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# Demented and Nondemented Patients with AIDS Differ in Brain-Derived Human Immunodeficiency Virus Type 1 Envelope Sequences

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**Human immunodeficiency virus (HIV) dementia is a common clinical syndrome of uncertain pathogenesis in patients with AIDS. In several animal models of retrovirus-induced brain disease, specific viral envelope sequences have been found to influence the occurrence of central nervous system disease. Therefore, to search for unique envelope sequences correlated with HIV dementia, we studied 22 HIV-infected patients who were neurologically assessed premortem and classified into demented (HIVD) ($n$ = 14) and nondemented (ND) ($n$ = 8) groups. Using DNA from autopsied brain and spleen, we amplified, cloned, and sequenced a 430-nucleotide region including the V3 loop and flanking regions. All brain-derived clones in both clinical groups showed marked homology to the macrophage-tropic consensus sequence within the V3 loop. Two amino acid positions within (position 305) and outside (position 329) the V3 region showed significant divergence between the two clinical groups. At position 305, a histidine was predominant in the HIVD group and was not observed in the ND group, but a proline was predominant in the ND group and was not observed in the HIVD group. Similarly, at position 329, a leucine was predominant in the HIVD group but rarely observed in the ND group, whereas an isoleucine was predominant in the ND group at this position. In addition, the HIVD group had 21 amino acid residues at specific positions that were unique relative to the ND group, whereas only 2 residues at specific positions were unique to the ND group. These data suggest that distinct HIV envelope sequences are associated with the clinical expression of HIV dementia.**

Human immunodeficiency virus (HIV) dementia is a major neurological complication in 20% of patients with AIDS (29, 34). This clinical syndrome is characterized by progressive motor signs, behavioral abnormalities, and cognitive deficits (34). At present, there is no definitive explanation for the variation in severity and clinical course of HIV dementia, although a number of hypotheses have been proposed. These include a role for HIV gp120 or quinolinic acid as a neurotoxic agent (6, 14, 50), complementation by defective viruses (24, 25), enhanced central nervous system (CNS) cytokine production (52, 56), and increased permeability of the blood-brain barrier (31, 41). Although some patients with AIDS may have CNS inflammatory changes, which have been termed HIV encephalitis (1), many patients with HIV dementia do not have any neuropathological changes (11, 33). Furthermore, although HIV antigens have been detected in the brains of some demented and nondemented patients, a clear relationship between the amount of virus and clinical symptoms has not been made (21, 46, 54, 59). These findings raise the possibility that different HIV strains vary in the potential for induction of clinical brain disease, as has been reported with retroviruses in other species such as mice, sheep, and monkeys (9, 27, 38, 39, 43, 44, 48).

In general, the symptoms associated with the outcome of viral infections of the CNS are determined by the type of cells infected and the damage that a particular strain of virus inflicts on an infected cell and its neighbors. Studies of several groups of viruses that cause neurologic disease, including retroviruses, suggest that the surface proteins are often major determinants of virulence. Single amino acid changes in the surface proteins of alphaviruses (51), flaviruses (19), rhabdoviruses (5), and murine retroviruses (48) lead to profound changes in the severity of the neurologic diseases induced. For most of these neurotropic viruses, the mechanism by which these amino acid changes affect virulence has not yet been determined, but suggested mechanisms include altered protein folding and processing (48), increased efficiency of viral spread between neurons (16), interaction with cellular proteins governing programmed cell death (23), and indirect neurotoxicity (26).

To date, several studies have analyzed HIV sequences from cerebrospinal fluid (20) and brain (7, 24, 25, 37) in attempts to detect tissue-specific viral strains, but no strong associations between particular viral sequence patterns and presence or absence of clinical CNS symptoms have been obtained. In part, this may be due to the extreme complexity of studying a heterogenous outbred human population infected by a highly variable infectious virus. These difficulties are further compounded by the problem of obtaining accurate clinical information on patients of interest.

In this study, we attempted to identify specific HIV envelope sequences associated with HIV dementia in a population of patients with AIDS prospectively studied at the Johns Hopkins University Department of Neurology (14, 28, 29, 41, 52, 56). Each patient was studied prior to death to determine by objective criteria whether the patient was or was not demented. HIV envelope sequences from the autopsied brains of

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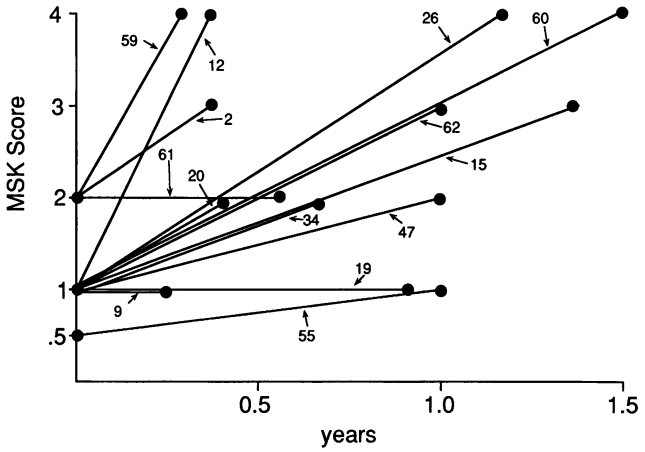


FIG. 1. MSK scores of HIVD patients plotted versus time for initial and final evaluations. The clinical status of each patient was assessed at the time of initial diagnosis of HIV dementia and the final clinical evaluation before death by the same clinician during the course of disease progression.

these patients were then compared to begin to look for associations with clinical HIV dementia. Unlike the case for previous studies, a control group consisting of nondemented patients with AIDS (ND group) was compared with a group of patients with HIV dementia (HIVD group). These studies indicate that the HIV sequences in the brains of AIDS patients with dementia are different from those of patients without dementia and suggest that the strain of virus infecting the brain may be an important determinant of neurologic disease.

Spleens and subcortical white matter from the mid-frontal gyrus of brains of 22 patients were selected from the AIDS Brain Bank at Johns Hopkins University on the basis of clinical status. The AIDS Brain Bank contains frozen and fixed brains from over 300 autopsied patients with AIDS, who were characterized clinically by the AIDS Neurology Group prior to death (11, 28, 41, 52, 56). Patients were defined as ND ($n$ = 8) and HIVD ($n$ = 14). Diagnostic criteria for HIV dementia included all of the following: (i) HIV-1 seropositivity, (ii) history of progressive cognitive decline, (iii) neurological and/or neuropsychological findings consistent with a decline from the premorbid baseline, and (iv) exclusion of CNS opportunistic infections by computed tomography or magnetic resonance imaging and cerebrospinal fluid analysis during the phase of clinical dementia (17). The dementia observed in the HIVD group is a primary HIV-induced syndrome and unlikely to be caused by other AIDS-associated CNS diseases such as CNS lymphoma and CNS opportunistic infections, which were excluded during the long clinical observation period before death. In contrast, in previous studies, complicating CNS opportunistic infections and malignancies were present in several of the patients whose brain HIV sequences were analyzed (37), making it difficult to determine whether direct pathogenic effects of HIV itself on the CNS were in fact present. The severity of HIV dementia was assessed by using a dementia rating scale (Memorial Sloan-Kettering [MSK]) (42). The MSK scale ranges from mild dementia or minimal cognitive and motor deficits (MSK = 1) to severe dementia or end-stage vegetative state (MSK = 4). Each patient's mental status was assessed over the course of disease on the basis of MSK scores (Fig. 1). The HIVD and ND groups were well matched for mean ages, CD4 counts, neuropathological findings, and antiretroviral drug therapy, without statistically significant differences between groups (Table 1). Multinucleated giant cells, typical of HIV encephalitis, were identified in only 3 of 14 HIVD patients (patients 2, 15, and 20) and in none of the ND patients. Thus, the 11 HIVD patients who lacked pathological abnormalities were typical of many HIV dementia cases which show a marked discrepancy between the severe clinical defects and mild or absent pathological findings (11, 33). Our patients differ from other patients with HIV dementia or encephalopathy in whom severe encephalitis was described and HIV DNA levels were unusually high, allowing direct molecular cloning without PCR amplification (24, 25). This diversity in pathological findings may imply that the pathogenic mechanisms involved in the development of CNS disease differ between patients with and without prominent HIV encephalitis.

Because of previous data suggesting that the V3 region of the envelope influences infectivity for macrophages, microglia, T lymphocytes, and CD4-positive HeLa cells (3, 15, 35, 36, 47, 49, 55, 57), we focused our initial efforts on sequence analysis of this region of the HIV genome. In addition, recent in vitro indirect neurotoxic effects have been attributed to this portion of the HIV genome (10). To avoid selection bias by in vitro viral isolation, we used DNA obtained directly from infected brains and spleens to examine the V3 and flanking regions. Sequences were amplified by using a nested PCR protocol, and the first and second PCRs were carried out with oligonucleotides 6575/7330C and 6813/7258C (7258C, 5′-TTTGCTAGC TATCTGTTTTAAAGT-3′) as described previously (4). This amplification yielded a fragment of 430 bases that included both the C2 and V3 regions of the HIV envelope gene. The DNA product was cloned in the pCRII vector (Invitrogen, San Diego, Calif.), and the sequence was analyzed by dideoxy sequencing.

TABLE 1. Clinical features in HIVD and ND patients

| Clinical group[a] | Mean age (yr) ± SD | Mean CD4$^+$ cells/mm$^3$ ± SD | No. with HIV encephalitis[b] | No. with opportunistic CNS infections[c] | No. receiving antiretroviral drug therapy[d] |
|---|---|---|---|---|---|
| HIVD ($n$ = 14) | 38 ± 8.8 | 15 ± 12 | 3/14 | 8 | 8 |
| ND ($n$ = 8) | 38 ± 13.3 | 44 ± 74 | 0/8 | 3 | 2 |

[a] Groups did not differ statistically ($P > 0.05$) for any of the features analyzed (ages and CD4 counts, Mann-Whitney $U$ test; HIV encephalitis, opportunistic infections, and antiretroviral therapy frequencies, Fisher's exact test).

[b] The diagnosis of HIV encephalitis was based on the presence of multinucleated giant cells in at least one of multiple sections taken from the brain of each patient.

[c] In the HIVD groups, cytomegalovirus (CMV) ventriculitis ($n$ = 1), progressive multifocal leukoencephalopathy ($n$ = 1), cryptococcal meningitis ($n$ = 1) and cortical microabscesses ($n$ = 1), CMV encephalitis ($n$ = 2) and myelitis ($n$ = 1), and *Toxoplasma* cerebritis ($n$ = 1); in the ND group, *Toxoplasma* cerebritis ($n$ = 2) and *Cryptococcus* meningitis and multifocal leukoencephalopathy ($n$ = 1). Opportunistic infections were not observed in any sections adjacent to the tissues used for extraction of DNA. Repeated clinical evaluations indicated that these opportunistic infections developed between the last clinical assessment and death and thus did not contribute to the signs and symptoms of HIV dementia.

[d] Number of patients receiving antiretroviral therapy (zidovudine, dideoxyinosine, and/or dideoxycytidine) within a year of death.

TABLE 2. Comparison of the number of sequence differences between paired brain- and spleen-derived clones

| Patient no. | Group | No. of differing amino acids in the 143-residue fragment (C2 and V3) | |
|---|---|---|---|
| | | Brain vs brain[a] | Brain vs spleen[b] |
| 10 | ND | 4 | 16 (16) |
| 14 | ND | 5 | 24 (22) |
| 9 | HIVD | 4 | 21 (21) |
| 15 | HIVD | 4 | 16 (16) |

[a] Two brain-derived clones from each patient were aligned and compared.
[b] A single spleen-derived clone was compared with each of the brain-derived clones from the same patient. Numbers in parentheses represent comparisons for the second brain clone.

In four patients, sequences from paired brain- and spleen-derived clones were compared to ascertain the diversity of HIV sequences between lymphoid tissue and brain (Table 2). In the 143-amino-acid fragment including the C2 and V3 regions of HIV envelope, spleen-derived HIV sequences appeared to differ significantly from brain-derived sequences in each of four patients analyzed. Regardless of clinical diagnosis, there were 4 to 5 differing amino acids between the sequences of paired brain-derived clones from each patient, whereas the brain-to-spleen comparison from the same individual showed from 16 to 24 differing amino acids (Table 2). These results indicated that the similarity between the brain-derived clones was much greater than that between the brain- and spleen-derived clones. This finding was similar to previous reports comparing multiple brain- and blood-derived HIV clones from two patients (37). Therefore, we concentrated on the brain-derived HIV sequences to study differences between the HIVD and ND groups in a larger group of patients with AIDS.

The V3 regions of all the brain-derived sequences from the 22 patients studied were grouped by clinical diagnosis and compared with blood-derived HIV isolates of known cell tropism (Fig. 2). A striking similarity was observed between a previously described macrophage-tropic consensus sequence derived from blood-derived viruses (4) and all the brain-derived sequences of both HIVD and ND clinical groups. In contrast, the brain-derived sequences differed extensively from sequences of blood-derived non-macrophage-tropic viruses. The similarity of brain-derived V3 sequences from both HIVD and ND patients to the macrophage-tropic V3 consensus sequence and to the blood-derived macrophage-tropic isolates was consistent with the previous findings of in vitro macrophage tropism of most brain-derived HIV isolates (22, 24, 45) and suggested that macrophage tropism might be necessary for CNS infection but not sufficient for the induction of HIV dementia.

Although some clones in the HIVD group were very homologous to clones in the ND group, the development of HIV dementia might be dependent on a few amino acid residue differences, as has been found for CNS diseases caused by Moloney murine retrovirus, rabies virus, Sindbis virus, and dengue virus (5, 19, 48, 51). To look for specific differences, sequences in each group were analyzed to determine whether conserved amino acids were found at any position in either group (Fig. 3). Between clinical groups, significant differences in the amino acid residues at two positions were identified (Table 3). A histidine at position 305 was identified in 17 of 25 HIVD clones and in none of the ND clones. At the same position, a proline was identified in 8 of 14 ND clones but not in any of the HIVD clones. At position 329, a leucine was found in 15 of 25 HIVD clones and in only 2 of the ND clones, whereas an isoleucine was present in 11 of 14 ND patients and in 9 HIVD clones. Although a histidine at position 305 and leucine at position 329 were most strongly correlated with the HIVD clinical group, these substitutions have been frequently found in blood-derived HIV isolates (Table 3), including strains with differing tropisms (4, 8, 15, 32, 57). Since many of these isolates probably came from ND patients, it is clear that the presence of His-305 or Leu-329 in virus derived from blood or lymphoid tissues is not sufficient to cause dementia. Viral strains bearing His-305 and Leu-329 may be pathogenic in the CNS but not pathogenic elsewhere in the body, as has been reported for neurotropic murine retroviruses which also infect various cells in the male and female reproductive tracts without any apparent pathogenic sequelae (40).

In our previous studies on in vitro tropism of HIV, we found that no single V3 amino acid controlled cell tropism, but instead the entire V3 sequence context was important (4). Other *env* regions besides V3 can also influence tropism (12, 13, 47, 58), which suggests that the three-dimensional folding of the entire envelope protein might be important in determining tropism. Similarly, other envelope amino acids might account for the lack of absolute correlation between HIVD

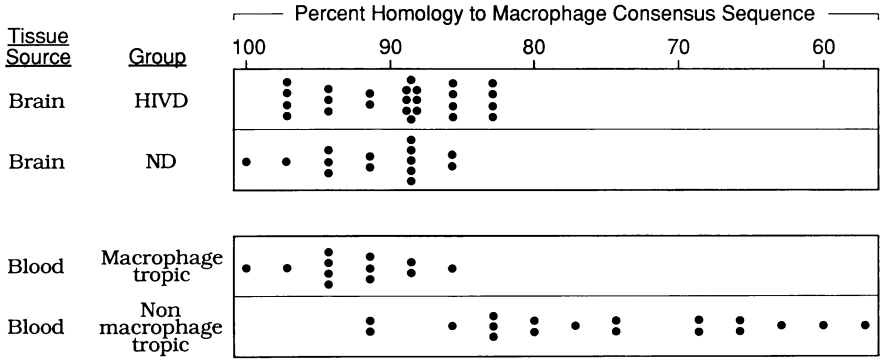


FIG. 2. Comparison of the homology of V3 sequences of brain-derived clones and previously reported blood-derived clones and isolates of known tropism with the macrophage-tropic consensus sequence (4). Each dot represents a different clone. Regardless of clinical group (HIVD or ND), brain-derived isolates were similar to blood-derived macrophage-tropic isolates and differed significantly from blood-derived non-macrophage-tropic isolates. Macrophage-tropic isolates included previously described macrophage-tropic clones (4) and isolates defined as nonsyncytium inducing in phytohemagglutinin-stimulated T-lymphocyte cultures (8). Non-macrophage-tropic strains included isolates capable of syncytium induction in phytohemagglutinin-stimulated lymphocytes (8) and clones capable of infecting clone 1022 CD4-positive HeLa cells (4).

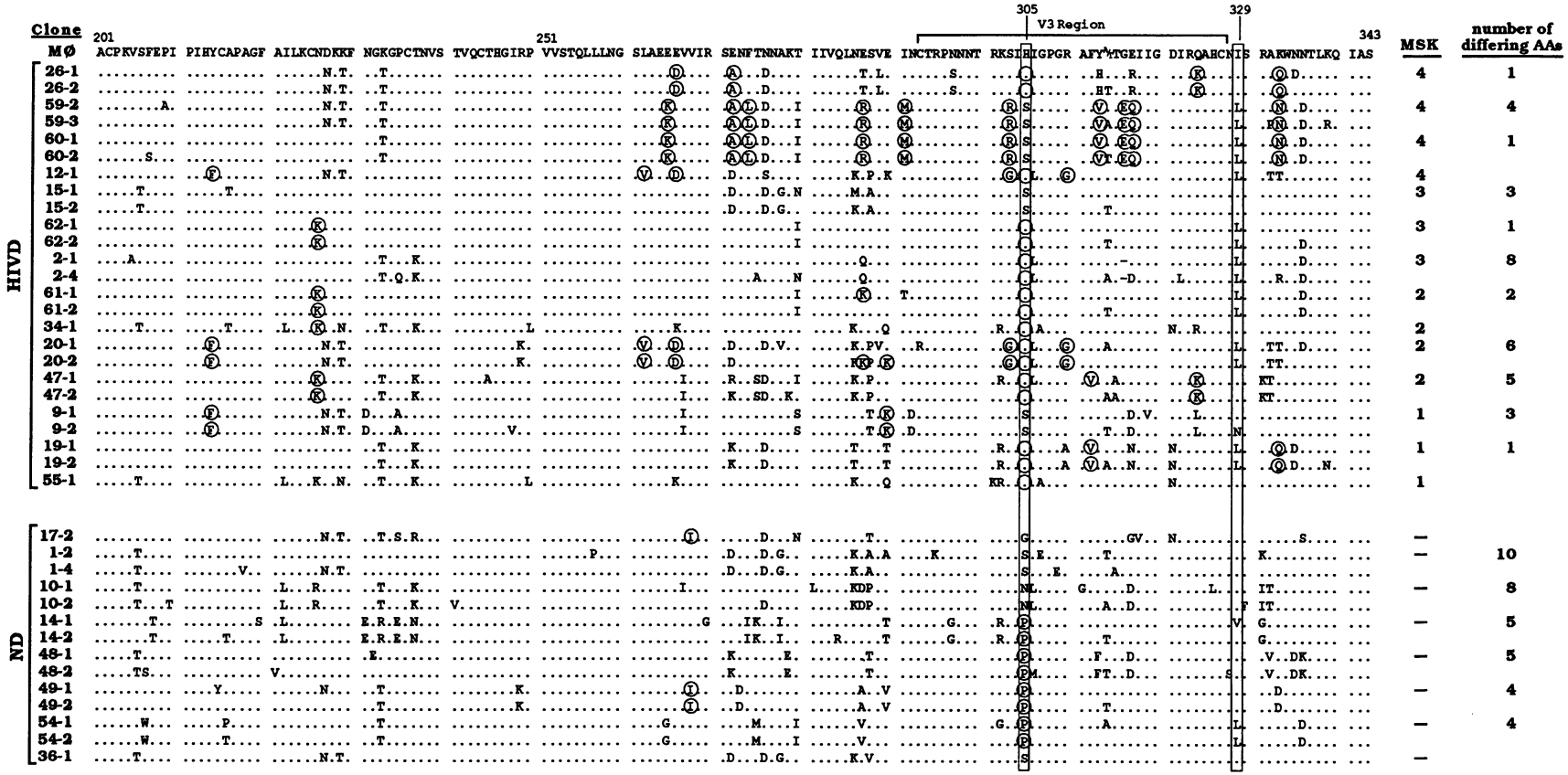


FIG. 3. Comparison of HIV envelope amino acid sequences from patients belonging to the HIVD and ND groups. Sequences are aligned with the macrophage-tropic consensus sequence (MØ) (4). HIVD sequences are presented in the order of severity of dementia according to the MSK score. Two clones were sequenced from each patient except patients 12, 34, 55, 17, and 36, and the number of differing amino acids between the two clones from the same patient is indicated at the right. In agreement with previous reports (24, 25, 37), clones from the same individual were closely related, showing an average of 4 amino acids differing within the 143-amino-acid fragment. In view of this homogeneity, we elected to sequence only one or two clones from each patient and instead study more individual patients. Positions 305 and 329, as indicated by boxes, show significant divergence in the residues between the HIVD and ND groups. Unique amino acids found at a particular position in two or patients in one clinical group and absent in all patients of the other group are circled. In many cases, only a single clone or single patient within a clinical group showed a unique amino acid at a particular position, and in this situation it was impossible to know whether this was due to a PCR-induced mutation. Even when one considers only those changes where unique amino acids were seen in two or more different patients, the HIVD group was found to have 21 unique amino acids and the ND group had 2 (Mann-Whitney, $U = 99.5$, $P = 0.0033$). Unique amino acids were significantly more frequent in clones from the severely demented patients (MSK = 4; patients 26, 59, 60, and 12) than in the other HIVD-derived clones (Kruskal-Wallis, $P = 0.027$).

TABLE 3. Comparison of amino acids at positions 305 and 329 for brain-derived HIV envelope clones

| Patient group or source | Frequency[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Position 305 | | | | | Position 329 | | |
| | His | Ser | Pro | Other | Total | Leu | Ile | Other |
| HIVD | 17 | 8 | 0 | 0 | 25 | 15 | 9 | 1 |
| ND | 0 | 3 | 8 | 3 | 14 | 2 | 11 | 1 |
| Data base[b] | 20 | 6 | 6 | 27 | 59 | 6 | 41 | 12 |

[a] The frequency of each amino acid at each position in the HIVD and ND groups was compared individually with the sum of all other amino acid frequencies in separate 2 × 2 contingency tables with Fisher's exact test. The following *P* values were observed: His-305, <0.0001; Pro-305, <0.0001; Leu-329, 0.0078; and Ile-329, 0.0187. Boxed values are significantly different between HIVD and ND groups.

[b] From reference 32.

and His-305 or Leu-329. For example, in the HIVD group, there were 21 amino acids at specific positions that were unique relative to the ND group, whereas only two residues at specific positions were unique to the ND group (Fig. 3). Furthermore, the most severely demented patients (MSK = 4) (patients 12, 26, 59, and 60) contained the highest frequency of unique amino acids (Kruskal-Wallis, $P < 0.027$) (Fig. 3). The findings of highly conserved amino acids at two specific positions and the high number of unique amino acids in the HIVD group indicated that the brain-derived HIV sequences differed between the HIVD patients and the ND patients.

The finding of highly significant differences in HIV *env* sequences between the two clinical groups suggests that distinct *env* sequences might account for the presence of dementia. Similarly, mouse retroviral *env* sequences have been shown to influence the development and course of CNS disease (2, 39, 48). More recently, *env* sequences have also been shown to alter the location of CNS pathological lesions and the sites of viral antigen detection within the CNS (38a). However, in these mouse models, *gag* and long terminal repeat sequences also influence clinical CNS disease, and this might suggest that other regions of the HIV genome are also involved in the induction of dementia. Thus, future efforts should include analysis of other areas of the HIV genome derived from both of these clinical groups.

Although our results show a strong association of certain HIV genotypes with two clinical groups, it is also possible that this correlation is related to other unknown factors differing in these two groups. For example, if the HIVD group had been infected for a much longer time before death than the ND group, there would have been more time to generate variable HIV genotypes. Similarly, other factors such as host genetic background and source of infection might influence the associations that we observed in this work. Thus, it will be important to extend this work to a larger group of patients from different geographic areas, using larger regions of the HIV genome to confirm or refute these striking early observations.

It will also be interesting to express some of the envelope sequences from each clinical group in our study in live HIV molecular clones to study their effects in different biological in vitro assays related to CNS tropism or pathogenicity (31, 45, 55). However, it is unclear which type of assay will give the most valid correlation with the generation of HIV dementia in vivo. Resolution of this problem will require a better understanding of the complex mechanisms of pathogenesis of HIV dementia. Therefore, at present it may be more fruitful to use in vivo approaches such as the transgenic mouse gp120 expression models or the HIV-infected severe combined immunodeficiency mouse model (18, 50, 53).

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