

Central Nervous System Complications of HIV Infection

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Issues relevant to the nervous system garnered substantial attention at the 18th Conference on Retroviruses and Opportunistic Infections. Several topics emerged as areas of importance both for informing current understanding of HIV-related neurologic disorders and their treatment, and for spurring future investigations. Measurable biomarkers of HIV-associated neurocognitive disorder (HAND) were a major theme, with studies ranging from new investigations of known laboratory and imaging markers to identification of novel molecules that might be investigated as potential means to follow disease activity as well as to better understand etiology of disease. Studies of pathogenesis of HAND and simian immunodeficiency virus–mediated neurologic injury added to prior understanding of lentivirus neuropathogenesis. Another broad area of investigation was the interplay between treatment with antiretroviral or adjunctive therapies and biomarkers of HAND. New data were presented on the potential importance of acute and early infection on the integrity of the central nervous system, complemented by studies of the effects of early treatment interventions.

Introduction—What’s in a Name?

Several recent studies indicate that HIV-associated neurocognitive disorder (HAND) persists in patients taking potent antiretroviral therapy, with a prevalence that ranges from 39% to 52% in varied settings.^{1,2} HAND is a potentially multifactorial condition defined by a combination of the patient’s reported symptoms and measured abnormalities on neuropsychologic (NP) test performance. Unlike other dementias, the diagnosis of HAND does not formally include additional biomarkers (either from the cerebrospinal fluid [CSF] or through neuroimaging).

In the current treatment era, HAND represents a broader spectrum of disease than that underlying the classic AIDS dementia complex (ADC) described in the era before potent antiretroviral treatment was widely available, perhaps with a greater heterogeneity in presentation, and importantly, etiology. Predominantly characterized by mild forms of disease that may either

be perceived by patients (mild neurocognitive disorder, or MND) or recognized only through NP testing (asymptomatic neurocognitive impairment, or ANI), HAND is frequently detected in patients receiving antiretroviral treatment. Even HIV-associated dementia (HAD), which overlaps in clinical presentation considerably with severe forms of ADC as described in the past, includes a spectrum of disease from inactive to active and may reflect neuropathologic events within the central nervous system (CNS) distinct from those associated with dementia that occurs solely in the absence of antiretroviral therapy.

Patients now assessed as having HAND likely have a complex interplay of factors affecting their neurologic status. Current HIV-1 infection and attendant immune activation within the CNS may be important. Past events (eg, CD4+ cell count nadir, prior exposure to neurotoxic antiretroviral drugs or drugs subject to abuse) may have increasing impact on a patient’s current status as individuals with HIV survive longer and have more time for such effects to accumulate. With longer-term survival of HIV-1-infected individuals, HAND is also perhaps influenced by distinct factors in the current era such as cerebrovascular disease, abnormal

glucose metabolism, prolonged exposure to therapeutic drugs including psychiatric medications and antiretroviral drugs, and natural neurodegeneration occurring with aging. Thus, in review of the data presented at the 2011 Conference on Retroviruses and Opportunistic Infections (CROI), it continues to be important to keep in mind the complex features that likely underlie HAND, the subject population examined in each study, and how HAND was assessed in each analysis.

Studies of animal models continue to provide a more controlled setting in which to investigate the direct effects of lentiviruses in the nervous system, and many important studies reported at CROI 2011 employed such models to inform an understanding of human disease. Future studies need to address rigorous characterization of subject populations, comorbid factors that may affect results, and detailed understanding and integration of systemic parameters into study analyses. Such definitions will allow the effects of HIV-1 in the CNS to be understood for patients in a variety of circumstances, including those with optimal viral suppression with treatment, virologic failure during treatment, treatment complicated by comorbidities such as mental illness and substance dependence, and delayed therapy resulting from limited access to treatment.

New Biomarkers of HIV-Associated Neurocognitive Disorder

Numerous studies presented at the conference sought to identify and validate new biomarkers of HAND. Some aimed to judge the relevance to the nervous system of biomarkers from blood or other clinical sources that are known to be important in systemic HIV progression. Others investigated biomarkers considered specifically relevant to the CNS, assessing their role

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in or association with the presence of HAND. A few studies employed means to generate new candidates for biomarkers relevant to the presence or prediction of CNS disease in lentiviral infection in primates. Although the following reports focused on identification of biomarkers, many studies investigating pathogenesis of HIV in the nervous system and effects of treatment in the CNS employed neurologic biomarkers as endpoints or further examined the mechanisms of such markers in producing HAND, indicating the central importance of such markers in the investigation and management of the neurologic complications of HIV.

New Insights Into Recognized Biomarkers

Detection and measurement of HIV-1 RNA. In the early epidemic, measurable levels of HIV-1 RNA within the CNS were ubiquitous in patients with classic HAD. Although presence of CSF HIV-1 RNA was not specific to dementia, and levels of HIV-1 RNA did not consistently associate with severity of disease, declines in HIV-1 RNA level could be observed with the initiation of therapy, and these changes correlated with improvements in neurologic performance. In the current era, HAND can be detected in patients whose treatment successfully suppresses plasma and CSF HIV-1 RNA levels to those below the level of detection by standard assays (< 50 copies/mL), rendering assessment of the “activity” of HIV infection in the context of neurologic disease difficult. A major question is the extent to which persistent, low-level HIV-1 RNA replication in the CNS may underlie ongoing neurocognitive impairment in treated patients.

Dahl and colleagues presented a novel application of the single-copy assay for HIV-1 RNA detection to paired blood and CSF samples in 17 treated, suppressed subjects before and after addition of raltegravir in an intensification study, as well as in 11 “elite controllers” (subjects with endogenous control of HIV) (Abstract 423). Very low levels of CSF HIV-1 RNA were detected in treated subjects, and there

was no change in HIV-1 RNA level after intensification with raltegravir. Elite controllers also had extremely low levels of HIV measured in the CNS despite slightly more detectable virus in the periphery. The single-copy assay thus can be applied to CSF and can measure low-level HIV-1 RNA in some cases in CSF in suppressed subjects. The subject population for the study was highly selected for treatment adherence and successful viral suppression measured by standard assays (requiring evidence of plasma HIV-1 RNA level < 50 copies/mL for at least 1 year and CSF HIV-1 RNA level < 50 copies/mL at baseline); it attracted highly treated subjects including those receiving 4- or 5-drug antiretroviral regimens. Future studies of subjects treated with more standard current regimens and under more routine circumstances may reveal the importance of low-level replication of HIV-1 RNA as detected by the single-copy assay in CSF.

Neuronal injury markers. Three studies focused on CSF markers utilized as markers of neurodegeneration. Krut and colleagues reported on a new, more sensitive assay for the light subunit of the neurofilament protein (NFL) with which they observed a broad spectrum of values within the lower range (Abstract 392). Whereas the prior CSF NFL assay had value as a predictor of dementia in some subjects not receiving treatment and indicated very high NFL values in patients with progressive HAND or HAD, the older NFL assay also showed NFL levels to be normal in most subjects in the neuroasymptomatic stages of HIV infection. This group demonstrated, however, that the new NFL assay has different “upper-limit of normal” values for HIV-uninfected subjects that vary according to age, discriminating between younger and older noninfected subjects.

Using this new assay, the investigators found NFL levels high for median age in neuroasymptomatic HIV-1-infected patients across the spectrum of CD4+ cell counts from below 50/ μ L to above 350/ μ L, suggesting that the new test is sensitive to subtle neural injury that may accompany asymptomatic

HIV infection. The percentage of abnormal values correlated inversely with current CD4+ cell count across the span of disease. At the ends of the spectrum, 79% of patients with CD4+ cell counts below 50/ μ L and only 7% of those with counts higher than 350/ μ L had abnormal NFL levels. NFL level correlated with level of CSF neopterin, a macrophage-activation marker, but not with CSF viral load. This new assay provides promise as a tool for assessment of “subclinical” progressive neurologic injury in people with HIV infection. These findings suggest that active injury in currently asymptomatic patients is especially prevalent in advanced immunosuppression and occurs in association with CNS immune activation.

Nath presented a complementary report investigating the potential utility of measures of the heavy chain of neurofilament (neurofilament H) in detecting neuronal injury in asymptomatic HIV infection (Anderson et al, Abstract 407). Density measurements of Western blot bands confirmed by antisera to neurofilament H were obtained in CSF samples from 44 HIV-infected subjects mostly receiving antiretroviral treatment and from HIV-uninfected subjects with other neurologic disorders or clinically stable multiple sclerosis. Neurofilament H level was higher overall in the HIV-infected group than in either HIV-uninfected group. Furthermore, when HIV-infected subjects were separated into diagnostic groups according to the clinical definitions of neurocognitively normal, ANI, MND, and HAD, neurofilament H level was elevated without variation across the spectrum of disease. Subjects who clinically deteriorated over 6 months had larger increases in neurofilament H levels during this period than did those with no change or with improvement in neurologic status.

These results corroborate the findings in the NFL report, in that CSF neurofilament H may indicate smoldering neuronal injury during neuroasymptomatic HIV infection. However, the finding that neurofilament H levels in subjects with overt HAD were similar to those in neurocognitively normal subjects is surprising. This may be ex-

plained by variable levels of treatment and viral suppression in the different diagnostic groups. That is, a larger number of subjects in the “normal” group may have had ongoing high levels of HIV replication and inflammation, driving active CNS injury, whereas subjects in the progressively more “abnormal” groups might have had more suppression of current pathologic processes, with performance based on prior irreversible injury. Alternately, HAD in the current era may represent processes not associated with enhanced detection of neurofilament H. Future studies should clarify the relationship between neurofilament H levels and active neuropathologic processes in people with HIV infection and in various treatment scenarios.

Tau proteins are microtubule-associated proteins that stabilize microtubules in axons. Total Tau (tTau) protein in CSF is elevated in neurologic disease associated with HIV infection, presumably reflecting disruption of neurons. However, a hyperphosphorylated Tau protein (pTau) is thought to promote neurofibrillary “tangles” in the brain, and recent studies have found CSF pTau level to be normal in HIV disease³⁻⁵ but elevated in Alzheimer disease and other “Tauopathies.”

Letendre and colleagues investigated the relationships between HIV infection, age, and pTau levels in 23 HIV-uninfected subjects, 53 HIV-infected subjects on antiretroviral therapy, and 17 HIV-infected subjects off antiretroviral therapy (Abstract 406). HIV-infected patients had elevated CSF pTau. Analyses of only the HIV-infected subjects indicated that CSF pTau levels correlated with older age, larger change in CD4+ cell count from nadir to current measure, antiretroviral therapy use, and worse prospective memory performance but not with worse global neurocognitive impairment. HIV-infected subjects in this study were substantially older than HIV-uninfected controls (median ages, 45 years and 36 years, respectively), suggesting that the differences observed in this study and in another by Brew, both of which contrast with others finding normal pTau levels in HIV infection, may be influ-

enced by age.^{5,6} Future studies might investigate whether alterations in CSF pTau levels reflect distinct HIV- or non-HIV-related neuropathologic processes occurring at different ages and further clarify the relationships between CSF pTau levels and comorbid factors.

Inflammatory markers. The variable utility of currently recognized plasma and CSF biomarkers of HIV-associated immune activation has fostered investigation into new biomarkers of inflammation relevant to CNS HIV disease. Plasma lipopolysaccharide (LPS), an indicator of microbial translocation, may contribute importantly to chronic systemic immune activation and immunopathogenesis in HIV infection. Prior work has shown a correlation between plasma LPS levels and presence of HAD,⁶ but assessment of a more mildly affected group with chronic HIV infection receiving antiretroviral therapy showed no correlation between degree of impairment and LPS level.⁷

To further investigate the utility of LPS level in predicting milder forms of HAND, Carsenti-Dellamona and colleagues performed a subanalysis of data from 179 subjects in the Neuroadapt study, measuring plasma LPS levels in association with NP test performance (Abstract 404). Among subjects with mostly mild neurocognitive impairment (only 3% with HAD), blood LPS level was associated with presence of impairment ($P < .0001$), and there were statistically significant differences between unimpaired subjects and each HAND subgroup. Impairment of attention, concentration, speed of information processing, and motor speed were associated with higher LPS levels. In multivariable analysis, plasma LPS levels higher than 120 pg/mL, plasma HIV-1 RNA levels greater than 40 copies/mL, and hepatitis C virus (HCV) coinfection were independent risk factors for neurocognitive impairment. Future studies should determine whether LPS level is a direct indicator of pathologic processes relevant to the CNS or a more nonspecific marker of ongoing systemic immune activation in patients at risk of HAND developing through other mechanisms.

The plasma level of soluble CD14 (sCD14), a protein secreted by trafficking monocytes and perivascular macrophages in response to LPS stimulation, is an independent predictor of systemic disease progression and mortality in chronic HIV infection. Prior studies of plasma sCD14 level and HAND in the current era have yielded inconsistent results; one group found associations with information processing speed but not global impairment,⁷ whereas another found an association with NP test performance.⁸

Lyons and colleagues compared plasma sCD14, LPS, and CC chemokine ligand 2 (CCL2) levels to neurocognitive test scores (normalized global and domain-specific T scores) from 97 subjects in the National NeuroAIDS Tissue Consortium (NNTC) and the CHARTER (CNS HIV Antiretroviral Therapy Effects Research) study with nadir CD4+ cell counts less than 300/ μ L (Abstract 405). Approximately three-fourths of the subjects were receiving antiretroviral treatment; the median CD4+ cell count (87 cells/ μ L) was low; and there was a broad distribution of HAND diagnoses. In this group, plasma sCD14 (but not LPS or CCL2) level was associated with worse global neurocognitive performance and impairment in specific domains (attention and learning T scores). Using area under the receiver operating characteristic curve analysis, plasma sCD14 level was also more closely associated with impaired scores than were plasma or CSF HIV-1 RNA levels or CD4+ cell count. It is possible that the interaction between plasma sCD14 level and cognition identified in this study was also influenced by variable antiretroviral treatment status or comorbidities, including use of narcotics or cocaine and HCV infection.

Markers of blood-brain barrier integrity. The blood-brain barrier provides a protective environment for the CNS by naturally restricting diffusion of large molecules and potential neurotoxins into the brain. Markers of blood-brain barrier disruption have been associated with severity of HIV-associated neurologic disease.^{9,10} However, data

presented at this year's conference by Letendre and colleagues introduced the suggestion that a less permeable blood-brain barrier might have a negative impact on HIV-1 RNA suppression within the CNS (Abstract 408). Using one measure of blood-brain barrier permeability, the CSF to serum albumin ratio (CSAR), this group assessed data from 190 treated CHARTER subjects, most of whom had normal CSAR for age. Using cutpoint analyses, which divided values within the normal range of CSAR values, lower CSAR values in patients treated with low-CNS-penetrating regimens was associated with higher CSF HIV-1 RNA level as well as with global neurocognitive impairment. These preliminary studies require further assessment of the biological importance of the spectrum of CSAR values within the normal range, their relationship to markers of neuroinflammation, and the potential specific relationships between lower CSAR values and levels and effects of antiretroviral drugs within the CNS.

Markers of vascular disease. Several groups have shown that cardiovascular disease (CVD) risk factors such as hyperlipidemia, hypertension, and past history of CVD are associated with reduced neurocognitive performance in HIV-infected subjects.¹¹⁻¹³ Fabbiani and colleagues reported on CVD risk indicators including carotid intima-media thickness (cIMT) in association with performance on NP testing (Abstract 403). This cross-sectional study enrolled 247 patients, with 87% receiving antiretroviral treatment. Subjects underwent NP examination via 11 tests, and assessments of CVD risk factors and common cIMT were also performed. The group had mild to moderate neurocognitive disease, with a median of 2 of 11 tests indicating "pathologic" scores. A total of 40% of patients showed at least 2 CVD risk factors. Having 2 or more CVD risk factors was associated with poorer neurocognitive performance on testing. Subjects with diabetes, hypertension, and abnormal cIMT values each showed lower cognitive performance than subjects without these risk fac-

tors. Multivariable linear regression analysis revealed that only diabetes and abnormal cIMT values were independently associated with poorer NP performance. Higher education levels and current abacavir use were associated with better NP performance.

Neuroimaging markers. The role of neuroimaging in understanding the pathogenesis of HAND continues to grow as evidenced by the burgeoning number of posters and talks at the 2011 CROI. Many presentations highlighted roles for morphometry, magnetic resonance spectroscopy (MRS), and diffusion tensor imaging (DTI) as possible noninvasive techniques to assess the effects of HIV infection in the brain.

Ragin and colleagues performed a cross-sectional study of subjects at a mean of 1 year after HIV transmission ($n = 43$) and compared results with those of age- and sex-matched HIV-seronegative control subjects ($n = 22$) (Abstract 55LB). Volumetric analysis using semiautomated tools demonstrated a statistically significant reduction in cortical structures in HIV-seropositive patients at this early stage of infection compared with HIV-seronegative control subjects. These results nicely complement posters by Tate and colleagues (Abstract 443), Kallianpur and colleagues (Abstract 441), and Ortega and colleagues (Abstract 442), who studied brain volumetric measures in chronically HIV-infected patients. Statistically significant decreases in brain volume were observed in HIV-infected patients and may be related to sex (female > male), race (African American > white), nadir CD4+ cell count, presence of detectable plasma or CSF HIV-1 RNA, or duration of antiretroviral therapy. Ragin and colleagues showed an association between plasma levels of monocyte chemoattractant protein-1 (MCP-1) as measured by multiplex assays and evidence of brain injury by analyzing brain volume fractions for a variety of regions in 10 HIV-infected subjects (Abstract 446). Larger longitudinal studies of both HIV-infected and -uninfected patients are needed to tease out the possible impact of each of these components.

Valcour and colleagues performed MRS in a cohort of acutely HIV-infected patients, chronically HIV-infected patients, and HIV-uninfected control subjects from Bangkok (Abstract 54). Acutely infected patients tended to have higher choline (Cho) ratios (a measure of cell turnover, as compared with creatine [Cr], a stable measure of energy metabolism) within the basal ganglia than those of either chronically infected patients or uninfected control subjects. A correlation was observed between MRS-measured metabolites and CSF biomarkers for acutely infected individuals. Cho:Cr ratios from the frontal lobe were positively correlated with MCP-1 values. These results suggest that even in acute infection, changes can be observed within subcortical structures; they also validate previous neuroimaging studies of early HIV-infected individuals.

Similar results were also observed in chronically HIV-infected individuals. Navia and colleagues showed that neuronal injury was present in chronically infected participants despite antiretroviral therapy (Abstract 56). Both current CD4+ cell counts and baseline MRS-measured metabolite values (specifically, decreases in *N*-acetylaspartate [NAA] and Cho levels in the basal ganglia) were good predictors of progression from unimpaired to subclinical impairment. Letendre and colleagues investigated associations between plasma and CSF biomarkers associated with systemic and CNS immune activation with imaging biomarkers in a group of chronically infected subjects. Subjects had a mean CD4+ cell count nadir less than 50/ μ L, with the majority receiving suppressive antiretroviral therapy (Abstract 444). A total of 212 subjects underwent blood sampling, lumbar puncture, and cerebral metabolite measurement by MRS. In plasma, findings included associations between MCP-1 levels and levels of markers of both neuronal integrity (ratio of NAA to creatine [Cr]) and inflammation (Cho:Cr ratio). In CSF, MCP-1 levels also directly correlated with lower NAA:Cr ratios in some regions, whereas fractalkine level inversely correlated with NAA:Cr ratios, and levels

of sCD14 and interferon-inducible protein-10 were directly correlated with Cho:Cr ratios. MRS-measured metabolite abnormalities were also observed in perinatally HIV-infected patients (Abstract 450). All of these MRS findings support the hypothesis that immune activation within the brain occurs soon after seroconversion and may persist even during effective systemic virologic control with antiretroviral therapy.

The conference also highlighted DTI, an imaging technique that measures the restricted diffusion of water within the brain in order to visualize neural fiber tracts. Results from HIV-monoinfected participants were compared with those from individuals coinfecting with HIV and HCV (Abstract 448). Gongvatana and colleagues demonstrated that the coinfecting patients had statistically significant decreases in white matter integrity (ie, decreased fractional anisotropy and increased mean diffusivity) in all lobes of the brain.

Identification of Novel Biomarkers

Witwer and colleagues used an accelerated simian immunodeficiency virus (SIV) macaque model of HIV, in which AIDS and CNS disease occur within 3 months of infection, to unveil new biomarkers potentially associated with the development of CNS disease in primate lentiviral infection (Abstract 409). This group previously demonstrated that levels of CSF HIV-1 RNA, CSF interleukin 2 (IL-2), and MCP-1 are predictive of CNS disease in this accelerated SIV model.¹⁴ MicroRNA (miRNA) was identified in plasma to seek molecules associated with progression to SIV encephalitis (SIVE). Profiles of miRNA were assessed in plasma sampled from animals before acute SIV infection and 10 days after infection. A total of 45 miRNA molecules were differentially expressed in acutely infected and uninfected samples, revealing a plasma miRNA “signature” of acute infection. Of these miRNA molecules, at least 6 were statistically significantly different between animals that did or did not develop encephalitis. Two of these latter miRNA molecules have previously

been linked to senescence and CNS disease.

Another study employed high-throughput proteomic methods to identify novel proteins expressed in the CSF in HIV infection and through the course of antiretroviral treatment. Price presented an analysis comparing CSF samples from HIV-uninfected and -infected subjects to identify peptides (> 2000 peptides per sample) with increased abundance in the HIV-infected patients, and to identify recognized proteins (> 300 proteins per sample) present in CSF (Angel et al, Abstract 61). This study also assessed peptides identified in 11 longitudinal sample sets from subjects at different stages of HIV-related CNS disease, before and after initiation of antiretroviral treatment. These investigators used correlation with CSF neopterin, a pteridine marker of macrophage activation, as a “probe.” Thus, proteins identified by the proteomic discovery that either positively (eg, complement) or negatively (eg, autotaxin) correlated with the known marker CSF neopterin were highlighted as potential novel biomarkers with mechanistic importance in the etiology of HAND.

These investigators also used Ingenuity Pathway Analysis (IPA) to map known interconnections between identified proteins, suggesting pathways of disease mechanism. IPA mapped 3 major networks of identified proteins that correlated with neopterin and were potentially associated with neurologic function and disease. Applying state-of-the-art proteomic technologies to CSF samples of well-characterized HIV-infected subjects provides a powerful discovery method for identifying new biomarkers and, potentially, the pathways of HIV-related CNS disease.

Neuropathogenesis of HIV and Simian Immunodeficiency Virus Infection

Whereas many biomarker and treatment studies presented at the conference had implications for understanding the pathogenesis of HAND across the spectrum from mild disease to HAD, a number of presentations fo-

cused directly on the identification of mechanisms of HIV- and SIV-related CNS injury. Studies examining properties of HIV associated with neurologic injury, including genetic analyses of CSF and blood species, were complemented by studies focusing on the host characteristics associated with the development of HAND, centering on host CNS immunologic characteristics in HIV and SIV infections.

Virologic Determinants of HIV-Associated Neurocognitive Disorder

Swanstrom reported on the *env* gene sequences of HIV-1 derived from paired CSF and plasma samples in a study to determine phylogenetic relationships between blood and CSF in subjects with HAD in the absence of antiretroviral treatment (Schnell et al, Abstract 58). The study also sought to evaluate entry properties of HIV-1 species associated with distinct decay characteristics. All subjects with HAD had highly compartmentalized HIV-1 between the CNS and the plasma, but HIV-1 species from subjects with rapid reduction in CSF HIV-1 RNA levels in response to antiretroviral treatment showed no infectivity in cells expressing low levels of CD4 receptors, suggesting that these species must be infecting and independently replicating within T lymphocytes in the CNS.

Viral species from subjects with slower decline in CSF HIV-1 RNA levels after initiation of antiretroviral therapy were more complex, suggesting that they had been growing as separate populations for a long period of time, and were able to enter cells with low levels of CD4 receptors, indicating replication within macrophages or microglia. These macrophage-infecting viruses were almost exclusively found in CSF viruses rather than virus from plasma. In one subject, a macrophage-tropic virus was detected within the CNS 2 years before the onset of dementia. This study provides novel evidence for 2 types of compartmentalized CNS infection in patients with HIV infection and dementia: a form supported by T-lymphocyte infection and another fostered by infection within

macrophages and microglia. These investigators also showed the first evidence of CNS compartmentalization of subtype-C HIV-1 species.

Hightower and colleagues presented data on the degree of genetic diversity within plasma HIV species in chronic infection (Abstract 57). They used a method derived from a population-based sequence that takes into account the number of mixed bases occurring in the sequence, normalized by the total sequence length, which produced a Total Mixed Base Index. They validated this approach as a measure of intra-individual HIV-1 population diversity by comparison of these findings with Shannon entropy and average pair-wise distance. Multivariable logistic regression was used to model the relationship between viral diversity and AIDS, and viral diversity and NP. Blood HIV-1 population diversity was found to be associated with both presence of AIDS and presence of neurocognitive impairment.

Diversity and compartmentalization of SIV variants were investigated in a more controlled setting through studies utilizing the accelerated macaque model of neuroAIDS (accelerated via infection with neurovirulent SIV_{mac251}) (Abstract 437). This group examined gp120 SIV sequences from plasma and postmortem brain tissue in 2 animals euthanized at 21 days postinfection and 4 animals with disease that progressed to meningitis or encephalitis and died. A V2-C2 amino acid variant associated with macrophage tropism unique to brain and lung tissue (and absent from all plasma samples) was identified in 4 animals, suggesting selective outgrowth of a macrophage-tropic variant in these tissues. These studies support the possibility that specific macrophage-tropic variants present in low quantities in transmitted HIV species in humans might selectively replicate within the CNS and be associated with predisposition to immune dysregulation and development of HAND.

Further investigation of the viral determinants of HAND will be fostered by the development of a novel, publicly available “HIV Brain Sequence

Database,” presented by Holman and colleagues (Abstract 436, <http://www.hivbrainseqdb.org/>). This group assembled a database of clonal HIV *env* sequences available in the National Institutes of Health genetic sequence database, GenBank, relevant to CNS HIV infection. Importantly, sequences included had to be either derived from the CNS (from the brain, meninges, or CSF; n=1272) or from other tissues from subjects for whom CNS sequences were also available (n = 1245). Numerous annotation details were assembled for each subject at each point of sampling, including antiretroviral drug history, current laboratory indices including CD4+ cell count and HIV-1 RNA levels, and cognitive and pathologic diagnoses. The database was designed to be readily searchable by all annotation criteria as well as sequence and tissue criteria.

Host Features Associated with HIV-Associated Neurocognitive Disorder

Schrier and colleagues investigated host genetic factors associated with development of HAND in a study of HIV-infected subjects in Anhui, China (Abstract 390). A prior study by this group found an association between the presence of the HLA allele HLA-DR*04 and a diagnosis of HAND in 191 participants recruited within the United States. The current investigation confirmed this finding in 178 HIV-infected Chinese subjects with a distinct genetic background and a different spectrum of comorbidities (including a 94% prevalence of HCV infection).

Furthermore, in subjects with detectable plasma HIV-1 RNA, cognitive decline was greater in the HLA-DR*04 group than in the non-HLA-DR*04 group over a 12-month interval. No individual class I HLA allele (A,B), recognized as associated with slower systemic HIV progression, had an apparent protective effect against HAND. However, subjects with at least 1 protective allele had better NP performance scores and less progression of HAND over 12 months. HLA alleles, which confer important characteristics

of host immune function, plausibly contribute to the systemic and CNS immune responses involved in HIV neuropathogenesis.

In an effort to understand the relationships among lentiviral primate infection, the CNS host immune response to infection, and the development of neuronal injury, Fell and colleagues examined blood, CSF, brain tissue, and patterns of cerebral metabolites in an accelerated (CD8-depleted) SIV macaque model wherein 85% of animals develop SIVE (Abstract 59). They quantified SIV RNA levels in CSF, plasma, and frontal cortex of the brain, and they obtained MRS results every 2 weeks in 24 animals, including 7 placed on minocycline for weeks 4 through 8 postinfection and 4 animals administered antiretroviral drugs for weeks 6 through 12 postinfection. They also quantified activated monocytes (identified as CD14+CD16+ cells) in the blood over time in these animals.

In the absence of treatment, NAA:Cr ratio steadily decreased through the early weeks postinfection, with a 20% reduction below baseline by 8 weeks postinfection. The percentage of activated monocytes correlated with plasma and brain SIV RNA levels but not with CSF SIV RNA levels. A statistically significant inverse correlation was identified between the percentage of CD14+CD16+ monocytes in blood and concentrations of NAA and Cr in the frontal cortex. Both minocycline and antiretroviral treatments prevented further decline in the NAA:Cr ratio and halted expansion of the monocyte subset, but neuronal recovery was not observed. These data suggest that neuronal dysfunction in the early stages of SIV infection in an accelerated macaque model is associated with the presence of activated monocytes in the periphery.

Cells of the monocyte and macrophage lineage were the focus of another study employing an accelerated macaque model of neuroAIDS (using the neurovirulent strain SIV_{mac251}), in this case in an investigation of the time course of monocyte recruitment into the CNS and of the characteris-

tics of perivascular macrophages accumulating during SIV infection and SIVE (Abstract 430). Nowlin and colleagues used intracranial injection of dextran conjugated to different fluorescent labels at distinct timepoints to demonstrate greater accumulation of perivascular macrophages in meninges and brain in animals with SIVE, occurring mostly between days 20 and 49 postinfection. Perivascular macrophage recruitment was estimated to be increased 6-fold in animals with SIV infection without encephalitis, and increased 8-fold in animals with SIVE. These studies also revealed that although CD163+ macrophages are a main component of perivascular macrophages in SIV-uninfected and -infected animals, in animals with SIV, a distinct population of macrophages lacking SCD163 is also recruited.

Treatment With Antiretroviral or Adjunctive Therapies and Biomarkers of HIV-Associated Neurocognitive Disorder

Effects of Standard Antiretroviral Treatment on Biomarkers

Important information on the effects of initiation of antiretroviral treatment on CNS function in antiretroviral-naïve subjects in diverse resource-limited settings was presented by Robertson and colleagues (Abstract 428, AIDS Clinical Trials Group [ACTG] 5199, The International Neurological Study). A total of 860 subjects recruited in Africa (6 sites), India (2 sites), and South America (3 sites) were randomly assigned to 1 of 3 antiretroviral regimens for initial therapy and monitored for changes in NP test performance every 24 weeks; final analysis was done at 192 weeks. Subjects had CD4+ cell counts less than 300/ μ L at entry and were a median of 34 years old; a majority (53%) were women. Randomization led to equal distribution among the 3 treatment groups: efavirenz/lamivudine/zidovudine, emtricitabine/atazanavir/didanosine (enteric coated), or efavirenz/emtricitabine/tenofovir. Results from a short battery of language-adjusted NP tests (grooved pegboard, timed gait,

finger tapping, and semantic verbal fluency) along with standardized neurologic examinations and dementia assessments were obtained at each visit.

Statistically significant improvements in NP test performance were detected in all treatment groups at each follow-up visit over the 192 weeks. Although practice effects from the repeat testing could have contributed to this improvement, antiretroviral treatment may also have positively affected the CNS through control of HIV replication and related processes. The proportions of patients with neurologic abnormalities, dementia, or milder forms of HAND also decreased over the study period.

This study confirms that in resource-limited settings, the initiation of antiretroviral therapy is associated with improvement in NP test performance. The fact that the regimens in the first and third treatment groups are currently initial treatment recommendations from the World Health Organization underscores the clinical importance of witnessing performance improvement in these 2 groups. Additionally, although the 3 different treatments comprised regimens with distinct levels of presumed effectiveness in penetrating the blood-brain barrier (with “clinical penetration effectiveness [CPE],” scores of 9, 6, and 7, respectively^{15,16}), similar improvement was observed in all groups.

Improved NP performance in patients receiving antiretroviral therapy in a broad variety of geographic settings was also observed in an analysis of data obtained from baseline to month 6 in the SMART (Strategies for Management of Antiretroviral Therapy) study (Abstract 402). A total of 292 participants with CD4+ cell counts greater than 350/ μ L were enrolled in Australia, the United States, Brazil, and Thailand and randomly assigned to either episodic antiretroviral treatment (if CD4+ cell count fell below 250/ μ L) or continuous antiretroviral treatment.

Five NP tests (grooved pegboard average, color trails 1 and 2, timed gait, and finger tapping, summarized as the quantitative NP z-score of the 5 tests [QNPZ-5]) were administered to sub-

jects at baseline and at 6 months, at which point the study was terminated because of increased AIDS and non-AIDS-related complications in the episodic treatment group. Subjects were a median of 40 years of age, were composed of 42% women, and had a baseline median CD4+ cell count of 513/ μ L and abnormal NP test performance (mean QNPZ-5, -0.7). During the 6-month follow-up period, 86% of subjects in the episodic treatment group had started antiretroviral treatment according to study indications; thus, the total average exposure to antiretroviral treatment in this group was 3.6 months versus 5.9 months in the continuous treatment group.

In both treatment-strategy groups, improvement in QNPZ-5 scores and in each individual test was noted between study entry and 6-month follow-up. There were no clear correlations between changes in NP test performance and CD4+ cell count (which declined in the episodic group and rose in the continuous group) or plasma HIV RNA level (which rose in the episodic group and declined in the continuous group). The authors note that because there were changes in both groups independent of the usual laboratory-based predictors of NP test performance, the performance improvement between baseline and follow-up might have been influenced by practice effect. The study did not find a difference in improvement between the 2 groups but was underpowered to detect a difference as a result of the early closure of the study. Further studies of treatment strategies that use repeated NP testing would be enhanced by inclusion of a well-matched control group to assess the effect of learning.

Ongoing low-level CNS immune activation during antiretroviral treatment may contribute to the persistence of HAND in patients receiving suppressive treatment in the current era.^{17,18} Yilmaz and colleagues sought to investigate the response of CSF neopterin level, as a biomarker of intrathecal macrophage activation, to initiation of antiretroviral treatment in chronic HIV infection (Abstract 429). Subjects ($n=114$) who were antiretroviral-naïve or had

been off treatment for at least 6 months underwent lumbar puncture before and after initiation of a variety of antiretroviral regimens that successfully suppressed plasma HIV RNA to levels below standard detection. A total of 107 neuroasymptomatic subjects had a median CD4+ cell count of 190/ μ L and abnormal median levels of CSF neopterin (26.3 nmol/L) and plasma neopterin (24.2 nmol/L) at baseline.

A mixed-effects model, which took into account each subject's serial neopterin values and the days of follow-up after treatment initiation, was used to examine the decay pattern and final "set point" of CSF neopterin for the group. After a median 89 weeks of suppressive treatment during follow-up, the estimated CSF neopterin set point was 7.7 nmol/L, statistically significantly higher than the upper normal limit of CSF neopterin in HIV-uninfected healthy subjects (5.8 nmol/L). Seven subjects with HAD were evaluated separately. Despite similar plasma levels, they had higher baseline levels of CSF neopterin, slower decay, and statistically similar elevations in final CSF levels compared with the neuroasymptomatic group. This longitudinal study provides a window into the dynamics of the intrathecal inflammatory response after initiation of standard antiretroviral treatment, and it provides further evidence that immune activation persists in the CNS for a prolonged period during treatment that reduces HIV-1 RNA below levels of standard detection.

Effects of Adjunctive or Intensified Therapy During Antiretroviral Treatment

Because residual neuroimmune activation during antiretroviral treatment may be a contributing mechanism of HAND, treatment with adjunctive therapies that directly suppress CNS inflammatory processes might provide additional benefit in amelioration of HAND. Sacktor and colleagues presented findings from an ACTG study investigating the effect of the addition of minocycline, an antiviral drug with possible immunomodulatory effects, to

the antiretroviral regimens of subjects with progressive HAND (Abstract 421). This study was conducted as a randomized, double-blind, placebo-controlled trial with an endpoint of change in NP test performance (on a battery of 8 tests, summarized as NPZ-8) after 28 weeks of therapy. The 107 subjects had a mean CD4+ cell count of 543/ μ L; 86% had suppression of plasma HIV RNA level to less than 30 copies/mL; and most had subclinical or mild neurocognitive disease at baseline. The subjects were equally divided between 2 treatment groups of oral minocycline or placebo.

Minocycline treatment showed no evident effect distinct from placebo on the NPZ-8 scores or on any individual scores except the grooved-pegboard test, which did not reach statistical significance after correction for multiple comparisons. After 50% of the targeted enrollment completed the 24-week treatment phase, an interim review by the study's data safety monitoring board recommended early termination for futility.

The concept that ongoing brain injury during antiretroviral treatment may be facilitated by low levels of persistent HIV-1 replication driving immune activation within the CNS provides rationale for attempted "intensification" of standard regimens to better suppress these potential residual processes. Price and colleagues presented a pilot study of raltegravir intensification, in which 18 subjects (mean CD4+ cell count, 513/ μ L) with evidence of sustained suppression of HIV-1 in the plasma and successful antiretroviral treatment of CSF HIV-1 to levels below 50 copies/mL were randomly assigned to receive either treatment intensification with the addition of the integrase inhibitor raltegravir or no treatment intensification (Abstract 424). Subjects maintained the assignment for 12 weeks, and those in the no-intensification group had the option to roll over to receive raltegravir intensification after that time. Sampling of blood and CSF and NP testing (timed gait, grooved pegboard, finger tap, and digit symbol; QNPZ-4) was performed for clinical and laboratory

outcomes at baseline and at weeks 4, 8 (blood only), and 12. A total of 14 subjects (including roll-over subjects), preselected for no detectable plasma or CSF viral burden by standard assays, were administered raltegravir intensification; their results were compared with those of 9 subjects without intensification. Overall baseline CSF neopterin levels were within the normal range (mean, 5.6 nmol/L; normal, < 5.8 nmol/L), and no change in CSF neopterin level was observed in either treatment group. Raltegravir intensification also had no effect on most other CSF markers of inflammation examined (white blood cell [WBC] count or CD38+ HLA-DR+ CD8+ T-lymphocyte percentages), blood-brain barrier integrity (CSAR), or QNPZ-4 score.

Early Injury

The possibility that the earliest phases of HIV infection set the stage for subsequent neurologic injury by seeding the CNS with persistent HIV infection, causing early injury to the nervous system, or initiating a cascade of neuroinflammation, has received increasing focus in recent years. Several presentations this year focused on the effects of early lentiviral infection in animal models and in human infection with HIV.

Natural History of Early Central Nervous System Infection

Dash and colleagues presented multimodal findings of the CNS effects of acute and early HIV-1 infection in a humanized mouse model, which was created by reconstituting immunodeficient mice with human CD34+ cells derived from fetal liver (Abstract 438). Longitudinal blood sampling indicated that HIV-1-infected humanized mice had changes in absolute CD4+ and CD8+ cell counts similar to those accompanying typical acute HIV-1 infection in humans, with peak elevation in plasma HIV-1 RNA levels at 4 weeks postinfection. Serial 7 Tesla brain MRS over the first 15 weeks of infection revealed reduced NAA concentrations in the cerebral cortex of HIV-infected mice at weeks 8 through 15 compared with

baseline measures, corresponding to reduced mean diffusivity and fractional anisotropy in this region as detected by DTI. Postmortem examination of animals euthanized at 15 weeks of infection showed human cells infiltrating meninges and perivascular spaces, activation of microglia and astrocytes, and decreased levels of neuronal and astrocyte markers (including synaptophysin, neurofilament, microtubule-associated protein 2 [MAP2], and glial fibrillary acid protein) compared with those of uninfected humanized mice.

Two studies employing neuroimaging biomarkers as crucial endpoints focused upon the effects of HIV infection in the human brain during the early stages of infection (Abstracts 54 and 55LB). Valcour and colleagues found not only CNS inflammation during acute infection by detection of elevated cerebral metabolites with MRS, but also documented some of the earliest evidence of HIV neuroinvasion in humans, detecting CSF HIV-1 RNA as early as 8 days after HIV transmission (Abstract 54). This group characterized the relationship between CSF and plasma levels of HIV-1 RNA in 17 subjects with acute HIV infection (a median of 15 days after transmission), finding that mean CSF HIV-1 RNA levels were approximately 2.5 log₁₀ copies/mL lower than levels in the plasma. Three subjects had undetectable CSF HIV-1 RNA despite having detectable virus in plasma that reached levels of almost 300,000 copies/mL. CSF cytokines correlated with neuroimaging indices of CNS inflammation in certain brain regions.

Additional studies sought to investigate the mechanisms underlying neurologic and behavioral characteristics present in the first months after HIV transmission. Lee and colleagues performed a cross-sectional study assessing the relationship between the occurrence of neurologic disorders during primary infection and blood, CSF, and clinical parameters assessed in a group of 85 subjects evaluated at a median of 75 days after HIV transmission (Abstract 411). Of the 85 participants, 17 were determined by a neurologist's history and physical examination to

have specific neurologic manifestations related to recent infection either before or at evaluation; manifestations included meningitis, encephalitis, facial palsy, and peripheral nerve disorders. Age, CD4+ cell count, sex, and number of days since HIV transmission were similar between the subjects with or without neurologic symptoms. Abnormalities in CSF were common in both neurosymptomatic and neuroasymptomatic subjects during primary infection. Of laboratory factors including blood and CSF HIV RNA level, CSAR, and CSF WBC count, only CSF WBC count was independently associated with the presence of neurologic symptoms in a logistic regression multivariable analysis. These results suggest that host CNS immune response (rather than primarily CSF viral burden) is a determining factor in the neurologic manifestations of HIV during primary infection.

Grill and colleagues investigated the association between reported fatigue and CNS changes as indicated by measures of systemic and intrathecal viral burden and inflammation in 43 men with recent HIV infection (at a median estimated 129 days after transmission) (Abstract 413). Degree of fatigue was measured by the "fatigue subscore" portion of the Profile of Mood States inventory and correlated with scores on the Beck Depression Inventory (BDI) as well as laboratory measurements that included blood and CSF HIV RNA levels, CSF WBC count, and CSF levels of neopterin, cytokines regulating cellular trafficking, interferon-gamma-inducible protein 10 [IP-10], and MCP-1. In linear regression analysis of fatigue subscore and individual markers, CSF MCP-1 levels were statistically significantly associated with degree of fatigue. Fatigue subscore also strongly correlated with levels of depression as measured on the BDI. In a multivariable regression model incorporating CSF WBC count (a marker of inflammation), CD4+ cell count, and HIV RNA levels in plasma and CSF as possible predictors of fatigue, only CSF WBC count was independently correlated with fatigue subscore. During the first months of HIV infection, the expe-

rience of fatigue, although tied closely to symptoms of depression, may be in part related specifically to inflammatory processes within the CNS.

Effects of Treatment During Early Infection

Several studies focused on the effect of early antiretroviral treatment on the CNS. Though numerous studies have evaluated the effect of early initiation of therapy on the course and outcomes of systemic HIV infection, these studies have not been conclusive regarding the value of early treatment for systemic outcomes. Whether early treatment may be effective in suppressing HIV RNA levels, preventing establishment of viral reservoirs, and controlling neuroimmune activation within the CNS has not been systematically investigated.

Graham and colleagues examined the effects of extremely early initiation of antiretroviral therapy in an accelerated SIV macaque model of neuroAIDS (Abstract 410). At 4 days after intravenous inoculation with SIV, 3 animals were treated with saquinavir, atazanavir, and an integrase inhibitor; 3 animals were treated with atazanavir and an integrase inhibitor; and 6 animals were untreated. The animals were euthanized at 21 days, and tissues were examined for presence of SIV RNA, SIV DNA, and cell and cytokine levels.

In treated animals, CSF SIV RNA was statistically significantly reduced compared with untreated animals at days 7 and 10 after inoculation, as was CSF MCP-1 (also known as CCL2) at day 10. Levels of CD4+ and CD8+ T lymphocytes were increased in treated animals, and SIV RNA levels were reduced in brain tissue at day 21. However, SIV DNA levels in brain did not differ between treated and untreated animals, and some measures of inflammatory responses within the CNS, including major histocompatibility complex class II molecules and glial fibrillary acid protein, were elevated in all infected animals. These findings in an animal model of lentiviral infection that leads to accelerated brain injury suggest beneficial effects of hyper-

acute treatment of SIV. Such effects include reducing the burden of viral replication (SIV RNA level), preserving elements of the immune response within the CNS, and reducing markers of immune activation associated with macrophage recruitment and the inflammatory cascade within the CNS. In a related presentation, Mankowski reported that maraviroc monotherapy given to rhesus macaques 24 days after SIV inoculation reduced brain and CSF levels of SIV RNA and levels of brain markers of macrophage activation (CD68+ cells) and neuronal degeneration (accumulation of amyloid precursor protein) in the brain in a similar accelerated model of lentiviral CNS disease in primates (Kelly et al, Abstract 60LB).

A study examining the effects of early initiation of antiretroviral therapy on the CNS in humans with recent HIV infection was presented by Muthulingam and colleagues (Abstract 412). Results from longitudinal blood, CSF, and NP (grooved pegboard, timed gait, finger tapping, and digit symbol; the NPZ-4) testing were obtained for 14 subjects with laboratory-confirmed primary HIV infection who initiated antiretroviral therapy at a median of 6.4 months after HIV transmission. Changes in laboratory values and NPZ-4 scores over time after initiation of therapy were examined in longitudinal follow-up over a median of 458 days; results were compared with those from a group of nondemented subjects who initiated antiretroviral therapy during chronic HIV infection (at a median of 93 months after diagnosis) with a median 328 days of follow-up. Although treatment regimens were variable, CPE scores were similar between the 2 groups. In response to antiretroviral therapy, primary-infection subjects demonstrated complete suppression of detectable HIV-1 RNA in plasma and CSF, but treatment in a subset of chronic-infection subjects failed to completely suppress plasma and CSF HIV RNA levels in follow-up. Median CSF WBC count, a nonspecific marker of inflammation, did not reach normal levels in either group by the end of the follow-up period, suggesting

that mild intrathecal immune activation may persist even in subjects who initiate antiretroviral therapy early during the course of HIV infection. NPZ-4 scores improved in response to antiretroviral treatment in the primary-infection subjects in parallel with expected improvements in the chronic-infection subjects. This phenomenon may have been the result of practice effect but also might indicate that treatment initiation even during primary infection has benefit to neurocognitive performance.

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