POSTER ABSTRACTS

P01
DETECTION AND LOCALISATION OF HIV-1 DNA IN BRAINS OF ASYMPTOMATIC HIV-1 POSITIVE INDIVIDUALS BY PCR-ISH.
Shu F. An, Franciscose Grey, and Francesco Scaravilli.

Objective: To identify the types of cells and their density in brains of HIV-1 positive asymptomatic patients by detection of HIV-1 DNA using PCR-ISH.

Methods: Formalin-fixed, paraffin-embedded brain tissue from 18 HIV-1 positive asymptomatic drug users and from suitable positive and negative controls was examined using routine, immunohistochemical, PCR, and PCR-ISH.

Results: All brains were morphologically normal except for mild and diffuse increase in astrocytes and microglia in most of the cases. None of them revealed positive with p24 antibody. All of them were PCR (tested) positive. HIV-1 DNA was detected by PCR-ISH in 5 brains. The signal was seen in cell nuclei and was higher in white than in grey matter. Positive cells represented 2-3% of the total. Using double staining PCR-ISH positive cells appeared to include microglia, astrocytes, and few endothelial cells. No positive neurons were seen.

Conclusions: Results reveal widespread presence of HIV-1 in various types of cells in brain from early stages of HIV infection with similar distribution to that previously observed in AIDS patients. The observation that glial and endothelial cells, in addition to microglia, are infected early only implies that microglia are not the only reservoir of the virus and suggests that damage to the CNS could be induced following different pathways.

This work was supported by the Brain Research Trust.

P02
TRANSMISSION ROUTES OF HIV-1 GP120 FROM BRAIN TO LYMPHOID TISSUES.
Mary Faith Cashion, William A. Banks, Kenneth L. Bost, and Abba J. Kastin.

Objective: HIV-1 within the CNS is sheltered by the blood-brain barrier (BBB) from bloodborne antiviral agents and so can act as a reservoir for reinfusion of peripheral tissues. The pathways used by HIV-1 within the CNS to reenter the periphery are, therefore, important. We characterized the efflux from brain of radioactively labeled HIV-1 (gp120) after intracerebroventricular (icv) injection in the mouse.

Methods: The rate of efflux from the CNS to blood for I-gp120 was characterized by the icv injection method (J Pharmacol Exp Ther 239:668-72, '86). The rate of uptake by the spleen and cervical nodes from blood after iv and icv injection was also characterized and compared.

Results: The half-time disappearance rate of I-gp120 from brain was 12.6 min, which was faster than could be explained by the reabsorption of cerebrospinal fluid into blood (half-time disappearance of 30-40 min in the mouse). The usual explanation for a faster disappearance rate is the presence of a saturable transporter, but the efflux of I-gp120 was not inhibited by unlabeled gp120. Furthermore, the amount of icv administered I-gp120 appearing in the serum was less than, not greater than, that predicted from CSR reabsorption. These discrepancies were explained by the sequestration of I-gp120 by the cervical nodes. The uptake of I-gp120 by the cervical nodes was much greater when the I-gp120 was given icv rather than by iv route, especially when compared to uptake by spleen, demonstrating that drainage from brain to the cervical lymph nodes occurs directly through the brain=s primitive lymphatic system.

Conclusions: The primitive lymphatics of the brain provide a pathway through which CNS reservoirs of HIV-1 could directly reinfest lymphoid tissue without being exposed to circulating antiviral agents.

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P03
TRANSPORT OF HIV-1 gp120 ACROSS THE BLOOD-BRAIN BARRIER.
William A. Banks.

Objective: The cellular mechanisms by which HIV-1 and HIV-1 infected immune cells cross the blood-brain barrier (BBB) are unknown, since the endothelial cells comprising the BBB are CD-4 and galactocerebroside negative. We tested the hypothesis that gp120 could induce adsorptive endocytosis (AE) in brain endothelial cells (BEC) in a manner similar to other glycoproteins such as wheatgerm agglutinin (WGA), which induces AE by binding to sialic acid and N-acetyl-B-D-glucosamine on the luminal surface of BEC. AE provides cellular pathways both into and across BEC.

Methods: In vivo studies measured pharmacokinetic parameters after the iv injection of gp120 labeled with 125I (I-gp120) in mice. In vitro, isolated murine BEC were used to characterize the binding characteristics of I-gp120.

Results: In vivo studies showed that I-gp120 crossed the BBB faster than serum albumin, demonstrating selective uptake for gp120 at the BBB. About 0.15% of the injected dose/g of brain crossed the BBB. Uptake of I-gp120, but not of serum albumin or monoglycosylated gp120, was increased about 17 fold by simultaneous administration of WGA. Other lectin glycoproteins had little or no effect on I-gp120 uptake suggesting that WGA and gp120 bind to similar or proximate sugars. I-gp120 uptake was enhanced by LPS, suggesting a role for cytokines, by an indomethacin-independent mechanism. I-gp120 both disrupted the BBB and stimulated AE with the latter predominating. WGA-enhanced uptake likely represents the initial phase of internalization into BEC and occurred at the cerebellum, ponti-medulla, midbrain, cortex, and thalamus but not at the hippocampus, striatum, and hypothalamus. Complete transport across the BBB was fastest at the hippocampus, pons-medulla, and hypothalamus and slowest at the striatum and cerebellum. Therefore, neither internalization into BEC nor transport across BEC is uniform throughout the brain. In vitro studies showed that internalization of I-gp120 into isolated BEC is stimulated about 17 fold by WGA and is partially dependent on glucose and potassium. I-gp120 binds to the cytoskeleton but internalization is not dependent on clathrin, caveolae, calcium channels, or endosomal acidification. Fusion of I-gp120 with the membrane after internalization via a protease sulfate dependent co-receptor suggests involvement of heparan sulfate.

Conclusions: In vivo and in vitro studies show that gp120 is internalized by and transported across brain endothelial cells by processes resembling adsorptive endocytosis.

Acknowledgements: Supported by ROI MHS4979.

P04
ACCELERATED NEURODEGENERATION IN IFN-γ (-/-) MICE INFECTED WITH LP-BMS.
Yelena Kustova, Michael G. Espey, Tom McCarty, Herbert C. Morse III, Yoshitaka Sei and Anthony S. Basil.

Objective: The contribution of the pro-inflammatory cytokine IFNγ to the pathogenesis of the encephalopathy and neurodegeneration observed in LP-BMS infected mice was investigated using IFNγ (+/-) mice on a C57BL/6 strain.

Results: Brain virus load, IL-2 receptor and Thy1 expression on lymphocytes in infected IFNγ (-/-) mice were comparable to infected wild type (wt) mice. However, the performance of LP-BMS infected IFNγ (+/-) mice in the Morris water maze reached minimal values 2 weeks before infected wt mice. The development of splenomegaly and lymphadenopathy was delayed in IFNγ (-/-) mice, and the levels of circulating ICAM were significantly decreased. At this time, perivascular spaces and venules of the brain were invaded by peripheral monocytes, with the development of multilaminated giant cells, NO induction in macrophages, apoptosis of stratal neurons and histological evidence of increased cortical neuron degeneration. CSF glutamate concentrations in infected IFNγ (-/-) mice were approximately twice that of infected wt (10.1 vs 5.1 µM, 0.9 µM for uninfected wt).

Conclusions: Neurodegeneration and cognitive loss is accelerated in LP-BMS infected IFNγ (-/-) mice, despite the lack of evidence of increased viral burden in the CNS. Thus, maintaining IFNγ levels is essential in the face of virus-induced immunodeficiency.
**Poster abstracts**

**P05**

**ACTIVATING, AMPA-SELECTIVE ANTIBODIES IN THE BRAINS OF LP-BM5 INFECTED MICE MAY CONTRIBUTE TO RETROVIRAL ENCEPHALOPATHY.**

Yelena Kustova, Peter Usherwood, Linda Fosson, Bradley Keeler, Michael Rogawski, and Anthony S. Basile.

**Objective:** LP-BM5 infected mice develop hypergammaglobulinemia with accumulation of IgG in the CNS and show a decrease in AMPA receptor density. The involvement of an activating, anti-AMPA receptor IgG in the neurodegeneration observed in these mice was investigated.

**Methods:** IgG was isolated from infected mouse brains with protein A/G columns. IgG inhibited [3H]AMPA binding to the high affinity component (IC50 = 200 ng/protein), while enhancing binding to the low affinity site, but had no effect on ligand binding to nicotine or GABA receptors. IgG activated kainate-like sodium currents in pyramidal neurons that are cyclothiazide-dependent and suppressed by NBQX and GYKI 52466. Finally, incubating primary granule neuron cultures with MK-801 and the IgG preparation kills neurons in a cyclothiazide-dependent and NBQX-suppressible fashion (IC50 = 400 ng/protein).

**Conclusions:** Anti-AMPA receptor antibodies arising from polyclonal B cell expansion or molecular mimicry can activate AMPA receptors in LP-BM5 infected mice and contribute to their chronic neurodegenerative syndrome.

**P06**

**ELEVATED GLUTAMATE LEVELS CONTRIBUTE TO INCREASED BLOOD-BRAIN BARRIER PERMEABILITY IN LP-BM5 INFECTED MICE.**

Yelena Kustova, Yoshitatsu Sei, and Anthony S. Basile.

**Objective:** To determine if the blood-brain barrier (BBB) is disrupted in LP-BM5 infected mice and how to treat it.

**Methods:** BBB permeability was determined histologically. MK-801 was administered by osmotic minipump (0.1 mg/kg/d SC) for 2 wks to mice 10 wks PI.

**Results:** Immunohistochemical staining for IgG (150 kD), albumin (69kD), transferrin (81kD) and α2-macroglobulin (798kD) was increased throughout the CNS, particularly in the cortex, cerebellum, choroid plexus, meninges, and ependyma. Following IV injection of Evan’s Blue, which quantitatively binds serum albumin, pale blue staining was observed throughout the brains of 30-40% of mice infected for 4-8 wks. Dye accumulation in the forebrain of mice infected for 12 wks was significantly increased (13±1.5 vs 45±10 ng/mg dry wt, et vs LP-BM5). Treating infected mice continuously with the NMDA antagonist MK-801 reduced brain levels of Evan’s Blue levels to control values.

**Conclusions:** The BBB of LP-BM5 infected mice is extensively disrupted, but can be effectively treated with MK-801. Activated glia are the probable source of the increased CNS levels of glutamate causing BBB disruption.

**P07**

**EXTRACELLULAR GLUTAMATE LEVELS ARE ELEVATED IN THE BRAINS OF LP-BM5 INFECTED MICE.**

Michael G. Espey, Yelena Kustova, Yoshitatsu Sei and Anthony S. Basile.

**Objective:** Mice infected with the LP-BM5 develop a progressive immunodeficiency with behavioral, histological and neurological alterations suggestive of excitotoxic hyperactivation.

**Methods:** Glutamate in the cerebrospinal fluid (CSF) and striatal microdialysates from the striatum of infected mice was quantified by HPLC.

**Results:** CSF glutamate in infected mice was significantly elevated (30-80%) at 4-12 wks PI. Concurrently, steady-state glutamate levels increased 3-fold in striatal microdialysates. Evoked glutamate concentrations increased to 167-210% above control in mice 4-8 wks PI. Suppressing synaptic transmission reduced evoked glutamate levels 88% in control mice but had negligible effects on infected mice. Striatal expression of the glutamate transporters EAAC-1 and GLT-1 decreased to 85 and 75% of control in mice 12 wks PI, but GLAST expression increased 125%. Glutamine synthetase activity increased throughout the CNS of mice 8-14 wks PI.

**Conclusions:** Glutamatergic hyperactivation contributes to the neurochemical and behavioral abnormalities observed in LP-BM5 infected mice.

**P08**

**GLUTAMATERGIC HYPERACTIVATION OF THE HYPOTHALAMIC-PITUITARY ADRENAL AXIS IN LP-BM5 INFECTED MICE AUGMENTS IMMUNODEFICIENCY.**

Michael G. Espey and Anthony S. Basile.

**Objective:** The mechanism of hypothalamic-pituitary-adrenal (HPA) axis activation and the role glucocorticoids (GC) play in chronic disease was investigated with the LP-BM5 infected mice.

**Methods:** ELISA kits were used to quantify cytokine and GC levels in plasma/serum. Drugs were administered by subcutaneous osmotic pumps.

**Results:** Coincident with a shift from the Th-1 (interferon-γ) to Th-2 (IL-4, -10) cytokine phenotype in the late stage of infection was a significant increase in plasma ACTH and corticosterone. No significant changes in either circulating IL-1β, IL-6 or TNF-α levels were observed. Administration of either IL-4 or -10 did not alter the HPA axis in normal mice. However, administering MK-801 to infected mice normalized plasma ACTH and corticosterone levels, indicating that glutamate was a major activator of the HPA axis. This treatment also reversed the Th-1 to Th-2 cytokine shift to a degree comparable to that of IL-4/46 therapy.

**Conclusions:** GCs exacerbate immunodeficiency in the late stage of infection with LP-BM5 and that hyperactivation of glutamatergic pathways in the hypothalamus is the primary mechanism for HPA axis activation in these mice.
**P09**

TIME COURSE OF MRI POST-CONTRAST ENHANCEMENT IN DEMENTED AND NON-DEMENTED HIV SEROPOSITIVE PATIENTS


**Objective:** To use the timecourse of post-contrast MRI fractional enhancement (FE) to examine the relationship between vascular changes in the basal ganglia (BG) and white matter (WM), and HIV in demented patients.

**Methods:** Seropositive patients fell into three groups: i) non-demented (ND) (n=4); ii) mildly demented (MD, MSK dementia rating < 1.0) (n=2); iii) moderate-severely demented (MSD, MSK ≥ 1.0) (n=5). Axial T1-weighted spin echo MR images covering the entire brain were collected at constant gain before and for up to 40 minutes following contrast administration (Magnevin™, 0.1 mmol/kg i.v.). Post-contrast FE timecourses were determined in BG, WM and in the sagittal sinus (SS).

**Results:** There was no significant difference between ND and demented patients in peak FE, or in the rate of FE decline in SS post contrast, confirming that there was no significant difference in contrast dose, or clearance rate. In all three groups there was an initial rapid BG enhancement which reached a maximum (FEmax) ~4 mins post-contrast. There was no significant difference in BG FEmax between ND (FEmax = 0.042 ± 0.007) and MD patients (FEmax = 0.041 ± 0.011). However BG FEmax was significantly greater (p < 0.05) in MSD patients. Furthermore, in the MSD patients, the decline in post-contrast BG FE was slower than in ND or MD patients, and was slower than the rate of SS FE decline. Thus 30 minutes post contrast, BG FE was 0.008 ± 0.002 in MSD, while it was 0.006 ± 0.003 in MD, and 0.016 ± 0.001 in ND patients. There were no significant differences in WM FEmax between the demented (FEmax = 0.020 ± 0.004) and ND (FEmax = 0.020 ± 0.001) patients, nor was there any significant difference in WM FE 30 minutes post-contrast (FEmax = 0.000 ± 0.001, ND; FEmax = 0.011 ± 0.006, demented).

**Conclusions:** The significantly greater BG FEmax seen in moderate to severe dementia is consistent with the increased-cSBV previously reported in these patients (Nasia & Gonzalez 1997). The greater FE 30 minutes post-contrast and the slower decline in BG FE suggest some compromise of the BBB in the BG in advanced stages of the disease.

**P10**

MICROGIAL ACTIVATION AND NEUROLOGICAL DEFICITS IN THE SIV MODEL OF NEUROAIDS.

Nancy J.E. Berman, Joanne K. Mancatto, Chi Yong, Leigh A.M. Raymond, Opendra Narayana, and Paul D. Cheney.

**Objective:** The quantitative relationship between microglial activation, evoked potential changes and behavioral deficits was examined in the simian immunodeficiency model of neuroAIDS.

**Methods:** Macaque monkeys were infected with SIV 239/R71/17E. Functional impairment was assessed by motor and auditory brain stem evoked potentials and by measurements of behavioral task performance. Brain tissue was examined by immunohistochemistry using two markers of microglia activation, MHC-II and matrix metalloproteinase-9 (MMP-9).

**Results:** Postinoculation disease progression in these animals followed two distinct trends: rapid progressors which survived 6-14 weeks and slow progressors which survived 87-109 weeks. In the rapid progressors, MHC-II expression in microglia was high and two patterns of MHC-II expression were present: a widely disseminated pattern of stained microglia in cortical gray matter and subcortical white matter, and a more focal pattern confined to white matter. Animals exhibiting both patterns of microglial activation showed both mild and severe deficits in behavioral task performance and evoked potentials. These changes were evident before the onset of SIV-related clinical signs. All rapid progressors showed expression of MMP-9 in microglia in subcortical white matter and typical SIV encephalitis. In addition, rapid progressors had increased total numbers of white matter glia relative to slow progressors. In the slow progressors MHC-II and MMP-9 staining was similar to uninfected control macaques, and there was little or no evidence of encephalitis. These animals showed no significant abnormalities in evoked potentials, and behavioral deficits occurred only late in the disease course after the appearance of SIV-related clinical signs.

**Conclusions:** In the SIV model, evoked potential changes and behavioral deficits are associated with rapid disease progression, expression of MHC-II and MMP-9 in microglia, and increased total numbers of white matter glia. Supported by NS32262.

**P11**

NEUROPSYCHOLOGICAL STATUS AS A FUNCTION OF CD4 COUNT, AND OTHER MEASURES OF HIV PROGRESSION IN HIV+ WOMEN.

Ronald Cohen, Robert J. Holland, David Moser, Pamela Schuman, Janet Moore, Eleanor Schoenhoffen, Dana Owsiewicz, Kathleen Morrow.

**Objective:** This study investigates whether neuropsychological (N-) impairments in HIV+ women vary with CD4 count, and whether whether aggressive antiviral (HAART) therapy improves N-status.

**Methods:** 435 women were examined. HIV+ women (who were part of a multi-site longitudinal study) with CD4 counts under 100 were assessed every 6 months. They receive a brief N- battery, psychosocial assessment, an assessment of substance abuse, and various medical and laboratory indices. Using linear regression, MANOVA, and time series analyses, we examined how N- performance varied in relationship to the illness-related factors described above.

**Results:** A strong relationship between N- dysfunction and CD4 level was found (R= .77). Color sequencing errors and verbal fluency performance accounted for the variance in CD4 level at visit 1. When depression severity (on the CES-D) was entered as an additional variable, N- performance still accounted for the majority of variance in CD4 levels, though the CES-D accounted for an additional 20% of the variance (R= .95, p<.001). N- performance was not retained as one of the predictors of CES-D status, as CD4 levels and severity of drug use accounted for most of the variance in CES-D. Patients on HAART therapy exhibited much stronger N- performance compared to those on alternative therapies (p<.01), and CES-D and drug history did not contribute to this effect.

**Conclusions:** The present results demonstrate that CD4 levels, in patients who have AIDS with CD4<100 are directly associated with N- status, suggesting that severity of immunocompromise affects brain function. The introduction of HAART therapy in this cohort appears to improve cognitive functioning, suggesting that the neuropathological changes in many of these patients may be reversible. (Support: CDC grant U64/CCU106795).

**P12**

EFFECTS OF EXTRACELLULAR HIV-1 VPR PROTEIN IN CELLS OF NEURONAL ORIGIN.

Ming-Bo Huang, Ophelia Weeks, Ling-Jun Zhao, Mary Sallarelli, and Vincent C. Rand.

**Introduction:** Evidence suggests that HIV-1 Vpr exists in soluble form in the serum and cerebrospinal fluid (CSF). Further, its abundance in the bloodstream, and the CSF, and its activity on other cell types suggest that Vpr could have an effect on brain activity.

**Objective:** We examined cells of neuronal origin for extracellular Vpr-induced cell death.

**Methods and Results:** Using mixed embryonic rat brain cultures as a model, Vpr-induced cell death was observed. Similar Vpr-induced effects were observed in enriched primary cortical rat astrocytes, as well as in the C6 glioma cell line. Vpr-induced cell death in astrocytes appeared to be caused primarily by a necrotic mechanism, although a few apoptotic nuclei appeared to also be present. We did not observe Vpr-induced cytotoxic effects on any primary cortical neurons, although Vpr-induced cytotoxicity was observed in hippocampal neurons and astrocytes. Finally, we observed no effects on the cell cycle due to extracellular Vpr protein.

**Conclusions:** This data suggests that different cell types are effected by the toxic effects of extracellular Vpr protein, and that differential toxic effects of extracellular Vpr protein are observed in similar cell types.

This project was supported in part by NIH Grant R01-R0000304, and by NINDS/NIDCD grant U54 NS34194-01.
P13

THE HIV-TRANSGENIC RAT: A NEW MODEL FOR THE ANALYSIS OF HIV/CNS DISEASE.

Frank J. Denaro, Odell Jones, Norman Hayes, William Reid, K. Houze and Joseph L. Bryan.

Objective: The development of an HIV transgenic rat model system to study the neuropathology, neurologic deficits and cognitive/behavioral changes associated with HIV transgene expression.

Methods: Standard transgenic techniques were used to create the first HIV-Transgenic Rat. A staining battery for the analysis of neuropathology was used to examine brain, peripheral nerve and muscle. Operant conditioning techniques were used to assess behavior, memory and a mini neurologic exam was used to evaluate gait, and sensory responses.

Results: A) Neuropathology: Analysis of CNS tissue reveals that neurons, astrocytes and endothelial cells are undergoing cell death. Apoptosis markers indicate that at least a subset of these cells are undergoing programmed death. Both, chronic and acute changes are evident. Stains for calcium reveal that neurons and endothelial cells are calcium positive in areas of chronic change. H&E reveal acute changes. Inflammation is noted in the parenchyma with perivascular cuffing. Hemorrhage and leakage of serum surrounding the capillaries is evident. This suggests BBB break down. Immunocytochemistry to HIV-1 transgene reveals very low GP120 in monocytes. Peripheral changes include inflammation of nerve and muscle. In later stage animals, severe hind limb paralysis has occurred. Muscles in these animals are severely dystrophic with intense inflammatory changes. B) Neurology: Gait irregularities, hind limb paralysis and some slight sensory loss were identified. C) Cognitive/Behavioral changes: Animals have been trained in operant conditioning paradigms to establish the feasibility of a learning and memory model. They can learn a number of operant tasks and studies are underway to identify deficits.

Conclusions: The HIV-Transgenic rat, replicate a number of changes of HIV/CNS disease. The transgenic rat offers the potential to study neuro-pathology, neurologic and behavioral cognitive changes in a single small animal model. This makes it a potential model for the testing of neuroprotectants. NS31857

P14

HUMAN MONOCYTE CHEMOTAXIS BY GP120 AND CHEMOKINES: INHIBITION BY PEPTIDE T.

Douglas J. Dietter, Courtney M. Peterson, Candace B. Pert Michael R. Ruff.

Objective: To develop small receptor-binding drugs which block gp120-mediated neuropathogenesis. To determine if the GP120 V2-region "peptide T" blocks gp120 and chemokine monocyte chemotaxis by acting at chemokine receptors.

Method: Chemotaxis of purified human monocytes was studied.

Results: Purified GP120 (LAV,MN,CM,SF-2) caused monocyte chemotaxis, 10^-11 M to 10^-8 M. Several peaks of activity were observed (0.1-10 pM), with a stimulation index (SI) of 3-5. Peptide T, (Gly-dle-peptide T-amine, Pert, CB et al., 1986), which defines a V2 region gp120 receptor binding epitope, also showed multiple peaks of chemotactic activity when tested over a broad range (-8M to -1M). Some activity peaks were reproducibly observed at -15M and -7M. Peptide T thus acts as a super-agonist for the gp120 chemotaxis receptor. Peptide T inhibited GP120 chemotaxis but was more a more potent antagonist of the M-ocept (CM) vs. the T-acute (LAV) env. Moreover, MIP2, monocyte chemotaxis, and RANTES and MIP3a chemotaxis on a CCR5 receptor transfected cell line was inhibited.

Conclusions: The peptide T epitope of gp120 comprises a gp120 receptor-binding domain which is important in AIDS neuropathogenesis. Peptide T pharmacologically acts as a mixed agonist/antagonist. When tested alone it is super agonist for monocyte chemotaxis, but also acts as a selective, potent antagonist for gp120 strains and chemokines which bind to CCR5 receptors. Peptide T is effective for cognitive endpoints in controlled trials (Heseltine, 1998), and these results support its anti-gp120 effects in Neuro-AIDS as brain viruses isolate all bound to CCR5 [Advanced Immunity].

P15

RELEVANCE OF GLUTAMATE LEVELS IN THE CEREBROSPINAL FLUID OF PATIENTS WITH HIV-1 ASSOCIATED DEMENTIA COMPLEX.

Michael Eapey, Ronald Ellis, Robert Heaton, and Anthony Basile.

Chronic hyperactivity of excitatory amino acid pathways in the central nervous system of patients infected with human immunodeficiency virus (HIV-1) may contribute to the pathogenesis of HIV-1 associated dementia complex (HADC). To determine its utility as a diagnostic marker for HADC, the glutamate level in cerebrospinal fluid (CSF) obtained by lumbar puncture was measured. Glutamate concentrations in the CSF (mean 3.3 ± 1.0 µM) were not elevated in either HIV-1 infection generally or HADC. While glutamate may play an important role in the pathogenesis of HADC, our results suggest that measuring the concentrations of glutamate in the CSF is not useful in determining the presence of excitotoxicity in the brain parenchyma of patients infected with HIV-1. The low microenol basal presence of glutamate in CSF suggests that quinoline does not play an excitotoxic role in HADC due to the millimolar EC50 requirement for quinoline in activating NMDA receptor-gated currents.

P16

SUSTAINED BENEFIT OF COMBINATION ANTIRETROVIRAL THERAPY FOR NEUropsychological PERFORMANCE IN HIV-1 INFECTION.

Stephen Ferrante, M.D., Martin McElhinney, Ph.D., Judith Rahbin Ph.D., and Wilfred van Gorp, Ph.D.

Objective: We have previously reported cross-sectional data from a naturalistic cohort of 130 non-demented HIV+ men, indicating that men taking highly active antiretroviral therapy (HAART, 3 or more antiretrovirals including a potent protease inhibitor) had superior neuropsychological (NP) function compared to men taking less potent antiretroviral regimens. We aimed to determine whether the initiation of or continuation of HAART or other combination therapy regimens resulted in improvement or sustained benefit for NP function after 1 year.

Methods: 110 (85%) of the cohort were tested at baseline and 1 year with a NP test battery assessing attention, concentration, psychomotor speed, memory and executive function. Subjects were divided into 5 groups based on antiretroviral treatment between the two assessment points: 1) no treatment (N=8), 2) 2-3 antiretrovirals, no protease (N=19), 3) continued HAART (N=40), 4) initiated HAART (N=19), 5) discontinued HAART (N=4). Groups were compared on proportion at each time point with NP impairment (2 SD in the impaired direction relative to published norms on 2 or more NP tests) and change over time in individual NP test scores.

Results: Subjects averaged 42 years; 56% were white, 19% black, 19% Hispanic, 6% other; 86% had post high school education; mean baseline CD4 was 291, mean log, HIV RNA was 3.7; 86% had AIDS. Group 1 compared to the rest of the sample had higher baseline mean CD4 (580 vs. 267cells/mm3, p<.01) and were less likely to have AIDS (38% vs. 87%, p<.01). Over 1 year, the rate of NP impairment for the whole cohort declined from 30% to 15% (p<.01), with the greatest improvement in groups 2 (42%-5%, P<.0001) and 4 (58% to 26%, P<.01). On individual NP tests, similar significant improvements on tests of attention, memory, psychomotor speed, and executive function were seen in groups 2, 3 & 4. Changes in NP test scores were not correlated with changes in CD4 or plasma HIV RNA for the group as a whole or any subgroup.

Conclusions: In this sample of HIV+ men, most of whom had AIDS, initiating or maintaining combination therapy with 2 or more antiretrovirals was associated with improvement or sustained benefit for neuropsychological function over 1 year.

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P17

HIV-1 INFECTION OF BRAIN ENDOTHELIAL CELLS (BMVEC) IS ABORTIVE BUT ALters THE BLOOD-BRAIN BARRIER (BBB) AND HIV NEUROINVASION.
Milan Finda, Chandrachur Gulati, Tatiana Pashkareva, Michael Bokrinsky, and David Looney.

Our objective has been to determine the effects of HIV-1 on BBB and HIV-1 neuroinvasion.

Methods: We have investigated HIV reverse transcription in BMVEC using PCR (using primers for early and late reverse transcription products), immunofluorescence, RNA copy (AmpliPrep) and p24 antigen assays. To investigate HIV-1 neuroinvasion, we constructed an in vitro BBB model with BMVEC and astrocytes. Chemokine receptor expression was determined by immunostaining, flow cytometry and RT PCR.

Results: BMVEC express CCR1 to CCR5, CXCR1, CXCR3 and CXCR4. In BMVEC cultures exposed to M-tropic, T-tropic or bi-tropic strains, we detected strong-stop HIV DNA (at a decreasing level for up to 4 days after infection) but not further progression of reverse transcription or virus replication, as shown by the absence of p24 gag and viral RNA, and p24 antigen. IL-6 pretreatment inhibited the strong-stop DNA synthesis. HIV-1 gag exposure of BMVEC induced IL-6 in a prolonged fashion. In the BBB model 1 h post-exposure, HIV-1 gag, penetration was inhibited by 99.9% (as measured by viral RNA), however, 24 to 48 h post-exposure virus penetration increased >60 fold, RANTES strongly inhibited viral penetration. Cocaine enhanced HIV-1 gag penetration 8.4 times.

Conclusions: Our results suggest that HIV-1 enters BMVEC and initiates reverse transcription, which does not proceed beyond the strong-stop DNA, but does not replicate. HIV also induces alterations in cytokine secretion and barrier function that may lead in vivo to the breakdown of the BBB. HIV-1 gag may penetrate BMVEC by transcytosis or percellular transport initiated by virus binding to CCR5 or CCR3. (Supported by NIH grants DA 10442, NS 56256, HL 48945).

P18

HIV PROTEIN TAT ACTIVATES APOPTOTIC CASCADES IN HIPPOCAMPAL NEURONS: PROTECTION BY ADNF-9 AND NF-AB ACTIVATION.

Progressive neuronal degeneration in brain regions involved in learning and memory processes is a common occurrence in patients infected with HIV-1. We now report that levels of Par-4, a protein recently linked to neuronal apoptosis in Alzheimer's disease (Nat. Med. 4:957-962 (1998)), are increased in neurons in hippocampus of human patients with HIV encephalitis, and in monkeys infected with a chimeric strain of HIV-1 and simian immunodeficiency virus. Par-4 levels increased rapidly in cultured hippocampal neurons following exposure to the neurotoxic HIV-1 protein Tat, and treatment of the cultures with a Par-4 antisense oligonucleotide protects the neurons against Tat-induced apoptosis. Par-4 participates at an early stage of Tat-induced neuronal apoptosis prior to caspase activation, oxidative stress and mitochondrial dysfunction. Gel-shift analyses suggest that Tat suppresses activation of the anti-apoptotic transcription factor NF-AB, and NF-AB decay DNA treatment enhances Tat-induced apoptosis suggesting an anti-apoptotic role for NF-AB. Pretreatment of neurons with activity-dependent neurotrophic factor peptide (ADNF-9) or tumor necrosis factor-a activates NF-AB and suppressor Tat-induced apoptosis. Our data suggest that Par-4 may be a mediator of neuronal apoptosis in HIV encephalitis, and that agents that suppress the Par-4 pathway or activate NF-AB (e.g., ADNF) in neurons may prove beneficial in preventing neuronal degeneration and associated dementia in patients infected with HIV-1. (Supported by the NIH).

P19

NEUROIMAGING IN HIV INFECTION.
Colin Hall, Murali Doraiswamy, Cecil Charles, Ranga Krishnan, Catherine Kapoor, Wendy Robertson, and Kevin Robertson.

Objective: Brain MRs and morphometric analysis of brain MRI could be potential early and sensitive indicators of nervous system involvement and progression in HIV infection.

Methods: Between-subject morphometric comparisons were conducted on 64 subjects. Volumes were adjusted by baseline total brain volume or baseline volume of the cerebral areas where appropriate. Reference normalized MRs variables of N-Acetyl Aspartate (NAA), NIT (NS), creatine (CR), and choline (Cho) were assessed in frontal, basal ganglia and hippocampal regions, then averaged into totals.

Results: Decreased white matter volume was found with advancing AIDS Dementia stage (r=33, p<0.05). A trend was found between increased plasma (r=33, p<0.09) and CSF (r=35, p=0.08) viral load (HIV RNA) and decreased white matter volume. Significant correlation was found between both decreased left hemispheric (r=-44, p<0.001) and right hemispheric (r=44, p<0.001) volume and increasing neurological dysfunction. Decreased parietal (r=-33, p<0.01) and parietal (r=-29, p<0.05) volumes were also correlated with increasing global neurological dysfunction. Increasing systemic disease stage was correlated with decreasing NAA (r=-38, p<0.01), CR (r=-31, p<0.05) and Cho (r=-29, p<0.05) and the ratio NAA/Cho (r=29, p<0.05). Trends were found between increasing CSF HIV RNA viral load and decreasing NAA, CR, and Cho levels (p<0.10). Presence of possible or early AIDS Dementia was significantly correlated with the MRs variables of NAA (r=32, p<0.01), Cho (r=39, p<0.01) and a trend for CR (r=24, p<0.10). Decreases in NAA (r=-34, p<0.005), CR (r=-36, p<0.05) and Cho (r=-36, p<0.05) were significantly correlated with increasing neurological dysfunction.

Conclusions: We have found correlations between HIV related neurological dysfunction and the MR based morphometric and spectroscopy variables. These preliminary data suggest trends of interest that will be followed longitudinally. Supported by NIMH R01- MH54566 03 & NINDS R01-NS 34240.

P20

CEREBRAL BLOOD FLOW VELOCITY DEFICITS IN HIV+ COCAINE ABUSERS: ANTIVIRAL MEDICATIONS REDUCE THE DEFICITS.
Ronald L. Herling, Kimberly Tate, Warren Better, and Jean Cadet.

Objective: The study examines the influence of HIV-seropositivity and antiviral medications on cerebral blood flow velocity in cocaine abusers.

Methods: Forty-eight HIV+ cocaine abusers (CD4: mean 386, SD 195), 36 control HIV-+ subjects and 7 HIV-+ control subjects (CD4: mean 372, SD 172) were studied. Blood flow velocity was determined for the anterior and middle cerebral arteries using transcranial Doppler sonography. Psychological assessments included the psychiatric distress (SCL-90R), hopelessness (Beck) and wellness (Ellisian) questionnaires.

Results: HIV+ cocaine abusers and unmedicated HIV+ cocaine abusers (n=13/22) had elevated pulsatility values, indicating increased resistance in the cerebral blood vessels, in comparison to control subjects, medicated HIV+ control subjects. HIV+ cocaine abusers (n=9/22) and HIV+ control subjects using antiviral medications had pulsatility values similar to HIV-+ control subjects. Medicated HIV+ cocaine abusers had higher SCL-90R scores than the other groups.

Conclusions: Although preliminary, our findings suggest that unmedicated HIV+ cocaine abusers have cerebrovascular deficits which are similar to HIV+ cocaine abusers. In addition, antiviral medications appear to reduce these perfusion deficits in HIV+ cocaine abusers. The increased intensity of psychiatric distress in HIV+ cocaine abusers does not appear to be related to the perfusion deficits. Further research is needed to clarify these issues.
P21

BIDIRECTIONAL INTERACTIONS BETWEEN HIV-1 AND SUBSTANCE P IN HUMAN IMMUNE CELLS.
Wen-Zhe Ho, Jian-Ping Lai, David R. Gettins, Dwight L. Evans, and Steven D. Douglas.


Methods: We used human immune cells (monocytes/macrophages, microglia, and lymphocytes). Using RT-PCR assay with specific primers, HIV gag gene and SP were amplified in cells infected with HIV (Bal or IIIB strains) or treated with SP.

Results: Similar to monocytes and macrophages, human brain microglial express SP mRNA. HIV infection enhanced expression of SP mRNA and protein. Levels of SP protein induced by HIV infection were positively related to the virus replication curve in macrophages and lymphocytes. Activation of HIV up-regulated SP expression in chronically infected promonocytic cells and T cells. An increase in HIV gag gene expression was observed when monocytes/macrophages were treated with SP. An SP receptor antagonist (SP600126) inhibited HIV gene expression in monocytes/macrophages.

Conclusions: HIV infection up-regulates expression of SP mRNA and protein by human immune cells. Reciprocally, SP enhances HIV gag gene expression in these cells. These data indicate that the functional interactions of HIV-1 and SP are likely to have in vivo relevance to psychiatric and neurologic complications of HIV-1 infection and AIDS. (NSH-MH 49981).

P22

RAMIFIED MICROGLIA AND LENTIVIRUS PERSISTENCE.
David L. Huo and James J. Vornov.

Objective: Clearance of cellular reservoirs that harbor HIV during prolonged HAART treatment is dependent on the turnover rate of reservoir target cell populations. Ramified, quiescent microglia in the CNS represent one of the most stable HIV target cells in the body, having an extremely slow turn-over rate. Whether or not replication-competent HIV provirus persists in the brain in ramified quiescent microglia and the effects of infection on ramified microglia function has important implications for HIV neuropathogenesis and persistence.

Methods: We created model culture systems in which to study factors affecting ramification of microglia and its relationship to lentivirus replication using primary neuronal-glial cultures.

Neurons and astrocytes in neuronal-glial cultures provided important signals that enhanced microglial ramification. Viraemia virus replicated productively in nonramified microglia, but was severely restricted under conditions that favored ramification. Neuronal-glial cultures became infected and harbored virus, but had markedly restricted virus replication. The infected cultures became highly productive for viraemia virus replication only following neuronal injury or treatment with thrombin, a glial mitogen. Finally, exogenous addition of infected microglia to neuronal-glial cultures resulted in down regulation of virus replication as the microglia incorporated into the culture. These results suggested that healthy neurons produce signals that promote the maintenance of an "anti-activation" environment favoring microglial ramification and persistent, restricted viraemia virus infection. Since nonramified microglia in the brain turn over at a much higher rate than ramified microglia, a better understanding of the regulation of the transition between ramified and nonramified microglia and their relationship to lentivirus infection are of importance in developing a mechanistic understanding of the brain as a site for HIV-1 persistence. (Supported by NS53693).

P23

NEGATIVE FEEDBACK BETWEEN PROSTAGLANDIN AND ALPHA- AND BETA-CHEMOKINE SYNTHESIS IN HUMAN MICROGLIAL CELLS AND ASTROCYTES.
Nazarl Janabi, Isabelle Hau, and Marc Tartour.

The understanding of immune surveillance and inflammation regulation in cerebral tissue is essential in the therapy of neuroimmunological disorders.

Objective: The role of chemokines and prostaglandin in several biological effects induced by proinflammatory cytokines prompted us to study a possible interaction between pathways of synthesis of these mediators.

Methods: The production of chemokines and Progs by either primary cultures of CNS cells or purified cultures of human microglial cells and astrocytes was tested by enzyme immunoassay after TNF-alpha, IL-1beta and IFN-gamma stimulation, alone or in combination (200 U/ml for each).

Results: We demonstrate that primary human glial cells are able to produce IFN-gamma, TNF-alpha and beta-chemokines (IL-1beta, RANTES, MCP-1a, MCP-1b) in parallel to Progs (PGE2 and PGF2alpha) after proinflammatory cytokine stimulation (i.e., TNF-alpha + IFN-gamma) induced all except RANTES which was induced by TNF-alpha + IFN-gamma. Purified cultures of astrocytes and microglial cells were also induced by the same combination of cytokines, to produce all these mediators except MCP-1a and MCP-1b, which were produced predominantly by astrocytes. The inhibition of PG production by indomethacin led to a 37-60% increase in RANTES, MCP-1a and MCP-1b, but not in GROa and IL-8 secretion. In contrast, inhibition of IL-8 and GROa activities using neutralizing Abs resulted in a specific 6-fold increase in PGE2 but not in PGF2alpha production by stimulated microglial cells and astrocytes, whereas Abs to beta-chemokines had no effect.

Conclusions: The production of Progs in human glial cells down regulates their beta-chemokine secretion, whereas beta-chemokine production in these cells controls PG secretion level. These data suggest that under inflammatory conditions, the intraparenchymal production of Progs could control chemotactic gradient of beta-chemokines for an appropriate effector cell recruitment or activation. Conversely, the elevated intracerebral beta-chemokine levels could reduce PG secretion, preventing the exacerbation of inflammation and neurotoxicity.

* This work was supported by Institut National de la Sante et de la Recherche Medicale CRI 96-12, by grants from the Agence National de Recherche sur le SIDA, Sidaction and Universite Paris XI.

P24

INTERACTION OF THE HIV-1 TAT PROTEIN WITH CELLULAR PROTEIN, PReA: ACTIVATION OF JC VIRAL DNA REPLICATION AND INHIBITION BY A SYNTHETIC PEPTIDE BASED ON A PReA-TAT-BINDING DOMAIN.

Objective: Certain effects of the Tat protein of HIV-1 in the CNS are mediated through its interaction with ubiquitous cellular protein, PReA. Tat and Purus cooperatively stimulate transcription of the HIV-1 genome in a Tat-dependent manner. In addition, Tat and Purus activate transcription of the late promoter of JC virus (JCV), which causes a neurodegenerative disease in brains of individuals with AIDS. PReA binds to sequence elements near the JCV origin of replication. We sought to test mechanisms by which HIV-1 might directly influence JCV replication.

Methods: Replication initiated at the JCV origin was determined by Dpmt resistance of full length plasmid DNA from either transfected U21MG glial cells or an in vitro system employing Hela cell extracts supplemented with JCV T-antigen.

Results: Tat enhances DNA replication initiated at the JCV origin in human glial cells. In an in vitro replication system maximal activation by Tat is achieved in the presence of PReA. The origin of the Mad-l strain of JCV, frequently detected in brains of affected people, is highly susceptible to DNA labeling and to activation of replication by Tat. In contrast, an archetypal strain of JCV, found in kidneys of both affected and non-affected people, is much less susceptible to activation. Since Tat freely diffuses across cell membranes, these results provide a mechanism by which HIV-1 can directly influence JCV infection. Analyses of mutant Tat proteins reveal that Cys22, in one of two Tat domains implicated in binding to PReA, is critical for activation of replication. Analysis of Purus mutants implicates two acidic leucine-rich rich repeats which are involved in binding to Tat. We have tested a synthetic peptide, based on a Purus T-antigen domain, that is an effective inhibitor of the Tat-Purus interaction.

Conclusions: Tat is capable of directly activating DNA replication initiated at the JCV origin in the presence of T-antigen. This activation is due, at least in part, to interaction with the cellular protein, PReA. Inhibition of this interaction could have therapeutic effects on both HIV-1 and JCV infections. Supported by R01 NS35009.
**P25**

**EXTRACELLULAR VPR MODULATES THE EXPRESSION OF ENDOTHELIAL CELL ADHESION MOLECULES (VCAM-1, ICAM-1) AND CHEMOKINES/MCP-1**

Seymouhous Joseph Ph.D., Kamel Klaftil, Ph.D., Alganamnew Srivivasan, Ph.D., and Ronald Cellman, M.D.

**Objective**: To study the impact of extracellular Vpr on the expression of endothelial cell adhesion molecules and chemokines that are involved in regulating HIV infected monocyte trafficking through the blood-brain barrier.

**Methods**: Human endothelial cells derived from brain, umbilical vein and skin were treated with varying doses of the fusion protein GSTVpr or GST control proteins. Subsequently, endothelial cell adhesion molecule expression and chemokine levels were monitored by flow cytometry and ELISA respectively. Transcriptional activation of adhesion molecule gene expression was studied by co-transfection of endothelial cells with VprDNA and ICAM-1 or VCAM-1 reporter plasmids. Functional studies examined the impact of GSTVpr on monocyte (THP-1) interactions with endothelial cells using adhesion assays.

**Results**: A dose dependent increase (maximum of 3.7 fold) in endothelial ICAM-1 and VCAM-1 expression was observed by flow cytometry following GSTVpr treatment. A three fold transcriptional activation of ICAM-1 and VCAM-1 promoter was induced by cotransfection with VprDNA. Monocyte adhesion to GSTVpr treated endothelial cells was increased greater than two fold relative to control. Significant levels of the chemokine MCP-1 was also released following treatment of brain or umbilical vein endothelial cells with GSTVpr.

**Conclusions**: Extracellular Vpr modulates the expression of endothelial cell adhesion molecules and chemokines which may play a role in regulating HIV infected monocyte transendothelial migration through endothelial cell barriers.

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**P26**

**INVOLVEMENT OF ATF/CREB FACTORS IN MONOCYTIC REGULATION OF THE HIV-1 LONG TERMINAL REPEAT**

Fred C. Krebs, John McAllister, Heather Ross, and Brian Wigdahl.

**Objective**: Regulation of HIV-1 expression in infected macrophage/monocyte and microglial cells is an important aspect of HIV-1-associated CNS neurologic disease. Our objective was to better understand the involvement of ATF/CREB factors in LTR-directed regulation of HIV-1 expression in cells of human monocyte origin.

**Methods**: Functional and biochemical analyses were conducted using the U-937 and THP-1 monocytic cell lines. LTR activity was assessed by luciferase expression during transient transfection under basal, activated, and Tat-transactivated conditions. Reporter constructs included the wild type LALLTR or the LALLTR incorporating selected mutations in the ATF/CREB binding site located between the LEE-1 and distal NF-kB binding sites. ATF/CREB factor binding to the clade B consensus site as well as several naturally occurring and artificially mutated binding sites was investigated using electrophoresic mobility shift (EMS) analyses.

**Results**: EMS analyses indicated that naturally occurring and artificially placed base pair changes in the ATF/CREB site increase or decrease factor binding. Within the context of the LALLTR, nucleotide changes that affect ATF/CREB recruitment also impact LTR function in transient expression analyses conducted in both cell types.

**Conclusions**: Our findings demonstrate the involvement of ATF/CREB transcription factor family members in regulation of HIV-1 in monocytic cells.

This research was supported by Public Health Service grants NS 32092 and NS 27405.

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**P27**

**ALTERATIONS IN BRAIN RECEPTORS AS A RESULT OF GP120 OR BMDM**

Anita H. Lewis, Herman Navarro, William P. Ross, and Mary Ellen Brust.

The tragedy of HIV-1 is frequently compounded by the associated cognitive/motor dysfunction termed HIV-associated Dementia (HAD). The HIV-1 envelope glycoprotein gp120 has been implicated in producing brain injury responsible for HAD.

**Objective**: Since gp120 leads to activation of NMDA receptors in retinal ganglion cells (Lipton, 1992), inhibits ligand binding at NMDA receptors in rat forebrain (Sweatt et al., 1993), and reduces dopamine transport in rat midbrain dopamine cultures (Bennett et al., 1995), we undertook to a) determine the effect of chronic gp120 on the NMDA and dopamine transporter receptors; b) to confirm the relationship between effects of gp120 on brain receptors and cognitive deficits; and c) to compare the effects on brain receptors, and on cognitive deficits, elicited by HIV and BMDM. Because HIV infection in the drug-addicted population continues to increase it was also of interest to evaluate the effects of CNS-active agents on HIV infection.

**Methods**: Male rats subjected to gp120 (i.c.v.) for 5 days were trained and tested in active avoidance, and then decapitated. Female C57BL/6 mice were allowed a choice between oral morphine and water; at 12 and 15 weeks post-infection with LPS-BMS MVM (MAIDS), morphine drinking and control mice were decapitated. Well washed, whole brain homogenates were used to evaluate radioligand binding at NMDA-regulated calcium ion channels, at NMDA-associated styrchino-insensitive glycine recognition sites, and at dopamine transporters.

**Results**: Binding at NMDA-regulated calcium ion channels and at dopamine transporters is reduced in both gp120 treated and MAIDS brains. No effects were observed in the brains of MAIDS mice orally self-administering morphine. Chronic gp120 treatment resulted in impaired response acquisition in active avoidance training.

**Conclusions**: The cognitive deficits associated with HAD may be related to decreases in NMDA and dopamine transported receptor densities; these effects may be modulated by CNS-active agents. The MAIDS model is useful in the determination of CNS effects associated with HAD.


**Acknowledgement**: Supported in part by Grant No. 5 R01 DA0 9038 from the National Institute on Drug Abuse.

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**P28**

**IMPACT OF SP FACTOR LEVELS, MONOCYTIC DIFFERENTIATION AND LTR G/C BOX ARRAY SEQUENCE VARIATION ON HIV-1 GENE EXPRESSION**

John M. McAllister, Scott Millhouse, Jean Conner, Heather Ross, and Brian Wigdahl.

**Objective**: Infection of monocytic cells by C5 (M-tropic) HIV-1 strains and migration of the infected cells into the brain is likely to play an important role in HIV-1-associated CNS disease. As a component of studies to examine HIV-1 replication in cells of the monocyte/macrophage lineage, we have tested the ability of the G/C box elements within the LTR to drive viral gene expression within a series of cell lines selected to approximate a number of stages of monocytic differentiation.

**Methods**: We utilized electromobility shift analyses, in conjunction with transient transfection assays to examine Sp factor affinity and LTR function, respectively.

**Results**: We have shown that the levels of Sp family activators (Sp1 and Sp3) increase relative to a Sp family repressor (truncated Sp3) as one moves toward a more differentiated state. Moreover, the absolute levels of Sp1 increases as the monocytoid differentiates. We have also shown a correlation between the absolute levels of Sp factors and relative Sp activator to repressor ratio and the ability of HIV-1LTR Luciferase constructs containing naturally occurring Sp site III alterations to drive basal and Tat-mediated gene expression across the spectrum of monocytic cell lines examined.

**Conclusions**: We propose that Sp factors and sequence variation in their binding array play critical roles in regulating HIV-1 replication during monocytic differentiation.

This research was supported by Public Health Service grant NS 27405 and NS 32092.
P30

CHEMOKINE RECEPTORS ON HIPPOCAMPAL NEURONS ARE COUPLED TO MULTIPLE INTRACELLULAR PATHWAYS AND REGULATE gp120 NEUROTOXICITY.

Olimpia Meucci, Alessandro Fatatis, Arthur A. Simon, Trevor J. Bushell, Patrick W. Gray* and Richard J. Miller,

Objective: The HIV-1 envelope protein gp120 induces apoptosis in rat hippocampal neurons. As chemokine receptors act as cellular receptors for HIV-1 and related viruses we examined hippocampal neurons for the presence of functional chemokine receptors and studied the intracellular pathways activated by chemokines and gp120.

Methods: Ca imaging, electrophysiology, biochemical and molecular biological paradigms were used to study short term and long term effects of chemokines/gp120 on rat hippocampal neurons.

Results: We found that MDC, RANTES, MIP-1α, TARC, I-309, IL-8, SDF-1α, and soluble fractalkine were able to induce [Ca2+]i transients in neurons. Ca transients were also induced by the HIV-1a gp120 and the SIIVm gp120. When neurons exhibited spontaneous Ca fluctuations chemokines reduced the amplitude and frequency of the Ca oscillations. Chemokines were also able to block the frequency of spontaneous glutamatergic excitatory postsynaptic currents recorded from these neurons. RT-PCR showed the expression of CCR1, CCR4, CCR5, CCR9/10, CXCR2, CXCR4 and CX3CR1, as well as the chemokine fractalkine in these neurons. Chemokines also activated other signaling pathways in hippocampal neurons. Both fractalkine and MDC produced activation of ERK1/2, whereas no activation of JNK/SAPK or p38 was evident. These two chemokines, as well as SDF-1α, also activated the Ca and cAMP dependent transcription factor CREB. We previously observed that upon removal of their glial feeder layer, cultured hippocampal neurons die by apoptosis over the next few days. Addition of MDC, fractalkine, RANTES, or SDF-1α to the culture medium slowed the rate of death considerably. The same chemokines were also able to block gp120 induced apoptosis of hippocampal neurons, both in the presence and in the absence of the glial feeder layer. Fractalkine also activated the Akt kinase, an enzyme directly involved in the activation of anti-apoptotic pathways in neurons and other cells types.

Conclusions: Our data show that hippocampal neurons possess a wide variety of chemokine receptors and suggests a role for AKT/P38 kinase pathway in the protection of neurons from gp120-induced apoptosis. (Supported by NIH Grants DA 01221, DA 02575, MH 40165, NS 33826, DK 42086, DK 44840).

P32

ASSOCIATION OF HUMAN HERPESVIRUS 6 WITH THE DEMYELINATING LESIONS OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY.

David J. Mock, James M. Powers, Andrew D. Goodman, Jeffrey V. Baker, Leon G. Epstein, and Benjamin M. Blumberg.

Objective: PML brain tissues were examined for the presence of HHV6 DNA and proteins in relationship to both the presence of JCV and the demyelinating lesions.

Methods: A two-step in-situ polymerase chain reaction procedure was developed to amplify and detect latent and active HHV6 genomes in archival brain tissues. Immunohistochemistry for HHV6 pp14 and p101 antigens and JC virus large T antigen were performed.

Results: A high frequency of cells containing human herpesvirus-6 (HHV6) DNA in PML white matter were found both within and surrounding the demyelinating lesions of PML. HHV6 genome was found mainly within oligodendrocytes. Lower amounts of HHV6 genome were detected in most normal, AIDS and other neurological disease control tissues. Immunohistochemistry for HHV6 antigens showed actively infected nuclei of oligodendrocytic morphology only within the demyelinating lesions of PML, but not in adjacent uninvolved tissue in 10 of 12 sections, examined from 11 brains. Four of these brains were from patients without HIV-1 infection. In addition, no HHV6 antigens were detectable in control tissues including brains of individuals with HIV-1 encephalopathy but without PML. Double immunohistochemical staining for JC virus, large T antigen and HHV6 antigens demonstrated co-labeling of many swollen intraleisional oligodendrocytes in the PML cases. Preliminary results also showed that less histologically involved areas had both higher frequencies of HHV6 DNA containing preserved oligodendrocytes and lesser amounts of HHV6 proteins than severely demylinitated areas.

Conclusions: HHV6 activation in conjunction with JCV infection is associated with the demyelinating lesions of PML independent of the presence or absence of HIV-1. A strong correlation was seen between lesional areas expressing relatively large amounts of HHV6 proteins and oligodendrocytic cell death.
P33

ROLE OF DIFFERENT NF-1 CLASSES IN THE PATHOGENESIS OF JC VIRUS, AN OPPORTUNISTIC INFECTION OF AIDS.
Maria Chiara G. Monaco, Stephan A. Frye, Linda C. Derham, and Eugene O. Major.

The human polyomavirus JCV is the etiologic agent of Progressive Multifocal Leukoencephalopathy (PML), a denervating disease of the central nervous system occurring almost exclusively in immunodeficient individuals. PML occurs in 5% of all AIDS patients. Previously, we demonstrated that human hematopoietic precursor cells (CD34+) are infectible by JCV. Moreover, we have shown that when hematopoietic precursor cells differentiate into cells with macrophage-like characteristics, they are no longer susceptible to JCV infection in vitro.

Objectives: In this study we explored the molecular mechanisms that are responsible for JCV replication within susceptible cells, comparing them with those of non-susceptible cells. We investigated the role of NF-1 during cellular differentiation that may convey loss of susceptibility to JCV infection in hematopoietic precursor cells when differentiated into macrophages.

Methods: We isolated cytoplasmic RNA from control primary astrocytes as well as human hematopoietic precursor cell, treated and non-treated with PMA. The RNA was used for both RT-PCR reactions, with class specific primers, and Northern hybridization analyses. We also extracted nuclear protein from all the cell types and used them in gel shift and super shift experiments.

Results: Our preliminary results show that cells treated with PMA, and no longer susceptible to JCV infection, had expression of mRNA for NF-1 class D down regulated as opposed to the non-treated parental cells. Supershift experiments, using antibodies specific for class C and class D, demonstrate supershifting of the NF-1-specific band in the presence of class C, but not by class D antibodies.

Conclusions: These data support the possibility that NF-1 class D can play a crucial role in the regulation of JCV in susceptible cell types. These studies are necessary to better clarify the pathogenesis of PML.

P34

LENTIVIRAL VECTOR-BASED GENE THERAPY OF NEUROVIRAL DISORDERS.
Muhammad Mubhair, Mohamad Bou Haddad, and Roger J. Pomerantz.

Several studies demonstrate human immunodeficiency virus type 1 (HIV-1) infection of central nervous system-based cells resulting in a series of devastating clinical conditions collectively termed as acquired immune deficiency syndrome (AIDS) demyelinating (ADD). Gene therapy of these neuroviral disorders necessitates utilization of vector system that can mediate in vivo delivery and long-term expression of transgene in non-dividing/post-mitotic neuronal elements. Our studies are focused on transfer of various anti-HIV-1 genes in primary isolated CNS-based cells and their effects in protecting these cells from viral infection. Utilizing a previously characterized HIV-1-based vector system, we have been able to efficiently transduce and maintain expression of a marker transgene, LacZ, in CNS microvascular endothelial cells, human fetal astrocytes, plus immature and mature(differentiated) NT2 cells. Currently, we are utilizing a transdominant negative form of Rev, i.e. Rev M10, in the HIV-1 based vector system and evaluating its potential in controlling retroviral infection of CNS-based cells. Initial studies demonstrate expression of RevM10 in all CNS-based cell types. Further studies are in progress to characterize the anti-HIV-1 potency of RevM10 in relevant primary isolated human CNS-based cells. Research supported in part by NIH grant AG09455.

P35

SYNERGISTIC INCREASES IN NEURONAL CELL DEATH AND LEVELS OF INTRACELLULAR CALCIUM BY THE HIV-1 PROTEINS TAT AND gp120 - NEUROPROTECTION BY MEMANTINE.
Avindra Nath, Norman Haughey, Carol Anderson, and Jonathan D. Geiger.

Objective: HIV-1 Tat protein can be detected in brain of patients with HIV encephalitis and serum of patients with AIDS. Tat has been shown to cause neuronal excitation, neuron cell death, and increases in levels of intracellular calcium as well as increased induction of cytokines and chemokines in glial cells. In this study, we determine if HIV-1 proteins, Tat and gp120, exacerbate neuronal responses to each protein alone and if the neurotoxic effects can be blocked using a panel of neuromodulators.

Methods: Cultures of human fetal neurons were simultaneously exposed to Tat and gp120 and various concentrations for variable durations of time and neuronal cell death was monitored 18 hours post exposure. In select experiments intracellular calcium was also monitored. In experiments using neuromodulators, the cultures were exposed to the drugs for the duration of the experiment.

Results: Subtoxic concentrations of Tat and gp120 when incubated together caused neuronal cell death and prolonged increases in levels of intracellular calcium - 10-times lower concentrations were required when compared with responses to the individually applied proteins. An exposure of only a few seconds was sufficient to cause neuronal cell death, but maximal levels of cell death was observed with application lasting 30 min. Memantine (2 µM) blocked completely the neurotoxicity while no significant effects of dipyridamole, flunarizine or vigitridazine were noted.

Conclusion: The neurotoxic effects of Tat and gp120 are synergistic, require only a transient exposure to neurons and can be blocked by memantine.

P36

OLIGOCNGLAL T CELLS ARE INFILTRATING THE BRAIN OF CHILDREN WITH AIDS: SEQUENCE ANALYSIS REVEALED HIGH PROPORTIONS OF IDENTICAL 8-CHAIN T-CELL RECEPTOR (TCR) TRANSCRIPTS.
Wan L. Lin, John Fincke, Christopher Schluter, Leroy Shaver, Chris D. Plattsoucas, and Emilia L. Oleszak.

Objective: We have recently described the presence of angio centric CD3+CD45RO+ T cells infiltrating the brain of children with AIDS (Clin Diagn Lab Immunol, 6: 105-114, 1999). To determine whether these infiltrates contain oligoclonal populations of T cells.

Methods: We amplified, by the non-palindromic adaptor-PCR method 8-chain TCR transcripts from autopsy brains of two pediatric patients with AIDS. The amplified transcripts were cloned and sequenced.

Results: Sequence analysis of 8-chain TCR transcripts from one patient revealed multiple identical copies of three TCR 8-chain transcripts. The clonal expansions were confirmed using an independent amplification method (V8-specific PCR). Sequence analysis of 8-chain transcripts from brain tissue of a second pediatric patient with AIDS, also revealed multiple identical copies of 8-chain transcripts. These sequences are novel. All 8-chain TCR transcripts from normal peripheral blood mononuclear cells were unique, as expected for polyclonal T cells.

Conclusions: The presence of oligoclonal populations of T cells in the brain of these two patients suggests that these T cells have undergone antigen-driven proliferation and clonal expansion. Although the specificity of the clonally expanded 8-chain TCR transcripts remains to be elucidated, none of them were identical to those specific for HIV-1 antigens that are currently reported in the GENBANK/EMBL databases. T cells using these transcripts may play a role in endothelial cell injury and the opening of the blood-brain-barrier of HIV-1-infected children.

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**P37**

**HIV-1 VPR INDUCED APOPTOSIS AND CYTOTOXICITY IN NT2 NEURONAL CELLS.**

Charvi A. Patel, Mohammad Mahfuz, and Roger J. Pomerantz.

HIV-1 infection in the CNS causes AIDS dementia complex (ADC) in some patients. Recent studies have suggested that patients with ADC have an increased incidence of neuronal apoptosis leading to a loss of neurons. Although, the underlying mechanisms of HIV-1 induced dysfunctions of the CNS remains elusive, the HIV-1 regulatory protein, Vpr, has been implicated in the induction of CNS-based apoptosis in HIV-1 infections. Stewart and coworkers have reported that Vpr induces apoptosis in human fibroblasts, T cells and peripheral blood lymphocytes following cell cycle G2 arrest. Furthermore, Levy and coworkers detected a higher level of Vpr accessory protein in the CSF of AIDS patients with neurological disorders. We designed these studies to elucidate the effect of Vpr protein in neuronal precursor NT2 cell line and retinoic acid differentiated neurons. In preliminary studies, recombinate Vpr was bacterially expressed as a GST fusion protein. GST Vpr fusion protein, GST protein as well as differentiation marker, retinoic acid, were extracellularly added to the growth media of NT2 cells and assayed for apoptosis at the end of 5 weeks. Preliminary data show that the treatment of NT2 cells with either GST Vpr 6ng/ml or retinoic acid 10mg/ml induced a unique differentiation phenotype. However, recombinate Vpr protein-induced differentiation led to apoptosis at a significantly greater rate when compared to retinoic acid-induced differentiation. Since, Vpr may be exerting selective cytotoxicity additional studies are required to clarify the pathways involved in Vpr-induced differentiation and apoptosis in the NT2 cell line. These studies using Vpr expressed as a free protein from the MEB expression and isolation system will be presented. Research supported in part by USPHS grant MH58526.

**P38**

**BLOOD-BRAIN BARRIER BREAKDOWN IN HIV-INFECTED PATIENTS CORRELATES WITH PERIVASCULAR AND INTRAMURAL T CELL INFILTRATES BUT NOT WITH HIV OR AIDS.**

Carol K. Pelto and Becky Adkins.

**Objective:** To determine the cause of the enhanced vascular permeability found in post-mortem brains of AIDS patients is not known. Although its widespread distribution and failure to correlate with HIV indicates possible systemic factors, our recent studies found that neither chronic systemic administration of pH120 nor of tumor necrosis factor altered the normal blood-brain barrier (BBB).

**Methods:** To determine the relationship between BBB breakdown and inflammation in AIDS (n=14) and asymptomatic infected (ASY) (n=7) brains and in AIDS with (n=12) and without (n=7) HIV. Immunohistochemistry identified T cells, macrophages and BBB leak (graded as 1-3+).

**Results:** BBB leak was similar in AIDS vs. ASY brains (2.2 ± 1.1 vs. 2.4 ± 0.7). HIV was not associated with the number of vascular T cells or with the number of T cell-positive blood vessels (BV) but was associated with increased parenchymal T cells (0.18 ± 0.35 vs. 0.05 ± 0.06) although the difference was not significant. BBB breakdown correlated with the number of vascular T cells/BV (5.0 ± 0.19 vs. 0.12 ± 0.1, p<0.05) and with the number of T cell-positive BV/high power field (0.13 ± 0.08 vs. 0.05 ± 0.04, p<0.05). Correlation between BBB leak and macrophage infiltrates was not observed.

**Conclusions:** These studies confirm prior reports that link brain inflammation with BBB leak. They suggest that T cell infiltration is a likely mechanism for the enhanced vascular permeability found in patients with AIDS.

This research was supported by a NIH grant to CKP (NINDS NS35331).

**P39**

**FRONTAL LOBE NEURONAL INJURY IS INDEPENDENT OF VIRAL LOAD IN HIV-INFECTED CATS.**

Michael Podell, Wayne R. Buck, Kazuyo Maniyama, Mark Smith, Kathleen Hayen, Debbie S. Ruchhilmann, and Lawrence E. Mathes.

**Objective:** The purpose of this study was to determine if neuronal injury detected by high spatial resolution proton magnetic resonance spectroscopy (MRS) in the frontal lobe of feline immunodeficiency virus, Maryland isolate, (FIV-MD) infected cats correlated to immune-status, peripheral and brain proviral or viral load, and neurologic function.

**Methods:** Proton MRS of the frontal lobe was compared in cats infected intravenously with FIV-MD at 8 wks of age (n=6) to age matched controls (n=6) at 8, 14 and 24 mo of age. Correlations to serial lymphocyte phenotype profiles, blood and lymph node proviral and viral load, quantitative electroencephalography (QEEG), and terminal brain viral load were analyzed.

**Results:** A significant reduction in both N-acetylaspartate (NAA) and the NAA/choline ratio was found in the FIV 14 mo old compared to the FIV 8 mo old cats and age-matched control cats only. In the FIV 14 mo old group, there was a significant negative correlation between NAA and CD4 lymphocyte counts (p=0.02) and a positive correlation to QEEG (p=0.004). No correlation was found between viral DNA and RNA copies in the blood and CD4 lymphocyte counts, frontal cortical NAA, or frontal cortical QEEG.

**Conclusions:** Peripheral viral load appears not to be related to early onset neuronal injury and functional change, further supporting our overall hypothesis that indirect mechanisms mediate neuronal injury during the early stages of this feline model of lentiviral encephalopathy.

**P40**

**NEUROAL LOSS IN HUMAN IMMUNODEFICIENCY VIRUS INFECTION. IS IT RELATED TO RISK GROUP?**


**Objective:** To clarify the relationship between substantial neuronal loss, seen in HIV/AIDS and risk group.

**Methods:** Frontal cortex tissue of individuals who died whilst asymptomatic infected with HIV or of AIDS were compared to uninfected control cases. Cases were available from London and Edinburgh; risk groups were denoted as men who have sex with men (MWSM) and injecting drug users (IDUs) respectively. Additionally IDUs cases from Paris, were used as a companion to the Edinburgh cases, these could not be directly compared due to fixation differences. Cell density estimates were used to look at differences in neuronal density between risk groups; bivariate analysis was used to look at the relationship between neurons and microglia/macrophages.

**Results:** A significant decrease was observed in neuronal density in the London AIDS cases (p = 0.011), this was not found in the Edinburgh AIDS cases. Thus, there were significant differences between the neuronal densities of the London and Edinburgh AIDS cases (p = 0.0051) but not the control cases. The neuronal density decrease in London was concurrent with an increase of microglia/macrophages in the area studied, these may contribute to neuronal loss. Neuronal density decrease was not found in the Paris AIDS cases.

**Conclusions:** This decrease in neuronal density decrease may be attributed to differences in neuropathology between risk groups.

Funded by an AIDS Education and Research Trust (AVERT) studentship.
P41
SEQUENCE VARIATION IN HIV-1LTR/CIHBP SITES PLAYS A CRITICAL ROLE IN VIRAL TRANSCRIPTION IN CELLS OF THE MONOCYTE/MACROPHAGE LINEAGE
Heather Ross, Michael Nonnemacher, and Brian Wigdahl.

Objective: To examine the importance of sequence variation in two cis-acting C/EBP elements within the HIV-1LTR, with respect to HIV-1 transcription within cells of the monocytic/macrophage lineage.

Methods: Electrophoretic mobility shift analyses were conducted to characterize monotypic proteins (from U-937 and THP-1 cell lines) which interact with naturally occurring C/EBP site sequence variants. The functional impact of these natural sequence variants was investigated using transient transfection analyses.

Results: Naturally occurring C/EBP site sequence variation leads to alterations in C/EBP binding affinity for its cognate binding site, which ultimately impacts LTR function. Furthermore, an adjacent ATG/CREB site can recruit C/EBP proteins to a commonly-encountered low affinity C/EBP site, which does not recruit C/EBP proteins alone, due to interactions with C/EBP binding protein (CBP). This ATG/CREB site also influences C/EBP-dependent transcription in transient expression analyses. Furthermore, sequence analysis indicates that two strong C/EBP sites are well conserved in brain-derived LTRs but not LTRs derived from blood.

Conclusions: Natural sequence variation at two LTR C/EBP sites impacts HIV-1 transcription in monocytic cells, which may be critical to infection of the brain and CNS.

This research was supported by Public Health Service grants NS 27405 and NS 32092.

P42
VIP AND PEPTIDE T RELEASE CHEMOKINES WHICH PREVENT HIV ENVELOPE PROTEIN-INDUCED NEURONAL DEATH

Objective: To determine if chemokines block gp120 neurotoxicity and if Peptide T, previously shown to block gp120 effects, causes release of anti-viral chemokines.

Methods: Rat hippocampal cultures were treated with gp120 and peptide T (Nature, Brenneman, 1988) and neuronal survival and chemokine release was determined.

Results: GP120-induced neuronal killing in rat hippocampal cultures was blocked by specific chemokines, as well as by peptide T, a gp120 receptor antagonist derived from a V2 binding epitope. The specificity of blockade for five gp120s tested (LAV/BRU, CMD43, RFR, SF-2, and MN) differed among the chemokines tested; RANTES had the broadest and most potent blockade (IC50 = 3pM for the RFI isolate). Only Peptide T completely blocked all five species of gp120 tested. Treatment with chemokines alone did not affect neuronal cell number, while antisera to chemokines reduced neuronal survival, suggesting that these naturally occurring brain substances exert a tonic effect on neuronal maintenance. The neurotropic effects of chemokines revealed a novel and important action for these molecules which occurred at concentrations a thousand-fold lower than those for chemotactic activity. Additional results showed that peptide T induced astroglial release of the β chemokines RANTES, MIPα, and MIPβ.

Conclusions: The anti-gp120 effects of peptide T in treating Neuro-AIDS pathogenesis (Heseltine, 1998) may be two-fold: CCRC3 receptor modulation, and release of anti-viral chemokines which provides broad protection against diverse brain viral isolates.

P43
CXCR4 EXPRESSION BY NEURAL CELLS IS MODULATED BY FIBROBLAST GROWTH FACTOR: IMPLICATIONS FOR NEURODEGENERATION IN HIV ENCEPHALITIS
Virginia J. Sanders, Ian P. Everall, and Elietze Maatlah.

Objective: Preliminary data suggest a correlation between neuronal fibroblast growth factor-I (FGF-I) levels and lack of neurodegeneration in brain tissue from patients with HIV. One potential mechanism of neurodegeneration seen in HIV is gp120- or SDF-1-induced CXCR4 signaling. The purpose of this study was to determine if, in vivo, FGF-I levels correlated with CXCR4 levels in brain tissue and, in vitro, if FGF modulated CXCR4 expression in neuronal and astroglial cells.

Methods: Frontal cortex and basal ganglia from HIV+ patients were immunostained for FGF1 and CXCR4. Labeling intensity (optical density) was quantitated. C6 astroglialoma and SH-SY5Y neuroblastoma cells were treated with FGF1 and FGF2, and CXCR4 protein levels were measured by Western blot analysis.

Results: In vivo, regression analysis revealed a negative correlation between neuronal FGF1 expression and neuronal CXCR4 expression. This was supported by in vitro neuronal CXCR4 level's decreasing in a dose dependent manner after exposure to FGF1. Conversely, glial CXCR4 was increased in a dose dependent manner after exposure to FGF. This increase was blocked by inhibiting the tyrosine kinase activity of the FGF receptor with 5'-methylthioadenosine (MTA).

Conclusions: One mechanism by which FGF may exert its many functions is modulation of CXCR4 levels; an increase in glial receptor expression would promote angiogenesis and a decrease in neuronal receptor expression may be neuroprotective. Since gp120 may promote apoptosis directly through neuronal CXCR4 binding or indirectly through glial or macrophage CXCR4 receptors, modulation of receptor expression may have potential therapeutic significance. Grant Support: MH 45294-10.

P44
SUPPRESSION OF HIV-1 TRANSCRIPTION AND REPLICATION BY A VPR MUTANT.

Ectopic expression of the HIV-1 accessory protein, Vpr, has been shown to modulate viral gene expression.

Objective: In this report, we have examined the efficacy of various mutants of Vpr in competing with the transactivating function of the wild-type protein. These mutants have scattered mutations in the predicted helical domain, the leucine-isoleucine-rich region, and the arginine conserved region of Vpr, and two such mutants, with substitutions at amino acid T73 (R/S and R/A) were shown to negatively regulate viral promoter activity.

Methods: To further examine these mutants in the context of their effect on viral replication, a chimeric virus harboring mutation R73/S was constructed.

Results: These results demonstrated that this mutation renders the protein incapable of transactivating the viral promoter, and that it acts as a competitive inhibitor of the wild-type protein. Since one of the approved functions of Vpr is its effect on cell cycle progression, the effect of its mutant derivative on G2 arrest was studied. The results indicated that the mutant fails to arrest the cell cycle in the presence of the wild-type protein.

Conclusions: The lack of cytotoxicity of the mutant and its capacity to oligomerize with the wild-type protein make it a suitable candidate for the suppression of viral replication. The utilize of such a mutant for designing therapeutic strategies to combat the wild-type Vpr will be discussed.
**Poster abstracts**

**P45**

CHEMOKINE RECEPTORS EXPRESSED ON HUMAN GLIAL CELLS AND THEIR ROLE IN HIV DEMENTIA.

Becky Schweighaeter and Walter I. Aitwood.

**Objective:** To investigate our hypothesis that chemokine receptors expressed on human glial cells play a fundamental role in HIV dementia by mediating both HIV-1 infection and HIV-1 induced bystander killing of glial cells.

**Methods:** Chemokine receptor expression on the human glial cell line, SVG, was analyzed by flow cytometry and RT-PCR. Susceptibility of SVG cells to HIV infection was determined by infecting the cells with HIV luciferase expressing reporter viruses pseudotyped with envs from different strains of HIV-1. Cytotoxicity was determined by propidium iodide staining followed by flow cytometric analysis.

**Results:** Our data demonstrate that SVG cells express low levels of the chemokine receptors CXCR-4 and CCR3, but do not express CCR5. We found that expression levels of CXCR-4 and CCR3 are significantly increased by DMSO treatment. Susceptibility to HIV-1 infection was not detected in untreated SVG cells or in SVG cells stably expressing CD4. However, once chemokine receptor expression was increased by DMSO treatment, significant levels of infection by a Tropic strain and a primary brain isolate of HIV-1 were detected in both SVG and SVG-CD4 cells. We also demonstrate that treatment of uninfected SVG cells with the combination of soluble gp120 (IIIb) and TNF alpha induces a high level of cytotoxicity, which is not observed in cell cultures treated with either reagent alone.

**Conclusions:** The human glial cell line, SVG, expresses low levels of CXCR-4 and CCR3. The paucity of chemokine receptors expressed on SVG cells is not sufficient to mediate infection by HIV-1 pseudotyped viruses. Increasing chemokine receptor expression on SVG cells increases susceptibility to infection by HIV-1. Uninfected SVG cells are susceptible to the synergistic toxic effect of soluble gp120 and TNF alpha.

This work was supported in part by a Grant-in-Aid of Research awarded to BS from Sigma Xi, the Scientific Research Society.

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**P46**

TAT-INDUCED DYSFUNCTION OF BRAIN ENDOTHELIAL CELLS.

Michal Toborek, Andrzej Malecki, Mark P. Mattoo, Bernhard Hentzig, Hans C. Bauer, and Avindra Nath.

**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, 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.

**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-κB (NF-κB) 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.

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**P47**

CHOROID PLEXUS HARBORS HIV DURING CLINICAL LATENCY DURING HIGHLY ACTIVE ANTI-RETROVIRAL THERAPY.


**Objective:** to evaluate the possibility that the choroid plexus (CPx) is a reservoir or a sanctuary for HIV during the period of clinical latency in asymptomatic HIV-infected patients (ASY) or in the setting of anti-retroviral therapy (ART).

**Methods:** We examined the immunoreactivity for HIV gp120 on deparaffinized sections of brain and CPxs and looked for HIV DNA by nested PCR in a subset of cases.

**Results:** Series One contained 14 AIDS cases and 7 HIV-infected asymptomatic cases (ASY); all died before 1994 and clinical information was otherwise available. 7 of 14 AIDS cases had HIV+ CPs; 3 of these had HIVE and 2 had HIV-positive cells in brain. 2 of the 7 ASY cases had HIV+ CPs but none had HIVE. 4 of 45 ASY cases had HIV sequences in the CPs but only 1 of 5 had sequences in brain. All 5 had FOX sequences in systemic organs and all specimens contained sequences for GAPDH. Series Two contained 17 AIDS patients who died in 1998; 3 had HIV+ CPs and HIVE; 7 had HIV+ CPs alone; 2 had HIVE alone and 5 had HIV+ CPs and brain. 3 of 4 with HIV+ CPs were on ART at the time of death. CD4 counts were < 200; plasma viral load was >750,000 in 2 and was undetectable.

**Conclusions:** These studies indicate that the CPxs may be a reservoir for HIV during the ASY period of HIV infection when productive brain infection is absent. They also raise the possibility that the CPxs is a sanctuary for HIV during anti-retroviral therapy since one patient on HAART had an undetectable plasma viral load but had HIV+ cells in the CPxs.

This study was supported by the NIH grant NS35331, CKP.

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**P48**

HUMAN BRAIN BALLS: A VISIBLE, STATIONARY 3-D FETAL BRAIN CULTURE MODEL TO INVESTIGATE HIV NEUROPATHOGENESIS.

Gusta Trillo-Pazos and Ian P. Everall.

**Objective:** Current models of HIV neuropathogenesis suggest that neuronal damage depends on complex 3-D cellular interactions between brain cells. The development of a stationary and visible 3-D culture model of human fetal brain was attempted.

**Methods:** Fetal human brain cell suspensions (0.5 – 5 x 10^6 cells/ml) were grown onto agarose coated 12 well plates to inhibit the attachment of cells to plastic. The effect of various culture media (human serum versus fetal calf serum, DMEM vs DMEM/F12) on cellular viability was tested as a measure of Alamar Blue conversion. Human brain balls were embedded in paraffin wax at 3 and 4 weeks in culture, sectioned at 7 μm and characterised by immunohistochemistry for cellular lineage specific markers.

**Results:** Human serum containing media significantly increased the viability of the human brain balls in suspension (p<0.05). Human brain balls have so far remained viable in culture for 78 days and reach a diameter of 1-2 mm from 2 weeks in culture. Human brain balls contain GFAP+ and Vimentin+ glial cells, with MAP2+ neuronal cells, and p91α-activated macrophages/microglia, as well as CNPase+ oligodendroglial cells.

**Conclusions:** Human brain balls are a stationary, visible 3-D in vitro culture model that can be used to investigate the complex cellular interactions that are thought to drive HIV neuropathogenesis in the human brain.

**Sponsors:** GTP is funded by Glaxo-Wellcome
**P49**

**IS THE NEUROTOXICITY OF NF-E2, TAT, AND gp120 HIV PROTEINS ON PRIMARY HUMAN NEURONES IN CULTURES A "HIT AND RUN" TOXICITY PHENOMENA?**

Gusta Trillo-Pazos, Geoffrey J. Pickfington, and Ian P. Everall.

**Objective:** Recently, a "hit and run" process of HIV protein toxicity has been proposed to underlie neuronal damage during HIV disease in the brain. In this study, primary human neuronal cultures were exposed to recombinant nef, tat and gp120 to test if toxicity of these proteins is a limited or progressive event in culture.

**Methods:** Mature, primary human neurons in culture were exposed to nef, tat and gp120 (100 ng/ml) for 3 days and 6 days. Neuronal viability was assessed at these times by MTT, LDH and Trypan blue assays. The toxicity of these proteins was compared by factorial ANOVA.

**Results:** Both a limited and a progressive form of neurotoxicity were observed. Gp120 and tat are toxic to human neurons after three day exposure (p<0.001), whereas nef mediated toxicity is not apparent until six days in culture.

**Conclusions:** Neuronal damage elicited by HIV proteins, at least in culture, may be mediated by a combination of "hit and run" and a progressive form of toxicity.

**Sponsorship:** GTP was funded by a scholarship from the John Ellerman Foundation, with additional funds for laboratory consumables from the Psychiatry Research Trust, the Elton John AIDS Foundation and the Wayne Foundation.

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**P50**

**EARLY EVENTS IN SIV NEUROPATHOGENESIS IN RHEUS MACAQUE NEONATES.**

Susan V. Westmoreland, Kenneth C. Williams, Colin Delbakker, Heather Knight, and Andrew A. Lackner.

**Objective:** To explore the pathogenesis of early events of SIV infection of the CNS in neonates as a model of the neuropathogenesis of pediatric AIDS.

**Methods:** Thirteen rhesus neonates were inoculated IV with molecularly cloned SIVmac239 within 24 hours of birth. Two animals were euthanized at 3, 7, 14, 21, and 50 days post-infection and an additional three animals were euthanized when terminally ill at 79, 141, and 209 days after infection. Three additional animals were infected with uncloned SIVmac251 and were euthanized or died at 21, 35, and 50 days post-inoculation, and two were infected with macrophage-tropic SIVmac239/316 and euthanized at 50 days post SIV. All animals received the same dose of virus (20ng p27/kg body weight).

**Results:** Histologic changes in the brain, although mild, resembled those seen in HIV-1-infected children including perivascular infiltrates of mononuclear cells, mineralization of vessels in the basal ganglia, and glions. The perivascular lesions and glions were associated with the presence of occasional infected cells that required in situ hybridization with radiolabeled riboprobes for detection. Using this technique, SIV-infected cells could be detected in the brain parenchyma by 7 days p.i. These findings were confirmed by nested PCR for SIV gag DNA. Together, SIV infection of the CNS was seen in 12/13 neonates infected with SIVmac239, 3/3 with SIVmac251 and 2/2 with SIVmac239/316.

**Conclusions:** The prevalence of CNS infection in rhesus neonates was indistinguishable from that of older animals infected with the same dose and stock of virus, but neonates appeared to have many fewer infected cells in the CNS and detecting them required more sensitive techniques. This observation was true regardless of inoculum and despite the fact that neonates had equal or greater viral loads in the periphery compared with older animals. This suggests that host factors, rather than viral factors, contributed to limiting SIV infection in the CNS of rhesus neonates.

This work was supported in part by NIH grant NS30769.

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**P51**

**BRAIN PERIVASCULAR MACROPHAGES AS THE TARGET FOR SIV INFECTION: THE BRAIN AS A RETROVIRAL RESERVOIR.**

Kenneth C. Williams, Susan V. Westmoreland, Doug Pauley, Heather Knight, and Andrew A. Lackner.

**Objective:** To investigate targets of SIV infection of the CNS in animals early after infection and in animals with SIV encephalitis.

**Methods:** Eight rhesus juveniles were inoculated with SIVmac251 and were sacrificed during peak viremia (2 weeks post infection) (n=2) or when they developed AIDS (n=5) and compared with non infected control CNS tissues (n=2). Animals were examined histologically for encephalitis and by immunohistochemistry using a panel of myeloid markers. Double label immunohistochemistry for myeloid cell markers and viral protein, immunohistochemistry followed by in situ hybridization for myeloid markers and viral RNA, and confocal microscopy studies were performed to define macrophage populations that are infected early and late in this model.

**Results:** Perivascular brain macrophages and the resident brain macrophage, microglia, are both CD11b and CD68 positive. The perivascular macrophages, but not microglia, are also positive for CD14, CD45, and CD86. Multi-nucleated giant cells (MNGC), when present, are CD11b and CD68 positive like parenchymal microglia and perivascular macrophages. The MNGC also are CD14 and CD45 positive, but express variable to non-detectable levels of CD4. Using combined immunohistochemistry for in situ hybridization and/or double label immunohistochemistry for myeloid cell markers followed by gp120, or confocal microscopy for myeloid markers and viral proteins, we demonstrate that the majority of gp120 or viral RNA positive cells are CD14 positive perivascular macrophages. The resident brain macrophage, microglia and CNS endothelium are not infected. The perivascular cells are the major target during peak viremia (2 weeks post infection) and in animals with SIVE.

**Conclusions:** Perivascular brain macrophages and not microglia are the major target of SIV infection at peak viremia and in animals with SIVE. These data question whether the brain is truly a reservoir for retroviral infection.

This work was supported in part by NIH grant NS37654

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**P52**

**HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 TAT PROTEIN INDUCES ICAM-1 AND VCAM-1 EXPRESSION IN HUMAN ASTROCYTES.**

Scott E. Woodman and Joan W. Berman.

AIDS encephalitis is a frequent consequence of CNS HIV infection, especially in children. One of its many characteristics is a leukocyte infiltrate that is believed to contribute to the production of cytokines, chemokines, and neurotoxic factors resulting in CNS damage. Entry of such leukocytes into the CNS is mediated in part by the expression of adhesion proteins by the blood-brain barrier (BBB) endothelial cells. Expression of these proteins by astrocytes, the other main component of the BBB, also serves to target these leukocytes to the CNS parenchyma.

**Objective:** Investigate if HIV-derived tat, a soluble protein secreted by infected cells, induces the expression of leukocyte adhesion proteins on human astrocytes.

**Methods:** Cell-based ELISA was used to determine the dose and time-dependent induction of VCAM-1 and ICAM-1. The functional role of tat in monocyte binding in vitro was established by adhesion assays using U937 cells (a human promyelomonocytic cell line).

**Results:** Tat (10 ng/ml and 100 ng/ml) induced the expression of VCAM-1 and ICAM-1 in a time-dependent manner with peak expression at 24 hours. Tat (100 ng/ml) significantly increased the number of U937 cells which bound to astrocytes after a 16 hr. exposure.

**Conclusions:** These data suggest that the presence of tat may be a significant factor in the trafficking of HIV-infected cells and inflammatory cells into the CNS via its effect on adhesion protein expression by astrocytes.
P53

IL-1 AND IFN-γ POTENTIAL ROLES IN HIV-1 INFECTION OF THE CENTRAL NERVOUS SYSTEM.
Dona T. Wu, Carrie McManus, and Joan W. Berman.

HIV-1 causes dementia in 20-30% of infected individuals. The exact mechanisms are unknown, but include infection of cells in the central nervous system (CNS) and the production of toxic substances. Human astrocytes, a key component of the blood-brain barrier (BBB), express CXCR4, an important receptor in HIV-1.

Objective: We determined the effects of IL-1 and γ-IFN on CXCR4 expression in human fetal astrocytes.

Methods: We used flow cytometry to examine the expression of CXCR4 on human fetal astrocytes treated with IL-1 and γ-IFN. We also determined the levels of CXCR4 mRNA in cytokine-treated astrocytes using RNase protection assay techniques.

Results: There was a significant increase in CXCR4 cell surface protein and mRNA when fetal astrocytes were treated with IL-1 (p<0.05). Maximal effects were seen at 16 hours and at 5 U/ml of IL-1. Astrocytes treated with IFN-γ (200 U/ml) expressed significantly less cell surface CXCR4, as well as mRNA, compared to untreated astrocytes (p<0.05). When astrocytes were treated with both IL-1 (2 U/ml) and γ-IFN (200 U/ml), the inductive effect of IL-1 was negated by γ-IFN (p<0.05), and the level of CXCR4 cell surface protein as well as mRNA was comparable to untreated astrocytes.

Conclusions: The modulation of CXCR4 expression on astrocytes by these cytokines may affect the susceptibility of these cells HIV-1-infection.

This work was supported by NIH Grant #526-2506. Dona Wu is supported by a Howard Hughes Research Training Fellowship for Medical Students.

P54

INHIBITION OF LTP BY HIV-1-INFECTED MONONUCLEAR PHAGOCYTE SECRETORY PRODUCTS: IMPLICATION FOR HIV-1-INDUCED PATHOGENESIS.
Huangui Xiong, Yong-chan Zeng, Jiabin Zheng, Michael Thylin and Howard E. Gendelman

In the infected human host, virus-infected immune competent mononuclear phagocytes (MPS) secrete bioactive molecules that mediate neuronal damage in HIV-1-associated dementia (HAD).

Objective: The objective of this study is to understand the mechanisms underlying the neuronal dysfunction/damage in HAD, by investigating the effects of virus-infected / uninfected MP culture fluids, without lipopolysaccharide (LPS)-stimulation on LTP in the CA1 area of rat hippocampal slices.

Methods: Experiments were carried out on rat hippocampal slices. Enhancement of synaptic strength induced by high frequency stimulation (HFS) was assayed by measuring the slope of field excitatory post-synaptic potentials (fEPSPs). Progeny virus free MP culture fluids were prepared from virus-infected and control (uninfected) cells, without LPS activation. The fluids were applied onto slices and incubated for 1 hr before electrophysiological recordings.

Results: Prolonged enhancement of synaptic strength was recorded following HFS in the slices incubated with culture media alone or uninfected MP culture fluids with an average increase of 167.8 11.9% (n=17) and 162.5 18.0% (n=4), respectively. In contrast, synaptic strength was reduced following incubation of slices with virus-infected / LPS-activated MP culture fluids, with an average increase of 115.81.3% (n=9). The differences are statistically significant (p<0.05), indicating a blockade of LTP. The enhancements on synaptic strength caused by both virus-infected, non-LPS-activated (128.915.1%, n=8) and uninfected, LPS-activated (127.811.6%, n=6) MP fluids were not significantly different in comparison with those recorded on either normal or control slices (p>0.05).

Conclusions: Our data suggest that both virus-infection and immune activation are required to induce alteration in synaptic function in HAD. Supported by research grants from NIH: P01NS31492-01, R01NS4239-01 and R01NS36126-01.

P55

EXPLORING THE LINKAGES BETWEEN INTRACELLULAR CXCR4 SIGNALING, NEURONAL APOTOSIS, AND NEUROPATHOGENIC MECHANISMS OF HIV-1-ASSOCIATED DEMENTIA.
Jielin Zheng, Michael R. Thylin, Anuja Ghepade, Huangui Xiong, Yuri Pervushky, Robin L. Cotter, Douglas Niemann, MyHahn Chei, Yong C. Zeng, Harris A. Gelbard, Robin B. Shepard, Jennifer M. Swartz, and Howard E. Gendelman

Objective: CXCR4 is both a principal part of neural development and a co-receptor for HIV-1. CXCR4 is expressed on macrophages, lymphocytes and neurons and its ligands, stromal cell-derived factor-1 (SDF-1α), affects neuronal viability. This study will explore the mechanism(s), by which CXCR4 affects neural injury in HIV-1-associated dementia (HAD).

Methods: We investigated GTP binding protein (G-protein) linked signaling and neuronal apoptosis after neuronal exposure to SDF-1α, virus-infected monocyte-derived macrophage (MDM) secretory products, and progeny virions.

Results: In both human and rat neurons, CXCR4 was expressed at high levels. SDV-1α was detected predominantly in astrocytes and at low levels in MDM. SDV-1β was expressed in HAD brain tissue and upregulated in astrocytes exposed to HIV-1 infected and/or immune activated MDM fluids (MDM). HIV-1-infected MDM secrete, progeny virus and SDV-1a induced a G inhibitory (Gi) protein linked decrease in cyclic AMP (cAMP) and increased inositol 1, 4, 5-triphosphate (IP3), intracellular calcium, neuronal apoptosis and caspase 3 activation. Such effects were partially blocked by antibodies to CXCR4 or removal of progeny virions in MDM fluids by filtration. Changes in G-protein-coupled signaling correlated with increased neuronal synaptic transmission and apoptosis.

Conclusions: These data, taken together, suggest that CXCR4 mediated signal transduction is a potential mechanism for neuronal apoptosis during HAD. Supported by NIH research grant: P01 NS31492.