Select resistance-associated mutations in blood are associated with lower CSF viral loads and better neuropsychological performance

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ABSTRACT

Background: When antiretroviral therapy does not fully suppress HIV replication, suboptimal levels of antiretrovirals can select for antiretroviral resistant variants of HIV. These variants may exhibit reduced replication capacity and result in lower viral loads in blood. Our study evaluated whether antiretroviral resistance was associated with viral loads in the cerebrospinal fluid (CSF) and better neuropsychological (NP) performance.

Methods: We enrolled 94 participants and each participant underwent a comprehensive neuromedical evaluation that used structured clinical assessments of medical history, ART and other medication use, comprehensive NP testing, and neurological and general physical signs of disease. Blood was collected by venipuncture, and all participants were offered lumbar puncture. Univariate and multivariate statistical methods were used to analyze the relationship between antiretroviral resistance, blood and CSF HIV RNA levels, substance use, and NP performance.

Results: Antiretroviral resistance, detected in blood, was associated with lower CSF viral loads (p<0.01) and better NP performance (p=0.04) in multivariate analyses, independent of past and current ARV use and blood viral loads (model: p<0.01). However, HIV RNA levels in CSF did not independently correlate with NP performance. Low viral loads in the CSF limited our ability to investigate the relationship between antiretroviral resistance detected in CSF and NP performance.

Conclusions: Even in the absence of ART, antiretroviral resistance-associated mutations correlate with better NP performance possibly because these mutations reflect reduced neurovirulence compared with wild-type HIV.

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Background

HIV-associated neurocognitive disorders (HAND) range in severity from disabling dementia to asymptomatic cognitive, motor, and behavioral changes. With the widespread use of antiretroviral therapy (ART) in economically privileged countries, the incidence of HIV-associated dementia (HAD), characterized by severe neuropsychological (NP) impairment and inability to perform activities of daily living, has significantly decreased (reviewed in Deutsch et al., 2001; Sacktor, 2002). Despite a decrease in incident HAD, less severe forms of HAND have persisted (Antinori et al., 2002; Giancola et al., 2006; Antinori et al., 2007; Tozzi et al., 2007) and may actually be increasing as HIV-infected individuals live longer (reviewed in McArthur, 2004; Ances and Ellis, 2007). Comorbidities that are common in individuals infected with HIV, like hepatitis C virus (HCV) infection and methamphetamine abuse, are also associated with NP impairment and may make it difficult to distinguish the contribution of each to NP impairment (Rippeth et al., 2004; Clifford et al., 2005; Letendre et al., 2005, 2007; Chernera et al., 2005; Richardson et al., 2005).

Effective ART, as assessed by suppression of blood plasma viral load, is considered the standard of care for HAND; however, poor penetration into the central nervous system (CNS) by some antiretroviral drugs suggests that suppression of blood plasma viral load may not be an adequate guide when selecting treatment options.
for HAND and raises the concern that suboptimal antiretroviral concentrations could select for resistance-associated mutations (Letendre et al., 2004; Antinori et al., 2005). Studies that have examined cerebrospinal fluid (CSF) viral loads in the setting of ART and antiretroviral resistance suggest that ART lowers CSF viral loads even in the setting of antiretroviral resistance; however, the clinical significance of these findings remains unclear (Antinori et al., 2005; Spudich et al., 2006).

In addition, resistance-associated mutations can affect HIV replication and fitness in the presence or absence of ART. Studies have examined the relationship between resistance-associated mutations, in vivo viral load, and HIV disease (Samir et al., 2000; Schmitt et al., 2000; Antinori et al., 2001; Deeks et al., 2001; Barbour et al., 2002; Campbell et al., 2003; Paredes et al., 2009). These studies, however, were limited to blood viral loads and focused on indicators of HIV disease in the blood, like CD4+ cell counts. Considerably less is known about the impact of antiretroviral resistance on CSF viral load and the brain. To this end, we investigated the relationships between resistance-associated mutations, viral loads in blood and CSF, and NP performance.

Methods

Eligibility

Our study consisted of 94 participants enrolled in a research study at the University of California San Diego's HIV Neurobehavioral Research Center. Participant blood was collected by venipuncture, and for sequencing purposes only, participants with at least 500 HIV RNA copies/ml in blood plasma were included in this study. All participants were offered lumbar puncture with 69 consenting and having a successful procedure. Participants were excluded if they had significant head trauma, brain surgery, cerebral palsy, seizure disorder, or history of CNS opportunistic infection or received treatment with interferon-alpha. All subjects provided informed consent according to a protocol approved by the UCSD Human Research Protections Program.

Study design and statistical analyses

To investigate the relationship between antiretroviral resistance detected in blood and HIV RNA levels in blood and CSF, we utilized univariate and multivariate analyses. First, we examined demographic and medical characteristics of study participants in association with the presence or absence of resistance-associated mutations. HIV RNA levels in blood and CSF were log transformed to stabilize variances. Fisher's exact tests were used to compare categorical or binary measures and Wilcoxon rank sum tests were used to compare continuous measures. Multiple regressions were performed using CSF HIV RNA or blood HIV RNA as the continuous outcome and the detection of antiretroviral resistance in blood as the main predictor of interest. Other variables used in this model included past and current ART use, methamphetamine dependence, estimated duration of infection, and plasma HIV RNA levels. Analyses of CSF HIV RNA levels were limited to the 69 participants with successfully completed lumbar punctures. These analyses included the use of Tobit analyses to adjust for censored data because HIV RNA levels are subject to limit of detection censoring (Tobin, 1958). In addition, the demographic and medical characteristics of study participants for whom CSF HIV RNA were available were compared with the other study participants to assess the informativeness of the missing data.

To examine the impact of antiretroviral resistance on the relationship between CSF HIV RNA levels and NP performance, we utilized both univariate and multivariate approaches. The univariate approach consisted of a series of univariate analyses with participants grouped by the presence or absence of antiretroviral resistance. The first examined the relationship between CSF HIV RNA levels and NP performance and the second examined the relationship between blood HIV RNA levels and NP performance. Because ongoing ART use can significantly alter CSF and blood HIV RNA levels (Spudich et al., 2006; Marra et al., 2009), we excluded participants currently receiving ART. This univariate analysis was limited by the number of participants (i.e., power) and it does not control for other potentially confounding variables. To more effectively address these concerns, we performed additional multivariate analyses that included all available data and incorporated models that allowed us to address current and past ART and a number of other important factors that may influence the relationship between CSF viral load and NP performance including methamphetamine dependence, current and nadir CD4 count, and estimated duration of infection. Using this multivariate approach, we were able to examine if resistance had an impact on NP performance independent of current and past ART use and if resistance had an impact on NP performance independent of CSF HIV RNA levels.

In exploratory analysis, we examined genotypic discordance between blood and CSF among those with blood and CSF resistance data available. Resistance discordance was defined as the presence of one or more resistance-associated mutation(s) in blood not present in the CSF or vice versa. We limited the analysis of resistance discordance to amino acids with a Stanford HIV Resistance Database “mutation score” of 30 or greater (http://hivdb.stanford.edu, March 2009). In further exploratory analyses of viral genetic discordance, Fisher's exact tests were used to compare categorical or binary measures and the Wilcoxon rank sum tests to examine if resistance discordance altered HIV RNA levels. As univariate analyses were not informative, multivariate analysis was not performed. Statistical analyses were performed using JMP (version 5.0 for Mac; SAS Institute, Cary, NC) and R version 2.3.1 (R Development Core Team 2006).

Neuromedical and neuropsychological testing

Participants underwent standardized NP assessments of seven ability domains (learning, delayed recall, verbal fluency, processing speed, attention/working memory, abstraction/executive functioning, motor speed), as previously described (Heaton et al., 2008; Rippeth et al., 2004). All NP tests were administered and scored by trained psychometrists using demographically corrected normative data. Results were summarized by a neuropsychologist using global ratings that range from 1 (above average) to 9 (severely impaired), based on the demographically adjusted test scores in the seven ability domains. A global score of 5 or higher denotes NP impairment that is present in at least two ability areas (Woods et al., 2004).

At the time of their NP testing and clinical sample collection, the urine of each participant was screened with a point-of-care test for common recreational drugs, including amphetamines, cocaine, barbiturates, tetrahydrocannabinol, opiates, benzodiazepines, and phencyclidines (Rapid Response; Biotechnostix, Inc., Markham, Ontario, Canada). In addition, participants received a Breathalyzer test to evaluate alcohol intoxication (Alcohol Countermeasure Systems, Toronto, Ontario, Canada). NP testing was rescheduled if alcohol was detected or if the participant's urine was positive for non-prescribed substances, with the exception of cannabis, given its long elimination period. Likewise, participants were not tested if they appeared to be intoxicated or in withdrawal.

Methamphetamine dependence was determined with the Structured Clinical Interview from the Diagnostic and Statistical Manual of Mental Disorders version IV (Spitzer, 1995). Inclusion in the parent study from which the methamphetamine users were drawn required lifetime dependence and evidence of use within the previous 18 months as well as a minimum of 10 days of abstinence prior to NP testing.
Laboratory measures and antiretroviral resistance genotyping

Blood was collected by venipuncture and CSF was collected by lumbar puncture. HIV and HCV infections were diagnosed by serology. HIV RNA levels in blood plasma and CSF were measured (Amplicor HIV-1 Monitor; Roche Diagnostics, Branchburg, NJ). The ultrasensitive assay was used for CSF (lower limit of detection of 50 copies/ml) and the standard assay was used for blood (lower limit of detection of 400 copies/ml). A fluorescence-activated cell sorter quantified CD4 lymphocytes. The ViroSeq HIV genotyping system (Applied Biosystems, Alameda, CA) was used for population-based pol sequencing of HIV RNA extracted from blood plasma per manufacturer instructions (Smith et al., 2007). Genotyping of the reverse transcriptase coding region of CSF-derived HIV RNA included CDNA synthesis with RETROscript kit (Applied Biosystems) using random decamers according to the manufacturer’s protocol. Followed by two rounds of amplification with Taq polymerase (Invitrogen, Carlsbad, California) as previously described (Koelsch et al., 2003), using primers CI-Pol 1 and 3RT at cycling parameters: 95 °C×2 min; 95 °C×30 s, 50 °C×1 min, 72 °C×1 min for 35 cycles; 72 °C×10 min. and primers 5RT and 3RT at cycling parameters: 95 °C×2 min; 95 °C×30 s, 50 °C×1 min, 72 °C×1 min for 35 cycles; 72 °C×10 min. All assays included negative controls with PCR products visualized by agarose gel electrophoresis and were conducted in conditions to minimize PCR contamination. Sequencing was performed on an ABI 3100 Genetic and sequences were manually reviewed using BioEdit software. The Stanford HIV Resistance Database (http://hivdb.stanford.edu, March 2009) was used to interpret antiretroviral resistance from genotypic data.

Results

Study participants and antiretroviral resistance

Participants were mostly Caucasian men in their mid-30s (median = 35 years). The median HIV RNA levels were 4.7 (blood) and 2.9 (CSF) log_{10} copies/ml. The median blood CD4+ cell count was 319/μL, and 25% of participants had a positive serology for HCV. As expected, methamphetamine use was common in the cohort with 57% of participants reporting a history of abuse or dependence (Table 1). Lumbar punctures were successfully performed on 73% of participants (69 of 94). Clinical and demographic characteristics of participants with successful lumbar punctures resembled participants without lumbar punctures (data not shown), except that participants who did not undergo lumbar puncture had longer estimated durations of HIV infection (mean = 8.5 vs. 5.5 years, p = 0.02).

At the time of study evaluation, 63% had a past history of ART use and 29% were receiving ART at the time of participation and sampling. One or more resistance-associated mutations were detected in the blood plasma of 48 of the 94 study participants (51%). The most common mutations, M184V and K103N, were detected in 22% and 16% of participants, respectively (Supplementary Table 1). Univariate analyses demonstrated that individuals with antiretroviral resistance (AR+) differed from those with no antiretroviral resistance (AR−) in duration of HIV infection, levels of CSF and blood plasma HIV RNA, past ART use, and current ART use (Table 1). Participants with resistant virus did not differ in current CD4+ cell count, CD4+ cell nadir, HCV serostatus, or diagnosis of AIDS (Table 1).

Resistance profiles for the reverse transcriptase coding region derived from HIV RNA from CSF were obtained for 26 participants. Median CSF viral loads for these participants were higher than for those participants for whom resistance profiles could not be obtained (3.16 vs. 2.6 log_{10} copies/ml, p = 0.013) but did not differ with regard to demographic characteristics, current CD4+ cell count, CD4+ cell nadir, HCV serostatus, or diagnosis of AIDS (data not shown). Resistance-associated mutations in the reverse transcriptase coding region from HIV RNA populations in the CSF were identified in 4 (15%) of these 26 participants. Genotypic discordance between blood and CSF, defined as the presence of one or more resistance-associated mutation in blood not present in CSF or vice versa, was found in one of these 4 participants (25%) (Supplementary Table 2). In all but one case, participants with a major resistance-associated mutation(s) in blood also had the mutation(s) in the CSF.

Table 1

<table>
<thead>
<tr>
<th>Participant demographics and clinical characteristics.</th>
<th>Overall</th>
<th>AR+ (range)</th>
<th>AR− (range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>94</td>
<td>48</td>
<td>46</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35</td>
<td>34 (23–39)</td>
<td>35 (21–51)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>92%</td>
<td>96%</td>
<td>88%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Ethnicity (non-Caucasian)</td>
<td>37%</td>
<td>38%</td>
<td>37%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12</td>
<td>12 (9–20)</td>
<td>12 (6–18)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Duration of HIV (years)</td>
<td>5.3</td>
<td>8.1 (0.52–16.4)</td>
<td>4.1 (0–16.9)</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>HIV RNA, CSF (log_{10} copies/ml)*</td>
<td>2.9</td>
<td>2.6 (1.7–4.4)</td>
<td>3.3 (1.7–6.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HIV RNA, plasma (log_{10} copies/ml)</td>
<td>4.7</td>
<td>4.4 (2.8–6)</td>
<td>4.8 (3.3–6.3)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD4 count, current (μL)</td>
<td>319</td>
<td>340 (4–1188)</td>
<td>308 (5–1296)</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>CD4 count, nadir (μL)</td>
<td>216</td>
<td>200 (0–772)</td>
<td>269 (0–1296)</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>AIDS diagnosis</td>
<td>47%</td>
<td>50%</td>
<td>43%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Past ARV use</td>
<td>63%</td>
<td>73%</td>
<td>51%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Current ARV use</td>
<td>29%</td>
<td>50%</td>
<td>7%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>– Adherence (&lt;95% in 4 weeks)</td>
<td>53%</td>
<td>50%</td>
<td>75%</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>HCV seropositive</td>
<td>25%</td>
<td>25%</td>
<td>24%</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Methamphetamine abuse ever</td>
<td>57%</td>
<td>51%</td>
<td>63%</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

Values are medians or proportions; n = 94.
The p-values are based on univariate analyses. Individuals with drug resistance (AR+) differed from those with no drug resistance (AR−) in duration of HIV infection, CSF HIV RNA, plasma HIV RNA, past antiretroviral (ARV) use, and current ARV use.

* Subgroup analysis.

Antiretroviral resistance and CSF viral loads

In univariate analyses, lower CSF viral loads were associated with the presence of antiretroviral resistance in blood-derived virus, lower HIV blood viral load, and current ARV use. Multivariate analyses, which included adjustments for current and past ART use and blood viral load, demonstrated that lower CSF HIV RNA levels were associated with the presence of antiretroviral resistance in blood (model: p = 0.01). This relationship was particularly strong for participants with the M184V mutation (model: p = 0.01). Among the 26 participants with completed CSF resistance profiles of reverse transcriptase, univariate analysis did not indicate differences in CSF viral loads between individuals with and without evidence of resistance in CSF.
individuals with antiretroviral resistance, although this difference was stronger in individuals without antiretroviral resistance than in individuals with antiretroviral resistance. To exclude any direct ART effect, this analysis excluded participants on ART at the time of the study.

**Antiretroviral resistance, methamphetamine dependence, and neuropsychological performance**

To examine the impact of antiretroviral resistance on the relationship between CSF viral load and NP performance, we conducted univariate analyses with participants stratified by the presence or absence of antiretroviral resistance. The first examined the relationship between CSF viral load and NP performance, and the second examined the relationship between blood viral load and NP performance. Because ART use can significantly alter CSF and blood viral load, we excluded participants currently receiving ART. In these analyses, the correlation between global rating and CSF viral load was stronger in individuals without antiretroviral resistance than in individuals with antiretroviral resistance, although this difference did not reach statistical significance. To exclude any direct ART effect, this analysis excluded participants on ART at the time of the study.

**Fig. 2.** Univariate analysis of blood HIV RNA levels and neuropsychological performance. Confidence ellipses (global rating vs. plasma viral load) for individuals without antiretroviral resistance (AR−) (rho = 0.25, p = 0.10) and individuals with antiretroviral resistance (AR+) (rho = 0.16, p = 0.28) demonstrate that although not statistically significant, the correlation between global rating and plasma viral load is stronger in individuals without antiretroviral resistance than in individuals with antiretroviral resistance. To exclude any direct ART effect, this analysis excluded participants on ART at the time of the study.

**Discussion**

We found that the detection of antiretroviral resistance in HIV populations in blood was significantly associated with both lower CSF HIV RNA levels and better NP performance. These findings may help to explain what appears to be a weakening relationship between CSF viral load and NP performance in the modern treatment era (Brew et al., 1997; Ellis et al., 1997; McArthur et al., 1997). Thus, in our study and in other more recent reports (Sevigny et al., 2004; Vitiello et al., 2007), CSF HIV RNA levels were not independently associated with better NP performance. Taken together, these studies provide evidence that HIV variants harboring resistance-associated mutations may be less replication competent and less neurovirulent than wild-type HIV.

Single mutations within pol can result in reduced replication capacity in vitro (Goudsmit et al., 1996; Harrigan et al., 1998; White et al., 2002; Collins et al., 2004; Cong et al., 2007). Single amino acid variations within reverse transcriptase (RT) and protease (PR) may result in a lower viral fitness and hence lower replication capacity. These variations can also result in a loss of neurovirulence in vivo, as seen in antiretroviral-naive patients and in patients who have not been exposed to ART for a long time. These findings suggest that antiretroviral resistance may be a factor in the development of NP performance in individuals with HIV infection.
substitutions in clade B virus that result in the greatest reduction in replication capacity include nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations (K65R, T215Y, and M184V) and non-nucleoside reverse transcriptase inhibitor (NNRTI)-associated mutations (V106A, Y188H, and G190S) (Iglesias-Ussel et al., 2002; Collins et al., 2004; Weber et al., 2005; Wang et al., 2006; Martinez-Picado and Martinez, 2008). Our study extends these observations by demonstrating that resistance-associated mutations known to reduce replication capacity in vitro, particularly M184V, are associated with lower CSF viral loads, independent of past and current ART use. In contrast, the NNRTI-associated mutation K103N was not associated with lower viral loads, as might be predicted from its negligible reduction in replication capacity in vitro (Nicastro et al., 2004; Koval et al., 2006).

Suboptimal ART may select for HIV that is less replication competent and less neuropathogenic, but ongoing HIV replication regardless of phenotype leads to brain injury in a substantial proportion of untreated individuals. Although this investigation suggests that resistance-associated mutations may benefit the nervous system, this benefit is unlikely to match that from virologic suppression. In addition, compensatory mutations may accumulate that restore replication capacity while maintaining antiretroviral resistance. Understanding how individual resistance-associated mutations contribute to HAND is difficult, given no standard in vitro method to characterize HIV phenotypes by neurovirulence exists. One approach that might yield additional insight is to use an in vitro assay that utilizes microglia or brain macrophages, the likely cell types productively infected by HIV in the brain (reviewed in Fischer-Smith et al., 2008), to assess the replication capacity of specific resistance-associated mutations (Perez-Bercoff et al., 2007).

Some limitations of this study should be acknowledged. It analyzed retrospectively an existing cohort with specimens only from participants with detectable blood viral loads. In addition, analyses of CSF viral load were based on a subset of participants with available lumbar punctures. While detailed analysis demonstrated that this subset did not significantly differ from the other participants in the study, selection bias is possible. Analyses of antiretroviral resistance also were primarily based on HIV RNA extracted from blood. We did investigate CSF resistance profiles for 26 participants, but genotypic assessment was limited because viral loads were low in the CSF, which limited sequencing of the reverse transcriptase coding region. In all but one case, participants with a major resistance-associated mutation(s) in blood also had the mutation(s) in the CSF. This degree of concordance in CSF and blood resistance-associated mutations is likely explained in part by regimen stability, as the median time on current ART regimen for these participants was 10 months. Although current ART therapy was relatively stable, different ART regimens among participants and a wide range in time on and off ART made it difficult to employ a more rigorous assessment of ART use. Even though our analysis was nested in a well-characterized cohort and is one of largest studies of its kind, these limitations dictate that our observations should be validated in larger prospective studies with more frequent sampling.

In conclusion, our findings suggest that antiretroviral resistance alters the relationship between CSF viral loads and better NP performance. In particular, the presence of antiretroviral resistance appears to be associated with better NP performance. This may be mediated by impaired viral fitness and is likely less beneficial than complete viral suppression by ART. If antiretroviral resistance alters CSF viral loads, this may in part explain what appears to be a weakening relationship between CSF viral load and NP performance in the modern treatment era. Further study of antiretroviral resistance and HIV neuropathogenesis may contribute to an improved understanding of HIV disease in the CNS and perhaps improvements in treatment options for HAND.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.virol.2009.08.007.

References


