Independent Evolution of HIV Type 1 in Different Brain Regions

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ABSTRACT

HIV-1-associated brain pathology exhibits regional variability and we therefore studied the genetic differences in the V1–V5 domains of the HIV env gene in up to four regions of brain (frontal lobe, basal ganglia, medial temporal lobe, and nonmedial temporal lobe) from three patients. We found that in each separate brain region HIV-1 forms different quasispecies and that there is little gene flow among these regions. In further support of brain region-specific evolution of HIV-1, we analyzed amino acid signatures in these clones. In addition to known amino acid signatures associated with macrophage tropism and the lack of syncytium formation, we found 15 majority amino acid signature patterns from the V1–V5 env sequences associated with the neuroanatomical regions analyzed from the three individuals. Furthermore, on average, intrabrain genetic distances for the HIV-1 env were estimated to be much smaller than genetic distances between brain regions. Specific strains of HIV-1 may be neurotropic or neuroinvasive (replication preference in brain tissue) and may contribute to pathology, cognitive loss, and neuropsychiatric disease.

INTRODUCTION

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) infection of the brain occurs soon after initial peripheral infection and is often associated with cognitive deficits or neuropsychiatric disease.1–6 Related yet distinct quasispecies of HIV-1 exist in brain, spinal cord, dorsal root ganglia, cerebrospinal fluid (CSF), spleen, lung, and peripheral blood.7–10 In addition, intratissue regional heterogeneity has been observed in the HIV-1 quasispecies derived from epidermal Langerhans cells and possibly spleen.11,12 Several reports have stated that there is low variation of HIV within the brain, although comparisons between different regions were not performed.7,9,10,12,13 However, sequencing of HIV-1 directly from brain and from culture of HIV-1 from six regions in one brain showed that clustering occurred in up to four of the regions (left frontal, occipital, parietal, and right parietal).14,15 We report on DNA sequencing of 62 clones directly from brain tissue without the intercession of growing virus in culture. We analyzed phylogenetic patterns, the effective rates of HIV migration (HIV gene flow), and amino acid signatures for the HIV-1 envelope (env) V1–V5 domains in up to four brain regions from three patients.

MATERIALS AND METHODS

Obtaining sequences

Postmortem brain tissues from three patients (cases 144, 196, and 222), accompanied by clinical records, were obtained from...
Medical Examiner Offices in southern Florida. Brain tissue was obtained 9, 13, and 8 hr postmortem, respectively. Analyses for HIV-associated dementia (HAD) were based on American Academy of Neurology criteria\textsuperscript{16} and were performed using the clinical records. The retrospective, postmortem psychiatric autopsies specific for HAD were performed by a standardized review technique.\textsuperscript{17,18} Neuropathological examination of brain tissue sections was performed using hematoxylin and eosin (H&E)-stained sections. Patient 144 was a 42-year-old female intravenous drug user (IDU), patient 196 was a 33-year-old male IDU, and patient 222 was a 27-year-old homosexual male. Patients 144 and 222 had HAD, and there was insufficient information for patient 196 for the diagnosis of HAD, though there was evidence for neuropsychiatric disease.\textsuperscript{17-19} All three subjects exhibited pulmonary disease (pneumonia or tuberculosis) and died with AIDS as previously defined.\textsuperscript{20}

Total DNA and RNA were extracted from tissue aliquots (110–150 mg), using the DNA/RNA isolation kit from United States Biochemical (USB, Cleveland, OH), according to the manufacturer instructions. The V1–V5 region of the HIV-1 env gene was amplified from DNA or cDNA (Perkin-Elmer, Foster City, CA). Two pairs of nested oligonucleotide primers were used in polymerase chain reactions (PCRs) with 0.5 \textit{mM} dNTPs, 1.5 \textit{mM} Mg\textsubscript{2+}, 0.25 \textit{mM} of each primer, 0.1 \textit{mM} of each dNTP, 100 ng of either total DNA or cDNA as described previously.\textsuperscript{21,22} Amplification consisted of an initial denaturation step of 95°C for 5 min, followed by 30 cycles of 94°C for 35 sec, 60°C for 1 min 35 sec, 72°C for 2 min 35 sec, with a final extension at 72°C for 10 min.\textsuperscript{21,22}

For DNA sequencing, the PCR products with a size of \approx 1040 bp (identified by ethidium bromide staining of agarose gels) were pooled from five PCRs for each brain region. The purified PCR product (40 ng) was cloned into the pCR\textsubscript{II} TA cloning vector (Invitrogen, San Diego, CA), using kit instructions, and confirmed by endonuclease restriction mapping and Southern blots. Sequencing was done as previously described.\textsuperscript{21-23}

**Phylogeny reconstruction**

Sequences were aligned using CLUSTAL W\textsuperscript{24} and imported into PAUP* for phylogenetic analysis.\textsuperscript{25} The model of evolution for this analysis was determined by the procedure outlined in Huelsenbeck and Crandall.\textsuperscript{26} This procedure uses a likelihood ratio test for testing alternative models of evolution, given a tree topology. This allows one to test statistically a number of hypotheses. Our analysis rejected the hypothesis of equal base frequencies (A = 0.390, C = 0.168, G = 0.192, and T = 0.250), equal rates of transitions and transversions (estimated ratio of ti/tv = 1.612), and equal rates among sites, using a likelihood ratio test (all \textit{p} values were less than 0.0001). Therefore, the HKY85+\Gamma model of evolution\textsuperscript{27} was used, taking into account these inequalities with a gamma shape parameter estimated by maximum likelihood to be 0.7225. We also tested the resulting relationships for robustness to changes in the model of evolution and also the phylogeny reconstruction optimality criterion employed (i.e., maximum parsimony, minimum evolution, and maximum likelihood analyses). Likelihood ratio tests and phylogeny estimations were performed using PAUP*.\textsuperscript{25} Confidence in the reconstruction of various clades was assessed using 1000 replications of the bootstrap procedure\textsuperscript{28} in conjunction with the neighbor-joining method and the HKY+\Gamma model of evolution. Average genetic distances were calculated for intrabrain regions and between brain regions, using the same model of evolution.

The statistical parsimony method\textsuperscript{29} was used to estimate relationships among sequences within individuals. This approach makes connections between sequences that are most similar to one another. The method has been shown to be superior to standard phylogeny reconstruction methods when sequence divergence is low.\textsuperscript{30-32} Using Eq. (8) in Templeton \textit{et al.},\textsuperscript{29} minimum connections were justified for up to 15 mutational steps, with a confidence limit of greater than 95%. Therefore, sequences that differed from their nearest neighbor by more than 15 mutational steps were not included in the analysis. Sequences that were less than 15 mutational steps from some other sequence were connected into networks. We then followed the nesting procedure outlined in Templeton and Singh\textsuperscript{33} to determine significant nesting relationships. Once phylogenetic relationships were established, estimates of the levels of gene flow among brain regions within patients were made, using the approach of Slatkin and Maddison.\textsuperscript{34} This method estimates \( N_m \), the effective number of migrants per generation in the population. If \( N_m \) is measured to be less than one, significant population subdivision is indicated.\textsuperscript{34}

**Signature analysis**

Signature pattern analysis was used to identify common amino acid motifs from different brain regions and/or from different patients, using the Viral Epidemiology Signature Pattern Analysis (VESPA) program.\textsuperscript{35} An amino acid majority signature requires that the most common (majority) amino acid residue at a specific position in the query sequence from each brain region differ from the most common amino acid at the same position in the other three regions. Signature analyses were performed for each case separately and for the three cases together. All case-specific signatures were then deleted from the 3-case combined analysis and 15 signatures remained.

**Quality control**

Several methods were used to exclude contamination or sample mix-up for the sequences analyzed in this study.\textsuperscript{23} Tissue lysis was performed in a class II biohazard hood in a BSL-2 laboratory using BSL-3 procedures. Four separate rooms were always used: (1) for the addition of reagents, (2) for the processing of sample DNA templates, (3) for the performance of PCR, and (4) for the analysis of PCR products.\textsuperscript{23} Each brain region specimen was processed separately to avoid sample mix-up and contamination during the processing and subsequent amplification and cloning steps. In addition, laboratory sentinel tubes were routinely evaluated for contamination on a weekly basis. Negative PCR controls consisted of reagent mixes with water instead of purified target DNA and RNA. For target RNA experiments reagent mixes were without reverse transcriptase.\textsuperscript{21-23} Additional DNA sequences were included in the phylogenetic analyses of the brain-derived DNA sequences for viral strain subtyping and to control for additional possible sources of contamination and sample mix-up. Furthermore, a BLAST search was conducted for each clone, using GenBank\textsuperscript{36} to identify known sequences of highest similarity, and several
FIG. 1. Phylogenetic reconstruction of the env sequences from brain regions of three individuals. Each brain-derived sequence is designated by the patient number (144, 196, and 222), brain region (F, frontal lobe; T, nonmedial temporal lobe; G, basal ganglia; M, medial temporal lobe), clone number, and nucleic acid target template (D, DNA; R, RNA). In addition to the brain-derived sequences, we have also included the five most similar sequences from a GenBank BLAST search,\textsuperscript{36} designated by their GenBank accession numbers. Finally, we have included common laboratory strain sequences and subtype A and D sequences as outgroups. Branch lengths are shown proportional to the amount of change on the branches. Bootstrap values indicate the relative amount of support for the indicated clades as a percentage of time that clade was estimated in 1000 pseudoreplications.\textsuperscript{28}
of these sequences were included in phylogenetic analyses. No evidence of contamination was found (Fig. 1). Known subtype consensus sequences for subtypes A and D were used as outgroups for the phylogenetic analysis, in addition to B subtype sequences (HXB3, MFA, and NL4-3) that are commonly used laboratory strains of HIV-1. Phylogenetic trees were also produced that included subtypes C and E and additional B subtype sequences (ADA, BH8, BH10, BRU, HXB2, MN, PV22, and SF2), and no additional associations were found (data not shown).

RESULTS

Sequences

Nested PCR was used to amplify the V1–V5 region of the HIV-1 gp120 envelope gene from target DNA and RNA. DNA and RNA targets were purified from up to four neuroanatomical regions from three postmortem brains. These regions were frontal lobe (F), basal ganglia (G), medial temporal lobe (M), and non-medial temporal lobe (T) and consisted of F, G, M, and T, from

FIG. 2. Phylogenetic relationships among sequences within individuals, estimated using the statistical parsimony method. Sequence designations are as in Fig. 1. Zeros represent missing intermediates. The numbers along the branches indicate the number of nucleotide substitutions among those branches. Boxes indicate nesting levels, labeled in italics, with the level first and the clade number second; i.e., nest 2-1 is the first clade at nesting level 2. Nesting levels are proportional to evolutionary time, such that lower nesting levels are evolutionarily younger than higher nesting levels. Individuals have been divided into separate plots: 144, A; 196, B; and 222, C.
patient 144, T, G, and M from patient 222, and F and M from patient 196. Clones could not be obtained from G and T of patient 196 or from F patient 222. Neuropathological examination of brain tissue sections from patients 144 and 222 showed HIV-encephalitis (HIVE) and patient 196 lacked HIVE. The sequence data have been deposited in GenBank (accession numbers AF125810–AF125874).

**Phylogeny reconstruction**

To test the hypothesis of regional specificity within the brain, we reconstructed evolutionary relationships among the *env* sequences (Fig. 1). This phylogeny was estimated using the neighbor-joining method with the HKY85+Γ model of evolution. Sequences from each patient form monophyletic groups (i.e., exclusive clusters of all the descendants from a common ancestor). Each patient cluster is strongly supported with a bootstrap value of 100%. Similarly, sequences from different brain regions predominantly fall within distinct clades. For example, clade 2 contains all of the sequences from patient 144 temporal lobe supported with a bootstrap value of 100%. None of the sequences cluster with the laboratory strain sequences or with the sequences resulting from the GenBank BLAST searches, indicating the absence of contamination. The use of several additional methods also excluded contamination and sample mix-up as explanations of our result. Neighbor-joining phylogenetic analysis of the deduced amino acid sequences gave a tree similar to the nucleotide tree shown in Fig. 1. Likewise, these major groupings were supported using maximum likelihood, minimum evolution, and parsimony methods. Thus, there is clear evidence of clustering by brain region of the V1–V5 portion of the HIV *env* gene for the patients studied.

To explore further the evolutionary relationships among sequences within an individual, we reconstructed network relationships using a nesting approach for each patient (Fig. 2A–C). In each individual there is significant clustering by brain region, as no nest included sequences from another brain region. Where different brain regions are found within the same clade (i.e., clade 8 in Figs. 1 and 2C), each brain region clearly nests together before the multiple regions nest together. This clearly represents extensive population subdivision within each brain region. The divergence within patient 196 appears to be more recent since ancestral sequences (located in the interior of the cladogram) have been sampled from this individual (196F6D, 196H3D, and 196H5D) (Fig. 2B), whereas all other sequences are at the tips of the phylogeny. Likewise, clustering of sequences occurs at lower levels within patient 196: one and two step levels versus three step levels for the other patients. There is a similarly recent origin for case 222 sequences.
in clade 9. The use of the statistical parsimony method gives us a conservative approach to exclude recombination among the sequences within individuals.29,33

We also examined genetic distances to quantify the extent of divergence within versus among brain regions. For patient 196, the within-brain region divergence averaged 0.5% for frontal lobe (F) and 0.3% for medial temporal lobe (M), while the between-tissue divergence averaged 3.6%. Similarly, for patient 222 the within-brain region divergence was 0.1% (M), 0.3% (G), and 1.2% (T), while the between-brain region divergences were (G–M) 2.9%, (G–T) 1.0%, and (M–T) 2.5%. Finally, for patient 144 two tissues had low divergences (M, 0.72%; T, 0.76%) while the other two tissues had relative high divergences (F, 3.9%; G, 2.1%). These divergences are on the order of the between-tissue divergences in the other two patients. The between-tissue divergences for patient 144 were as follows: (F–G) 6.5%, (F–M) 6.5%, (F–T) 9.5%, (G–M) 1.8%, (G–T) 7.3%, (M–T) 6.7%. The extent of divergence within brain regions varied from 0.1 to 3.9% (average, 1.1%) and varied between brain regions from 1 to 9.5% (average, 4.8%). The average divergences within brain regions for cases 196 and 222 were 0.4 and 0.3%, respectively; however, for case 144 the average was 1.9%. The average divergences between brain regions for cases 196 and 222 were 3.6 and 2.1%, respectively; however, for case 144 the average was higher again at 6.4%.

**Gene flow estimates**

To quantify further the extent of tissue tropism within the brain, we used the standard population genetic parameter $N_m$, the effective number of migrants per generation.34,41,42 We estimated $N_m$ for different brain regions in patients 196, 222, and 144. Their $N_m$ values were 0.10, 0.25, and 0.78, respectively, all less than one. These values indicate significant population subdivision between the different brain regions and a significant chance for genetic drift to play a role in the divergence of these subpopulations.34,41,42 This could be due to a bottleneck effect of populations on penetrance into a specific brain region, resulting in a reduction of effective population size and separate genetic variation and quasispeciation.42,43

**Signature analysis**

An alternative explanation for the formation of distinct clades of virus associated with different brain regions is via natural selection. We tested if specific strains of HIV-1 with conserved amino acid sequences were present in different neuroanatomical regions of the brain. To do this, we performed an amino acid majority signature pattern analysis.35

Using the VESPA program,35 significant signature patterns were detected for each of the four neuroanatomical regions across the three patients (Table 1). Fifteen (4.3%) majority signatures out of a total of approximately 350 amino acids sequenced in the V1–V5 domains (including the C2–C4 conserved blocks of amino acid sequences) of the HIV env product were identified for the four brain regions. These 15 signatures are not case specific but reflect contributions from more than one case. These signatures were arrived at by calculating the regional majority signatures present for each brain alone (patients 144, 196, and 222), and these case-specific signatures were then deleted from the list of signatures calculated on the basis of all three cases together. Fifteen signatures then remained. Of these 15 signatures, each brain region has the following number of majority amino acid signatures: frontal lobe, 2; nonmedial temporal lobe, 1; medial temporal lobe, 1; and basal ganglia, 1. There are six amino acid signature sites in the V1 region, two signature sites in the C2 region, and seven signature sites in the V3 region of the Env protein.

**DISCUSSION**

The distinct populations of HIV-1 identified in each neuroanatomical region of brain in this study demonstrated clustering and independent evolution in up to four regions of the brain. These results are consistent with a prior study utilizing one brain, which showed clustering of sequences in four of six regions studies.14,15 There are several explanations of regional clustering. Spread of the virus among these different regions could occur subsequent to infection of the brain at some initial site. On the basis of neuroimaging studies, basal ganglia are

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**Table 1. Fifteen Regional Majority Amino Acid Signatures from Three AIDS Brains (144, 196, and 222)**

<table>
<thead>
<tr>
<th>Region of brain</th>
<th>No. of clones</th>
<th>Site</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 33 35 36 37 39 88 93 129 171 182 184 235 237 247</td>
<td></td>
</tr>
<tr>
<td>F 12 33 42 42 42 42 42 42 42 42 42 42 58T 42 42 43 42 42F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G 14 43R 29 29 29 29 29 29 29 29 29 29 7 40 29 29 29 29 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 20 3 50– 50– 50– 50– 50– 50– 50S 50V 50T 30 50N 50S 0 45V 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T 16 25 31 31 31 31 31 31 31 31 6 31 31 100K 31 31</td>
<td></td>
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</table>

aThe frequency of each signature residue in each brain region is shown as the percentage of all clones from that region with the identical residue. As described in text, these signatures are not case specific. The regional majority signatures are determined at each position using the program VESPA, as described by Korber and Myers.35 This requires that the most common amino acid be more abundant in that region than in the other three regions. These signatures are produced when at least two cases have the majority amino acid at each site. Signatures due to single cases are excluded (data not shown). HIV-1 clones were obtained from four different neuroanatomical regions as in Fig. 1: F, frontal lobe; T, temporal lobe (neocortex); G, basal ganglia; M, medial temporal lobe. No. of clones, The total number of clones isolated and sequenced for each neuroanatomical region from all of the brains; ±, deletion is the signature at that site. The amino acid sequence numbering is determined relative to the starting position of all of the brain clones. Amino acids are depicted using single-letter abbreviations. HIV env regions V1 includes sites 10–39 (six signatures); C2, sites 88 and 93 (two signatures); and V3, sites 129–247 (seven signatures). By brain region, signatures are as follows: 2 in F, 1 in G, 11 in M, and 1 in T.
most likely to be an early site of HIV-1 infection with potential subsequent spread to other brain regions (e.g., frontal lobe) resulting in region-specific clustering. The occurrence of HIV-1 variants in each brain region may be subsequent to evolution as adaptations to selection in specific microenvironments. These processes include variation in chemokine coreceptor specificity and abundance, local immunity, specific antigen stimulation, restricted cellular tropism, diversity of evolutionary pressures on different HIV genes including the HIV long terminal repeat (LTR) (which interacts with both host and viral proteins), and altered cellular trafficking regulated by cytokine and chemokine expression. An additional possible explanation is that virus infection of different brain regions could occur at different times owing to multiple breaks of the blood–brain barrier over time. However, if this were the case, the tree would then show a nested series of phylogenetic offshoots for the different regions examined and each invading virus would reflect peripheral HIV-1 temporal evolution of the HIV-1 infection. (Blood sequences of HIV-1 were not available to test this hypothesis.) The different regions of the human brain in our study evolved at different times and had different phylogenetic origins. Correspondingly, the viruses in our study were subjected to different biochemical and structural microenvironments for virus evolution.

Additional support for regional variation of HIV-1 in the brain was derived by calculation of genetic distances. Intrabrain genetic distances of HIV-1 sequences have been shown to be 0.2–1.2% and 0.1–2.1%. These studies demonstrated a low variation of HIV-1 within the brain although different regions were not analyzed. Our studies were performed among different regions of brain as well as for intraregional distances, and we found wider genetic distances. Our results indicate heterogeneity of HIV-1 infection in different regions of brain. Furthermore, on average, intrabrain genetic distances for the HIV-1 env gene were estimated to be much smaller than genetic distances between brain regions. Distances for case 144 were greater than for the other two cases. These results reflect the relative age of infection of HIV-1 in patient 144, which was longer than in the other two patients. This is consistent with the intraclide nest analysis that similarly showed a more recent origin for sequences from cases 196 and 222 than for case 144.

The variation of virus load in the brain is an additional indication of heterogeneity of HIV-1 infection. Studies based on immunohistochemical detection of HIV proteins showed maximum virus load in basal ganglia. We have shown that virus load varies nonrandomly among these regions of brain in a study of nine cases, supporting the notion of regional variation of virus load. In patient 144 the HIV-1 proviral DNA load was uniform and high. In patient 196 proviral DNA load was lower and varied; it was highest in the medial temporal lobe and lowest in basal ganglia and nonmedial temporal lobe. For DNA sequencing, isolation of PCR amplicon bands from the gels requires a visible band for efficient cloning and this is dependent on the levels of HIV-1 load. (This is the reason for sequences absent for patients in our study.) Cases 144 and 222 were diagnosed with HAD and showed HIVE, whereas there was an absence of HIVE for patient 196 (this patient showed neuropsychiatric impairment although there was insufficient information for a diagnosis of HAD). Although our study has a small number of cases it is consistent with a possible relationship of HAD, HIVE, and virus load, as indicated by some published reports, as well as with the possible existence of neuropathogenic virus strains. In the Bratanich et al. study, clusters of single-clone representatives occurred for patients grouped separately (i.e., with HAD and without HAD). This occurred for the env gene (V3 loop), partially for the tat gene, and not at all for the reverse transcriptase (RT) gene. Differences among the various studies could be due to the regions of the HIV genome studied and numbers of representative clones used. In addition, our finding of brain regional clusters of env sequences was subject to analysis to exclude potential sample mix-up and contamination, as pointed out above.

The majority of amino acid signatures show a preference for medial temporal lobe tissue and for the HIV-1 env V1 and V3 domains. The phenotypic properties associated with these signatures are unknown but could be related to region-specific selection pressures that occur in these regions of the brain, the structure and function of which differ. The signature amino acids may also reflect increased requirements or restrictions for virus replication, structure, and/or survival, unlike the other amino acid sites that contain few signatures. It should be noted that clones from case 144 did not dominate the signatures even though this patient contributed most of the clones in the overall analysis. Of the 15 signatures calculated using sequences from all three patients, cases 196 and 222 governed 12 signatures, cases 144 and 196 governed 1 signature, and cases 144 and 222 governed 2 signatures. Amino acid signatures have previously been reported for specific phenotypic properties of HIV-1, such as macrophage tropism, and our clones had macrophage-tropic signatures consistent with the 14 signatures that were proposed in the literature. None of the clones had a lymphotropic amino acid signature. The macrophage tropism signature amino acids differed by patient. This implies variability in the selective forces associated with macrophage tropism and perhaps an increased roll of genetic drift in the regional isolation of populations in different brain regions. Clearly selection is working in conjuction with population isolation, as signatures are evident within patients. Amino acid signatures have also been suggested for fusion activity, in that positively charged amino acids at positions 210 and 229 are found in the viral syncytium-inducing (SI) phenotypes, whereas non-syncytium-inducing (NSI) phenotypes have negatively or uncharged amino acids at the same positions. All of the 62 brain-derived clones correspond to the SI phenotype. This is consistent with the early penetration of the brain by macrophage-tropic NSI strains of HIV-1, which are the predominant viral strains during the early phase of infection and with the fact that macrophages and microglia are the primary reservoir of HIV-1 in the brain. (There was no preference for NSI amino acids when analyzed by neuroanatomical site or by subject.)

The presence of brain region-specific HIV-1 env clusters and amino acid signatures described in this article implies that regional selective forces operate in the HIV-infected brain and that the HIV we characterize may be associated with pathogenicity in brain. This laboratory has provided evidence of heterogeneity of macrophage surface marker expression in the human peripheral and central nervous systems in individuals with AIDS. In addition, there is evidence of heterogeneity of
macrophage function in various tissues in the HIV-1-infected individual.63 Since macrophages are the primary reservoir of HIV-1 infection in the brain,31,66,67 macrophage heterogeneity may contribute to the clustering of HIV-1 sequences. In addition, as mentioned above, we have shown that virus load varies nonrandomly among these regions of brain, with highest HIV-1 DNA load in the temporal lobe, supporting the notion of regional heterogeneity.19,58,59,68 Regional heterogeneity has also been demonstrated using immunohistochemical detection of HIV-1 proteins and brain imaging techniques.44,57,58

The spectrum of neuropsychiatric impairments in AIDS may ultimately be related to the pathology and virus infections of these different regions of brain in HIV-infected patients and to the interaction of these regions and HIV-1 signatures. Although individuals are initially infected with macrophage-tropic HIV-1, the virus generally evolves into lymphotropic virulent strains in the periphery,69 whereas the brain infection remains macrophage tropic with corresponding signature amino acids as described above.6,9,13,49,62 Thus, there may be restricted pathways for HIV-1 to evolve and cause pathology in the brain.70 HIV-1 forms different quasispecies in each separate brain region and there is little gene flow among these regions.

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