ORAL PRESENTATIONS

O1

NEUROPSYCHOLOGICAL AND PSYCHOLOGICAL CHANGES WITH DISEASE PROGRESSION. D. Cooke, S. Lunn, I. Weller, M. Harrison and S. Newman, Department of Psychiatry and Behavioural Sciences, Royal Free and University College London Medical School, London, UK.

The aim of the study was to examine neuropsychological test performance, mood and psychiatric disturbance over the course of HIV illness from asymptomatic to symptomatic disease. Forty-four participants, who changed disease status during the study period, were assessed at 7-monthly intervals over four years. At each assessment cognitive functioning was measured using a comprehensive battery of neuropsychological tests. The Beck Depression Inventory, the state measure of anxiety from the State-Trait Anxiety Inventory, and the Clinical Interview Schedule were also completed. There was no evidence of a significant decline in cognitive functioning from before AIDS diagnosis compared to after diagnosis. Following AIDS diagnosis, patients reported more somatic difficulties, significantly more patients were classified as being depressed and significantly more patients fell into the medium-high anxiety category compared to pre-AIDS diagnosis. In the case of depression this seemed to reflect the inclusion of somatic items. The proportion of patients classified as cases on the Clinical Interview Schedule, a measure of psychiatric disturbance, remained high throughout the study period. The data suggest most individuals do not show cognitive decline following a diagnosis of AIDS. Cut-off scores indicated an increase in the severity of anxiety and depression.

O2

CEREBROSPINAL FLUID (CSF) ANTIRETROVIRAL DRUG PENETRANCE AND TREATMENT OF HIV-ASSOCIATED COGNITIVE IMPAIRMENT. NC Sacktor1, RL Skolasky2, J Becker3, B Cohen1, EN Miller2, and JC McArthur1. Depts. of Neurology1 and Epidemiology2, Johns Hopkins Univ., Baltimore, MD, 3Univ. Of Pittsburgh, Pittsburgh, PA, 4Northwestern Univ., Chicago, IL, 5Univ. Of California, Los Angeles, CA USA.

Background: Highly active antiretroviral therapy (HAART) is associated with improved psychomotor speed performance in HIV+ patients with cognitive impairment. Objective: To determine if psychomotor slowing, a sensitive index of HIV dementia, has greater improvement with HAART containing multiple CSF penetrating agents, compared to HAART with a single CSF penetrating agent. Methods: 404 HIV+ homosexual men in the Multicenter AIDS Cohort Study (MACS) were studied. CSF penetrating drugs were defined as zidovudine, lamivudine, abacavir, nevirapine, or indinavir. Psychomotor speed tests (Grooved Pegboard, Trail Making, and Symbol-Digit Modalities test) were performed on HIV+ men with cognitive impairment (1 SD below the mean of age/education matched HIV- men) on HAART with either multiple or a single CSF penetrating drug. Results: Relative to subjects on a single CSF penetrating HAART (n=83), subjects on a multiple CSF penetrating HAART (n=321) showed no difference on any of the tests. Both groups showed an increased CD4 count and decreased plasma HIV RNA. Conclusion: HAART with multiple CSF penetrating drugs may be equivalent to HAART with a single CSF penetrating drug for treating HIV dementia. These data suggest that the ability of a HAART regime to penetrate the CNS is less critical than the overall systemic efficacy in virological suppression.
O3

HAART AND NEUROPSYCHOLOGICAL IMPAIRMENT. Mark Halman and Sean B. Rourke, Department of Psychiatry, Saint Michael's Hospital, University of Toronto, Toronto, Canada.

Objective: to examine rates of neuropsychological (NP) impairment in a sample of HIV+ subjects taking a variety of antiretroviral regimens.

Method: Cross sectional analysis of 109 subjects evaluated from 1996 -1999 using a comprehensive NP battery examining learning efficiency, processing speed, attention, executive function, verbal skills and memory.

Antiretroviral regimens were classified as HAART [2 NRTI’s + 1(or 2) PI’s or 1 NNRTI] vs. Non HAART (including no antiretrovirals, mono or dual therapies).

Salvage & Mega HAART regimens were treated as a third group.

Results: 41% of subjects on HAART and 58% of subjects not on HAART were classified as displaying global NP impairment. Significant differences between the groups were observed in the domains of learning (p<.05) and processing speed (p<.05). Among the PI-containing HAART regimens, there was no difference in impairment rates between putative brain penetrating and non-penetrating agents.

Conclusions: HAART regimens were associated with lower rates of impairment and significant advantage for domains of psychomotor speed and learning efficiency. There was no significant advantage for regimens containing Indinavir over other PI’s. Longitudinal evaluation is necessary to validate the impact of HAART on NP function.

O4

REDUCTION OF CSF HIV RNA LEVELS CORRELATES WITH REVERSAL OF HIV-INDUCED COGNITIVE DYSFUNCTION. S. Letendre, R. Ellis, J. Ripplst, and A. McCuishan, HIV Neurobehavioral Research Center and Depts. of Medicine and Neurosciences, Univ. of California, San Diego.

Background HIV RNA levels in cerebrospinal fluid (CSF RNA) correlate with current and predict future cognitive dysfunction and might help monitor therapy.

Objective To understand the determinants of cognitive improvement during antiretroviral (ARV) treatment by correlating and modeling changes in cognition, HIV RNA in CSF and plasma, and CD4 counts.

Methods Patients (n=15) with detectable levels of HIV RNA in CSF and plasma and symptomatic cognitive impairment started new, individualized ARV regimens and were assessed at baseline and 12 weeks. Cognitive impairment was assessed by neuropsychologic tests and summarized as an average deficit score (ADS), based on impairment measured by demographically adjusted performance on each test.

Results Patients had clinically significant reductions in cognitive impairment (ADS) and in plasma and CSF HIV RNA levels over 12 weeks of ARV treatment (see table).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 Wks</th>
<th>Change</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>ADS (deficit)</td>
<td>1.31</td>
<td>0.36</td>
<td>- .75</td>
<td>0.04</td>
</tr>
<tr>
<td>CD4 count</td>
<td>14.9</td>
<td>22.9</td>
<td>+ 8.0</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma RNA</td>
<td>4.7</td>
<td>3.5</td>
<td>- 1.2</td>
<td>0.007</td>
</tr>
<tr>
<td>CSF RNA</td>
<td>3.8</td>
<td>3.3</td>
<td>- 0.5</td>
<td>&lt;0.001</td>
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</tbody>
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Baseline CD4 count and undetectable (<50 copies/ml) CSF HIV RNA at 12 weeks were the strongest correlates of improved ADS in a multivariate linear regression model.

Conclusions Therapy for HIV should target suppression of CSF HIV RNA as a marker of adequate activity of ARV in the CNS.

O5

HIV-1-ASSOCIATED MOTOR DYSFUNCTION AS AN OUTCOME PREDICTOR ONLY INCOMPLETELY INFLUENCED BY HAART.

G. Arendt and H.J.v. Giesen, Department of Neurology, Heinrich-Heine-University, Dusseldorf, Germany.

We examined the possible influence of highly active antiretroviral therapy (HAART) on motor dysfunction assessed both clinically and with refined electrophysiological motor tests of motor function on the course of HIV-1 disease. In a prospective study, 1482 HIV-1 seropositive (+) individuals were examined clinically and electrophysiologically every three months over the last decade. Survival of HIV-1 (+) subjects with clinical and / or electrophysiological signs of motor dysfunction was significantly shorter than survival of HIV-1 (+) subjects without such central nervous system deficits (Kaplan-Meier non-parametric survival statistics, post hoc tests p < 0.0001). Pathological motor performance (reaction and contraction times > mean + 2 SD) preceded clinically manifest basal ganglia dysfunction and overt dementia. HAART had a significant prophylactic value both on the first manifestation of pathological motor performance and the subsequent clinical manifestation of basal ganglia deficits. A second and or more sustained type of pathological motor performance was not positively influenced by any therapy regimen currently available. The outcome of these patients has to be followed-up. We conclude that pathological motor performance is a predictor of clinical basal ganglia dysfunction in HIV-1 encephalopathy. Clinical basal ganglia dysfunction in HIV-1 (+) subjects is predictive of death. The course of the HIV-1 associated brain disease in patients with a more sustained and markedly pathological motor performance is not influenced by HAART.

O6

MODIFIED HIV DEMENTIA SCALE AND GROOVED PEGBOARD: ACCURATE STAGING OF HIV-ASSOCIATED DEMENTIA. H. F. Davis, R. Skolsky, O. Selnes D. Burgess and J. C. McArthur, Department of Neurology, Johns Hopkins University, Baltimore, MD, USA

Objective: The modified HIV dementia scale (M-HDS) has been shown to be sensitive in identifying persons with HAD. We found the modified HDS can accurately categorize individuals at various stages of dementia. Additionally, the Grooved Pegboard (GP) is sensitive to early cognitive/motor impairment. We sought to determine whether the M-HDS and the GP were comparable in assessing and staging dementia in HIV-seropositive individuals.

Methods: Data from 455 HIV-seropositive individuals were analyzed. demented (N=144) and non-demented (N=311). Of 144 with HAD, 13 had severe dementia (MSK 3), 55 had moderate dementia (MSK 2), 51 had mild dementia (MSK 1), and 25 had MCMD (MSK 0.5). We correlated dementia severity with the M-HDS total score and GP (non-dominant hand). Kruskal-Wallis chi-square analyses were used for all comparisons.

Results: The M-HDS (z=4.32, p<.05) and the GP-non-dominant (z=4.08, p<.05) differentiated demented from non-demented individuals. Furthermore, the M-HDS (z=2.95, p<.05) and GP non-dominant (z=2.68, p<.05) discriminated equally well the stages of dementia severity.

Conclusions: The M-HDS and the GP (non-dominant) are equally sensitive and specific in categorizing and staging HAD. The M-HDS has the advantage of requiring no equipment and so may be more useful for screening by non-neurologists.
C/EBP FACTOR BINDING SITE SEQUENCE VARIANTS COMMONLY ENCOUNTERED IN HIV-1-INFECTED BRAIN TISSUE AND THEIR IMPACT ON LTR ACTIVATION IN MONOCYTE/MACROPHAGE CELL POPULATIONS. M. Nonnermacher, H. L. Ross, J. R. Corboy, S. Gartner, J. McArthur, and B. Wigdahl1, Department of Microbiology and Immunology, Penn State College of Medicine, Hershey, PA, 170331, Department of Neurology, The University of Colorado Health Sciences Center, Denver, CO2, and Department of Neurology, The Johns Hopkins School of Medicine, Baltimore, MD, USA3

Recent studies have shown that two C/EBP sites are critically important for efficient HIV-1 replication within mononuclear cell populations, which serve as important vehicles for transport of virus to the CNS. Given the important role that monocyte tropism plays in HIV-1 infection of the brain and the general impact of LTR sequence variation on viral replication, we examined C/EBP site sequence variation within brain-derived LTR populations. Brain-derived LTRs commonly possess a C/EBP site I (−107 to −118) configuration (guanine substitution at position 6 of the clade B consensus sequence, designated G6) that leads to enhanced binding of C/EBP factors over that observed with the HIV-1 clade B consensus sequence. In contrast, the G6 configuration appears infrequently within PBMC-derived LTRs. In addition, the clade B C/EBP site II (−167 to −175) consensus sequence has a very high affinity for C/EBP factors, and is conserved in over 85% of brain-derived LTRs examined, but not in PBMC-derived LTRs. Given the prevalence of C/EBP binding sites that are highly reactive toward C/EBP factors in mononuclear cellular extracts, we have investigated the relevance of each C/EBP site with respect to IL-6 stimulation, Tat trans-activation and basal LTR activity in transient expression analyses. We have also investigated the relevance of these sites in different LTR backbones (LAI, YU-2, and 89.6) which are lymphotropic, monocytotropic, and dual tropic in nature.

CELL CYCLE PROTEIN RESPONSE TO CHEMOKINES AND NEUROOTRPHINS IN AN HIV MODEL. K. L. Jordan-Scots, S. Brown, B. Murray, L. RadhaKrishnan and C.L. Achim, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA

Striatal neurons degenerate in areas of abundant macrophage infiltration and activation in HIV encephalitis (HIVE). Among the factors implicated in neuronal death in HIVE are chemokines (RANTES, MCP-1, MIP-1) and, as we have recently reported, neurophins like BDNF. Many investigators believe that neuronal death in AIDS is the result of programmed cell death. We hypothesize that conventional caspase-dependent programmed cell death, usually occurring at a fast pace, can not account for the dynamics of chronic neuronal degeneration seen in HIVE. In support of our hypothesis recent reports have suggested that a prolonged, caspase-independent programmed cell death may play a role instead. Proteins found to be upregulated during this delayed programmed cell death include the cell cycle proteins p53 and E2F1. We have recently begun to study the role of these proteins in the neurodegeneration associated with presence of HIV infected macrophages and the factors secreted by them. Our in vitro system is based on second trimester human fetal neuro-glial cultures grown in serum free conditioned media. In these cultures we found abundant expression of p53 and E2F1. By double-label immunofluorescent confocal microscopy, p53 is found in the cytoplasm of astrocytes and in the nucleus of neurons. E2F1 is exclusively nuclear in neurons. We are currently assessing the changes in E2F1 and p53 subcellular localization in response to treatments with supernatants from HIV infected macrophages, neurophins, chemokines, and synthetic analogs like NNY and AOP-RANTES.

TAT-INDUCED OXIDATIVE STRESS AND DYSFUNCTION OF BRAIN ENDOTHELIAL CELLS. M. Toborek, A. Malecki, M.P. Matson, B. Hennig, H.C. Bauer, A. Nath, University of Kentucky, Lexington, KY 40536 and Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria.

Objective: Impaired function of brain vasculature might contribute to the neurodegenerative changes associated with HIV infection. Disruption of the blood-brain barrier (BBB) is more frequent in AIDS patients with dementia, as compared to non-demented AIDS patients or seronegative controls. Injury or dysfunction of brain microvascular endothelial cells (BMEC) can lead to the breakdown of the BBB and thus provide entry for the virus into the CNS. Evidence indicates that the protein Tat is present in macrophages in the CNS of patients with AIDS. Because Tat is released from the infected macrophages, BMEC can be exposed to high concentrations of this protein. We hypothesize that Tat could be responsible for BMEC injury and impaired normal function of the BBB.

Methods: Cloned BMEC were exposed to recombinant Tat,2 and several oxidative stress-related mechanisms of cell injury were measured in the treated cultures. In addition, barrier function of BMEC and cell viability were determined after exposure to Tat.2

Results: Exposure to Tat resulted in a significant dose-dependent decrease of total glutathione. In addition, treatment of BMEC with Tat activated oxidative stress-responsive transcription factors, such as nuclear factor-xB (NF-xB) and activator protein-1 (AP-1). Tat also diminished BMEC viability, as measured by mitochondrial MTT conversion assay, increase of cellular release of LDH, and compromised barrier function of BMEC.

Conclusion: Tat can disrupt BBB properties and thus can play an important role in the development of detrimental vascular changes in the brain of HIV-infected patients.
O11

LRP-MEDIATED UPTAKE OF HIV-1 TAT PROTEIN DISRUPTS NEURAL METABOLIC BALANCE OF LRP LIGANDS. Y. Liu, M. Jones, C. M. Hingigen, G. Bu, N. Larabee, R. E. Tanzi, R. D. Moir, A. Nath, and L. J. He, Department of Microbiology & Immunology Indiana University School of Medicine, Indianapolis, IN 46202

HIV-1 Tat protein, secreted from HIV-1-infected cells, is neurotoxic. However, the precise role of Tat on HIV-1-associated neuropathogenesis remains largely unclear. Here we report that binding of Tat to the low-density lipoprotein receptor-related protein (LRP) promotes efficient uptake of this protein into neurons. LRP-mediated uptake of Tat is followed by translocation to the neuronal nucleus. We also show that Tat binding to LRP potently inhibited neuronal uptake and degradation of LRP physiological ligands such as amyloid precursor protein (APP). LRP-mediated metabolism of these ligands plays an important role in maintaining normal neuronal function, and elevated accumulation of these LRP ligands is linked to neurodegenerative diseases, such as Alzheimer’s disease. Using macaques infected with a chimeric strain of simian/human immunodeficiency virus as a model, we provide in vivo evidence that Tat expression dramatically increases the number of APP-positive neurons in the brain of SHIV-infected macaques. Taken together, our data suggest that HIV-1 Tat may mediate HIV-1-induced neuropathology through a pathway involving both activation of neuronal genes, as well as disruption of metabolic balance of LRP physiological ligands. These findings also raise the possibility that AIDS-associated dementia and other neurodegenerative diseases may share a common final pathway that eventually leads to dementia.

O12

TRADD IS AN IMPORTANT INTRACELLULAR SIGNAL THAT MEDIATES THE EFFECTS OF TNF-α IN THE HIV-1 INFECTED BRAIN


In vivo evidence for the pathogenetic effects of TNFα in the HIV infected brain has been lacking although elevations of TNFα have been reported to correlate with ADC severity. Further, TNFα has been shown to induce neuronal apoptosis independent of NFκB. We have recently shown that TRADD, which mediates the effects of TNFα, is significantly elevated in AIDS brains compared to controls. To examine TNF-TRADD signaling in HIV brain injury, we have investigated TRADD and its associated proteins FADD and TRAF2, which mediate apoptosis and NFκB activation respectively, in SKNMC cells. Transfection with a TRADD overexpression vector resulted in apoptosis, while overexpression of a DN (dominant-negative) TRADD mutant incapable of NFκB activation resulted in greater cell death suggesting that NFκB may be neuroprotective. Overexpression with DN-FADD following treatment with TNFα resulted in reduced apoptosis compared with mock transfected cells, whereas overexpression of DN-TRAF2 increased apoptosis. Finally, overexpression of an IκB mutant, IκBαN, resulted in an increase in TNF-mediated cell death nearly equal to that observed with the TRAF2 mutant. This is the first report to show activation of the TRADD signaling pathway in the HIV infected brain and in a neuronal culture system in response to TNFα, a key factor in ADC. Further, NFκB activation in neurons may confer a protective effect against TNF-α and thereby provide a potential venue for therapeutic intervention.

O13

High levels of soluble Fas and Fas ligand in CSF of patients with AIDS-related dementia.

Sahbi F1, De Millo A1, Price R2, Elowaara I1, Cinque P1, Hagberg L1, Chiodi F1, 1 Microbiology and Tumorbiology Center, Karolinska Institute, Stockholm, Sweden; 2. Neurology Service, San Francisco General Hospital, 3. Neurology, Tampere University Hospital, 4 Department of Infectious Diseases Clinic, San Raffaele Hospital 5 Department of Infectious Diseases, University of Gothenburg,

Objective: Fas ligand (Fasl) and Fas are important mediators of apoptosis. Our aim was to assess the levels of the soluble forms of Fas (sFas) and FasL (sFasL) in CSF and serum of HIV-1 infected patients presenting with AIDS-related dementia and controls.

Materials and Methods: The level of sFas and sFasL in CSF and serum were assayed with an ELISA method. Specimens were obtained at one occasion from 64 HIV-1 infected individuals (29 presenting with AIDS related dementia) and 52 HIV-negative individuals. The influence of HAART on the levels of sFas and sFasL in CSF and serum was also studied in 10 infected subjects.

Results: CSF sFasL levels differed significantly between patients presenting with AIDS related dementia and other groups of HIV-infected patients (P=0.0061) and non infected individuals (P=0.0001). Also the serum sFasL levels were different between patients with dementia and other groups of HIV-infected patients (P=0.0128). Statisticaly significant difference in CSF sFas levels were also observed between HIV-infected patients presenting with and without dementia (P=0.0001) but not between HIV-patients without dementia and HIV-non infected individuals (P=0.317). The levels of sFas in serum differed significantly between HIV infected subjects and non infected controls with no correlation to AIDS dementia.

HAART therapy reduces the levels of sFas and sFasL in CSF.

Conclusion: The measurement of sFas and sFasL in CSF and serum of HIV-infected patients might be a useful marker for diagnosis and follow-up of AIDS-related dementia. Our findings indicate a role for Fas/Fasl pathway in tissue damage during AIDS related dementia.

O14

ENHANCED EXPRESSION OF FRACTALKINE IN HIV-1 ASSOCIATED DEMENTIA

Cláudia F. Pereira1, Joana Middel1, Gerard Janssen2, Hans S.L.M. Notter1, Eijkman-Walker Institute1 and Department of Pathology2, University Medical Center, Utrecht University, The Netherlands

Background: Fractalkine, the only member of the newly described class of CX3C chemokines was found to be up-regulated in the brain during inflammatory processes. Neurons, endothelial cells, astrocytes and microglia were found to express fractalkine. Fractalkine receptor, CX3CR1 is expressed in leukocytes, microglia and astrocytes. It is reported that fractalkine mediates communication between brain macrophages and astrocytes and between neurons and brain macrophages. In this study we tried to assess the role of fractalkine in HIV-1 associated dementia (HAD).

Methods: mRNA was isolated from brain tissue from demented and non-demented AIDS patients and from HIV-1 seronegative patients. Also, mRNA was isolated from cocultures of primary human astrocytes and uninfected macrophages or HIV-infected macrophages. Samples were used for fractalkine mRNA quantification by RT-PCR. Brain samples were also analysed by immunohistochemistry to determine fractalkine localisation.

Results: Fractalkine mRNA expression is up-regulated in the brains of demented AIDS patients when compared to the two other groups. Fractalkine immunoreactivity was mainly detected in astrocytes. Fractalkine mRNA expression was found to be up-regulated in cocultures of astrocytes and HIV-infected macrophages when compared to cocultures of astrocytes and control macrophages. Discussion: We propose that fractalkine produced during interactions between astrocytes and HIV-infected monocyte cells plays a role in HAD possibly by recruiting monocyctic cells to the brain and by regulating the trafficking of these cells in the brain parenchyma.
O15

NEUROANAL EXPRESSION OF SDF-1α IN PATIENTS WITH AIDS-DEMENTIA-COMPLEX (ADC)

K Rosasoy, M Kneissl, C Yiannoutsos, J Hedeen, B Navia, Boston, MA

Several in vitro studies have shown that SDF-1α and its chemokine receptor CXCR4 play an important role in neuronal apoptosis and viral entry. We recently identified astrocytes as the primary source of SDF-1α in the human brain, but also observed its expression in neurons of HIV subjects and controls. To further understand the significance of the latter finding, we examined the cellular and regional distribution of SDF-1α in the frontal cortex (FC), deep white matter and basal ganglia (BG) of 17 patients with ADC, and five normal controls by immunohistochemistry and in differentiated SKNMC cells. Staining for SDF-1α was localized to astrocytes and neurons in all patient groups and was confined to the gray matter area of the FC and BG. SDF-1α expression was also detected in SKNMC cells. Increased neuronal staining was observed in BG compared to FC in the AIDS subjects. The number of SDF-1α positive neurons in AIDS subjects with moderate/severe brain disease (ADC stage and/or pathology >1) was 24.9, compared to subjects with absent/mild disease, 3.67 and controls, 9.00 (p=0.3). The constitutive expression of SDF-1α in the human brain suggests an important cellular function in vivo. Further, the greater abundance of SDF-1α positive neurons with disease severity suggests that this chemokine may exert an important pathogenetic effect in the HIV infected brain.

O16

CSF 5-HYDROXYTRYPTAMINE & 5-HYDROXYINDOLEACETIC ACID IN HIV-1 INFECTION

Adarsh M. Kumar, M. Kumar, J B Fernandez, D. Waldrop, K. Goodkin, J. Berger. Department of Psychiatry and Behavioral Sciences, University of Miami School of Medicine, Miami, FL & Dept. Neurology, University of Kentucky, Lexington, KY, USA

Objective: Although 5-HT deficiency has been associated with a number of behavioral disorders including depression, suicide, aggressive behaviors, and sleep, the status of 5-HT in individuals with HIV-1 infection has not been delineated.

Method: CSF samples of patients with HIV-1 infection (n=22) and normal subjects (n=34) were analyzed for 5-HT and 5-HIAA using (HPLC-ECD).

Results: Concentration of 5-HT in CSF of HIV-1+ individuals was significantly decreased when compared with that of normal subjects and HIV-1 serostatus, was found to be significantly correlated with CSF 5-HT status (Spearman correlation coefficient, r=452, p<0.001).

Conclusions: The data showing a significant decrease in CSF level of 5-HT in HIV+ subjects suggest that pathways involved in the synthesis of 5-HT may be affected by viral infection. Clinical importance of our results is under investigation.

O17

STRESS ENDOCRINES AND CYTOKINES IN HIV-1 INFECTION: ROLE IN NEUROAIDS

Mahendra Kumar, A M. Kumar, J B Fernandez, K. Goodkin, C. Eisdorfer. Department of Psychiatry & Behavioral Sciences, University of Miami, School of Medicine, Miami, FL (U.S.A)

Background: Emerging evidence suggests that HPA activity is abnormal in HIV-1 infection. However, its interaction with TH2 cytokines and possible role in neuroAIDS has not been investigated.

Methods: The catecholamine, ACTH and cortisol responses to an adrenergic challenge (cold pressor/mirror star tracing) were investigated in HIV-1+ and HIV-1- individuals. Since cytokines and hormones of the HPA axis are interrelated, plasma levels of IL-6 and TNF-a are also being quantified.

Results: Our results show an attenuation in catecholamines, cortisol and ACTH responses to both the challenges. While the levels of cytokines are being investigated, response to mirror star tracing show abnormalities suggestive of visual-spatial abnormalities among HIV-1+ participants.

Conclusion: These results suggest that the abnormalities in HPA axis activity may be involved directly in the development of neuroAIDS. Alternatively, they may exacerbate the neurodegenerative properties of cytokines.
O19

HIPPOCAMPAL INJURY IN AIDS. CK Petito, J Torres-Munoz, B Roberts, J Cantando, A Rabinstein, N Taconorte, M McCarthy. Pathology and Neurology, U Miami School of Medicine, Miami, FL

We correlated hippocampal (HP) HIV chemokine coreceptor (CC) expression with quantitative counts of reactive gliosis in the HP of 22 AIDS and 10 HIV-brains. Four AIDS HP had local HIV encephalitis (HIVE) and one had CA3 neuronal loss. CXCR4 immunoreactivity was greater than CCR5; both were minimal in absent in CA1 neurons and intense in CA3 and 4 neurons; intergroup differences were absent. Reactive gliosis increased with AIDS and with HIVE, was minor in the CA1 region, and significantly different only in HIVE cases (see below). These studies suggest that HP injury is common in HIVE and correlates, in part, to regional CC expression. Because of the established correlation between HIVE and AIDS dementia, we suggest that hippocampal injury plays a role in this disorder and may be mediated via HIV chemokine coreceptors. RO1 NS27416 and 31977 (CKP), some controls from HD83284.

O20

ASSOCIATION BETWEEN HIV ENCEPHALITIS, NEURODEGENERATION AND NEUROCOGNITIVE DYSFUNCTION M. Chernier, E. Munshi, R. Heaton, R. Ellis, T. Marcotte, J. A. McCutchan, I. Grant and the HNRC Group, Departments of Psychiatry, Neurosciences, Pathology, and Medicine, School of Medicine, University of California San Diego, USA

Background: Studies have found inconsistencies in the association between HIV encephalitis (HIVE), neuronal damage, and cognitive deficits. We have previously shown a relationship between dendritic simplification and even mild degrees of cognitive impairment. Objective: To investigate further the relationship of in vivo cognitive functioning to postmortem evidence of HIVE and dendritic simplification. Methods: The cognitive functioning of 39 HIV infected study participants was assessed during life with a neuropsychological battery and characterized as representing HIV associated dementia (HAD), minor cognitive motor disorder (MCMD), sub- syndromic (deficits that do not appear to affect everyday functioning) NP impairment (NPI), or normal functioning. Subjects were divided into 4 groups based on the presence or absence of HIV encephalitis (HIVE+/-) and neurodegeneration (ND+/-) from post-mortem histopathologic analyses (macrophagic examination of fronto-temporal cortex, hippocampus, basal ganglia, cerebellum, and midbrain; MAP 2 immunoreactivity in frontal cortex), and quantitative virology (immunocytochemical detection of gp120 in frontal neocortex, basal ganglia grey matter, and subcortical white matter). The resulting group sizes were: HIV+ ND+ = 18; HIV+ ND- = 9; HIV- ND+ = 4; HIV- ND- = 8. Results: 85% of Ss in the HIV+ND+ group showed NP impairment, compared to 33% of the HIV+ND- 0% of the HIV-ND+ and 13% of the HIV-ND- group. All Ss with HAD and 82% of Ss with MCMMD were in the HIV+ND+ group. Interestingly, there were I (15%) cognitively normal Ss in this group. The HIV+ND+ group performed worse on measures of verbal functioning, abstraction, attention/spread of information processing, perceptual-motor skills, and learning of new information, typically followed by the HIV+ND- group in terms of mean scores. Conclusions: The current results use a larger sample size to expand earlier findings indicating that syndromic NP impairment is more likely to occur in the presence of HIVE and dendritic simplification together. However, there is not a 1 to 1 correspondence between these factors and cognitive dysfunction.

O21


The metabolic/anatomic substrates underlying neuropsychological (NP) impairment in HIV infection remain uncertain. In an AIDS Clinical Trials Group study, magnetic resonance spectroscopic (MRS) and NP evaluations (NPZE) were performed with 57 ADC patients, 35 HIV-positive and 39 HIV-negative controls. Most HIV-positive subjects were treated with combination antivirals. N-acetyl aspartate (NAA), Choline (Cho) and Myo-inositol (MI) were measured in the basal ganglia (BG), frontal centrum semiovale (CSO) and parietal cortex (PAR). The NPZE is a total of eight age- and education-adjusted tests, sensitive to the effects of ADC, that reflect functional domains of gross and fine motor skill, psycho-motor function and speed of information processing. In the BG, NPZE was associated positively with Cho and positively with NAA/Cho, driven by indices of fine motor skill (r=0.47) psycho-motor function (r=0.35) and information processing speed (r=0.35). In the CSO, NPZE correlated positively with all NAA ratios such as NAA/Cr (gross and fine motor indices; r=0.31-0.41), NAA/Cho (all domains including timed tasks; r=0.30-0.45), NAA/MI (fine motor; r=0.42 and psycho-motor indices; r=0.42). No associations were seen in PAR. These analyses identify region- and metabolite-dependent effects, specific for cognitive and motor domains impaired in HIV infection further supporting their subcortical origin. MRS provides a powerful approach to explore the metabolic anatomy of ADC. This research was supported by the ACTG, the NIMH, NINDS grant RO1NS36524, and NIMH grant RO1MH60565.

O22


Despite extensive research in the role of individual metabolites in HIV-related brain injury, metabolic patterns of injury have received limited attention (Salvan et al., 1997). These researchers defined a Choline (Cho) and a N-acetyl aspartate (NAA) pattern as higher Cho levels than the highest control level and NAA lower levels than the lowest control level respectively (a multiple NAA-Cho pattern was also defined). We used data on five metabolite ratios (NAA/Cr, Cho/Cr, NAA/Cho), including myo-inositol (MI) related indices (MIPR, NAA/MI), measured in three brain regions (basal ganglia, centrum-semiovale and parietal cortex). We compared metabolite levels among 57 ADC patients, 39 HIV-negative and 35 HIV-positive controls. Our observations indicate that brain injury represents a continuum, with considerable overlap between groups. We extend previous work in defining patterns of injury by more flexible cutoff points than those previously suggested and include MI-related measures. The sensitivity of tests distinguishing between subject groups can be dramatically improved when tests are based on flexible definitions of injury, include MI-related indices and are concentrated in the subcortical brain regions (i.e., basal ganglia and centrum semiovale).

This research was supported by the ACTG, the NIMH, NINDS grant RO1NS36524, and NIMH grant RO1MH60565.
O23

ROLE OF MONOCYTE ACTIVATION AND TRAFFICKING IN THE DEVELOPMENT OF HIV DEMENTIA

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In a previous case study, we found that HIV gpl60 sequences from the deep white matter (dwm) of brain clustered with those from bone marrow (bm), and were most similar to sequences recovered from blood monocytes collected 5 months earlier. Sequences from the T-lymphocyte population of the same blood specimen did not cluster with the dwm/bm/monocyte group. These findings suggest that in late-stage infection, bone marrow-derived monocytes, but not T-lymphocytes, bring HIV into the brain parenchyma.

Additional phylogenetic analyses have now been performed. In both patients studied, gpl60 sequences from the dwm or the caudate (another subcortical region of brain) clustered with sequences from bm. We also examined sequences recovered from the monocyte and T-lymphocyte fractions of blood samples taken from HIV patients with and without dementia, and found that the two cell types harbor different viral species, although the number of infected circulating monocytes is generally low. Immunophenotyping using the whole blood lysis technique, and cytokine assays were also performed on sequentially collected blood samples. As seen in our preliminary studies, the presence of cognitive impairment continued to be associated with increased numbers of CD16+ monocytes and elevated levels of M-CSF, a known inducer of CD16 expression on monocytes, suggesting a link between cognitive impairment and monocyte activation. For some patients, these elevations persisted for more than one year. Interestingly, almost all patients with elevated serum M-CSF also had elevated serum neopterin, but a significant number of those with elevated neopterin had normal levels of M-CSF.

O24

SOLUBLE CD23: A MARKER FOR BRAIN AIDS RELATED LYMPHOMAS. S. Bososlocco1,2, A. Nilsson1, A. De Millo1, A. Lazzarin1, A. Linde2, P. Cinque1, F. Chiodo2, S. Raffaele Hospital1 Milan, Italy, Swedish Institute for Infectious Disease Control1, A. Lindgren Childrens Hospital1, and Karolinska Institute4, Stockholm, Sweden.

Aim: To evaluate soluble CD23 (sCD23) as a tool for diagnosis and follow up of AIDS related non Hodgkin lymphomas (NHL) in the brain.

Materials and method: Cerebrospinal fluid (CSF) and plasma were collected from 50 patients with AIDS related NHL: 14 primary central nervous system lymphoma (PCNSL), 10 systemic NHL with brain involvement and 26 systemic NHL at the time of NHL diagnosis and after chemotherapy. The samples were examined for sCD23 levels by an ELISA test. As controls, CSF and plasma from HIV-1 infected patients presenting with other neurological symptoms associated to HIV-1 infection were tested.

Results: In patients with PCNSL at the time of diagnosis, median sCD23 levels in CSF and plasma were 19 ng/ml and 16 ng/ml, respectively. In patients with brain involvement of systemic NHL, median levels were 49 ng/ml in CSF and 9 ng/ml in plasma and in patients with systemic NHL, 4 ng/ml and 35 ng/ml, respectively. In patients with other neurological symptoms related to HIV-infection, the median sCD23 levels in CSF and plasma were 5 ng/ml and 9 ng/ml. The level of sCD23 in CSF are significantly different in patients with primary and secondary brain localization of AIDS related lymphomas as related to control specimens (p<0.04 and p<0.01).

Conclusion: Our results suggest the possibility of using sCD23 as an additional and simple method for diagnosis and follow up of NHL in HIV infected patients.

O25

CD8+ T LYMPHOCYTE DEPLETION RESULTS IN RAPID AND CONSISTENT DEVELOPMENT OF SIVE: THE ROLE OF THE IMMUNE SYSTEM IN SIV NEUROPATHOGENESIS.

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HIV and SIV are consistently detected within the CNS of humans and monkeys 7-14 days post infection (p.i.). Yet only about 30-40% of humans and macaques infected develop HIV/SIV encephalitis. Mechanisms thought to contribute to the development of HIVE/SIVE include viral sequences or “neurotropic viruses” and host factors. We have studied the CNS of rhesus macaques that were treated with a humanized anti-CD8 mAb that results in either acute (<21 days) or persistent (>28 days) CD8 T lymphocyte depletion. We report that monkeys that are persistently CD8 depleted developed AIDS, have a shorter survival time than acutely depleted animals, and have a high incidence (3/3) of SIVE. In contrast, animals that are acutely CD8 depleted have an accumulation of perivascular macrophages some of which are viral infected, but no SIVE. SIVE found in the persistently CD8 depleted animals is essentially identical to that seen in non-depleted animals and includes the accumulation of perivascular macrophages, some of which are infected. These data demonstrate a model of CD8 lymphocyte depletion that results in rapid and consistent CNS disease and underscores the role of the immune system in the development of SIVE.

O26

VIRAL-HOST INTERACTIONS IN SIV NEUROAIDS

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The central nervous system (CNS) repercussions of HIV infection can be modeled by simian immunodeficiency virus (SIV) infection of rhesus monkeys. We have shown that neurophysiological measurements provide reliable markers for CNS dysfunction induced by infection. One such test, assessment of CNS neuronal circuitry through the measurement of sensory evoked potentials, revealed delayed latencies following infection that could be reversed by lowering viral load, and enhanced by raising viral load. Other measures, such as movement, were not affected by modulating viral load. Following infection, macrophage activation is found in the brain, but CNS histopathology did not reveal a correlate of CNS dysfunction. However an acute increase in the level of the chemokine macrophage chemotactic protein 1 (MCP-1) was found in the cerebrospinal fluid (CSF) relative to plasma in the infected animals at the peak of acute viremia, likely contributing to an early influx of immune cells into the CNS. Examination of different anatomic regions of the CNS early in infection revealed distinct areas which were more susceptible to viral infection. Interestingly, a discordance could be identified between the viral load and the host immune response in the CNS. This may lead to some regions of the CNS suffering more from the effects of the virus, whereas others being preferentially damaged through the actions of the immune system. The continual interplay between the virus and the host response in the brain can lead to accumulation of injury, resulting in long-lasting changes in CNS-mediated functions.
ROLE FOR CELL CYCLE REGULATORS IN SIV ENCEPHALITIS. K.L. Jordan-Sciutto, G. Wang, M. Murphy-Corb, and C.A. Wiley, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Neurodegeneration associated with HIV encephalitis (HIVE) has been associated with the secretion of direct and indirect neurotoxins by infiltrating, HIV-infected or activated macrophages including chemokines, cytokines, etc. We hypothesized that the barrage of signals present in the extracellular milieu of HIV infected striatum causes inappropriate activation of neuronal cell cycle machinery. To test this hypothesis we examined 3 members of the cell cycle control machinery; p53, pRB, and E2F1 in the SIV encephalitis (SIVE) model. Compared to non-infected and SIV-infected non-encephalitic controls, we observed increased expression for E2F1 and p53 by immunoblot and found aberrant cellular localization of E2F1 and pRB by immunohistochemistry (IHC). Using double label immunofluorescent confocal microscopy (DICM), E2F1 was found to localize to the cytoplasm of astrocytes and astocytes of SIVE basal ganglia in areas showing SIVE pathology including multi-nucleated giant cells and activated macrophages. E2F1 was also found in the cytoplasm of cortical neurons. By DICM pRB staining was observed in neuronal nuclei and cytoplasm in SIVE cortex adjacent to microglial nodules. The cytoplasm of cortical neurons also stained positive for phosphorylated pRB, consistent with pRB staining. These data suggest that in SIVE, cell signaling results in increased phosphorylation of Rb and subsequent alteration in E2F1 activity. As increased E2F1 and p53-activities have been linked to cell death, these data suggest that the neurodegeneration in SIVE may in part be due to changes in expression and activity of cell cycle machinery.


Neonatal rhesus macaques were inoculated within one day of birth with either SIVmac239 or SIVmac251 as a model of pediatric AIDS. Neuroinvasion could be detected within 3 days by PCR of the brain and 7 days by in situ hybridization. To examine neuronal injury, frontal cortex of each animal was analyzed by proton MRS spectroscopy for N-acetylaspartate (NAA), a neuron-specific metabolite shown to be decreased with injury. NAA values were reduced below normal as early as 14 dpi in animals inoculated with SIVmac251 and by 50 dpi in those inoculated with SIVmac239. Terminal animals inoculated with either strain had NAA levels as low as 43% below normal. Consistent with these data were decreased immunoreactivity of Bet-2 in hippocampal neurons and decreased brain weights. Additional evidence of neuropathology included astrocyte (GFAP+) and microglial (CD16+) activation. Although 75% of cases developed mild CNS lesions consistent with those seen in HIV infected children, there was less virus (despite abundant virus in the periphery), fewer activated CD16+ macrophages, and no MNGCs when compared to older macaques inoculated with the same viruses. These data suggest that neuronal injury may occur with minimal virus and inflammation in neonates. Also, these animals may serve as an important model to investigate the regulation of viral entry into the CNS.

THE VPU GENE IS NOT REQUIRED FOR NEUROPATHOGENESIS OF SHIV. C. McCormick-Davis, S.B. Dalton, D. M. Pierson, N. E. J. Bernari, S. Wong, and E. B. Stephens, Departments of Microbiology1 and Anatomy and Cell Biology2, University of Kansas Medical Center, Kansas City, Kansas. 2Oregon Regional Primate Center, Beaverton, Oregon, USA.

We have used a molecular clone of simian-human immunodeficiency virus (SHIV) known as SHIV145721 to analyze the role of the vpu gene product in the CD4+ T cell loss caused by this virus in pig-tailed macaques. A mutant was constructed (ΔvpuSHIV145721) in which 42 of the 82 amino acids of Vpu were deleted, including the first α-helical domain and the first phosphorylation site. This virus was used to inoculate four macaques. One macaque developed a severe decline in CD4+ T cell numbers within one month and by 35 weeks post-inoculation had developed signs of neurological disease (blindness and tremors). At necropsy, histological examination of the lymphoid tissues revealed lymphoid depletion characteristic of pathogenic SHIV. Examination of the brain and spinal cord revealed perivascular cuffing, microglial nodules and neuronal loss. PCR analysis revealed high levels of ΔvpuSHIV145721 in all 14 regions of the brain and spinal cord examined. Sequence analysis of the virus from the brain of this macaque revealed significant amino acid changes in Env and in-frame deletions in Nef. The other macaques inoculated with this virus developed a moderate or no loss of CD4+ T cells and no neurological disease. The virus sequences isolated from these macaques did not show any conserved amino acid changes in Env or Nef. These results indicate that Vpu is not required for the CD4+ T cell loss and neurological disease in pig-tailed macaques and its absence can be compensated for by changes in other viral genes.

THE EFFECTS OF DRUGS OF ABUSE IN ANIMAL MODELS OF NEUROAIDS. S.J. Henrikson, T. Phillips, S.Huitron-Resendez, J. Criado, M. Barr, J-N Billaud, and H.S. Fox, Department of Neuropathology., The Scripps Research Institute, La Jolla, CA, USA.

Illicit drug use is an increasingly important vector for the transmission of the HIV-1 virus in many cultures. However, the role of these drugs, particularly opiates and amphetamines, as co-factors in disease progression is poorly understood. We have recently employed three animal models of NeuroAIDS: the SIV infected macaque; the FIV-infected feline; and transgenic mice overexpressing AIDS-related cytokines in drug studies designed to evaluate NeuroAIDS progression during acute or chronic drug administration. Preclinical studies have suggested that both morphine and methamphetamine are able to enhance viral replication, in vitro, providing support for the potential of increased viral load during episodes of drug abuse. In our animal models we, and others, have consistently observed delays in electrophysiologically monitored brainstem evoked potentials as an early sign of NeuroAIDS. We have used this measure and other neurophysiological tools in the monkey and the cat to assess disease progression while correlating these measures with immune status, CNS viral load, and brain pathology. Initial findings in on-going investigations suggest that multiple acute morphine exposure delays disease progression in the feline model, while methamphetamine administration shortens CNS disease progression in both the monkey and cat model. Data will be presented that correlates these CNS findings with other markers of disease progression in these animal models of NeuroAIDS.