Human Immunodeficiency Virus–1 RNA Levels in Cerebrospinal Fluid Exhibit a Set Point in Clinically Stable Patients Not Receiving Antiretroviral Therapy

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To characterize, over time, cerebrospinal fluid (CSF) virus-load change in clinically stable patients, human immunodeficiency virus (HIV)–1 RNA levels were measured in serial CSF samples and in plasma samples obtained, during periods of 20 days–6 years, from 17 HIV-infected individuals not receiving antiretroviral treatment. Longitudinal trends were analyzed by linear regression and restricted maximum-likelihood techniques. CSF HIV-1 RNA levels varied within a restricted range (±0.5 log10 copies/mL) around each subject-specific mean. Although 16 of the 17 subjects had CSF slopes not significantly different from zero, slopes that were more positive were associated with lower CD4 counts. In an individual patient, a CSF virus-load change >0.5 log10 copies/mL may be clinically significant. Furthermore, our data suggest that, if the CSF virus load reflects the size of the reservoir of infected cells in the central nervous system, this reservoir may increase in those individuals with advanced immunosuppression but is stable, over several years, in patients without AIDS.

Research involving human immunodeficiency virus (HIV)–1 RNA quantitation in body tissues/compartments other than blood, such as cerebrospinal fluid (CSF), has been limited. The potential value of longitudinal characterization of CSF virus loads is illustrated by comparison with findings of plasma virus loads obtained repeatedly either the same day or within a few days to weeks vary within ∼3-fold (±0.5 log10 copies/mL) of the initial measurements [1–3]. This degree of deviation probably reflects measurement variability plus normal biological fluctuations in virion production and clearance. Over longer periods (months to several years), plasma virus loads typically remain stable in clinically stable patients without AIDS [4]. Such patient-specific virus-load “set points” vary markedly between patients and are important clinically, since higher set points reliably predict more rapid rates of CD4+ cell loss, progression to AIDS, and death [5]. Thus, virus-load measurements are used by clinicians to evaluate whether patients with HIV infection require antiretroviral drug therapy and to monitor the efficacy of treatment.

Most reports of virus loads in CSF are based on single measurements. One study [6] examined the difference between CSF HIV-1 RNA levels at 2 times, 1.7–5.7 years apart, in 15 patients not receiving antiretroviral therapy. That study reported a statistically significant increase in CSF RNA levels between the first and the second visit. Yet the mean difference between visits was <0.5 log10 copies/mL, a difference that could be attributed to random variability, rather than to a clinically significant change. Because only 2 CSF samples/subject were considered, rates of change could not be reliably estimated.

In the present study, we obtained ≥3 serial CSF samples from each patient. We hypothesized that, among clinically stable patients, CSF virus loads would show restricted short-term variability, as well as a patient-specific set point—that is, no consistent, durable tendency to increase or decrease over time. Because other studies have shown that both CSF leukocyte counts and blood CD4+ lymphocyte counts strongly influence the relationship between CSF virus loads and plasma virus loads [7–10], we evaluated the effects of these parameters.

Methods. Study participants had laboratory-confirmed...
HIV-1 infection and were enrolled in longitudinal studies at the San Diego HIV Neurobehavioral Research Center (HNRC). Subjects met the following criteria: (1) CSF sampled on ≥3 occasions (each occasion ≥6 days apart), (2) initial CSF and plasma HIV-1 RNA levels above the nominal detection limit of the assay (see below), (3) no antiretroviral medication during the observation period, and (4) no active central nervous system (CNS) disease process, other than HIV, that might affect CSF parameters.

To separate cells, fresh plasma and CSF samples were centrifuged. Supernatants were aliquoted and were stored at −70°C. We measured HIV-1 RNA levels by reverse transcriptase–polymerase chain reaction, using the Amplicor HIV-1 Monitor Test (Roche Molecular Systems). For CSF, a nominal detection limit of 50 copies/mL was used; for plasma, a nominal detection limit of 400 copies/mL was used. CD4+ lymphocyte counts were quantitated by a fluorescence-activated cell sorter.

Plasma and CSF HIV-1 RNA levels were \( \log_{10} \) transformed before statistical analysis. CSF white blood cell (WBC) counts were classified according to the presence or absence of pleocytosis (CSF WBC counts >4/\mu L) [9, 11]. Crude estimates of slope (change over time) for each individual’s plasma and CSF virus loads were obtained by performance of separate linear regression models. These analyses generated residual values (observed minus predicted) for each study visit, values that were used to characterize variability. For change, over time, in the plasma-CSF gradient, slopes were similarly calculated (\( \log_{10} \) plasma HIV-1 RNA – \( \log_{10} \) CSF HIV-1 RNA). Restricted maximum likelihood (REML) regression analysis using subject as a covariate was performed to determine the effect of time on CSF HIV–1 RNA levels. Power estimates were calculated post hoc, as described below. Statistical analyses were performed by JMP software (version 4.0.4.; SAS Institute); power calculations were performed by S-Plus software (version 6; Insightful) [12].

A separate analysis was performed to estimate our data set’s power (2-sided test; \( P = .05 \)) to detect a common CSF viral growth rate (positive or negative slope) different from zero (the null hypothesis). By use of linear mixed-effects (LME) modeling, 1000 random simulations were performed according to the following procedure. Seventeen normally distributed set points (mean, 3.7 \( \log_{10} \); SD, 0.6 \( \log_{10} \) [based on the observed data set]) were generated. True virus loads for each patient were generated, based on both the times for each subject (total observations, 59) and an assumed common linear rate of viral growth. Simulated virus loads were produced by the addition of normally distributed random variables (mean, 0; SD, 0.4).

An LME model was fitted with normally distributed subject-specific set points, with and without a common growth rate, under maximum-likelihood assumptions. Analysis of variance was performed to calculate a \( P \) value, for growth versus no growth, in the LME model.

**Results.** Four women and 13 men met the study-inclusion criteria. Table 1 provides detailed clinical information on the participants. The mean (SD) age at the initial study visit was 38 (7.2) years; the median (interquartile range [IQR]) CD4 count at the initial study visit was 465 (240–578) cells/\mu L. Subjects were classified by use of the Centers for Disease Control’s HIV Disease Classification system [13], a system in which HIV-infected adolescents and adults are categorized on the basis of clinical conditions associated with advanced immunosuppression. In our study cohort, 7 subjects were classified as stage A, 7 were classified as stage B, and 3 were classified as stage C.

The mean initial virus loads in CSF and plasma were 3.57 and 4.55 \( \log_{10} \) copies/mL, respectively. Initial virus loads in CSF and plasma were not significantly correlated (\( r = 0.19; P = .49 \)) and were not significantly related to baseline CD4+ lymphocyte counts, CDC stage, sex, or age. The mean (SD) difference between log-transformed plasma and CSF virus loads (the plasma-CSF gradient) was 0.92 (0.84) \( \log_{10} \) copies/mL. Larger plasma-CSF gradients were significantly related to both lower CD4 counts (\( r = −0.58; P = .23 \)) and absence of pleocytosis (1.61 vs. 0.463; \( P = .004 \)).

Data from 57 CSF-plasma pairs, plus data from 2 CSF samples without corresponding plasma samples, were analyzed (figure 1). Of 59 residual values derived from the independent regression fits for each subject, 58 (98%) were \( \log_{10} \) plasma HIV-1 RNA, and plasma were not significantly correlated (\( r = 0.49 \)).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, years</th>
<th>CDC</th>
<th>CD4 count, cells/\mu L</th>
<th>HIV-1 RNA, copies/mL</th>
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<tr>
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<td></td>
</tr>
<tr>
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<td>B</td>
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<td>17 (F)</td>
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<td>B</td>
<td>191</td>
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</table>

**Table 1. Baseline characteristics of subjects.**

**NOTE.** CDC, Center for Disease Control HIV Disease Classification; CSF, cerebrospinal fluid; HIV, human immunodeficiency virus; WBC, white blood cell.
Slopes differed significantly from zero. Median crude P
the median CSF slope was slightly positive, (+0.175 log10 cop-
were performed on data from each subject. By this method,
calculated by 2 different methods. Separate linear regressions

to detect a viral growth rate of 0.15 log10 copies/mL/year; we
(details of which are described above), we estimated 80% power
for individual subjects. By this second method, the common
evidence from comparisons of HIV dynamics in both
CSF and plasma suggests that extra-CNS trafficking contributes
ly, changes larger than this might be clinically
significant. Furthermore, linear modeling demonstrated, during
intervals of up to several years, no evidence of consistent, durable
CSF virus-load increases or decreases. Our findings support the
hypothesis that CSF virus loads exhibit patient-specific set points,
similar to what previous studies have described for plasma virus
loads.

These results also provide insight into the dynamics of CNS
HIV replication and immune control. The CSF set point reflects
a dynamic equilibrium, or steady state, in which (1) the rate
of infection of new cells equals the rate of death of infected
cells, and (2) the rate of viral replication equals the rate of viral
elimination [14]. It has been speculated that the virus repeatedly
"reseeds" CNS tissues over time, resulting in a gradual expa-
sion of the reservoir of infected cells [15]; this expansion, which
is partially sheltered from antiretroviral drug penetration and
immune surveillance, could diminish the prospects for infec-
tion eradication by vaccines or antiretroviral therapy. If the
population of infected CNS cells grows with time, then CSF
virus loads should increase. We did not find such an increase
in our subjects.

It is possible, however, that a subject’s degree of immuno-
suppression may influence whether expansion of the CNS res-
ervoir occurs and whether this expansion could be detected
through examination of CSF. Few investigations have attempt-
ed to identify, in CSF, the cell and tissue sources of HIV virions.
Indirect evidence from comparisons of HIV dynamics in both
CSF and plasma suggests that extra-CNS trafficking contributes
significantly to CSF virus loads, especially in patients who are
relatively immunocompetent [9]. By contrast, individuals with
advanced immunosuppression show more discordant CSF dy-

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**Figure 1.** Cerebrospinal fluid (CSF) virus loads in subjects not receiving antiretroviral therapy. Serial human immunodeficiency virus RNA levels in CSF from the 17 study subjects are plotted on a log10 scale. The abscissa (time in days) is interrupted to allow for plotting of 1 subject with particularly long follow-up.

was 24 months (range, 0.7–75 months). Duration of observation was not related to the extent of variability in HIV-1 RNA levels in either CSF (P = .24) or plasma (P = .47). The CSF–plasma gradient did not change significantly over time (P = .15).

For CSF HIV-1 RNA, rates of change over time (slopes) were calculated by 2 different methods. Separate linear regressions were performed on data from each subject. By this method, the median CSF slope was slightly positive, (+0.175 log10 copies/mL/year [IQR, −0.037 to 0.661]). One outlier slope was significantly different from zero (+13.1 log10 copies/mL/year; P = .001). Among the remaining subjects, none of the crude slopes differed significantly from zero (P > .05). Median crude slopes for plasma virus loads also were slightly positive (+0.153 log10 copies/mL/year [IQR, −0.219 to 0.281]). In 2 patients, the plasma slope differed significantly from zero.

Calculating slopes independently for each subject results in limited power to detect an overall nonzero slope, causing the absence of a stable set point. To account for this limitation, we performed LME modeling of the pooled data points, adjusting for individual subjects. By this second method, the common estimate of slope (0.081 log10 copies/mL/year [IQR, −0.024 to 0.19]) did not differ significantly from zero (P > .13). In addition, we performed a post hoc analysis to estimate our data set’s power (2-sided test; P = .05) to detect a common CSF viral growth rate (positive or negative slope) different from zero (the null hypothesis). By using LME modeling (REML) (details of which are described above), we estimated 80% power to detect a viral growth rate of 0.15 log10 copies/mL/year; we estimated 45% power to detect a smaller growth rate, 0.1 log10 copies/mL/year; and we estimated 96% power to detect a larger growth rate, 0.2 log10 copies/mL/year. Because this analysis indicates an ability to detect a common slope substantially smaller than the “random” (measurement + biological) variability inherent in virus-load quantitation, we believe that our results reflect a reasonable capacity to detect a clinically meaningful virus-load change over time.

To evaluate factors associated with virus-load slopes, we performed secondary analyses of their relationships both to initial CD4 counts and to virus loads. Subjects with initial CD4 counts less than the median (465 cells/µL) had slopes slightly, but not significantly, more positive than did subjects with higher initial CD4 counts (0.31 vs. 0.093 log10 copies/mL/year; P = .23, Wilcoxon test). Initial CSF virus loads were not significantly related to CSF virus-load slopes, and plasma virus-load slopes were not significantly related to either CD4 counts or initial plasma virus loads (P > .15).

**Discussion.** We have found that, among individuals with established HIV infection who are not receiving antiretroviral therapy, HIV-1 RNA levels in CSF show restricted variability over time. Because random variation in CSF virus loads was almost always <0.5 log units, changes larger than this might be clinically significant. Furthermore, linear modeling demonstrated, during intervals of up to several years, no evidence of consistent, durable CSF virus-load increases or decreases. Our findings support the hypothesis that CSF virus loads exhibit patient-specific set points, similar to what previous studies have described for plasma virus loads.

It is possible, however, that a subject’s degree of immunosuppression may influence whether expansion of the CNS reservoir occurs and whether this expansion could be detected through examination of CSF. Few investigations have attempted to identify, in CSF, the cell and tissue sources of HIV virions. Indirect evidence from comparisons of HIV dynamics in both CSF and plasma suggests that extra-CNS trafficking contributes significantly to CSF virus loads, especially in patients who are relatively immunocompetent [9]. By contrast, individuals with advanced immunosuppression show more discordant CSF dy-
namics. In addition, the subject-specific gradient between plasma and CSF virus loads may be an indicator of the extent to which CSF virus load is either dependent on (in the case of smaller gradient) or independent from (in the case of larger gradient) plasma virus load [8, 9]. In other studies, as in the present one, larger gradients were associated with both lower CD4 counts and absence of pleocytosis, a finding suggesting that these factors are related to independence of the CSF virus load from plasma virus load. Overall, however, CD4 counts in the present study were relatively high (median, 465 [range, 92–905]). Thus, if we had studied more individuals with advanced HIV disease, we might have been more likely to find evidence of gradual expansion of the CNS reservoir, since a greater proportion of CSF virions would be of local (i.e., CNS) origin. Although this study was not powered to evaluate relationships between CD4 count and CSF virus-load slopes, we did find that subjects with lower CD4 counts had slopes that were nonsignificantly more positive than those of subjects with higher CD4 counts.

An additional limitation in generalizing the findings of this study relates to our exclusion of subjects with initially undetectable virus loads. This approach was taken to avoid the potential slope estimate–calculation bias that might result from making statistical assumptions about virus loads below the limit of quantitation of the measurement assay. Calculated by linear regression, such slopes would be mathematically restricted to a range between zero and infinity; that is, negative slopes would not be possible. Excluding such subjects from the analysis diminishes the possibility of spuriously concluding that slopes tend to be positive, but it also restricts the subject sample.

San Diego HNRC Group members. The San Diego HNRC Group is affiliated with the University of California San Diego, the Naval Hospital, San Diego, and the San Diego Veterans Affairs Healthcare System and includes Igor Grant (director); J. Hampton Atkinson (codirector and clinical trials component); J. Allen McCutchan (codirector and neuromedical and clinical trials components); Thomas D. Marcotte (center manager); Mark R. Wallace (the Naval Hospital, San Diego); Ronald J. Ellis and Scott Letendre (neuromedical and clinical trials components); Rachel Schrier (neuromedical component); Robert K. Heaton and Mariana Cherner (neurobehavioral component); Terry Jernigan and John Hesselink (imaging component); Eliezer Masliah (neuropathology component); Daniel R. Masys and Michelle Frybarger (data systems manager) (data management component); and Ian Abramson, Reena Deutsch, and Tanya Wolfson (statistics component).

References