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CME

HIV in the brain

RNA levels and patterns of zidovudine resistance

D.R. McClernon, BS; R. Lanier, PhD; S. Gartner, PhD; P. Feaser, BS; C.A. Pardo, MD; M. St. Clair, BS; Q. Liao, PhD; and J.C. McArthur, MBBS, MPH

Article abstract—*Objective:* To examine the association between HIV RNA levels, patterns of antiretroviral resistance, and neurologic status. *Methods:* Autopsy samples from 13 HIV-infected subjects were examined for HIV-1 viral RNA (vRNA), and viral reverse transcriptase (RT) genotype was determined. All subjects had been clinically characterized using standard instruments before death. *Results:* The median HIV-1 vRNA level in brain samples from subjects with moderate dementia was 7.79 log₁₀ copies/g (range 5.56 to 9.75 log₁₀ copies/g) compared with 5.44 log₁₀ copies/g (range 3.51 to 9.32 log₁₀ copies/g) for mildly demented subjects and 4.87 log₁₀ copies/g (3.51 to 6.86 log₁₀ copies/g) for those obtained from nondemented individuals. There were differences between subjects with moderate dementia and nondemented subjects ($p = 0.0002$) and between subjects with moderate and mild dementia ($p = 0.0128$). No significant differences among the groups were observed for vRNA levels in peripheral tissues. Some demented subjects had relatively low levels of HIV-1 vRNA, and paradoxically some nondemented subjects had high vRNA brain levels. Little subject effect in vRNA was noted in peripheral regions, but high regional variation in vRNA was noted within the brain. Patterns of the major zidovudine (ZDV) RT mutations in brain and peripheral tissues were concordant in most subjects. Subjects with longer duration of exposure to ZDV tended to have lower brain vRNA levels and a greater number of RT mutations than those with limited to no exposure. *Conclusions:* The presence and severity of HIV dementia correlates with the levels of productive HIV replication within the brain. Other pathophysiologic events (including macrophage activation) probably also contribute to neurologic dysfunction.

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HIV-1 infection of the CNS is believed to occur shortly after initial systemic infection, although in most individuals, sustained viral expression occurs only after immunosuppression. Levels of HIV-1 viral RNA (vRNA) in CSF have been shown to correlate

with the severity of HIV dementia, both in adults and in children.¹⁻³ The relationship between neurologic disease (specifically HIV-associated dementia) and the level of expression of HIV-1 within the brain is, however, still largely unknown. Most studies agree that the pathologic substrate for HIV-associated dementia is a chronic encephalitis with HIV-1 replication predominantly in macrophages and microglial cells and increased numbers of acti-

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From Glaxo Wellcome, Inc. (Drs. Lanier and Liao, and D.R. McClernon and M. St. Clair), Research Triangle Park, NC; and Department of Neurology (Drs. Gartner, Pardo, and McArthur, and P. Feaser), Johns Hopkins University School of Medicine, Baltimore, MD.

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Address correspondence to Dr. J.C. McArthur, Johns Hopkins Hospital, 600 N. Wolfe St., Meyer 6-109, Baltimore, MD; e-mail: jm@jhmi.edu

Table Clinical features of patients and antiretroviral exposure

Subject no.	Dementia status (MSK score)	CD4 count (closest to death)	Zidovudine exposure	Other antiretrovirals	Postmortem delay, h
416	No (0)	30	> 3 y AZT (d/c 8 mo PTD)	DDC	13
468	No (0)	200	18 mo AZT (d/c 13 mo PTD), DDC	DDC	25
474	No (0)	300	No ARV	None	24–36
433	No (0)	32	Remote AZT (d/c y PTD)	None	22
296	No (0)	700	No ARV	None	20
470	No (0)	30	No ARV	None	30
417	No (0)	39	AZT 36 mo (d/c 15 mo PTD)	None	7
429	Mild (0.5)	12	AZT 2 y (d/c 3 y PTD)	DDI	24–36
512	Mild (1)	9	AZT 2 y (d/c 4 y PTD)	DDC, DDI, ritonavir	9
525	Mild (1)	95	AZT 7 y (d/c 2 y PTD)	DDI, D4T	42
403	Moderate (2)	6	AZT 18 mo (d/c 18 mo PTD)	None	15
507	Severe (3)	8	AZT 18 mo (d/c 40 mo PTD)	DDC, 3TC, D4T	19
407	Moderate (2)	108	AZT 5 mo (d/c 1 mo PTD), DDI	None	13

MSK = Memorial Sloan-Kettering HIV Dementia Severity Scale; CD4 = cellular determinants; AZT = zidovudine; PTD = prior to death; d/c = discontinued; DDC = zalcitabine; ARV = antiretrovirals; DDI = didanosine; D4T = stavudine; 3TC = lamivudine.

vated macrophages.⁴ We⁵ studied HIV-1 DNA in mid-frontal gyrus from demented and nondemented subjects and found a very wide range of DNA levels, with no significant relationship to neurologic status. A stronger relationship exists between the severity of dementia and the abundance of HAM56 (a marker of macrophage activation) than for the levels of viral proteins.⁶ Whether this reflects the relative insensitivity of these early techniques, limited sampling, or a true discordance between brain levels of replication and neurologic disease is uncertain. Subsequent studies, in a small number of autopsies, demonstrated a good correlation between DNA levels and the pathologic severity of encephalitis.^{1,7}

The compartmentalization of resistance mutations also remains poorly understood. Unchecked HIV-1 replication within the CNS could potentially reseed the systemic compartment. This would have relevance if resistant mutants were selected within the brain and then emerged to repopulate the periphery with CNS-derived strains of HIV-1. Early studies of proviral DNA obtained from blood and CSF showed comparable positions and frequencies of zidovudine (ZDV) mutations in isolates from both compartments.⁸ Similarly, complete correspondence was found between plasma and CSF specimens in 15 plasma/CSF pairs from pediatric subjects for the 215Y mutation and determined that the presence of this mutation was associated with a poor neurologic outcome.² By contrast, in sequential samples of plasma and CSF of subjects receiving long-term ZDV therapy, in four of six subjects, the patterns of mutations in CSF RNA samples were different from those found in blood.⁹ Concordance was observed for the lamivudine resistance mutation M184V in plasma and CSF samples.¹⁰ Resistance mutations to nucleo-

side analogues were compared by clonal analysis in peripheral and brain tissues from four subjects who had received ZDV.¹¹ Differences were noted between the subjects: One harbored the resistance mutations M41L and T215Y both in spleen and in brain, and one had a putative transitional clone T215H only in spleen but otherwise had wild type in all tissues. Two showed discordance between brain and peripheral tissues: One had M41L and T215Y uniformly in all clones from spleen (15/15) and lymph node (13/13) but not brain clones (4/15), and one had T215Y in the majority of spleen and all lymph node clones but none in brain. In one subject in whom CSF- and brain-derived cDNA was analyzed, there was general concordance.¹² The inference from this and a phylogenetic analysis of *pol* sequences was that there were independently evolving quasi-species.

In an earlier work,³ we measured brain tissue RNA copy numbers and corresponding CSF values from 22 paired samples from midfrontal gyrus and basal ganglion (i.e., the study was limited to just these two brain regions in contrast to the current study, which is more comprehensive). Brain tissue HIV RNA levels were inter-correlated for these two regions, and taking HIV RNA levels from both of these regions, we noted a weak correlation with CSF HIV RNA levels (obtained antemortem) ($r = 0.33$).

We examined autopsy samples from clinically well-characterized patients, sampling multiple brain regions to determine brain HIV-1 RNA levels and presence of reverse transcriptase (RT) mutations using viral RT sequencing.

Methods. *Subjects.* Patients were clinically characterized from antemortem assessments performed within 90 days of death. (About half of these subjects had been in-

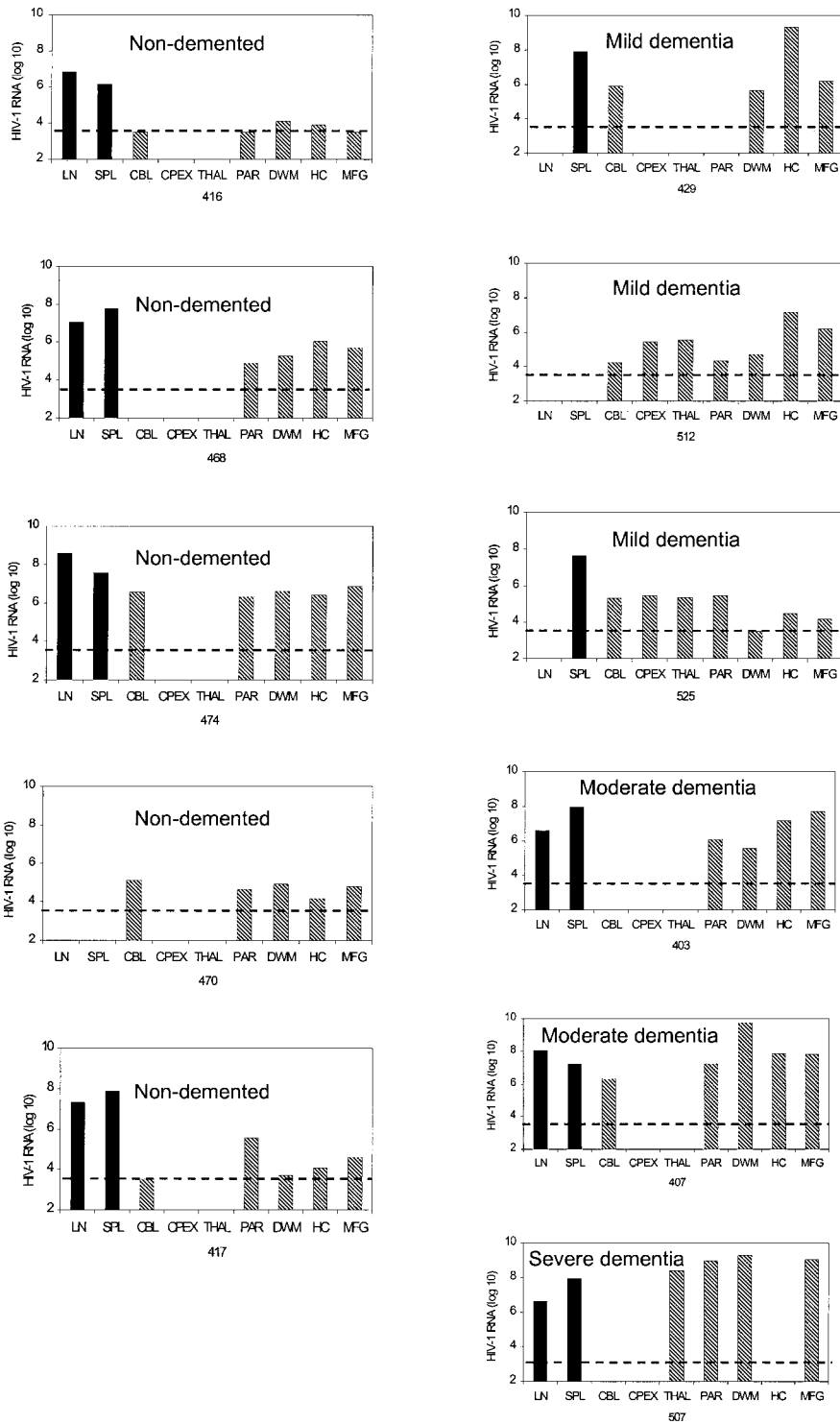


Figure 1. Individualized graphs of HIV RNA levels derived from peripheral (filled columns) and brain (hatched columns) tissues for nondemented and demented subjects. Horizontal line indicates the limit of detection. For clarity of presentation, two nondemented subjects are not displayed. LN = lymph node; SPL = spleen; CBL = cerebellum; CPEX = choroid plexus; THAL = thalamus; PAR = parietal lobe; DWM = deep white matter; HC = head of caudate; MFG = midfrontal gyrus.

cluded in the earlier 1997 publication.³) Neurologic and neuropsychological abnormalities were defined using the American Academy of Neurology criteria.¹³ The Memorial Sloan-Kettering (MSK) dementia scale¹⁴ was used to stage the severity of neurologic disease. Thirteen subjects were categorized a priori by their individual neurologic status: HIV-1 positive nondemented (27 brain specimens from seven subjects with MSK scores of 0), HIV-1 positive with mild dementia (20 brain specimens from three subjects with MSK scores of 0.5 to 1), and HIV-1 positive with

moderate/severe dementia (13 specimens from three subjects with MSK scores of 2 to 3).³ Patterns of antiretroviral use were obtained from chart abstraction and are displayed in the table. Remote use was defined as use >12 months from autopsy. Three subjects had never used antiretrovirals, and the remainder had used ZDV for varying periods before death (5 months to 7 years), with discontinuation of ZDV from 1 to 48 months before death and subsequent use of other nucleoside analogues. Protease inhibitors were used in only one subject (no. 512).

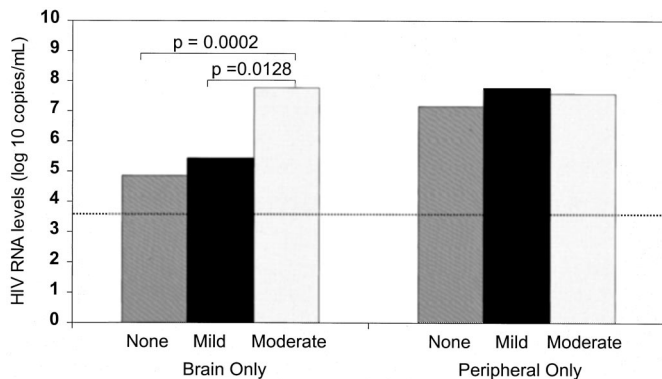


Figure 2. Median HIV RNA levels for brain (for all available brain regions) and peripheral tissues stratified by neurologic status: nondemented, mild (Memorial Sloan-Kettering score [MSK] = 0.5 to 1), and moderate/severe (MSK = 2 to 3). Significant differences among neurologic groups were noted only for brain tissues. Horizontal line indicates the limit of detection.

Assay technique. Autopsy samples from 13 subjects were examined for vRNA levels (log₁₀ copies/g of tissue; NASBA QT assay, Organon Technika, Durham, NC) using previously described methods.³ No appreciable effect of postmortem times on brain levels of HIV RNA was identified. Viral RT genotypes were determined using dideoxy dye terminator sequencing (Perkin Elmer/AB, Foster City, CA). The limit of vRNA detection for this study was determined by the amount of tissue used, giving a final limit of 3.51 log₁₀ copies/g. Regions analyzed included the cerebellum, choroid plexus, deep white matter, head of caudate, midfrontal gyrus, parietal lobe, thalamus, spleen, and lymph node.

Statistical analysis. vRNA levels of brain tissues and peripheral tissues were analyzed, and summary statistics were generated for the three different neurologic status groups, for groups with and without ZDV-associated RT mutations, and for specific brain tissue sites. Owing to the small sample size, median and range rather than mean and standard error of vRNA levels were presented in Results. These summary statistics were calculated using all vRNA levels regardless of the correlation of vRNA levels in different regions in each subject. However, to take into account within-subject variability, we used mixed effect models to make inferences on vRNA levels between brain tissues and peripheral tissues, between groups with and without ZDV-associated RT mutations, and among the three different neurologic status groups for brain tissue and peripheral tissues. In fitting the mixed effect models, the tissue type, dementia status, and presence of ZDV-associated RT mutations were three separate fixed factors, and correlation of vRNA levels within subject was modeled through the use of random effect. Here, the subject effect (random effect) was assumed to be normally distributed with some unknown variance, and vRNA levels in the model were also assumed to be normally distributed. The calculation of all vRNA levels was made under log₁₀ transformation, and the assumption of normality of log₁₀ vRNA levels was valid.

Statistical analyses were performed using SAS (version 6.12; SAS Institute, Cary, NC). A *p* value <0.05 was con-

sidered significant. No type I error adjustment was made for multiple comparisons.

Results. Neurologic status, CD4 counts, and other clinical features are presented in the table. Supplementary material on the *Neurology* Web site provides the vRNA levels and dominant RT genotype at specific regions. The vRNA levels varied widely among the different brain regions and subjects studied (figure 1). The vRNA levels obtained from peripheral tissues were higher than from brain tissues for the majority of the subjects, with the exception of three subjects with dementia who had higher vRNA levels in brain than peripheral tissues (nos. 507, 429, and 407). Taking all samples, the vRNA levels obtained from peripheral tissues were higher than from brain tissues (*p* = 0.0001). The median vRNA level from brain samples from subjects with moderate dementia was 7.79 log₁₀ copies/g (range 5.56 to 9.75 log₁₀ copies/g) compared with 5.44 log₁₀ copies/g (range 3.51 to 9.32 log₁₀ copies/g) for mildly demented subjects and 4.87 log₁₀ copies/g (3.51 to 6.86 log₁₀ copies/g) for those obtained from nondemented individuals. Using a mixed effect model, we found that the difference between subjects with moderate dementia and nondemented subjects was highly significant (*p* = 0.0002) (figure 2). The difference between moderate and mild dementia was also significant (*p* = 0.0128). However, the results from a mixed effect model showed that the vRNA levels for peripheral tissues were not statistically different among the three clinical groups (*p* = 0.652).

In fitting the above mixed effect models, the subject effect variability of vRNA within brain was estimated to be 0.878, whereas the residual variance was 0.761. The estimated common correlation between any two different brain regions from the mixed effect model was 0.536. For peripheral tissues, the subject effect variability of vRNA was estimated to be 0.072 and the residual variance was 0.385. The estimated correlation between lymph node and spleen from the mixed effect model was 0.158. There was thus little subject effect in vRNA in peripheral regions but higher subject effect within brain.

ZDV resistance mutations were analyzed from peripheral and brain tissues, as displayed in the supplementary material on the *Neurology* Web site. Genotypic data could not be obtained from 14 tissue samples. Twenty brain tissue samples had virus with no evidence of ZDV-associated RT mutations; the median vRNA from these samples was 7.0 log₁₀ copies/g (range 3.51 to 9.75 log₁₀ copies/g). Thirty brain tissue samples contained virus with at least one ZDV-associated mutation (41L, 67N, 70R, 210W, 215F/Y, and/or 219Q); the median vRNA from these samples was 5.6 log₁₀ copies/g (range 3.51 to 9.3 log₁₀ copies/g). The vRNA levels between the two groups were statistically different (*p* = 0.0046). Fifteen of the 30 brain tissue samples had HIV-1 RNA sequences with two or more ZDV-associated RT mutations; the median vRNA from these samples was 4.98 log₁₀ copies/g (range 3.51 to 7.17 log₁₀ copies/g).

In general, we detected similar patterns of RT mutations in brain and peripheral tissues for a given individual. In one set of tissues from a subject with moderate dementia (no. 403), the 215Y mutation was detected in lymph node, caudate, and deep white matter, whereas wild-type virus was present at other sites. In two other subjects, the 215Y mutation was detected in spleen and other tissues

contained wild type. One individual who had started ZDV 5 months before death had 215Y in lymph node but wild type in brain regions. As expected, there was a tendency for subjects with a longer duration of exposure to ZDV to have a greater number of RT mutations. Thus, four subjects with two or more ZDV RT mutations had used ZDV from 18 months to 7 years, whereas those with no or remote use had zero to two RT mutations.

Discussion. In this study, we show that brain HIV-1 RNA levels are, in general, significantly higher in subjects with moderate/severe levels of dementia than in nondemented or mildly demented individuals. In contrast, levels of HIV RNA in peripheral tissues (spleen and lymph nodes) did not distinguish neurologic status. There is, however, much more variability in the regional levels of brain HIV RNA. Patterns of ZDV resistance mutations were concordant for the majority of peripheral and brain tissues, and the presence of two or more RT mutations in brain tissue appeared to correlate with the duration of ZDV therapy.

One of the interesting observations from our study is that the highest levels of HIV RNA were observed in the caudate and deep white matter. Other investigators have also noted that this region is frequently affected pathologically. Several investigators have documented atrophy of the caudate region in HIV-associated dementia.^{15,16} Early immunohistochemical studies showed high levels of HIV antigens in this region¹⁷ or in the adjacent globus pallidus.¹⁸ A strong relationship was observed between the presence of HIV encephalitis and high levels of HIV-1 DNA and HIV p24.¹⁹ With use of quantitative PCR, the basal ganglia was found to have higher HIV-1 DNA levels than subcortical white matter or cortical gray matter.^{17,20} Quantification of HIV-1 proviral DNA in different brain regions demonstrated the highest levels in the medial temporal lobe (which included subcortical white matter and hippocampus).²¹ The basal ganglia had slightly lower levels than the medial temporal lobe, although the differences were not significant.⁷ Both Quantiplex (Chiron Diagnostics, Emeryville, CA) and Amplicor (Roche Diagnostics, Indianapolis, IN) techniques were used in postmortem brain tissue and CSF, and there was a good correspondence between the two techniques. Although clinical details in these cases were lacking, high levels of brain HIV-1 RNA corresponded with the pathologic features of HIV-1 encephalitis. In a rapid progressor model of simian immunodeficiency virus (SIV) encephalitis,²² tissue levels of SIV RNA correlated with the pathologic severity of encephalitis.

The relationship between CSF and parenchymal HIV-1 RNA levels is not addressed by this study. In earlier work, we found only weak correlations between brain and CSF levels of HIV-1 RNA.³ There was little agreement when CSF levels were $<10^5$ copies/mL,⁷ but a significant correlation when CSF levels exceeded 10^6 copies/mL.

Previous studies have examined ZDV and lamivudine resistance mutations in paired plasma and CSF specimens and, in general, have found concordance for the presence or absence of major resistance mutations.⁸⁻¹⁰ One group, however, has suggested that there may be important differences between the blood and CSF compartments.²³ Using the line probe assay, these investigators found concordance in 10 of 22 subjects. Six had wild-type or mutant variants in CSF but not plasma, and three had resistance mutations only in the CSF compartment. One caveat is that CSF samples failed to amplify in 35%. Our results conflict with earlier studies in a group of children with HIV infection.² These investigators found that the detection of the codon 215Y mutation in CSF correlated with progression of encephalopathy, whereas children with wild-type codon 215 remained stable neurologically. Relatively little information is available, however, on patterns of resistance mutations in brain tissue. Genetically distinct viral populations, which may display different patterns of drug resistance, have been found in the CNS,¹² lymphoid tissue,²⁴ and genitourinary tract.^{25,26}

It is uncertain why we detected lower HIV-1 RNA levels in brain specimens in which resistance mutations were detected. The acquisition of RT mutations might lead to a reduction in viral fitness, resulting in lower vRNA levels. In addition, antiretroviral therapy might suppress systemic replication, resulting in fewer infected cells trafficking into the brain and lower detectable tissue HIV RNA levels. Alternatively, the presence of mutations associated with antiretrovirals may simply be a surrogate marker of duration of exposure to antiretroviral therapy. Numerous differences were noted in the RT genotype from different anatomic regions, suggesting that viral replication and evolution may be compartmentalized between peripheral and CNS compartments.¹² It is also possible that these differences are due to differential amplification of quasi-species during PCR. Determination of this possibility will require analysis of multiple clones from each region.

One of the potential limitations of our study is whether blood contamination of tissues could have affected the assay results. This problem exists in all human studies where nonperfused tissues must be used. This issue was addressed in the examination of regional levels of HIV-1 DNA, where PCR-positive results were found only in regions with antigen-positive cells on immunocytochemistry.²⁰ This suggests that vascular contamination is not a major problem.

HIV dementia is associated with productive HIV-1 replication within the brain, and absolute levels of vRNA correlate with the severity of neurologic disease. There is, however, significant regional variation in brain HIV RNA levels. This suggests that other pathophysiologic events (e.g., macrophage activation) also contribute to the development of neurologic dysfunction.

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