | 92.2 | 90.0 | 91.1 | 87.5 | 90.1 | 89.9 | 93.4 | 90.1 |
| HXB2 | 92.8 | 91.2 | 91.5 | 91.9 | 90.6 | 92.2 | 91.4 | 91.1 | 93.8 | 90.2 |

[a] Patient numbers correspond to group 1 and 2 patient numbers as presented in Table 2. The results of the percentage identity from sequencing more than one lymphoid organ from a particular patient are all shown separately. The TCF-1α duplication, when present, is excluded from the sequence comparison. Patient 13 had HIV-1 DNA in his lymph nodes but not in his brain. Mean ± SEM for JR-CSF, JR-FL, and HXB2: 91.2% ± 0.4%, 90.6% ± 0.5%, and 91.7% ± 0.3%, respectively.

[b] The sequence data available for the LTR of JR-FL is incomplete.

TABLE 4. Summary of histological, immunohistochemical, HIV-1 DNA, and clinical features of the groups defined

| Feature | Result for: | |
|---|---|---|
| | Group 1 | Group 2 |
| Histological | | |
| Multinucleated giant cells | + | − |
| Other pathology | − | + |
| PCR | | |
| HIV-1 DNA | + | + |
| 2- to 8-kb unintegrated HIV-1 DNA | + | ±[a] |
| 1-LTR circles | + | − |
| Immunohistochemical | | |
| gp41 and p24 | + | − |
| gp41 or p24 | + | ±[b] |
| Clinical | | |
| Dementia | + | − |
| Cerebral atrophy | + | Not available |

[a] 2- to 8-kb unintegrated HIV DNA was only present in those brains with macrophage-microglial nodules. These nodules are histologically distinct from multinucleated giant cells, and they are usually found in association with CMV encephalitis or cerebral toxoplasmosis.

[b] All positive cases (gp41) had a diagnosis of CMV encephalitis.

in macrophages is thought to confer resistance both to the cytopathic effects of HIV-1 and to superinfection by HIV-1 (34, 35). Berman et al. (4) have also reported that monocyte-derived macrophages infected with HIV-1 at a low multiplicity of infection in vitro do not show a cytopathic effect and that they remain viable for at least 3 months; infection by HIV-1 promoted the long-term survival of these macrophages. Although culture supernatants from these chronically infected macrophages contained significant amounts of p24 antigen, the infectious titer of virus was very low. Purified cultures of primary microglia infected in vitro have also been shown to produce viral proteins over a period of several months (18).

Unintegrated linear and circular forms of HIV-1 DNA were found in 22 of the 31 brains. Pang et al. reported that high levels of unintegrated HIV-1 DNA were present in the brains of patients with AIDS and dementia (26); the mean ratio of unintegrated to integrated HIV-1 DNA was $11.5 \pm 1.8$ (range, 6 to 19). Their analysis did not distinguish between all the different forms of HIV-1 DNA, and they did not demonstrate a correlation between unintegrated HIV-1 DNA and viral antigen (p24). In our study, the mean ratio of unintegrated to integrated HIV-1 DNA was $1.9 \pm 0.6$ for group 1 and $1.1 \pm 0.3$ for group 2 ($P = 0.17$). However, unintegrated, 1-LTR circular forms of HIV-1 DNA and strongly positive IH for viral proteins were associated only with the presence of giant cells. The sequence of these 1-LTR circles did not differ significantly from that of integrated HIV-1 DNA. This could mean that these circles can serve as a template for the synthesis of viral proteins without them being able to integrate into genomic DNA, from where they would be able to give rise to infectious virus. Our further attempts to detect and quantitate HIV-1 RNA by reverse transcriptase PCR in all 31 cases have not been successful because of the rapid degradation of RNA which follows the often long delay between death and autopsy. However, in situ studies have previously demonstrated HIV-1 RNA in multinucleated giant cells and in some mononuclear macrophages in brains from patients with AIDS (12, 21).

Most of the patients with giant cells in their brains also had a clinical diagnosis of HIV-1-associated dementia, with radiological evidence of cerebral atrophy. This contrasted with the findings obtained with patients with other pathology. Wiley and Achim also found that 35% of patients with HIV-1-associated dementia had giant cells, compared to 6% of patients without giant cells in a study of 148 patients with AIDS ($P < 0.0001$) (39). These observations suggest that replication of HIV-1 and not just the presence of HIV-1 DNA within giant cells makes the important contribution to central nervous system damage.

Most of the HIV-1 found in the brain is of a non-syncytium-inducing–macrophage-tropic genotype (11). Several recent studies have failed to find any evidence of conserved differences in the V3 loop of viruses obtained from brain and lymphoid tissue (10, 11, 30, 33). Corboy et al. (9) addressed the role of the HIV-1 LTR in tropism by constructing transgenic mice which contained the LTR regions from either central nervous system-derived isolates or a T-cell tropic isolate of HIV-1. Only those mice with LTRs derived from a central nervous system isolate showed viral gene expression in neurons. Because Ait-Khalid et al. (1) have also reported that there is a unique LTR sequence which determined central nervous system tropism in one patient, we sequenced the LTRs from both integrated and unintegrated forms of HIV-1 DNA in brain and from integrated LTRs in lymphoid organs for 12 patients.

Our results do not confirm the results of Corboy et al. obtained with transgenic animals (9) or those of Ait-Khalid obtained with a single patient with AIDS (1). We have found no pattern in the differences between the HIV-1 LTR sequences in brain and lymphoid organs except for a TCF-1α duplication in the lymphoid tissues from those patients who had giant cells in the brain. Although the significance of this duplication to the pathogenesis of HIV-1 infection is not clear, it does suggest that the virus replicates under different selection pressures in different tissues. It also demonstrates that the HIV-1 PCR-amplified signals obtained from frontal lobe tissue were not due to the presence of peripheral blood mononuclear cells in the cerebral circulation. Furthermore, we found very few differences between the LTR sequences of the different forms of HIV-1 DNA either within a given brain or between the 12 brains. It is therefore unlikely that there are specific base differences within the LTRs which can either explain the tropism of HIV-1 for the brain or which could be responsible for the different patterns of viral protein expression in brains with and without giant cells.

Until recently, multinucleated giant cells had only been described in brain, spinal cord (12), and lung (11), but Frankel et al. have now also demonstrated their presence in adenoidal lymphoid tissue from asymptomatic HIV-1-positive patients (14). These giant cells expressed the markers of dendritic cells, stained strongly for p24, and were found in close association with $CD3^+$ T cells. Ulvestad et al. have reported that human microglia (which have the ultrastructural, phenotypic, and functional properties of monocyte-derived macrophages) also share some properties with dendritic antigen presenting cells; these included providing the focus for $CD3^+$ T-cell clustering and the initiation of a mixed lymphocyte reaction (38).

Frankel et al. (14) suggested that giant cells are an important source of infectious virus in lymphoid tissue. Our observations also suggest that giant cells are an important source of infectious virus in brains from patients with AIDS. Furthermore, as our study and other recent studies have not demonstrated that a unique V3 loop or LTR sequence determines neurotropism, the phenotype of the macrophage in the brain rather than the genotype of the virus infecting the brain seems to be the crucial determinant of whether high levels of viral replication take place.

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