WORKSHOPS

W1

CORRELATION BETWEEN DISEASE PROGRESSION AND ASTROCYTE APOPTOSIS IN HIV-ASSOCIATED DEMENTIA.
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HIV non-productive infection of astrocytes has been identified in HIV-associated dementia (HVID). As astrocytes participate in the inactivation of neurotoxins, we hypothesise that HIV non-productive infection of astrocytes leads to increased astrocyte apoptosis and a consequent increase in the levels of neurotoxins. Post mortem brain tissue was examined from clinically well characterised HIV positive demented, HIV positive non-demented and HIV seronegative non-demented subjects. The HIVD group was further categorised into subjects with rapid, medium and slow progression of dementia. Tissue was paraffin embedded and 6 µm sections, from the basal ganglia and mid-frontal gyrus, were processed to detect apoptosis by in situ TUNEL. Astrocytes were co-localised using immunohistochemistry. In situ PCR was utilised to detect HIV DNA in astrocytes and correlated with apoptotic astrocytes. Results were quantified by Video Image Analysis. The prevalence of apoptotic astrocytes was significantly greater in the HIVD groups compared with the HIV non-demented and HIV negative groups (p<0.05). The HIVD rapid progressors also had a significantly greater number of apoptotic astrocytes compared to the HIVD medium and slow progressors (p<0.01). Additionally, there was a greater number of HIV DNA positive astrocytes in the HIVD groups compared with the HIV non-demented and HIV negative groups. The results demonstrate a correlation between the number of apoptotic astrocytes and HIV-associated dementia and emphasise the importance of understanding the role of HIV infection of astrocytes in the neuropathogenesis of HIV-associated dementia.

W2

INVOLOEMENT OF QUINOLINIC ACID IN CHEMOKINE PRODUCTION AND CHEMOKINE RECEPTOR EXPRESSION IN ASTROCYTES.
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We hypothesised that Quinolinic acid (QUIN) could initiate brain inflammation in ADC by increase chemokine production and chemokine receptor expression in astrocytes.

Chemokine production and chemokine receptor expression were respectively determined using ELISA kits and RT-PCR associated with immunocytochemistry in highly purified primary human foetal astrocyte cultures.

We showed that QUIN: 1) induces to produce large quantities of MCP-1, RANTES, fractalkine, HuMIG, IL-8, 2) upregulates chemokine receptor CXCR4, BONZO, BOB, ChemR23 mRNA expression; 3) significantly increases GFAP expression.

We also demonstrated that TNFα and IFNγ upregulate expression of several chemokines including MIP-1α, RANTES, IP-10, MCP-1, fractalkine and HuMIG, and chemokine receptors including CXCR4, CCR5, BONZO, BOB, and ChemR23 in astrocytes.

This study represents the first evidence that QUIN induces chemokine expression and chemokine receptor expression in astrocytes. The ability of QUIN to induce chemokine receptor expression such as CXCR4 on astrocytes is a critical factor for HIV entry into these glial cells. The resulting persistent infection can lead to a dysregulation of the astroglial neuroprotective functions. These results suggest that early astrocyte stimulation by QUIN is the “switch on” in the brain that initiates the inflammatory phenomena in ADC.
Abstracts

W3

VPR-MEDiated ACTIVATION OF THE HIV-1 LTR: RELEVANCE TO CNS INFECTION. J. Hogan, J. J. McAllister, K. E. Steimer, S. Nonnemacher, H. Ross, and B. Widrich. Department of Microbiology, Penn State College of Medicine, Hershey, PA, USA

Human immunodeficiency virus type 1 (HIV-1) replication is regulated, in part, by interactions between cellular transcription factors and gag/protease promoter elements within the long terminal repeat (LTR). LTR sequence heterogeneity influences both basal and activated LTR activity. Previous studies have shown C/EBP site I (−120 to −109) and site II (−178 to −159) are required for viral replication in monocytoids but not CD4+ T lymphocytes. We have previously demonstrated that C/EBP site I often contains a guanine substitution at position 6 (6G) leading to a high affinity configuration in 31% of brain-derived LTRs. A well-conserved high affinity C/EBP site II is found in 85% of brain-derived LTRs. HIV-1 Vpr has been shown to activate basal transcription through interactions with members of the Sp transcription factor family and the G/C box array of the HIV-1 LTR as well as by mediating cell cycle arrest at the G2/M interface. Utilizing electrophoretic mobility shift (EMS) analysis, we have shown a direct association between C/EBP site I and an HIV-1 89.6-derived Vpr-GST protein. This association was enhanced by the use of a lower affinity C/EBP site sequence variant. In addition, we examined the effect of 89.6 Vpr on a panel of recombinant HIV-1 LTR-luciferase constructs containing high and/or low affinity C/EBP sites. These studies indicate that a low affinity C/EBP site enhances both the magnitude and fold-activation of the LAI LTR in the presence of 89.6 Vpr. Conversely, variants of C/EBP site II did not affect Vpr-mediated LAI LTR activation. To further these observations, we have cloned additional Vpr constructs from the brain-derived M-tropic YU-2, and the blood-derived T-tropic LAI molecular clones and are currently investigating their interaction with C/EBP site configurations that are preferentially encountered in HIV-1-infected brain tissue in the context of a brain-derived (YU-2) and a blood-derived (LAI) LTR.

W4

HUMAN ASTROCYTES SHOW IMPAIRED HIV-1 REV MEDIATED TRANSMISSION BUT SUPPORT THE NUCLEAR ARM OF REV FUNCTIONS. Jutta van Empel, Eva Ludwig, Jürgen Rochlitz, Volker Erfe, Markus Neumann and Ruth Brack-Werner. GSF-Institute for Molecular Virology, D-85758 Neuherberg, e-mail: brack@gsf.de

The HIV-1 Rev protein is an essential stimulator of viral structural protein synthesis and therefore necessary for efficient virus production. Rev is imported into the nucleus, where it binds to incompletely spliced viral RNAs coding for structural viral proteins. This specific binding occurs on a secondary RNA structure termed the Rev responsive element (RRE) where Rev forms multimers and chaperones bound transcripts into the nucleus for efficient translation. After RNA release Rev shuttles back into the nucleus.

Astrocytes represent target cells for HIV infection in the central nervous system, but the infection is only poorly productive. We have shown that the trans-activation function of Rev is impaired in astrocytes and that this inhibition is linked to aberrant cytoplasmic accumulation of Rev in astrocytes. In order to find out which aspects of the Rev-functional cycle may be compromised in astrocytes, we compared nuclear activities of Rev in astrocytes and control cells using a transient transfection assay designed to monitor binding of Rev to RNA in living cells (Tiley et al., 1992). Our data showed similar binding capacity of Rev to SLIB, the essential Rev binding site of the RRE, in three astrocytoma cell lines and two permissive control cell lines. Preliminary data suggest that Rev is less capable of binding to its target RNA in a fourth astrocytoma cell line (85GH66). A variation of this assay was used to assess oligomerisation capacity of Rev. No significant differences in oligomerisation behaviour were observed between astrocytic and control cell lines. The localisation of Rev-dependent RNA in astrocytes is currently under investigation. These results indicate that crucial nuclear activities of Rev are not impaired in astrocytes. Together with the aberrant cytoplasmic localisation of Rev they suggest that disruption of posttranscriptional control of HIV-gene expression occurs at steps involving cytoplasmic and/or trafficking activities of Rev.

W5

Convection-Enhanced Delivery of Cytosine Arabinoside (araC) for the Treatment of HIV-1 Related Progressive Multifocal Lenkencephalopathy (PML). Robert Levy, M.D., Ph.D. and Dennis Grotthuis, M.D., Departments of Neurological Surgery and Neurology, Northwestern University Medical School, Chicago, IL, 60611

Recent clinical trials suggest that araC is ineffective for treating PML when given intravenously or intraventricular routes. AraC, however, does not appear to enter the brain in high concentrations when given systemically. It also has significant systemic toxicity, limiting the amount that can be given. Convection-enhanced intraparenchymal drug delivery (CEDD) is a novel drug administration technique where drug in infused directly into the brain, bypassing the blood brain barrier and resulting in widespread distribution of high concentrations of drug. Based upon our earlier research, we have obtained FDA approval to begin a Phase I trial of the safety and efficacy of AraC delivered by CEDD in patients with HIV-1 related PML. 12 HIV infected patients will undergo stereotactic brain biopsy to confirm the diagnosis of PML. A specially designed catheter will be inserted directly into this target site, attached to a drug infusion pump (Medtronics, Neurological, Minneapolis, MN) and to infused 3.5 µ/min. The infusion will increase the amount of water in the brain extracellular space, which will be visualized on T2-weighted MRI scans. Saline will be infused for 48 hours, and an MRI scan performed to document the volume of distribution, and that the CEDD infusion is not causing significant mass effect. Infusions of AraC will be started at 1 mg/hr, far below the concentration that causes neurotoxicity in animal studies. Subjects will be evaluated by neurological examination, CSF analysis, and MRI scans every two days as the AraC dose is increased logarithmically. At 100 mM, local brain concentration will be roughly 20 mg/ml, a concentration that suppresses greater than 90% of JC virus replication in cell culture. The infusion will be continued indefinitely, and followup performed twice weekly. The primary endpoint of this study is length of survival. Secondary outcome measures include serial neurological examinations, MRI scans, viral CSF DNA, and after death, postmortem neuropathology. Thus, this is the first human clinical study of chronic CEID in humans and may ultimately be of significant importance for the treatment of a broad range of neurologic disorders.

W6

AIDS MORTALITY IN THE HAART ERA: THE ROLE OF SUBSTANCE USE AND DEPRESSION. David Dorfman, Mubashar Naseer, Pieter Gerits, Yvonne Brown, Susan Morgello, Departments of Psychiatry and Pathology, Mount Sinai School of Medicine, New York, NY.

The role of substance use and psychiatric disorders in the progression and mortality from HIV disease has been controversial since the start of the epidemic. The Manhattan HIV Brain Bank was established in 1998 to recruit advanced stage HIV patients to a longitudinal neurologic and neuropsychiatric study with an endpoint of autopsy and organ donation. To identify patients with a high risk of mortality within 12 months, we developed clinical criteria based on retrospective review of patients coming to autopsy since the introduction of HAART. Of 67 enrolled patients receiving complete neuropsychiatric evaluation, 54 (81%) had at least one DSM-IV alcohol, cocaine, or opioid substance use disorder. Of these 54, 52 (96%) also had DSM-IV defined major depression or dysthymia. In no instance were these mood disorders explainable solely by the patient’s current medical condition. This pattern also is true for gay men: of the 21 gay men receiving the full evaluation, 17 (81%) have both a substance use disorder and a mood disorder. These data suggest that the constellation of substance use and mood disorder is an important risk factor for AIDS mortality among all risk groups. Chart review of 21 HAART era patients passing to autopsy prior to the establishment of the brain bank are consistent with this conclusion. Possible explanations for this phenomenon include reduced compliance with medical regimens, reduced immunological function mediated by psychoimmunological mechanisms, and the immunosuppressive properties of drugs such as cocaine.
W7

HIV DEMENTIA PERSISTS DESPITE HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART). NC Sacktor1, M McDermott2, G Schiffito2, K Maruder3, S Albert4, B Cohen5, J McArthur1, L Epstein6, and the NorthEastern AIDS Dementia Consortium. Depts. of Neurology, 1Johns Hopkins Univ., Baltimore, MD, 2Univ. of Rochester, Rochester, NY, 3Columbia University, NY, NY, 4Northwestern Univ., Chicago, IL

Background: HAART has decreased the incidence of HIV dementia, but the effect on neurocognitive abnormalities is unclear.

Objective: To evaluate baseline neuropsychological testing abnormalities in 2 longitudinal cohorts of individuals recruited for their high risk of HIV dementia (CD4 <200 or cognitive symptoms with CD4 <300) before and after the introduction of HAART.

Methods: Baseline clinical data from 1994-5 in 272 HIV+ subjects in the Dana Consortium on Therapy for HIV Dementia (Dana Cohort) and (as of 12/1/99) 254 HIV+ subjects in the NorthEastern AIDS Dementia (NEAD) Consortium were compared. Clinical, neuropsychological testing, and functional assessments were identical.

Results: Dana subjects were predominantly on monotherapy (43%) or no therapy (43%), whereas NEAD subjects were predominantly on HAART (53%) or double therapy (24%). Dementia classification was similar (probable dementia in Dana, 27%, NEAD, 31%). There was no difference in the proportion of abnormal results (1 or 2 SD below age and education matched norms) for tests of psychomotor, motor, verbal and visual memory, language, or executive functioning.

Conclusion: Despite the introduction of HAART, HIV dementia remains a prevalent condition in advanced HIV disease. Analysis of longitudinal data and correlation with virological suppression are planned.

W8

THE NATIONAL NEUROAIDS TISSUE CONSORTIUM (NNTC): A NEW RESOURCE TO SUPPORT STUDIES ON NEUROPATHOGENESIS OF HIV. I Grant1, B.B. Gotman2, S. Morgello3, E.J. Singer2, P. Kozlowski2, University of California, San Diego and VA San Diego Healthcare System1, University of Texas Medical Branch, Galveston, TX2, Mount Sinai Medical Center, New York, NY3, University of California, Los Angeles, Los Angeles, CA4, New York State Institute for Basic Research4

A rate-limiting step in research on HIV neuropathogenesis has been lack of availability of high-quality human neuropathologic specimens which can be linked to detailed antemortem medical, neurological, and neurocognitive data. To address this need, the U.S. National Institutes of Health (NIH) has established four regional neuroAIDS tissue banks that are accruing specimens from HIV-infected persons and a limited number of uninfected controls from whom systematic clinical data are gathered before death. The NNTC sites are centered in Galveston, Texas, Los Angeles, California, New York, New York, and San Diego, California. Operations of the four sites are linked through a separate National Coordinating Office (NCO) that is prepared to receive requests for tissues and data.

This presentation will describe the objectives of the NNTC, provide details on the types of historic, neuromedical, neuropsychiatric, laboratory, and treatment information that is being prospectively acquired, and will provide details on the types of neuropathologic specimens being archived as well as the potential research uses of such materials. With approximately 1,000 living persons in late stage disease being assessed regularly, and with up to 100 neurologic specimens being accrued on an annual basis, the NNTC promises, over the next five years, to develop into the largest source of neuroAIDS specimens gathered from individuals well characterized before their death that has yet to be established. Ways in which investigators can access these resources will be described, and can be accessed through the NNTC public website <hivbrainbanks.org>.