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Sequence heterogeneity and viral dynamics in cerebrospinal fluid and plasma during antiretroviral therapy

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Understanding of human immunodeficiency virus (HIV) neuropathogenesis has been greatly enhanced by careful analyses of postmortem brain tissue, as well as by primate models of infection with related lentiviruses. However, brain tissue from persons living with HIV infection is rarely available for study, and elegant observations from primate systems may require confirmation in humans. Because of these inherent limitations, it is important to learn as much as possible by studying cerebrospinal fluid (CSF) during HIV infection. The present discussion considers selected issues relevant to the study of CSF from HIV-infected individuals. These include a strategy to intensely sample CSF to better understand viral dynamics and the role of the brain as a pharmacologic sanctuary site, evidence for HIV sequence diversity in CSF and plasma during HIV therapy and the implications of such diversity, and the importance of host genetics relevant to studying HIV neuropathogenesis. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 33–37.

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The value of analyzing paired cerebrospinal fluid (CSF) and blood samples in the context of controlled clinical trials is demonstrated by the Prometheus study in which 208 protease inhibitor-naive subjects received ritonavir and saquinavir, while one half also received stavudine (Gisolf *et al*, 2000). More than 20 subjects underwent CSF and serum sampling at baseline and again at week 12 of therapy. Although subjects in both arms experienced a mean serum human immunodeficiency virus type 1 (HIV-1) RNA decrease of approximately 2.0 $\log_{10}$ copies/ml, only subjects who also received stavudine experienced a virologic response in CSF. Stavudine penetrates relatively well into the central nervous system (CNS) (Haas *et al*, 2000a), in contrast to most HIV-1 protease inhibitors (Kim *et al*, 1998). This study demonstrated that discordant virologic responses may result from suboptimal drug penetration into the CNS. There is also evidence that CSF viral decay is slower when treatment in initiated during advanced HIV disease, suggesting productive viral replication in CNS tissues (Ellis *et al*, 2000). Important insights clearly arise from such studies.

Although HIV causes a chronic infection, many questions may be addressed by intensive study over short intervals. Seminal HIV pathogenesis studies by Ho *et al* (1995) based on careful analysis of viral kinetics in plasma helped define the cell types that produce virus, and the variable turnover rates of such cells. Applying a similar approach to the CNS, we used antiretroviral agents as tools in a proof of concept study to interrupt viral replication and characterize viral dynamics in CSF and plasma (Haas *et al*, 2000a). An integral component of this study was determination of viral sequence relatedness in CSF and plasma. We also assessed diurnal variability of HIV-1 RNA in CSF, and quantified disposition of selected antiretroviral agents into CSF. An ultraintensive CSF sampling approach was applied whereby lumbar intrathecal catheters were inserted and maintained for prolonged continuous sampling (Haas *et al*, 2000a). Collecting 150 precisely timed CSF samples over 48-h intervals using a peristaltic pump and automatic fraction collector provided nearly 300 ml

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of CSF for analysis. Blood samples were obtained in parallel. During study screening of 11 asymptomatic antiretroviral naive adults with plasma HIV-1 RNA greater than 25,000 copies/ml, 4 had fewer than 200 copies/ml in CSF. Considerable interindividual variability in CSF HIV-1 RNA concentrations confirmed that the amount of virus in CSF cannot be predicted from peripheral samples. Four subjects with greater than 2500 HIV-1 RNA copies/ml in CSF underwent two 48-h sampling periods, at baseline and again starting on day 3 of three-drug therapy with stavudine, lamivudine, and nelfinavir (Figure 1). After each catheterization period, 17 CSF samples collected at 3-h intervals were assayed for HIV-1 RNA concentrations. Plasma samples obtained at these time points were also assayed. In contrast to the interindividual variability previously noted, each individual's viral load in CSF remained remarkably constant over the 48-h baseline interval (Haas *et al*, 2000a). Lack of temporal variability is critical if change in HIV-1 RNA concentration in response to therapy is to be used to understand HIV pathogenesis. After initiating antiretroviral therapy, HIV-1 RNA decreased in both CSF and plasma, but the magnitude of response was discordant (Haas *et al*, 2000a). In two subjects, viral decay was significantly less in CSF as compared to plasma. Minimal estimates of HIV-1 RNA entry into CSF in these untreated patients ranged from 74,000 to 2 million copies/day (Haas *et al*, 2000a). Such levels of viral replication provide ample opportunity for viral evolution over time. Further examination of kinetics of viral decay indicated that, in these asymptomatic subjects, HIV-1 RNA concentrations decreased most rapid during the first 3 days of treatment. This is important because it suggests that in future studies, careful characterization of viral dynamics during the first 3 days will broaden the range of questions that can be addressed by this approach, and the types of antiretroviral combinations that may be safely administered over brief intevals. Although we studied only four subjects, we explored the ability of various CSF and plasma parameters to predict the magnitude of viral decay in CSF. Interestingly, only plasma pharmacokinetic parameters (peak and area-under-the-curve [AUC] values) for nelfinavir and its active M8 metabolite significantly correlated with CSF viral decay. This correlation was strengthened by considering viral decay in CSF relative to viral decay in plasma (Haas *et al*, 2000a). This preliminary observation suggests not only that effects of individual agents in multidrug regimens may be discerned, but also hints at a potential role of host factors.

It is likely that HIV neuropathogenesis involves complex interactions between viral genetic, host genetic, immunologic, and therapeutic factors. Regarding host and therapeutic factors, P-glycoprotein, the 170-kDa drug efflux pump encoded by the *MDR1* gene, may be particularly important during HIV-1 infection and its therapy. Expression of P-glycoprotein in capillary endothelial cells of the brain-brain barrier limits entry of structurally diverse drugs into the brain, and a seminal study by Kim *et al* (1998) using knockout mice showed that P-glycoprotein markedly reduced brain levels of HIV-1 protease inhibitors. The effect was most pronounced for

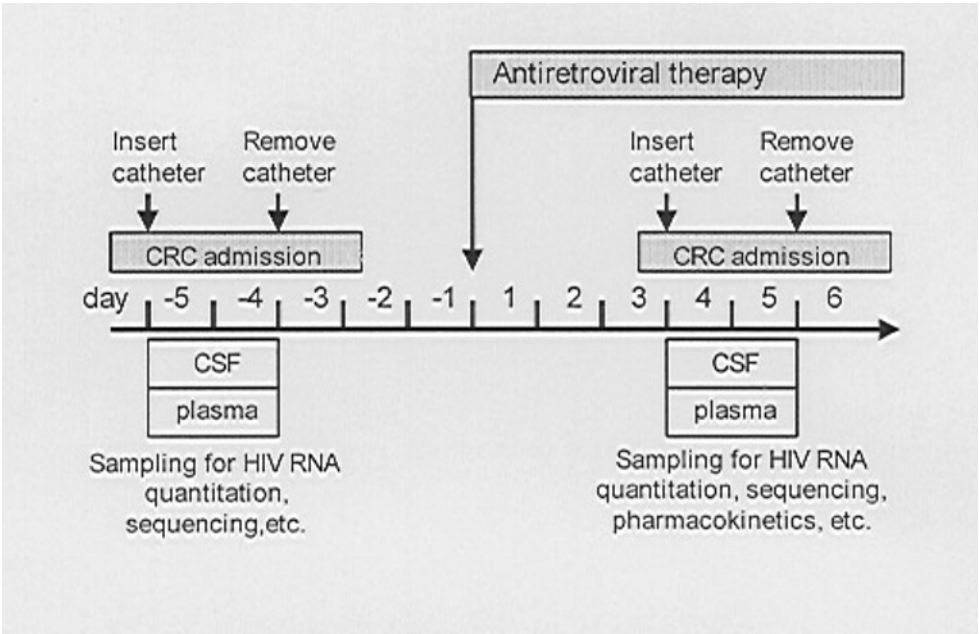

Figure 1 Schedule of events for ultraintensive CSF sampling. Timing of insertion and removal of intrathecal lumbar catheter for continuous sampling is shown. Durations of admissions to the Clinical Research Center (CRC) are also shown. Each 48-h sampling yields nearly 300 ml of CSF.

nelfinavir, with a 37-fold reduction in brain concentrations (Kim *et al*, 1998). There is considerable interindividual variability in P-glycoprotein activity. Many *MDR1* single-nucleotide polymorphisms have been identified (Hoffmeyer *et al*, 2000; Kim *et al*, 2001), and common polymorphisms have been implicated in genotype-phenotype relationships (Kim *et al*, 2001), including plasma drug concentrations after oral administration of nelfinavir (Fellay *et al*, 2002). We suspect the observed correlation between plasma nelfinavir pharmacokinetics and CSF viral decay may relate to genetically determined differences in P-glycoprotein activity in the blood-brain barrier, with higher plasma nelfinavir concentrations, reflecting decreased P-glycoprotein activity in the gut and blood-brain barrier, and subsequent enhanced nelfinavir penetration. Further studies are warranted to address this issue.

To use antiretroviral agents as tools to probe HIV pathogenesis in the CNS, it is important that disposition of these agents be characterized. In subsequent studies, we used serial CSF and plasma sampling approaches over entire dosing intervals to characterize indinavir pharmacokinetics, both without and with pharmacokinetic boosting by ritonavir. Because ritonavir can inhibit P-glycoprotein *in vitro* (Drewe *et al*, 1999), we were also interested in exploring whether ritonavir disproportionately enhanced indinavir penetration into CSF. In the absence of ritonavir, trough steady-state CSF indinavir levels modestly exceeded concentrations required to inhibit wild-type HIV-1 *in vitro* (Haas *et al*, 2000b). Coadministered ritonavir markedly increased both CSF and plasma indinavir concentrations (Haas *et al*, 2002), without altering the CSF-to-plasma AUC ratio for indinavir, suggesting little effect of ritonavir on P-glycoprotein in the blood-brain barrier. Although studying CSF from patients receiving indinavir in clinical practice might increase understanding of HIV replication in the CNS, this drug is now uncommonly initiated without concomitant ritonavir. A practical lesson is that observational studies relying on antiretroviral use in clinical practice to dissect HIV pathogenesis may be thwarted by rapidly evolving treatment guidelines and drug availability. In the previously described ultraintensive sampling study of patients initiating antiretroviral therapy, HIV-1 nucleotide mutations were characterized. Reverse transcriptase and protease gene sequences in CSF and plasma were determined in samples from baseline and day 5 of antiretroviral therapy, in comparison to a standard laboratory HIV-1 strain (Tang *et al*, 2000). As expected, resistance mutations were not observed in this treatment-naive population. However, sequences in paired plasma samples (baseline and day 5) were more closely related to each other than to sequences in CSF. Similarly, sequences in paired CSF samples (baseline and day 5) were more closely related to each other than to sequences in plasma. There was no evidence that virus translocated from plasma to CSF, as would be suggested had baseline sequences in plasma resembled day 5 sequences in CSF. The finding that HIV-1 RNA sequences in CSF and plasma differed significantly provided further evidence that HIV-1 arose from distinct compartments or cell populations.

A number of investigators have characterized relationships between viral sequences in CSF and peripheral blood during antiretroviral therapy. Because of concerns that inadequate drug penetration into CSF might foster emergence of resistant virus, most studies have focused on mutations associated with drug resistance. In an early study that relied on HIV coculture, Di Stefano *et al* (1995) showed that among six subjects receiving at least 1 year of zidovudine monotherapy, mutation patterns in CSF and plasma differed in four subjects. Chien *et al* (1999) showed that, among nine adults receiving antiretroviral therapy, two had evidence of a mixed population of virus in plasma that included the characteristic M184V lamivudine resistance mutation, but only wild-type virus in CSF. In a subsequent study of 24 adults, many of whom were by receiving antiretroviral therapy, Venturi *et al* (2000) showed that mutation patterns differed considerably in CSF and plasma in many subjects. A limitation of this study was that sequences could not be amplified from many CSF samples, suggesting selective amplification. In a study of 15 adults initiating antiretroviral therapy, Cinque *et al* (2001) noted concordant resistance patterns at baseline in both CSF and plasma, and that sequence diversity did not predict discordant viral decay in response to antiretroviral therapy. In a study of 11 children treated with nucleoside reverse transcriptase inhibitors, McCoig *et al* (2002) reported that only 1 subject had discordant resistance mutations in CSF and plasma at baseline, but after 48 weeks of therapy eight children had discordant resistance patterns. Tashima *et al* (2002) recently noted discordant CSF and plasma resistance patterns in three of five treatment-experienced patients. In one efavirenz recipient, the characteristic K103N efavirenz resistance mutation was detected only in CSF. Such observations strongly suggest that discordance between HIV-1 nucleotide sequences in CSF and plasma, including mutations associated with drug resistance, is common during antiretroviral therapy. However, the reasons for such discordance are uncertain, as are implications for neuropathogenesis and patient care. A common opinion is that the CNS and periphery represent different compartments of viral replication, and that virions generated in each compartment have differential access to CSF and plasma. However, although sequence in CSF and plasma differ, the extent of such differences have generally been modest, suggesting incomplete compartmentalization at best. An alternative explanation relates to the turnover rates and activation states of the cell populations infected with HIV. Sequences analysis of cell-free virus provides a snapshot of viral sequences

in the cells that produced that virus, whereas kinetics of viral decay in response to therapy largely reflect turnover rates of infected cells. The average half-life of actively infected $CD4^+$ lymphocytes, the major source of plasma virus, is approximately 1 day. In contrast, the half-life of productively infected monocyte/macrophages is approximately 2 weeks. Differences in turnover rates of different cells types or subpopulations may result in apparent sequence discordance in cross-sectional analyses. For example, in some patients, it is possible that perivascular monocyte/macrophages in the CNS produce much of the virus found in CSF (Gartner, 2000). In this case, if selective drug pressure causes resistant virus to predominate throughout both lymphocytes and monocyte/macrophages, withdrawal of such selective pressure may result in more rapid reversion to predominantly wild-type virus in plasma than in CSF, because productively infected lymphocytes turn over more rapidly than monocyte/macrophages. Infection with HIV is associated with vigorous anti-HIV immune responses, but the extent to which this influences viral replication and sequence diversity in the CNS is uncertain. As noted above, antiretroviral agents also differ in their abilities to achieve effective concentrations in the CNS, and such differences may be magnified as accumulation of resistance mutations incrementally reduces drug susceptibility. It is therefore important to consider not only viral susceptibility, but also local tissue drug concentrations and immune responses in studies of viral neuropathogenesis.

A limitation of the ultraintensive CSF sampling approach described previously was that dynamic events occurring during the first three days of therapy were not fully captured (Figure 1). Based on evidence that rapid HIV-1 RNA decay in CSF occurs during this interval, future studies may employ a 96-h continuous sampling interval, from 12-h pretherapy (to define baseline) to 3.5 days of therapy (to define rapid viral decay). Brief administration of carefully selected agents, such as drugs with limited CNS penetration, may better discern the cellular source of virus in CSF and plasma. In addition to studies of viral heterogeneity and dynamics, studies of anti-HIV immune responses in CSF and plasma may provide a more complete picture of interactions between the virus and host. Better understanding of complex interactions between the virus and host will certainly have implications beyond HIV infection. Continued pursuit of such investigations will improve our understanding of other neurological diseases caused by viral pathogens. In addition, the study of other inflammatory diseases of the CNS is also likely to benefit from these efforts.

In summary, using ultraintensive CSF sampling to generate detailed viral kinetic data complemented by pharmacokinetic, viral sequence, and immunologic data will help define the tissue and cellular sources of HIV, and the role of the immune system in controlling replication. This will increase understanding of HIV pathogenesis during various stages of HIV disease, and may lead to improved treatment strategies for HIV infection and its complications.

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