J. Neurovirol. (2012) 18 (Suppl 1):S1–S132
DOI 10.1007/s13365-012-0101-6

P1
HTLV-I/II seroindeterminate western blot patterns and correlated serological antibody responses

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Human T-cell lymphotropic virus type I (HTLV-I) was the first retrovirus associated with both a human malignancy, adult T cell leukemia, and a chronic, progressive neurodegenerative disease, HTLV-I-associated myelopathy/tropical spastic paraperesis (HAM/TSP). Serologic screenings for antibodies to HTLV-I/II have been implemented by blood banks to reduce the risk of HTLV-I transmission via exposure to infected blood products. Donated blood in the United States is screened by enzyme immunoassay (EIA), and repeatedly reactive specimens are confirmed by western blot (WB). However, there is a subset of samples that shows some WB reactivity without fulfilling the serological profile of HTLV-I infection and is considered HTLV-I/II seroindeterminate (IND). The clinical significance of these IND reactivities is unclear although IND reactivities have been widely reported in many blood bank screens and in subsets of patients with other neurological disease. We have reported on a novel, high-throughput, highly sensitive and specific luciferase immunoprecipitation systems (LIPS) assay as an independent test of HTLV-I specific antibody responses. In this study, we examined antibody responses against three HTLV-I immunodominant proteins (Gag, Env and Tax) in serum/plasma samples from 220 subjects in Jamaica (167 HTLV-I-seronegative donors and 53 IND). The LIPS assay was able to detect anti-Gag, anti-Env, or anti-Tax antibody responses in 60% of IND samples. We also found the immunoreactivity against HTLV-I Gag by LIPS correlated with the reactivity of p19 on HTLV WB. In addition, we show a case study of persistent indeterminate HTLV serology in an individual with a clear family history of exposure to HTLV-I. These results support the hypothesis that an HTLV-I/II indeterminate serology may reflect prior exposure to HTLV-I suggesting that this virus may be more widespread than previously thought.

P2
Effects of Morphine on Ferritin Subunits and Resulting Inhibition of CXCR4-Mediated Neuroprotection

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CXCR4 is a chemokine receptor constitutively expressed in the central nervous system, well characterized for its homeostatic pro-survival effects on developing, as well as

mature neurons. Recent studies identified morphine as a negative regulator of neuronal CXCR4 function, an effect relying on an increase in levels of the heavy chain subunit of ferritin (FHC). Although FHC is best known for its role in the iron-sequestering functions of ferritin, a protein complex formed with its partner subunit ferritin light chain (FLC), FHC has been shown to interact specifically with chemokine receptors, including CXCR4, and to inhibit endogenous CXCR4 signaling. This inhibition alters the neuroprotective function of the CXCL12/CXCR4 axis, likely contributing to neuropathology in drug abusers, including HIV + opiate users. In order to better characterize the mechanisms involved in FHC regulation by opiates, we investigated the relationship between iron-binding and CXCR4-regulatory functions of FHC. Here we demonstrate a morphine-induced increase in both FHC and FLC in vitro and in vivo, although the effect on FLC is modest compared to that on FHC. We also show increased neuronal iron accumulation in morphine-treated animals, which has been associated with a disruption in the cellular oxidative balance that may promote neurotoxicity. We then investigated the role of FHC's iron-binding activity on CXCR4 signaling, by using FHC mutants, and demonstrate an ability of both the ferroxidase-deficient mutant H-222 and wild-type FHC to inhibit CXCR4 signaling. A specific physiological consequence of altered FHC levels is suggested by data showing that activation of CXCR4 leads to an increase in dendritic spine density; therefore, future and ongoing experiments aim to analyze the effects of FHC on dendritic spine density. Together these data suggest a neuropathological outcome of increased FHC, which may disrupt multiple cellular processes, and may mediate neuronal dysfunction as a consequence of opiate abuse.

P3

Neuronal sestrin-2response to oxidative stress in HIV-associated neurocognitive disorders

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Sestrin-2 is involved in p53-dependent antioxidant defenses and in the maintenance of metabolic homeostasis. We hypothesize that sestrin-2 expression is altered in the brains of subjects diagnosed with human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) due to neuronal oxidative stress. We studied sestrin-2 immunoreactivity in 42 isocortex sections from HIV-1-infected subjects compared to 18 age-matched non-HIV healthy controls and 19 advanced Alzheimer's disease (AD) cases. With HIV infection, the sestrin-2 immunoreactivity pattern shifted from neuropil predominance (N) to neuropil and neuronal-soma co-dominance (NS) and neuronal-soma predominance (S; $P<0.0001$, Chi-square test for linear trend). Among HIV cases showing the NS or S pattern, HAND cases were preferentially associated with the S pattern (n=10 of 20) compared to cognitively intact cases (n=1 of 11; $P=0.047$, Fisher's exact test). In AD brains, sestrin-2 immunoreactivity was mostly intense in the neuropil and co-localized with phospho-Tau immunoreactivity in a subset of neurofibrillary lesions. Phospho-Tau-immunoreactive neurofibrillary lesions were rare in HIV cases and their occurrence was not associated with HAND. Levels of isocortical 8-hydroxy-deoxyguanosine (marker of nucleic acid oxidation) immunoreactivity were not significantly altered in HAND cases compared to neurocognitively intact HIV cases. In conclusion, the sestrin-2 immunoreactivity redistribution to neuronal soma in HAND suggests unique involvement of sestrin-2 in the pathophysiology of HAND, which is different from the role of sestrin-2 in AD pathogenesis. Alternatively, the difference in sestrin-2 immunoreactivity distribution between HAND and AD may be related to different degrees of severity or stages of oxidative stress. Supported by NIH grants P50 DA026306 and U01 MH83506 to IG, VS, CLA, BS, BG and DJM; P50 AG016570 and U01 AI035040 to ST and HVV.

P4

Raltegravir effects on macrophage-mediated inflammation in HIV infection

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Integrase inhibitors are a new class of promising antiretroviral drugs in our armamentarium against chronic HIV infection. They are especially valuable in long term combination treatments due to their reported low neurotoxicity. The main goal of this study was to understand the effects

that Raltegravir (RAL) has on human monocyte-derived macrophage (MDM) production of immune-mediators when exposed to HIV. We compared RAL effects with that of other antiretroviral (ARV) compounds like Efavirenz (EFV) and combination therapy, including Tenofovir (TDF) and Emtricitabine (FTC). Pro-inflammatory cytokines, IFN-γ, IL-10, IL-12-p70, IL-1, IL-8, TNF-α, and IL-6 were measured simultaneously in tissue culture supernatants from primary MDM. We tested RAL alone and in combination with TDF and FTC, and compared to EFV, alone and in combination with TDF and FTC. We found that RAL treatments resulted in the lowest levels of IL-8 five days after exposure to HIV compared to the other ARV formulations. Also, at earlier time points (days 2 and 3) IFN-γ and IL-10 were lower in the formulations that included RAL. Although all the ARVs decreased TNF-α production at day 2 in MDM exposed to HIV when compared to non-ARV treated controls, each ARV combination led to increased TNF-α at day 4, with RAL alone the lowest, indicating the possibility that the EFV or TDF/FTC caused the increase. The most significant effect of RAL, both in combination and alone, was on MDM prodution of IL-8. Since IL-8 functions as a potent chemoattractant, this may be relevant in reducing overall inflammation that may further impact on blood-brain-barrier permeability. Exploring the effects of RAL on pro-inflammatory molecule production in brain macrophages may also contribute to designing ARV neuroprotective strategies in chronic HIV infection. This work was supported by NIH grant R01 MH94159 to CA, BS, and ET, and a Merck IISP grant to CA.

P5

Increased cerebral beta amyloid deposition in HIV positive subjects with neurocognitive impairment

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Our previous autopsy studies showed increased brain amyloid deposition in HIV + subjects. We have now performed a controlled in vivo pilot study with 8 male HIV + subjects, four with HAND and 4 without. PET scans were acquired using the 11C-Pittsburgh Compound B (11C-PiB) ligand and structural MRI data were obtained. An unpaired, one-tailed t-test was performed between the two groups to determine if a statistically significant difference in 11C-PiB binding was present. Correlation analysis was then performed between the regions of increased 11C-PiB and neurocognitive data. Similar correlations were calculated between regions of increased 11C-PiB and CSF beta-amyloid (Aβ) levels.11C-PiB binding was significantly greater ($p<0.05$) in the impaired group, predominantly within the low to mid-convexity frontal lobes, orbitofrontal cortex, lateral and medial anterior temporal lobes and cerebellum. A significant positive correlation ($r>0.62$, $p=0.05$) was found between the global deficit score (GDS) and the regions of increased 11C-PiB binding in the impaired group. Additionally, while no remarkable correlation was found between 11C-PiB binding and Aβ42 and Aβ40 alone, a significant negative correlation ($r>0.5$, $p=0.1$) was present between 11C-PiB and the ratio Aβ42 / Aβ40. None of the 8 subjects in our study carried the ApoE e4 allele. This preliminary study suggests that Aβ deposition is greater in the brains of HIV+individuals with neurocognitive impairment. A significant negative correlation was found between 11C-PiB binding and CSF Aβ42 / Aβ40 suggesting these markers may be a useful adjunct in the identification of individuals with HAND. This work was supported by an HNRC Developmental Grant to MVC, and P30 MH62512 to RKH, CLA, IG, CFN, SA, TDM, MSB, and SA.

P6

Inverse Relationship Between Peripheral and CSF HIV DNA

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Background: In our previous work we demonstrated a relationship between peripheral activated monocyte HIV DNA (CD14+CD16+) and an individual's neurocognitive status. The current study focuses on identifying these cells in the cerebrospinal fluid (CSF) and determining if the same relationship occurs.
Materials and Methods: Six HIV-infected individuals enrolled in the Hawaii Aging with HIV Cohort were studied, three of whom were diagnosed with Normal Cognition (NC) and three with HIV-Associated Dementia (HAD). CSF cells were separated into three cellular subsets (activated monocytes, CD14+ CD16+; non-activated monocytes, CD14+CD16-; and non-monocytes, CD14-). The DNA were extracted from the sorted cells and HIV DNA copy numbers were determined using quantitative real-time PCR. Total burden of HIV DNA copy number in the CSF was compared to peripheral CD14+CD16 + HIV DNA copy numbers.
Results: Negative correlations between peripheral CD14+ CD16+ HIV DNA copy numbers and total burden of HIV DNA in CSF for both CD14+CD16+ cells (r=-0.81) and CD14+CD16- cells (r=-0.86) were seen. In addition, medians of total burden of HIV DNA in the CSF cellular subsets were higher in patients with NC than those with HAD.
Conclusions: The inverse relationship between HIV DNA copy numbers in peripheral CD14+CD16+ cells and total HIV DNA burden in CSF in all patients is consistent with compartmentalization between the periphery and CSF. The data support a hypothesis that the peripheral CD14+CD16+ cells traffic the HIV into the CNS in HAD and the cells released into the CSF harbor less HIV DNA where the CSF might represent an independent HIV reservoir.

P7

Co-selected single nucleotide polymorphisms in the human immunodeficiency virus type 1 viral promoter precede the onset of neurocognitive impairment

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The long terminal repeat (LTR) regulates gene expression of HIV-1 by interacting with multiple host and viral factors. Cross-sectional studies in the pre-HAART era demonstrated that single nucleotide polymorphisms (SNPs) in peripheral blood-derived LTRs [a C-to-T change at position 3 of C/EBP site I (3T) and/or at position 5 of Sp site III (5T)] were identified SNPs and were often encountered together in the LTR core enhancer region of the integrated provirus in late stage HIV disease. Additionally, the 3T variant correlated with HIV-1-associated dementia. LTR sequences derived from longitudinal sampling of peripheral blood from a single patient in the DREXELMED HIV/AIDS Genetic Analysis Cohort resulted in the detection of the 3T and 5T co-selected SNPs before the onset of neurologic impairment, suggesting that these SNPs may be useful in predicting HIV-associated neurological complications. The relative fitness of the LTRs containing the 3T and/or 5T co-selected SNPs as they evolved in their native patient-derived LTR backbone structure demonstrated a spectrum of basal, activated, and Tat-mediated transcriptional activities. In silico predictions utilizing co-linear envelope sequence suggested that the patient's peripheral blood virus evolved from an X4 to an R5 swarm prior to the development of neurological complications and more advanced HIV disease. These results suggest that the viral swarm evolves during the course of disease in response to selective pressures that lead to changes in prevalence of specific polymorphisms in the LTR and/or the envelope gene that could predict the onset of neurological disease and result in alterations in viral function.

P8
Humoral immune response against HTLV-I HBZ in HTLV-I-infected individuals

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HTLV-I infection can lead to development of Adult T cell leukemia/lymphoma (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). While the cellular and humoral immune responses against HTLV-I play crucial protective roles in HTLV-I infection, chronically activated immune responses have been also associated with the pathogenesis of HAM/TSP. Therefore, characterization of HTLV-I-specific immune responses may provide evidence of immune dysregulation during disease progression, or targets for novel immunotherapy of HTLV-I-related diseases. Recently it has been reported that HTLV-I basic leucine zipper factor (HBZ) gene, which is encoded in the minus strand, has a critical role in HTLV-I infectivity and the development of ATL and HAM/TSP. However, little is known about immune response against HBZ in HTLV-I-infected individuals. In this study, we examined antibody responses against HBZ in serum/plasma samples from 436 subjects including HTLV-I seronegative donors, asymptomatic carrier (AC), ATL, and HAM/TSP patients by the luciferase immunoprecipitation system. The immunoreactivity for HBZ was detected in AC (10.34%), ATL (12.36%) and HAM/TSP patients (13.46%), which is at a much lower frequency compared with antibody responses against HTLV-I immunodominant proteins, Gag, Env and Tax (99.3%, 92.3% and 93.0%, respectively) in HTLV-I-infected individuals. In ATL patients, the frequency of subjects with antibody response against HBZ was significantly higher in chronic subtype (24.14%) compared to lymphoma subtype (1%) ($p=0.0257$). In HAM/TSP patients, antibody responses against HBZ were confirmed by the presence of peripheral memory B cells producing HBZ-specific antibody, and also detected in cerebrospinal fluid of HAM/TSP patients with anti-HBZ in serum. However, antibody responses against HBZ did not correlate with proviral load and HBZ mRNA expression in HAM/TSP patients. This is a first report demonstrating the presence of humoral immune responses for HBZ in HTLV-I-infected individuals, but did not show any association with HTLV-I-related disease outcome.

P9
Antiretroviral Drugs Induce Oxidative Stress and Neuronal Damage in the Central Nervous System

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HIV-associated neurocognitive disorder (HAND), characterized by a wide spectrum of behavioral, cognitive, and motor dysfunctions, continues to affect approximately 50% of HIV (+) patients despite the success of combination antiretroviral drug therapy (cART) in the periphery. Of note, potential toxicity of antiretroviral drugs in the central nervous system (CNS) remains remarkably underexplored and may contribute to the persistence of HAND in the cART era. Previous studies have shown antiretrovirals (ARVs) to be neurotoxic in the peripheral nervous system in vivo and in peripheral neurons in vitro. Alterations in lipid and protein metabolism, mitochondrial damage, and oxidative stress all play a role in peripheral

ARV neurotoxicity. We hypothesized that ARVs also induce cellular stresses in the CNS, ultimately leading to neuronal damage and contributing to the changing clinical and pathological picture seen in HIV-positive patients in the cART era. In this report, we show that ARVs are neurotoxic in the CNS in both pigtail macaques and rats in vivo. Further, in vitro, ARVs lead to accumulation of reactive oxygen species (ROS), and ultimately induction of neuronal damage and death. While ARVs alone caused some activation of the endogenous antioxidant response in vitro, augmentation of this response by a fumaric acid ester, monomethyl fumarate (MMF), blocked ARV-induced ROS generation and neuronal damage/death. These findings implicate oxidative stress as a contributor to the underlying mechanisms of ARV-induced neurotoxicity and will provide an access point for adjunctive therapies to complement ARV therapy and reduce neurotoxicity in this patient population.

P10
Primary and Recurrent Herpes Simplex Virus-2 Meningitis

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The objective is to provide descriptive analysis of patients with herpes simplex virus type 2 (HSV-2) meningitis, confirmed by polymerase chain reaction (PCR) in spinal fluid, clinically examined and followed at a single institution with longitudinal follow-up.
Design: Retrospective case series.
Setting: Mayo Clinic, Rochester, MN, USA, 1995-2008.
Patients: Seven hundred sixty four medical records were reviewed that met search criteria for index words "herpes simplex" and "meningitis" or "encephalitis". Patients were included if they had clinical meningitis and/or HSV-2 detected by PCR in the cerebrospinal fluid (CSF).
Main Outcome Measures: Clinical and CSF characteristics, treatment and outcomes over time.
Results: Twenty eight patients with 33 episodes were identified that had typical signs and symptoms of viral meningitis. No patients were on oral anti-viral treatment at the time of presentation. Every episode, except one, was treated with IV acyclovir and then oral valacyclovir. There were no seizures or deaths in these patients. Nine patients without clinical meningitis, but with symptoms of encephalitis had HSV-2 detected in CSF.
Conclusions: HSV-2 meningitis is a common benign form of primary and recurrent meningitis with minimal sequelae. Recurrence is common but not universal. Genital herpes does not have a reliable enough association with central nervous system involvement by HSV-2 to identify this infection on clinical grounds alone. HSV-2 detection in the CSF is required for diagnosis. Medically complicated patients accounted for a 27% of the CNS HSV-2 infections found, and had encephalitic illness from HSV-2 infection, rather than simple meningitis.

P11
The Nurr1/CoREST Transrepression Pathway Impairs HIV Reactivation in Latently Infected Microglial Cells

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CNS infection by HIV leads to neurologic dysfunctions characterized by a compendium of motor and cognitive disorders known as HIV-associated dementia (HAD). Whereas the incidence of HAD is declining due to treatment with anti-retrovirals, its prevalence is in rise. We have established a model of HIV latency in microglial cells (CHME-5/HIV), the major reservoir for HIV in brain. In CHME-5/HIV cells, HIV is induced by HDAC inhibitors, suggesting that latency is associated with chromatin-mediated silencing, similar to latency in T-cells. However, in T-cells HIV silencing is mediated primarily by chromatin-remodeling enzymes of the polycomb repressive complex-2 (PRC-2) whereas in microglial cells silencing involves a distinct set of enzymes. For example, HIV in latently infected T-cells (Jurkat/HIV) was induced by DZNep, an inhibitor of EZH2, a component of PCR2, but not by chaetocin, an inhibitor of SUV39H1/HKMT. By contrast in microglial cells, HIV was partially reactivated by chaetocin, but not by DZNep. Surprisingly, we found that LPS-mediated HIV reactivation was severely impaired, even though LPS activates NF-κB in this system. We hypothesized that the Nurr1/CoREST transrepression pathway, which limits over-activation of NF-κB-dependent pro-inflammatory genes, may play a role in limiting HIV over-reactivation in infected microglial cells. In support of this hypothesis, we found that phenelzine, BIX01294, or UNC0638, inhibitors of CoREST repression complex enzymes, reactivated HIV in CHME-5/HIV cells, and sensitized them for LPS-mediated reactivation of HIV. Finally, shRNA-mediated knockdown of Nurr1, LSD1, or CoREST partially induced proviral reactivation in CHME-5/HIV cells. Our results indicate that the Nurr1/CoREST transrepression pathway plays a role in the maintenance of HIV latency in microglial cells, but not in T cells, and works to

limit HIV over-expression during inflammation-mediated activation of infected microglial cells in brain. Exploitation of this pathway may lead to unique induction/eradication strategies to eliminate HIV reservoirs in the brain.

P12
Characterization and behavior of T regulatory cells in HTLV-1 associated myelopathy/ tropical spastic paraparesis

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HTLV-1 is a human retrovirus that is associated with adult T-cell leukemia/ lymphoma as well as several inflammatory disorders including Sjögrens syndrome and the neuroinflammatory disorder HTLV-1 associated myelopathy/ tropical spastic paraparesis (HAM/TSP). HTLV-1 primarily infects primarily CD4+CD25+ T cells leading to proliferation and activation. As a subset of these cells are regulatory (typically CD4+CD25+hi -Tregs), we have shown decreased functional capacity of T regs in HAM/TSP patients as compared to normal donors (NDs). It was also shown that HAM/TSP patients have decreased FoxP3 protein expression by FACS and mRNA in their CD4+CD25+ subsets. However, due to the inflammatory component of HAM/TSP, markers normally used to characterize T regs, such as CD25, CTLA4 and GITR are limiting in identifying T regulatory cells. Indeed, FoxP3, thought to be a T reg lineage marker and master regulator of Treg function, is also up regulated in activated T cells. To more precisely characterize Treg cells, we used a novel method that analyzes the methylation status of specific CpGs in the FoxP3 locus in CD4+CD25+ T cells that stably express FoxP3. Tregs stably express FoxP3 which is associated with a completely demethylated locus. CD4+CD25+ cells were enriched as the most likely subset to contain these cells and then the DNA of these cells were analyzed by qPCR with primers directed against the Treg cells specific demethylation region (TSDR). We show that there is decreased demethylation in this population from HAM/TSP patients as compared to NDs, despite the increased CD4+CD25+population size in HAM/TSP. Further we show that this correlates with an overall expanded population of CD4+CD25lo cells with no change in the CD4+CD25hi group between NDs and HAM/TSP patients. We postulate that this imbalance is likely contributing to the decreased T reg function seen in these patients.

P13
CXCL10 plays a crucial role in the pathogenesis of human T lymphotropic virus type-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP)

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Background/Objective: To identify a key chemokine for the pathogenesis of a chronic inflammatory neurological disease termed human T lymphotropic virus type-1 (HTLV-1) associated myelopathy/tropical spastic paraparesis (HAM/TSP). Methods: We measured the levels of 9 pro-inflammatory chemokines, which are ligands of major T-cell chemokine receptor (CXCR3, CCR4, CCR5, and CCR6), in cerebrospinal fluid (CSF) samples from twenty-nine HAM/TSP patients and eight HTLV-1-infected non-HAM/TSP individuals. As to the chemokines which showed higher levels in HAM/TSP CSF, we compared the concentration of chemokines between CSF and serum to identify chemokine which concentrate is higher in CSF than in serum. Since CXCL10 was the only chemokine which demonstrated higher concentration in CSF, we performed chemotaxis assay to test whether neutralizing monoclonal antibodies against CXCL10 and its receptor CXCR3 can inhibit the chemotaxis of PBMC from HAM/TSP patients (n=30). We collected the migrated cells and measured the absolute provirus load using real-time PCR. Furthermore, we analyzed the expression of CXCR3 in CSF cells from HAM/TSP patients (n=13) by FACS.

Results: The levels of CXCL9, CXCL10, and CCL5 in CSF from HAM/TSP patients were significantly elevated ($p<0.0001$, $p<0.0001$, $p<0.0131$, respectively). Importantly, the level of CXCL10, but not of CXCL9 and CCL5, was significantly higher in CSF than in serum ($p<0.0001$). We demonstrated that anti-CXCL10 antibody significantly inhibited the cellular migration of HAM/TSP PBMC, while anti-CXCR3 antibody did not. Furthermore, the absolute provirus load in the migrated cells was lower when anti-CXCL10 antibody was used compared to control antibody. Notably, the expression of CXCR3 was demonstrated in more than 90% of CSF cells from HAM/TSP patients.
Conclusion: We demonstrated that CXCL10-CXCR3 interaction plays a crucial role in the pathogenesis of HAM/TSP, and antibody against CXCL10 might be a candidate for molecule targeting therapy for HAM/TSP patients.

P14
Interactions of HIV-1 with Amyloid Beta Peptide at the Blood-Brain Barrier Level

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An increase in the older population infected with HIV-1 is an emerging development in HIV-1 epidemiology. Aging is connected with increased deposition of amyloid beta peptide (amyloid beta) in the brain. In the current study, we propose that amyloid beta and HIV-1 can potentiate their toxic effects at the blood-brain barrier (BBB) level. To address this notion, we employed an in vitro model of human brain microvascular endothelial cells (HBMEC) directly exposed to HIV-1 or co-cultured with HIV-1 infected human monocytes. Exposure of HBMEC to amyloid beta (1-40) in the presence of HIV-1 resulted in a markedly increased amyloid beta binding/entry into HBMEC. We then hypothesized that HIV-1 may either increase binding/entry of externally added amyloid beta or elevate the amount of endogenously produced amyloid beta. The receptor for advanced glycation end products (RAGE) is known to be involved in the transport of amyloid beta across the BBB into the brain. RAGE immunoreactivity was stronger and RAGE protein levels were elevated in HBMEC exposed to HIV-1 as compared to control. In contrast, exposure to HIV-1 decreased expression of lipoprotein receptor related protein-1 (LRP1) which is the main receptor that transports amyloid beta from the brain to blood. These results indicate that HIV-1 can decrease the ability of the BBB to transport amyloid beta from the brain and thus predispose the brain to increased amyloid beta accumulation. Supported by MH072567, MH63022, and NS39254

P15
Immunomodulatory activity of THC and CB2 expression on hematopoietic cells

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Use of cannabis is reported to be as high as 81% among HIV-1-infected adolescents. The major psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol [THC], mediates anti-inflammatory effects through interaction with cannabinoid 2 [CB2] receptors on immune cells. As most studies of THC and immunity have been conducted in rodents, the role of THC in modulating human immunity and HIV-1 immune pathogenesis remains unclear. The impact of cannabis use on immune activation was investigated by a study combining in vivo and ex vivo approaches.
Expression of CB2 and the global effect of THC on human monocyte and macrophage activation were evaluated ex vivo. Total RNA and protein were isolated from human monocytes, monocyte derived macrophages, and undifferentiated or PMA differentiated THP-1, HL-60 and U937 monocytic cell lines. Nested reverse transcriptase PCR and sequencing identified CB2 mRNA in all cell types. However, CB2 protein was only detectable by western blot in undifferentiated monocytic cell lines and primary monocytes.
Next, impact of THC on inflammation was evaluated. THC pretreatment reduced TNF-induced ICAM-1 protein expression in undifferentiated THP-1 cells and monocytes. IL-6 levels were also significantly reduced by THC or the CB2 agonist JWH133 in LPS-activated peripheral blood mononuclear cell and monocyte cultures.
A panel of biomarkers of immune activation associated with neurocognitive impairment or overall morbidity/mortality was measured in plasma samples from a cohort of 78 HIV-1-infected and 35 uninfected subjects. In the HIV-

1-infected group 35.8% tested positive for cannabinoids compared to 37.1% in the control group. For the vast majority of biomarkers, cannabis use failed to correlate with immune activation in either group. Significantly decreased levels of sCD27 were observed in cannabinoid-positive subjects compared to cannabinoid-negative subjects in the control group [p=0.014]. THC reduces inflammatory outcomes in monocytes ex vivo and lymphocytes in vivo in the absence of HIV-infection Funded by NIH R01DA031017.

P16
Chronic Brain inflammation in HIV-patients is not positively influenced by cART

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Introduction: International cohort studies reveal that neurocognitive deficits rise in prevalence and incidence among HIV patients despite combination antiretroviral therapy (cART) and fully suppressed viral loads in different body compartments. This is due to viral as well as immunological factors. So far there are no markers for chronic inflammation in the central nervous system except for beta-2-microglobuline. Thus additional biological disease markers are warranted. Free caspase -3 and -7 activity can be measured in cerebrospinal fluid (CSF). It is elevated in neurodegenerative diseases like Creutzfeld Jakobs disease and may also serve as a surrogate parameter for neuronal degeneration in HIV.
Methods: In 35 HIV-positive patients paired plasma and CSF samples were drawn after approval by the local ethics authorities. Patients were subdivided into 4 groups (early CDC-stages=A1, A2, B1 and B2 with and without cART and AIDS-defined stages=A3, B3 and C1-3 with and without cART). We analysed: age, duration of HIV-1-positivity, CD4+-cell count, viral load (VL) in plasma and CSF, neurocognitive parameters (psychomotor speed, Trail-Making-Test 1+2 and Grooved Pegboard-Test) and compared these to the free caspase-3 and -7 activity in CSF measured by luminometric assay.
Results: Caspase -3 and -7 activity was highest in late stage patients independent of cART medication and significantly lower in early stages (p<0,05), independent of cART. In patients without medication caspase activity correlated with CSF, but not with plasma-VL. In late stage patients neuropsychological performance was worse in patients with high caspase activity independent of cART.
Conclusions: Free caspase activity in CSF of HIV-patients as surrogate parameter for neuronal degeneration is uninfluenced by cART, correlates with CSF-VL and with neuropsychological deficits, especially in late disease stages, thus pointing to HIV neuropathogenesis.

P17
Opiates, proBDNF, and HIV

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It is estimated that a third of human immunodeficiency virus type 1 (HIV)-infected individuals in the USA are intravenous opioid drug abusers. Opiates render these individuals more at risk to developing HIV-associated Dementia (HAD), a neurological disease characterized by synaptic simplification and neuronal apoptosis. It is currently believed that opiates may intrinsically alter the progression and pathogenesis of HAD. A better understanding of the mechanisms mediating HIV-1 neurotoxicity in the presence of opiates is crucial for developing effective therapies against HAD. In this work we have explored the novel hypothesis that opiates and HIV synergize in altering the trophic environment neurons and glia cells rely on to survive. Using rat primary neurons we have observed that the HIV protein gp120 reduces the length of neuronal processes similarly to the proneurotrophin pro brain-derived neurotrophic factor (proBDNF). Intriguingly, the effect of both proBDNF and gp120 was blocked by inhibitors of the p75 neurotrophin receptor, suggesting that proBDNF and gp120 share a similar mechanism of neurotoxicity. In fact, we observed that gp120 promotes a time-dependent intracellular and extracellular accumulation of proBDNF concomitantly with a decrease in mature BDNF. This effect is due to the ability of gp120 to alter proBDNF processing. A similar imbalance in the ratio proBDNF/mature BDNF was confirmed in postmortem brains of HIV positive subjects cognitive and motor impaired with no history of drug abuse. Interestingly, while cocaine in human brains did not affect the levels of BDNF, opiates seem to increase BDNF in the cortex. However, the number proBDNF positive neurons was also increased in the brains of HAD subjects with history of opiate abuse, suggesting that although opiate increase the synthesis of BDNF in the brain, the altered

BDNF processing caused by HIV leads to an increase in proBDNF rather than BDNF, thus exacerbating the HIV neurotoxic effect.

P18
MEMRI: A biomarker for neuroAIDS

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Introduction: The clinical and pathologic correlates of the most severe form of HIV-associated neurocognitive disorders is HIV-associated dementia and HIV-1 encephalitis (HIVE), respectively. HIVE takes varying forms and detecting it early could improve disease outcomes. To this end we used Mn2+ enhanced MRI (MEMRI) to evaluate early pathobiologic events in HIVE, notably astrogliosis and neuronal responses to infection. Methods: Thirty two five-week-old male NOD.Cg-Prkdcscid Il2rgtm1Wjl/Sz(NSG) mice were divided into three groups. In groups 1 (n=12) and 2 (n=12) HIV-1ADA -infected monocyte-derived macrophages (MDM), 5×10^5 cells were injected in a 5 μl volume intracerebrally into the caudate/putamen by stereotactic measures to induce HIVE. In group 3 (n=12) uninfected MDM were injected. MnCl2(50 mM) was injected in Group 1 and 3 i.p. for 8 days before magnetic resonance imaging (MRI) at 30 mg/kg/day. Mice were scanned by MRI at 4, 8, 15 and 28 days after MDM injection using T1 mapping and T1-wt imaging. Animals were euthanized for immunohistological validation; brains were removed and embedded in paraffin. Five μm thick sections were labeled with mouse monoclonal antibodies for GFAP, HLA-DR, HIV-1p24 and Iba1. Results: On day 4, 8 and 15 signal enhancement was observed at the injection sites in Group1 mice on T1-wt MRI. Limited to no signal enhancement at parallel sites was observed in Groups 2 and 3.The immunohistological results showed human MDM in and around the lesion sites with measures of HIV-1 infection and glial activation. In laboratory, in vitro comparative tests of Mn2+ uptake by glial cells and neurons showed increased neuronal Mn2+ uptake paralleled inflammation and no significant effect on glial cells Mn2+ uptake. Based on these, we hypothesize that the signal enhancement results from the increased neuronal activity as a result of gliosis stimulating neuronal Mn2+ uptake. MEMRI may be developed as a neuroAIDS biomarker.

P19
Processing and trafficking of amyloid-b peptides are altered by HIV

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It is controversial if amyloid-β (Aβ) deposition is accelerated in brains of HIV-infected individuals. In human brain tissues we found evidence that Aβ1-42 accumulated at a rate accelerated by 20-30 years in HIV-infected subjects compared with age-matched seronegative controls. We then used in vitro and in vivo approaches to study the potential mechanisms for altered amyloid precursor protein (APP) processing in the setting of HIV-infection. In cell culture experiments we determined that gp120 or combinations of gp120 (X4, R5 and dual), Tat, and heat inactivated supernatants from HIV-infected macrophages (MDS) increased the amount of amyloid precursor protein (APP) expression through a post-transcriptional stabilization that involved the heterogeneous nuclear ribonucleoprotein C. The amount of β-secretase (BACE1) expression was also increased through enhanced transcriptional activity. HIV-1 proteins increased the size and stabilized the structure of lipid rafts and increased and the co-localization of APP and BACE to lipid rafts. BACE activity was enhanced with a resultant increase in Aβ formation. However, very little of this Aβ was exported from the cells, and accumulated in intraneuronal compartments. Aβ

accumulated in lysosomes and appeared to remain stable in this compartment for prolonged periods of time. We then created a triple transgenic model expressing knock-in mutations in human APP and PS1 with HIV-gp120. In APP/PS1/gp120 mice there was an increase in the number and size of Aβ-containing plaques compared with APP/PS1 mice. Moreover, there was a striking accumulation of intraneuronal Aβ in APP/PS1/gp120 mice that began early (within 3 months), and worsened with age. Levels of lipid raft associated ceramides were increased in gp120 and in APP/PS1/gp120 mice but not in APP/PS1 mice suggesting that gp120 expression drive this biochemical effect. Thus, in the setting of HIV-infection a defect of protein trafficking may perturb neuronal function by trapping Aβ in intraneuronal compartments.

P20
Ethanol increases the surface expression of calcium permeable AMPA

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HIV-infected subjects who abuse alcohol exhibit greater declines in cognitive tasks that assess frontal lobe function compared to HIV-infected subjects who do not abuse alcohol. Here we provide evidence that alcohol impairs the traffic of calcium permeable AMPA receptors. Acute ethanol administration perturbed the structure of plasma membranes with a resultant collapse of GM1 gagliosides into focal microdomains. Ethanol selectively increased the surface expression of calcium permeable AMPA receptors that clustered in these membrane microdomains. Although surface expression was increased, activity of these calcium permeable AMPA receptors was blunted in the presence of alcohol. Immediately following alcohol withdrawal AMPA receptor activity was enhanced, consistent with increased surface expression. The enhanced surface expression of AMPA receptors following ethanol involved increased phosphorylation of GluR1 at serine 831 and serine 845 by PKC and PKA respectively, and was reversed by the addition of soluble cholesterol back into the membranes. To further investigate the effects of ethanol on AMPA receptor trafficking in an HIV setting, we developed a binge-drinking model in HIV Tat transgenic mice. Induced expression of Tat or ethanol self-administration increased total GluR1 and the amount of GluR1 phosphorylated on s845. During ethanol withdrawal in Tat mice the phosphorylation of GluR1 at s845 remained high although total GluR1 levels returned to levels not different from control mice. These data suggest alcohol increases the surface expression of calcium permeable AMPA receptors through increased phosphorylation of C-terminal serines. The activity of these receptors is actively blocked by ethanol, but become over activated during ethanol withdrawal. Moreover, HIV Tat may prolong the phosphorylation and hence surface localization of calcium permeable AMPA receptors during ethanol withdrawal.

P21
HIV-1 Tat protein activates the c-Abl/p73 pro-apoptotic pathway in neurons

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Involvement of HIV-1 Tat protein in neuronal deregulation and in the development of HIV-1 associated neurocognitive disorders has been shown by several groups including ours, however the mechanisms used by Tat to cause such deregulation are not fully understood. Previously, we demonstrated that Tat functions through a pathway that implicates p73 and p53 proteins. In here, we showed that Tat deregulates the levels of miR-196a that leads to phosphorylation of p73 by c-Abl as well as its accumulation. p73 accumulation is the result of a series of posttranslational modification of p73 and not the activation of its relatively silenced promoter. Further, we found that in Tat-treated neurons, c-Abl phosphorylates p73 on tyrosine residue 99 (Tyr-99). Tat lost its ability to promote accumulation and phosphorylation of p73 in the presence of miR-196a mimic. These results led to the conclusion that Tat protein induces neuronal deregulation through a well-established pro-apoptotic pathway that is under the regulation of miR-196a. Additional studies are needed to understand the degree of involvement of this mechanism in the overall picture of HAND, which can lead to the development of therapeutics improving the quality of life in individuals with such disorders.

P22
Differential regulation of miR-146a and EAAT2 by IL-1beta and glutamate induced NFkB activation in astrocytes

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The astroglial EAAT2 glutamate transporter is essential for clearing the critical neurotransmitter glutamate in the central nervous system and protecting against excitotoxicity. The transcription factor NFkappaB is a positive regulator of microRNA 146a and a negative regulator of EAAT2 expression. Glutamate is known to induce the transcription factor NFkappaB. In the present study we investigated the effect of glutamate-induced activation of NFkappaB in glial cells of the CNS, including primary astrocytes on the regulation of mir-146a and EAAT2 promoter regulation. Our studies demonstrate that glutamate mediated NFkappaB activation induced EAAT2 promoter and had no affect on mir-146a promoter. In contrast, HIV induced pro-inflammatory cytokine IL-1beta induced NFkappaB activation activated mir-146a promoter but inhibited EAAT2 promoter activity. We also demonstrate that glutamate induced p65-IkappaBalpha dissociation in the absence of IkappaBalpha phosphorylation or degradation. Furthermore, glutamate induced the rapid phosphorylation of p65 at Ser536. In contrast, pro-inflammatory cytokine IL-1beta induced rapid phosphorylation of p65 at Ser536 and IkappaBalpha phosphorylation or degradation. Our results provide insight into a glutamate-induced regulatory pathway distinct from that described for cytokine-induced NFkappaB activation in regulation of mir-146a and EAAT2 in astrocytes.

P23

SILAC Proteomics analysis of neurons exposed to cell free HIV-1

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Human immunodeficiency virus 1-associated neurocognitive disorders (HAND) remain the most common CNS disease linked to advanced viral infection. Viral and host cell products released from HIV-infected cells have been implicated as inducers of neurologic disease through chronic secretion of neurotoxic factors. Since HIV-1 does not infect neurons, the direct effects of HIV neuronal dysfunction remain uncertain. To better understand the global molecular mechanisms and to identify the pathways involved in the HIV-associated neuronal cell damage, we exposed SHSY neuronal cells to cell free HIV and used state of the art proteomics technology, stable isotope labeling by amino acids in culture (SILAC) to assess changes in neuronal proteins. Using this approach we identified >1,200 differentially expressed proteins, 51 of which were upregulated more than 1.5-fold, and 17 of which were down-regulated at least 2-fold. Ingenuity Pathway Analysis of the differentially expressed proteins that were identified by mass spectroscopy highlighted signaling clusters with center nodes in p53, retinoic acid and AKT pathways. Some of the differentially expressed proteins and other downstream proteins related to these signaling pathways were further validated by Western blot analysis. Network analysis of the differentially expressed proteins indicated associations with three major networks: 1) cellular function and maintenance, 2) lipid metabolism and 3) molecular transport. In conclusion, these data indicate that exposure to cell free HIV induces biologically relevant changes in neuronal signaling proteins. Pathways in which these proteins function can be further studied to better understand the pathogenesis associated with HAND and may represent novel targets for interventions aimed at improving neuronal functioning in HIV patients.

P24

SILAC-based quantitative proteomic approach to identify HIV-1 Vpr mediated changes in macrophage metabolic pathways

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Macrophages are the major viral reservoirs during HIV-1 infection. HIV-1 viral protein R (Vpr) mediates many processes that promote HIV-1 infection, evasion of the immune system, and persistence in the host. Although Vpr mediated pathogenesis in macrophages has been extensively investigated, the global molecular mechanism associated with the metabolic pathways has not been investigated. We used stable isotope labeling by amino acids in culture (SILAC) based proteomics technology to determine the differentially expressed proteins in U937 derived macrophages transduced with adenoviral Vpr construct. Using this approach we identified >1,000 differentially expressed proteins, 44 of which were up regulated more than 1.5-fold, and 46 of which were down-regulated at least 2-fold. Ingenuity Pathway Analysis of the differentially expressed proteins that were identified by mass spectroscopy highlighted some

metabolic pathways including: pyruvate metabolism, pentose phosphate pathway and citrate cycle. Some of the differentially expressed proteins and other downstream proteins related to these signaling pathways were further validated by Western blot analysis. Network analysis of the differentially expressed proteins indicated signaling clusters with NFkB(complex) as a center node in associations with three major functions: nucleic acid metabolism, small molecule biochemistry, cell-to-cell signaling and interaction. In conclusion, these data indicate that HIV-1 Vpr induces biological relevant changes at the protein level with several metabolic consequences in macrophages infected with HIV-1. Further studies of these metabolic pathways can offer a better understanding of the mechanism responsible for the viral reservoir persistence in macrophages and can be use as a novel targets for drug discovery to reduce the viral load.

P25
MEG-identified Recovery of CNS Functional Connectivity in HIV Disease after HAART

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Objectives: To document the brain functional and structural changes during the course of recovery from HIV-Associated Dementia (HAD).
Design: Single subject, observational.
Methods: Patient PG was treatment-naïve when he presented with pneumocystis pneumonia. At the first visit, PG met criteria for HAD with a distal polyneuropathy and myelopathy; cognition was impaired in all spheres. He underwent detailed neuropsychological testing, a magnetoencephalographic (MEG) measurement and magnetic resonance imaging of the brain. His data were compared with those from 16 other individuals (9 HIV infected, 7 controls) at baseline and after six months of treatment.
Results: After six months of HAART, PG showed viral suppression with improvement in CD4+ cell counts. Cognitive functions improved, except for Memory and Learning. MRI whole brain volume measure with SIENA showed a 5% increase over the 6-month interval. Functional connectivity in the MEG data was quantified using the mean mutual information (MI) between critical sensor pairs. At study entry PG had a MI score at the mean of HIV-infected subjects; after treatment, his MI score improved to the level of the uninfected controls.
Conclusions: HAD can still be one of the presenting syndromes of HIV disease. Aggressive treatment not only controls peripheral viral replication and results in immune reconstitution but also appears to induce a partial restoration of brain volume and leads to significant improvements in brain functional connectivity. These data also suggest that MEG measures of functional connectivity may serve as a useful biomarker for HIV-Associated Neurocognitive Disorder, and for tracking the response to pharmacotherapy.

P26
Rearranged polyomavirus JC non-coding control region sequences as a possible marker of virulence within a cohort of pediatric Crohn's disease patients treated with infliximab

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The recent introduction of monoclonal antibodies in Crohn's disease (CD) management has been associated with the

development of serious complications, such as the progressive multifocal leukoencephalopathy (PML), caused by JC Polyomavirus (JCV) reactivation and with a high incidence in HIV-positive subjects. Therefore, the aims of our study have been the investigation of the possible JCV reactivation in pediatric CD patients treated or not with infliximab, performing quantitative PCR in urine, plasma, and intestinal biopsies at the time of recruitment (t0) and every 4 months in 1 year of follow-up (t1, t2, and t3), and the analysis of the JCV Non-Coding Control Region (NCCR) sequence to detect cellular transcription factors binding site mutations. Results obtained showed that, in urine and ileal specimens, JCV load significantly increased in infliximab treated patients after 1 year of treatment (t3), while viremia was significantly higher at t1. JCV NCCR sequence analysis showed a structure similar to the non-pathogenic variant CY archetype in 65/80 analyzed sequences, but the remaining 15/80, obtained exclusively from plasma and biopsies, evidenced a CY NCCR organization with two recurrent nucleotide changes, the 37-T to G transversion in boxA Spi-B binding site and the 217-G to A transition in boxF, and a boxD deletion. These rearrangements were always found at t3 within seven infliximab-treated CD patients, who presented a very severe disease at t0. After 18 months of treatment, we observed in 2/7 colon biopsies of these patients a particular NCCR sequence, with a structural organization that resembles the pathogenic Mad-1 variant. We can conclude that these rearranged NCCR sequences could be considered a marker of JCV virulence within 12-24 months of mAb treatment, although none of our examined patients developed PML, and further studies on a larger cohort of patients should be performed.

P27

Neurofibromatosis Type 2 tumor suppressor protein, NF2, induces proteasome-mediated degradation of JC virus T-antigen in human glial cells

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The human polyomavirus, JC virus (JCV), is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) and displays a strong tropism toward glial cells. JCV typically replicates under immunosuppressive conditions, making this virus a threat to AIDS patients and those undergoing immunomodulatory therapies. Understanding the molecular mechanisms responsible for viral replication can have a pivotal affect on deterring viral resurgence in these patients. Expression of the viral early promoter is the initiating step in the virus life cycle and leads to the production of the major viral regulatory protein, T-antigen. To promote viral replication, T-antigen must hijack the cell cycle machinery of the host to enhance expression of itself and other JCV proteins. Studies on JCV T-antigen transgenic mice, which develop tumors resembling the malignant peripheral nerve sheath tumors (mpnst), have led to the identification of neurofibromatosis type 2, NF2, as a binding partner for T-antigen. NF2 is a cytoplasmic scaffolding protein affecting cell motility and morphology which has tumor suppressor properties and has also shown to be present in the nucleus. We have found that NF2 downregulated expression of both large and small T-antigen proteins and activity of the JCV bidirectional promoter. Upon further characterization of this interaction, we determined that NF2 utilizes a novel approach to suppress T-antigen expression, whereby it promotes the accumulation of mature T-antigen mRNA and the proteasomal degradation of T-antigen protein. Collectively, these results show that NF2 is a negative regulator of JCV T-antigen expression, and suggests a novel role of NF2 as an inhibitor of JCV reactivation. The utility of NF2 as a potential strategy to block JCV replication in glial cells is discussed. This work was supported by grants awarded by NIH to JG. SB was supported by an NIH Ruth L. Kirchstein National Research Service Award (1T32MH079785).

P28

The HIV glycoprotein gp120 impairs fast axonal transport through an axon-autonomous mechanism

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Distal sensory polyneuropathy (DSP) is a prevalent neurological complication associated with human immunodeficiency

virus (HIV) infection. DSP is characterized by progressive dying-back degeneration of dorsal root ganglion (DRG) neurons projecting to the distal extremities. Gp120, a neurotoxic glycoprotein overproduced and shed by HIV-infected macrophages, has been linked to DSP pathogenesis. However, the exact role that gp120 plays in promoting degeneration of DRG axons in DSP remain uncertain.

We hypothesized that gp120-induced neurotoxicity might involve alterations in the DRG cytoplasm. To test this, we first determined whether gp120 is internalized into neurons. We found that gp120 was indeed internalized by F11 cells, a hybrid cell line generated from rat dorsal root ganglion and mouse neuroblastoma cells. In addition, we determined specific endocytic pathways through which gp120 is internalized with immunolocalization studies with specific endosomal markers. How might internalized gp120 be toxic to neurons? Since dying-back neurodegeneration has been linked to deficits in fast axonal transport (FAT), we hypothesized that intracellular gp120 might affect FAT. To test this idea, recombinant gp120 was perfused in isolated squid axoplasm. These experiments indicate that gp120 inhibits FAT at nanomolar concentrations. Further, coperfusion of gp120 with pharmacological inhibitors of specific kinases and phosphatases delineated the signaling pathways that mediate the effect of gp120 on FAT. Finally, the role of CXCR4 gp120 internalization and kinase activation was also established. The unique reliance of neurons on FAT mechanisms suggests that axon-autonomous activation of phosphotransferases by gp120 might represent a critical pathogenic event in DSP.

P29

HIV-1 Tat protein variants: critical role for the cysteine region of Tat in producing neuronal dysfunction

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The transactivating protein (Tat) of the HIV-1 virus is essential for viral replication. However, the neural responses to the Tat protein in the CNS are important aspects in consideration of therapeutic approaches to HAND. In the current study, HIV-1 Tat 1-101 clades B and C, and Tat variants commonly used in the laboratory as well as novel peptide lengths (truncated 47-57, D31-61, and 1-86 with a mutation at cys22) were examined to determine their ability to induce neuronal apoptosis and synaptodendritic injury. A standard cell culture LIVE/DEAD assay was used to determine neuronal apoptosis. F-actin positive punctae (spines) were counted along MAP-2 labeled neurites and compared to controls to examine neuronal network integrity. Tat proteins 1-72 and 1-86 induced significant apoptosis and network damage; however, all variants with a mutation in the cysteine region did not induce neuronal apoptosis or cause significant neuronal network damage. Moreover, HIV-1 Tat 1-101 Clade B was neurotoxic, whereas HIV-1 Tat 1-101 clade C had no detectable effects on neuronal cells. These results indicate that the highly conserved cysteine region of Tat is required for apoptosis and neuronal network damage, and the presence/absence of mutations in the cysteine region is a critical factor for neurotoxicity.

P30

Molecular mechanisms involving sigma receptor-mediated induction of MCP-1: implication for increased monocyte transmigration

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Cocaine abuse hastens the neurodegeneration often associated with advanced HIV-1 infection. The mechanisms, in part, revolve around the neuroinflammatory processes mediated by the chemokine monocyte chemotactic protein-1 (MCP-1/CCL2). Understanding factors that modulate MCP-1 and, in turn, facilitate monocyte extravasation in the brain is thus of paramount importance. We now demonstrate that cocaine induces MCP-1 in rodent microglia through translocation of the sigma receptor to the lipid raft microdomains of the plasma membrane. Sequential activation of Src, mitogen-activated protein kinases (MAPKs), and phosphatidylinositol-3' kinase (PI3K)/Akt and nuclear factor kappaB (NF-kappaB) pathways resulted in increased MCP-1 expression. Furthermore, conditioned media from cocaine-exposed microglia increased monocyte transmigration, and thus was blocked by antagonists for CCR2 or sigma receptor. These findings were corroborated by demonstrating increased monocyte transmigration in mice exposed to cocaine, which was attenuated by pretreatment of mice with the sigma receptor antagonist. Interestingly, cocaine-mediated transmigratory effects were not observed in CCR2 knockout mice. We conclude that cocaine-mediated induction of MCP-1 accelerates monocyte extravasation across the endothelium. Understanding the regulation of MCP-1 expression and functional changes by cocaine/sigma receptor system may provide insights into the development of potential therapeutic targets for HIV-1-associated neurocognitive disorders.

P31
Human ß-Defensins 2 and 3 inhibit HIV in macrophages: implications for HIV infection in the CNS

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Human ß-Defensins (hBD) are secreted, broad-spectrum antimicrobial peptides that we and others have shown to inhibit HIV in primary CD4+ T cells. The inhibitory activity is mediated in T cells by CCR6, a receptor that binds both the chemokine MIP-3α and hBD2 and -3, and eventuates in transcriptional induction of the antiretroviral cellular factor APOBEC3G. Intriguingly, our data show that ß-defensin 2 is expressed in the CNS, but very little is known about the role played by defensins in the brain. We hypothesized that, besides T cells, hBDs could protect microglial cells and perivascular macrophages from productive HIV infection. Our data on primary monocyte-derived macrophages (MDM), which we used as an in vitro model for microglial cells show that hBD2 and -3 inhibit HIV at an early stage of infection in association with increased levels of APOBEC3G. Flow cytometry analyses show that MDM express CCR6 and CCR2, another shared chemokine-defensin receptor, although with high variability among donors. Since agonist and antagonists of chemokine receptors are becoming available, our data could lead to the development of new therapeutic approaches to treat HIV infection systemically and in the CNS. In addition, decreased defensin production that we observed in HIV-infected subjects could be associated with increased occurrence of HIV-associated symptoms.

P32
Enhancement of Rift Valley fever encephalitis following vaccination

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Rift Valley fever virus (RVFV) is a vector-borne zoonosis causing periodic disease outbreaks in livestock and humans in Africa and the Middle East. In humans, infection is associated with fever and liver damage, but a subset of patients progress to hemorrhagic fever, encephalitis, ocular disease or death. RVFV is considered a category A agent because it has the potential to be easily disseminated. We have examined RVFV ZH501 infection by intraperitoneal or aerosol routes in BALB/c mice. Intraperitoneal infection led to acute onset hepatitis and was lethal by day 4 post-infection. There was no evidence of brain infection by this route. Aerosol infection resulted in a different course of infection. Hepatitis was still observed but it was less prominent and delayed by 1-3 days. Brain infection was detected in some animals by day 6 post-infection. By day 10 post-infection, mice developed severe encephalitis with the majority of neurons infected. Vaccination of BALB/c mice led to protection from infection upon intraperitoneal challenge and resulted in less liver infection after aerosol challenge. Surprisingly, aerosol challenged, vaccinated mice showed enhanced brain infection. This suggests that depending on the route of challenge, vaccination may enhance rather than protect from encephalitis.

P33
Common Transcriptional Signatures in HIV-1-Associated Neurocognitive Disorder, Alzheimer's Disease and Multiple Sclerosis. Comparison with a Mouse Model of HAND

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HIV-Associated Neurocognitive Disorder (HAND) is a common manifestation of HIV infection that afflicts about 50% of HIV-positive individuals. As people with access to antiretroviral treatments live longer, HAND can be found in increasing segments of the population at risk for other chronic, neurodegenerative conditions such as Alzheimer's disease (AD) and Multiple Sclerosis (MS). This raises the possibility that brain diseases of diverse etiologies may overlap in utilization of similar biological pathways. To test this proposition, we used meta-analysis to compare gene expression signatures and biological pathways in brain tissues of HAND, AD and MS. In pair-wise and three-way analyses, we found a surprisingly large number of dysregulated transcripts and biological processes common to all three diseases. All three diseases shared up-regulation of inflammatory and immune response processes and down-regulation of processes implicated in signal transduction and synaptic function. The results obtained in human meta-analysis were then compared with microarray data from brain tissues of mice infected with chimeric HIV (EcoHIV). Common down-regulated transcripts in human and mouse were implicated in synapse vesicle transport, glutamate receptors, and learning and memory processes. This approach could facilitate identification of common disease mechanisms in complex neurocognitive disorders. EcoHIV-infected mice may serve as a useful system for empirical validation of molecular and physiological changes in the brain underlying HAND.
Supported by grants DA017618 and MH083627 from the National Institutes of Health, US Public Health Services.

P34

National NeuroAIDS Tissue Consortium: Recent Updates to the Investigator's Resource

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The National NeuroAIDS Tissue Consortium was founded with funding from NIMH and NINDS in 1998 to fill the research community's need for well-characterized tissue and fluids from HIV-infected individuals. The NNTC has since established itself as a valuable resource to the research community by providing quality pre and ante mortem clinical data and tissue to qualified investigators. Since its launch the NNTC has continued to refine its structure and processes in response to the research community's needs, expanding the scope of available clinical data, implementing a bioinformatics platform, and standardizing data. New resources have been made available via the NNTC website (www.NNTC.org) to assist investigators in mining the NNTC holdings. User feedback is solicited from all users of the NNTC resources for input on NNTC future directions. Currently, the NNTC cohort includes 2331 HIV positive participants, of which 2056 contributed data to the Longitudinal Cohort, 609 are currently in the active cohort, and 782 are deceased with autopsy. In addition, the bank has data and tissue from 237 HIV negative participants. The clinical data collection includes demographics, neuromedical data, neuropsychological assessments, cognitive diagnoses, neuropathologies, PRISM/CIDI, and clinical labs. Recent revisions/additions include improved ARV/concomitant medication histories, comorbidities, and supplemental neuropathologies. The NNTC bioinformatics platform consolidates microarray and other bioinformatics data with clinical annotations to provide an integrated view of the available NNTC resources. Currently SNP and Gene Array data are available for selected cases. The impact of the NNTC is demonstrated by the highly effective and efficient use of high quality tissue and data to as many investigators as possible. The NNTC resource has been used by 203 investigators, with over 315 reported journal publications by external as well as internal investigators.

P35

Glutamate excitotoxicity is involved in motor dysfunctions and paralysis following infection by a human respiratory coronavirus

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Human coronaviruses (HCoV) are respiratory pathogens. We have reported that strain HCoV-OC43 can infect human neuronal and glial cells, activate neuroinflammatory and neurodegenerative mechanisms, thus possibly be involved in neurological disease of unknown etiology, such as multiple sclerosis (MS). Using an animal model, we reported that a viral persistence-associated point mutation in the viral spike S glycoprotein (Y241H) modified neuropathology from encephalitis to MS-related hind-limb paralysis. Viral CNS infections may induce excitotoxicity, a pathological process by which neurons are damaged and die following an excessive stimulation by the glutamate neurotransmitter on its specific ionotropic receptors (AMPAr and NMDAr). We have previously shown that the paralytic disease induced by the HCoV-OC43 S mutant involves (AMPAr)-mediated glutamate excitotoxicity.
Given that overactivation of the N-methyl-D-aspartic acid receptor (NMDAr) may also lead to excitotoxicity, pharmacological research has focused on the development of NMDAr antagonists. Memantine is one such molecule: it modulates glutamate excitotoxicity and is widely used for treatment of human neurological disorders such as MS. We now show that a low dose of memantine (1 μg/g body weight) improved clinical scores related to paralytic disease in mice infected by the HCoV-OC43 S mutant, without affecting viral replication. However, a 10-fold higher dose attenuated both the clinical scores, related to paralysis and motor dysfunctions, and mortality rates. Memantine attenuated virus replication in a dose-dependent manner in cell culture, compared to dizocilpine (MK-801), another NMDAr antagonist, which neither affected virus replication nor reduced mortality rates. Studies are currently underway to investigate the mechanisms underlying such dose-dependent neuroprotective and antiviral activities of memantine. (Supported by operating grant MT-9203 from Canadian Institutes of Health Research (CIHR) to Pierre J. Talbot, who is the holder of the Tier 1 (Senior) Canada Research Chair in Neuroimmunovirology award. Elodie Brison acknowledges a doctoral studentship from the MS Society of Canada).

P36
Analysis of JCV regulatory region in the urine of PML patients with different clinical outcomes

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Introduction: The JC virus(JCV) regulatory region(RR) exhibits sequence variability determining viral pathogenicity. The stable archetype RR is found in the urine of healthy and immunosuppressed individuals. Conversely, the RR found in CNS of progressive multifocal leukoencephalopathy(PML) patients contain rearrangements. We analyzed JCV RR sequences in urine samples of PML patients with different clinical outcomes.
Methods: We collected urine from 33 patients, including 12 PML-survivors(alive more than one year after disease onset), 14 PML-progressors(deceased within one year of disease onset), and 7 HIV + patients. Two urine samples were collected(mean of 10 months apart) from 13 patients(12 PML-survivors, 1 PML-progressor). JCV DNA was quantified by qPCR and RR was amplified from positive samples by nested PCR. A total of 127 RR clones were sequenced. Rearrangements were defined as duplication within the 98 bp element while any other duplication or deletions compared to archetype were classified archetype-like.
Results: JCV DNA was detected in 24/39(61%) of PML patient samples(median 4.7×104copies/ml, range 4.2×101-4.4×108), including 7/15(47%) PML-progressor samples (median 2.59×105copies/ml, range 2.54×102-4.39×108) and 17/24(71%) PML-survivor samples(median 1.51×104copies/ml, range 4.16×101-9.79×107). JCV was detected in 4/7(57%) of HIV + samples(median 3.78×104copies/ml, 1.35×102-5.96×107). Archetype-like RR was found in 11/33 (33%) clones from six PML-survivors and 2/31(6%) clones from four PML-progressors($p=0.01$). RR rearrangement was found in 5/5(100%) clones from one PML-progressor. Archetype RR was found in all 15 clones from three HIV + patients. Of four PML-survivors with urine samples at two time points, three kept stable JCV RR(2 archetype, 1 archetype-like) and one had archetype-like RR that transformed to archetype.
Conclusions: RR rearrangement was found in 1/11(9%) of PML patient samples or 5/69(7%) of clones. Archetype-like JCV RR was found more frequently in PML-survivors than in PML-progressors. These data suggest that minor alterations in archetype RR are not detrimental for survival and indicate that RR rearrangements may be found in urine of PML patients.

P37
Nonmuscle myosin light-chain kinase mediates microglial migration induced by HIV Tat: Involvement of β1 integrins

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Introduction: HIV Tat released from HIV-infected cells has been shown exhibit chemotactic activity. Activated microglia are a hallmark feature of HIV-associated neurological disorder (HAND). These cells can migrate to the signal elicited by HIV Tat released from infected cells. We hypothesized that the microglial adhesion/migration mediated by Tat will involve actin rearrangement and the activation of its upstream non-muscle myosin light chain kinase (MYLK) and integrins.
Methods: Microglial migration was assessed by both Boyden and Dunn Chamber. Role of Cdc42 was assessed by GLISA. F-actin polymerization was examined by palladin staining and flow cytometry. HIV Tat was stereotactically injected in the mice hippocampus followed by transplantation of AAV-GFP transduced mice microglia in the corpus calloseum.
Results: Intra-hippocampal injection of HIV Tat1-72 into mice resulted in migration of AAV-GFP transfected microglia to the Tat-injected site. Molecular mechanisms of this process involved Tat-mediated activation of VEGFR1 receptor, leading to inside-out activation of MYLK & β1 integrin, resulting subsequently in outside-in activation of the downstream Pyk2, Src, and Cdc42. This ultimately culminated into actin rearrangement and the ensuing migration of microglia.
Conclusions: Our findings for the first time have identified two novel phenomena: a) RGD-independent activation of β1 integrin by HIV Tat and, b) Involvement of MYLK in Tat-mediated microglial migration.

P38
"Communication is life": Elucidating the role of synaptic microRNAs in chronic Methamphetamine associated CNS dysfunction

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Drug abuse notably, methamphetamine (METH) abuse continues to ravage the United States as well as globally, damaging the individual user and increasing societal economic burden. Our studies focus on defining how chronic METH abuse apart from leading to neurodegeneration, has an array of devastating effects such as predisposing individuals to stroke, seizures, movement disorders and behavioral changes. Importantly, the behavioral changes can persist for years after drug use is discontinued and can precipitate relapse. Studies in our lab are focused on understanding the changes occurring at the level of the synapse- key structures involved in neurotransmission and neuroplasticity. In the brain, microRNAs are emerging as important regulators of synaptic plasticity especially in the dendritic synthesis of proteins, whereby synaptic stimulation activates the local translation of mRNAs stored close to the synapse. Our preliminary studies on synaptosomes (isolated synapses containing the pre and post synaptic components) from autopsied brains have identified a set of miRNAs enriched in the synaptic fraction. We hypothesize that the underlying epigenetic state of that gene is a crucial determining factor. One key question we are interested is what controls the formation and maintenance of distinct epigenetic states at particular genes especially at the synapse? Importantly, in a pathological state such as chronic METH abuse, what are the underlying intracellular signaling cascades that transduce the initial drug action at the neurotransmitter-receptor level to the neuronal nucleus to regulate the epigenetic state of specific subsets of genes? We believe as clues on transcriptional and epigenetic mechanisms of chronic drug abuse (here METH) accumulates, integrating this information with that obtained from post-transcriptional (translational and post-translational) regulation will be vital to obtain a complete understanding of how chronic METH abuse changes the brain thus leading to neurodegeneration as well as behavioral changes.

P39
Disparities in neuroAIDS: a training institute responds

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The current landscape of HIV infection requires that neuroAIDS research respond to a vastly underserved reality: health disparities and comorbid medical conditions. There is a pressing need to train investigators in the fundamentals of neuroAIDS disorders and to raise awareness of comorbidities prevalent in HIV + racial and ethnic minority (REM) communities. To this end, the NIMH-funded Mount Sinai Institute for NeuroAIDS Disparities (MSINAD) was deployed as a training program to develop disparities-focused investigators, particularly those from REM backgrounds.
Recruitment occurred through direct communication with neuroAIDS investigators and directors of minority training programs, print ads, listserve postings, solicitation at conventions and through our website. Selected applicants were invited to the 6-week institute for intense multi-disciplinary didactic seminars, a translational research experience, individualized career development sessions and coaching, a mentoring team with follow-up for two years, and a $20,000 - $25,000 pilot

grant. MSINAD has held 3 institutes since 2008 and received a total of 27 applications from junior investigators at 16 institutions. We experienced a significant increase in applications from REM applicants: from 43% in 2008 to 71% in 2012. There was significant diversity in applicants' training level, discipline and MD/PhD orientation. We have graduated 12 scholars, who have persisted in neuroAIDS disparities research with great success: 75% applied for external funding, 50% were awarded funding (e.g., pilot grants or NIH K-series awards), and there have been 35 manuscripts published or submitted for review.

The MSINAD provides an innovative and comprehensive approach to training in neuroAIDS disparities and has demonstrated feasibility, sustainability, and the successful development of junior investigators, particularly from REM backgrounds. Over the course of its funding cycle, it is expected that MSINAD will continue to contribute to the diversity of a new neuroAIDS workforce, stimulate widespread interest in the field and heighten attention to underserved populations and conditions.

P40
CXCL12-Mediated T Cell Transmigration Across the BBB Is Increased By Dopamine

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Antiretroviral therapy has increased the life span of HIV-infected individuals but the prevalence of neurocognitive impairments is increasing as infected individuals live longer. A major contributor to HIV-associated neurocognitive disorders (HAND) is chronic neuroinflammation in response to viral infection of the CNS. A significant number of HIV-infected individuals are also drug abusers and HAND in many of these individuals often occurs at earlier time points and is more severe than in the non-abusing infected population. Drugs of abuse, including cocaine and methamphetamine, increase extracellular dopamine in the CNS. In HIV-infected drug abusers, increased CNS accumulation of T cells was reported. The chemokine CXCL12 (SDF-1), a T cell chemoattractant, is constitutively expressed in the CNS and is increased with HIV infection. We hypothesize that dopamine increases CXCL12-mediated T cell transmigration across the blood brain barrier (BBB). Using our in vitro model of the human BBB, we showed a significant increase in resting human T cell transmigration in response to CXCL12 and dopamine when compared to CXCL12 alone. Dopamine by itself does not increase transmigration and does not alter T cell expression of the CXCL12 receptor, CXCR4, as determined by flow cytometry (FACS). The migratory phenotype of T cells in response to CXCL12, as detected by actin and tubulin staining, is increased by dopamine. D4 dopamine receptor (D4R) is detected on the surface of T cells of all donors analyzed by FACS and is significantly decreased with PHA and IL-2 activation. In some donors, D5R is detected on resting T cells and D1R and D5R on activated T cells. The contribution of specific dopamine receptors to resting, as well as activated, T cell transmigration will be determined. Thus, dopamine may increase CXCL12-mediated T cell influx into the CNS, contributing to increased neuroinflammation in HIV-infected drug abusers.

P41
CCL5 expression in the rat brain is mediated by morphine abuse, withdrawal and maintenance

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Intravenous drug abuse is a common practice observed in HIV infected individuals. The use of opioids such as heroin and morphine can cause distinct physiological states including tolerance, dependence and withdrawal. Each state may have differential effects on chemokine and cytokine expression, thereby modifying disease progression. The relationship between these states and how they may affect HIV neurotoxicity remains undefined. To address this, we incorporated three morphine treatment paradigms to emulate drug abuse, withdrawal, and maintenance therapy in rats. The in vivo expression of CCL5, a chemokine possessing both pro-inflammatory and protective properties, was examined in the cortex, striatum, hippocampus and cerebellum. Additionally, because CCL5 is a chemoattractant for microglia and astrocytes, antibodies against Iba-1 and GFAP were used to determine cell number and morphology. The morphine abuse paradigm resulted in a two-fold increase in CCL5 protein and mRNA expression in the cortex and striatum. This treatment, however, did not affect classical pro-inflammatory cytokines IL1-β and TNF-α. Microglia and astrocyte number and morphology remained comparable between control and morphine abuse. The morphine withdrawal paradigm resulted in a significant decrease of CCL5 expression in cortex, with a return to basal levels in the striatum. Additionally, a reduction of GFAP labeled

astrocytes within the cortex was observed. The morphine maintenance paradigm failed to alter CCL5 levels or induce a glial response. Finally gp120Bal, a neurotoxic protein from the HIV envelope, was injected into the striatum of rats undergoing the morphine abuse paradigm. There was no increase in caspase-3 immunoreactivity in morphine treated animals compared to control, suggesting that CCL5 does not potentiate neuronal loss caused by gp120Bal. These data suggest that each paradigm possesses a unique profile of CCL5 and glial responses which may affect the pathogenesis of HIV within the CNS.

P42

JC virus load in cerebrospinal fluid and transcriptional control region rearrangements may predict the clinical course of progressive multifocal leukoencephalopathy

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Progressive multifocal leukoencephalopathy (PML) is a severe disease of the central nervous system (CNS), caused by infection with the Polyomavirus JC virus (JCV). Because there are no known treatments or prognostic factors, we performed a long-term study focusing mainly on cerebrospinal fluid (CSF) samples from PML patients to describe the virological features akin to the different forms of the disease. Twenty-eight PML patients were enrolled: 10 HIV-1+ patients with classical PML (CPML), 9 HIV-1+ patients with slowly progressing or stable neurological symptoms (benign PML), 3 HIV-1+ asymptomatic patients and 6 HIV-1-negative patients. CSF, urine and blood samples were collected at the enrollment (baseline) and every six months afterwards when possible. The JCV DNA and HIV-1 RNA loads were determined, and the JCV strains were characterized.

At baseline , the mean CSF JCV load was log 6.0±1.2 copies/ml for CPML patients, log 4.0±1.0 copies/ml for benign PML patients, log 4.2±0.5 copies/ml for asymptomatic PML patients and log 5.8±1.3 copies/ml for HIV-1-negative PML patients (CPML versus benign: $p<0.01$; CPML versus asymptomatic: $p<0.05$; HIV-1 negative versus benign: $p<0.01$). Organization of the JCV transcriptional control region (TCR) showed unusual archetype structures in 2 long-term survival patients; the NF1 sequence was found most commonly, whereas the Sp1 binding site was the most common for both CPML patients and HIV-1 negative patients. Our results suggest that the JCV load in the CSF and the organization of the TCR should be considered as indicators of PML clinical outcome.

P43

Buprenorphine, a therapy for opiate addiction reduces the inflammatory response of monocytes characteristics of NeuroAIDS and alters the BBB

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Human immunodeficiency virus 1 (HIV-1) enters the CNS early in the course of infection and results in greater than 50% of HIV infected people´s having neurocognitive impairment, called HIV-Associated Neurocognitive Disorders (HAND), despite successful antiretroviral. Many HIV infected opiate abusers have increased inflammation that contributes to HAND. Buprenorphine (bup) is used to treat opioid addiction. The impact of bup on the transmigration of HIV infected monocytes across the Blood Brain Barrier (BBB) that contribute to inflammation are unknown. We are studying the effects of bup and CCL2, a chemokine elevated in the CNS of HIV infected people, on junctional proteins of human monocytes and brain microvascular endothelial cells (BMVEC) necessary for monocytes to cross the BBB. We hypothesize that bup will alter CCL2mediated changes in junctional proteins on monocytes and BBB to reduce the ability of monocytes to transmigrate into the CNS across the BBB. We showed that CCL2 increased HIV infected monocyte transmigration across BBB. We found that CCL2 increases JAMA phosphorylation while CCL2 + bup decreases this effect. As this phosphorylation is associated with migration, bup could decrease neuroinflammation. CCL2 induces chemotaxis and cell projections

on monocytes which are associated with a migratory phenotype, CCL2 + bup decrease these effects. By proteomics we quantified membrane peptides of monocytes and BMVEC and showed differences in phosphorylation of leukosialin and cavin-2, respectively, between CCL2 and bup + CCL2 treatment. Our data demonstrate important mechanisms by which buprenorphine may impact NeuroAIDS.
This work was supported by NIDA, Grant # 5P20DA026149-02 Einstein Proteomics Center for Study of the Neurological Consequences of HIV and Substance Abuse.

P44
Distinct interferon responses in neurons may contribute to survival during viral infection

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Despite advances in immunization, viral infections of the central nervous system (CNS) remain a devastating cause of encephalitis and neurodegeneration, particularly in the young, elderly, and immunocompromised. Neurons are principally non-renewable and traditional mechanisms of viral clearance that are employed in the periphery, such as cytolysis of infected cells, could prove detrimental if targeted towards CNS neurons. While it is clear that the immune system can limit viral spread in the brain, the mechanisms by which infected neurons respond to the inflammatory environment created by immune infiltration into the brain remain largely undefined. Interferon-gamma (IFNγ) and interferon-beta (IFNβ) are potent anti-viral cytokines that classically signals through activation of STAT-1 (IFNγ) or STAT-1 and STAT-2 (IFNβ). Recently, we have shown through western blotting that basal STAT-1 levels in primary mouse hippocampal neurons are drastically reduced as compared to control mouse embryonic fibroblasts (MEFs). Consequently, phosphorylation and nuclear localization of STAT-1, as detected by western blot and immunofluorescence, is substantially muted and delayed in hippocampal neurons treated with IFNγ. Nevertheless, IFNγ treatment protects primary neurons from measles virus (MV) infection. Conversely, the neuronal STAT-1 and STAT-2 responses to IFNβ do not differ significantly from that in MEFs. However, while IFNβ has an inhibitory effect on MV replication in neurons, qPCR analysis has revealed the pattern of interferon-stimulated gene (ISG) expression in neurons in response to interferon and poly(I:C) treatment differs significantly from that in MEFs. These data suggest that neurons utilize distinct signaling responses to anti-viral cytokines. We propose that these cell type-specific differences in type I interferon responsiveness play a key role in the ability of neurons to survive viral infection.

P45
Unveiling the Roles of Autophagy in Mouse Hepatitis Virus Induced Demyelination

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Neurotropic Mouse hepatitis virus (MHV) infection in mice causes meningoencephalitis, myelitis with subsequent axonal loss and demyelination. Direct virus-mediated axonal damage can occur concurrently with and independently of demyelination and serve as a virus-induced model of multiple sclerosis. Our current studies using MHV-A59 or its isogenic spike protein recombinant strain, RSA59, exhibit that CNS demyelination involves a mixed population of inflammatory cells, predominantly macrophages/microglia and a smaller population of T lymphocytes. Evaluation of axonal loss and demyelination in the spinal cord, demonstrates that neurotropic MHV infection begins in the neuronal cell body and propagates centripetally to the axon with subsequent axonal degeneration and demyelination. Neurotropic MHV strains also induce optic neuritis by retrograde spread from the brain through the optic nerve into the eye after intracranial inoculation. Migration and activation of macrophages/microglia in the white matter of the optic nerve and spinal cord following viral spread suggests that recruitment of these cells mediates demyelination. During chronic infection, macrophages/microglia remains present within areas of demyelination. Ultrastructural studies reveal macrophages can surround myelinated axons with the myelin unravelling, yet the axon is completely intact. The macrophage cell membrane is in intimate contact with the outer portion of the myelin sheath and engulfs the myelin sheath. Therefore,

one mechanism of demyelination involves macrophage-mediated myelin stripping through an autophagy pathway. Affymetric Microarray analysis of mRNA expression from MHV infected tissues reveals upregulation of several molecules of classical signalling pathways that regulate autophagy and interferon (IFN)-gamma production. IFN-gamma can activate microglia by promoting phagolysomes maturation and autophagy. The autophagic sequestration of viral components can also fuel MHC Class-II presentation of MHV antigen and regulate production of Type I IFNs. Evidence of molecular pathways driving autophagy in MHV infection advances our understanding of mechanisms of how viral induced demyelination can occur without involving conventional αβT cells.

P46

Programming of neurotoxic cofactor CXCL-10 in HIV-1 associated dementia: Abrogation of CXCL-10 –induced neuro-glial toxicity in vitro by PKC activator

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More than 60% patients undergoing antiviral treatment for prolonged HIV-1 infection develop HIV-1 —associated neurocognitive disorders (HAND). Neurological complications during HIV-1 infection are the result of direct neuronal damage by proinflammatory products released from HIV-1 infected or uninfected activated-macrophages, -microglia and -astrocytes. In the current study, Bioplex array showed elevated levels of signatory chemokines/cytokines (—IL-6, IFN-γ, CXCL10, MCP-1 and PDEGF), in the cerebro-spinal fluid (CSF) of HIV-1 infected demented (HIV-D) patients (n=7) but not that of HIV-1 infected non-demented (ND) patients (n=7). Consistently, HIV-infected macrophages or astrocytes treated with HIV-1 and TNF-α, induced the signatory molecules. Among the signatory cytokines and chemokines, CXCL10 was distinctly upregulated over 1000- fold in HIV-1 activated astrocytes and HIV-1 infected macrophages. CXCL10 in combination with HIV-1 synergistically enhanced neuronal toxicity and showed chemotactic activity (~ 40 fold) for peripheral blood mononuclear cells (PBMC). This suggested the intersection of signaling events imparted by HIV-1 and CXCL10 after binding to their respective surface receptors CXCR4 and CXCR3, present on neurons. In accordance, blocking CXCR3 and its downstream MAP kinase (MAPK) signaling pathway, suppressed neurotoxicity. Bryostatin, a PKC modulator and suppressor of CXCR4 (Mehla et al., 2010) conferred neuroprotection against HIV-1 and CXCL10. Bryostatin also suppressed HIV-1- and CXCL10- induced PBMC chemotaxis. In conclusion, we have demonstrated induction of CXCL10 and other chemokines/cytokines during HIV-1 infection in the brain, as well as synergism of CXCL10 with HIV-1 in neuronal toxicity, which was dampened by bryostatin

P47

Relationships between genetic markers of neuroinflammation, neurodegeneration and neurocognitive impairment in controlled HIV infection

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Background: The role of neuroinflammatory genetic markers in HIV-related neurocognitive impairment may inform on new pathogenic mechanisms.
Methods: 103 HIV + individuals aged >45 years were enrolled with historically advanced disease. All were stable on HAART and 98 % had undetectable plasma HIVRNA. All underwent a standard neuropsychological (NP) assessment, assessing 7 cognitive abilities. Two NP summary scores were generated, the global Mean-T score to capture overall neurocognitive performance, the Global Deficit Score (GDS) to capture degree of cognitive impairment. NP-impaired versus NP-normal were classified according to the GDS ≥.05 method (21% were NP-impaired). DNA was extracted from saliva and thirteen SNPs were genotyped using TaqMan assays (Applied Biosystems). Repeats and non-template controls were included in each run. Median (range) genotyping success rates were 99% (98%–100%). Carriage of the minor allele of each SNP was compared against the neurocognitive scores.
Results: APOE allele 2 (12%) was associated with better overall neurocognitive performance ($p<0.01$). CCR2V64I allele 2 carriers (19%) were slightly more NP-impaired (35% vs 18%; $p=0.09$) than those with allele 1. IL1A +4845 allele 1 carriers (63%) were more likely to be NP-impaired (28% vs 10.5%; $p=0.04$) than allele 2 carriers. IKBL +446 allele 1 (71%) was associated with greater NP-impairment based on the GDS ($p=0.02$). IL1A +4845 allele 1 carriers had slightly lower nadir CD4 than allele 2 carriers (164 vs 209; $p=0.07$). IKBL +446 allele 2 carriers had higher CD8 counts (1070 vs 855; $p<0.03$) and had more AIDS-defining illnesses ($p<0.05$).
Conclusion: The involvement of APOE and CCR2 V64I minor alleles are consistent with increased risk for cognitive

progression in non-HIV and HIV-dementia cohorts. SNPs in TNF and adjacent genes have been associated with other inflammatory diseases (diabetes and asthma) and may be linked to increased co-morbidities role in HAND pathogenesis. As the TNF block has high linkage disequilibrium, haplotypic analyses will be conducted.

P48
The role of Bim in virus-induced apoptosis within the central nervous system

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Reovirus infection of the mouse brain and spinal cord provides a classical model system for the study of viral pathogenesis within the central nervous system (CNS). Reovirus infection of the mouse CNS results in neuronal apoptosis leading to tissue injury and disease. Bax is a pro-apoptotic Bcl-2 family protein present on the mitochondrial membrane. When activated Bax forms homo (Bax-Bax) or hetero (Bax-Bak) dimers which create a pore in the mitochondrial membrane through which pro-apoptotic factors, including cytochrome c and second mitochondrial-derived activator of caspase (SMAC) are released. We have recently shown that Bax is activated in the brain following reovirus infection and contributes to viral pathogenesis. Bax can be activated by members of the Bcl-2 homology domain 3 (BH3) only family of Bcl-2 proteins. We now show that the BH3 only Bcl-2 family protein Bim (Bcl-2 interacting mediator of apoptosis) is up-regulated in the brain following infection with reovirus. An increase in Bim mRNA can be detected in the brains of mice infected with reovirus. In addition, Bim is up-regulated at the protein level and migrates to the mitochondrial membrane in infected cells where it co-localizes with Bax. Bim is also up-regulated in the brains of mice infected with West Nile virus suggesting that the up-regulation of Bim is a common pathway for initiating neuronal apoptosis following virus infection of the CNS.

P49
Dopamine mediated changes in the blood brain barrier and neuroinflammation in the context of CNS HIV infection and substance abuse

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CNS complications due to HIV infection persist, even with current antiretroviral therapy. HIV enters the CNS within two weeks of primary infection by the transmigration of infected monocytes across the blood brain barrier (BBB). Once in the CNS, these cells produce virus and neurotoxic factors that infect and/or activate perivascular macrophages, microglia and astrocytes. The resulting neuroinflammation leads to HIV- associated neurocognitive disorders (HAND), which encompasses CNS abnormalities including forgetfulness, inability to follow directions, and impaired decision making. A more mature monocyte expressing CD14 and CD16 is permissible to HIV infection and preferentially transmigrates across the BBB. Many drug abusers have higher monocyte and T-cell infiltration into the CNS, which may contribute to altered neurocognitive deficits. Drugs of abuse such as methamphetamine and cocaine increase extracellular dopamine in the CNS. In addition, SDF-1, a chemokine expressed in the CNS that is chemotactic for T-cells and monocytes, is also increased during HIV infection. Our lab demonstrated that dopamine increases SDF-1 mediated transmigration of CD14+CD16+ monocytes across an in vitro BBB model. We hypothesize that increased extracellular dopamine in the CNS of HIV-infected drug abusers increases SDF-1 mediated transmigration of CD14+CD16+ monocytes, facilitating entry of HIV into the CNS and contributing to the chronic neuroinflammation that causes HAND. We are determining the concentrations of SDF-1 and dopamine for maximal transmigration of HIV infected and uninfected monocytes across our tissue culture model of the human BBB. The expression of dopamine receptors on monocytes is being determined using flow cytometry and qRT-PCR. We are also examining brain microvascular endothelial cells for the effects of SDF-1 and dopamine on junctional proteins JAM-A, Occludin, Claudin-5, ALCAM and PECAM that maintain BBB integrity and facilitate transmigration.

P50
Endoplasmic reticulum (ER) stress in cocaine induced microglial cell death

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Although it has been well documented that drugs of abuse such as cocaine cause enhanced progression of human immunodeficiency virus (HIV)-associated neuropathological disorders, the underlying mechanisms mediating these effects remain poorly understood. The present study demonstrated that exposure of microglial cell line-BV-2 cells to cocaine resulted in decreased cell viability as determined by MTS and TUNEL assays. Microglial toxicity of cocaine was accompanied by an increase in the expression of cleaved caspase-3 as demonstrated by western blot assays. Furthermore, increased microglial toxicity was also associated with a concomitant increase in the production of intracellular reactive oxygen species, an effect that was ameliorated in cells pretreated with NADPH oxidase inhibitor apocynin, thus underscoring the role of oxidative stress in this process. A novel finding of this study was the involvement of ER signaling mediators such as Elf2α, PERK, and CHOP, that were upregulated in cells exposed to cocaine. In conclusion these findings underscore the importance of a robust microglial innate immune response, which in presence of cocaine is dampened due to microglial toxicity. Understanding the link between ER stress, oxidative stress and apoptosis will lead to the development of therapeutic strategies targeting cocaine-mediated microglial death/dysfunction.

P51
Web-based collection of antiretroviral medication histories

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Background: In the HAART era, collection of longitudinal antiretroviral medication (ARV) histories from HIV-infected individuals has become increasingly important. Using technology, it is possible to increase the efficiency with which medication histories are obtained and summarized.
Methods: The HIV Neurobehavioral Research Program (HNRP) DMIS Group developed a web-based touchscreen-enabled iPad application. The interface displays medication use reported at previous visits and uses actual pill images to facilitate accurate participant reports. Data is subjected to real-time QC checks, accounting for clinically unlikely regimens, patient history, and medication availability. The raw data is then dynamically transformed into medication instances (MI = uninterrupted period of use for a particular drug). These MI represent drug use as a set of overlapping epochs that can be presented graphically or linked to other data (such as neurological or medical complications). Because this application has been developed for the web, hardware and software requirements are minimal and training time should be greatly reduced. Availability of this information via the web could potentially extend the use of this application beyond research to clinical settings.
Results: Development of the application is complete and existing ARV data has been transformed into the new data structure. This data includes the lifetime medication histories of 3,911 patients in support of 24 studies. The application is currently in use at the HNRP, replacing traditional CRFs. With this release, we anticipate accuracy and efficiency of data collection to improve dramatically, in terms of time spent by participants, accuracy of neuromedical assessment, data entry and analysis. Please explore the application through the attached kiosk or by visiting http://hnrp.hivresearch.ucsd.edu/arvweb/.
Conclusions: Using real-time data checks, a flexible data storage model and user-friendly visuals, we have been able to provide an easily deployed, accurate, low-cost solution for the collection of medication history data.

P52
Macrophage infiltration of the brain in Theiler's virus infection contributes to the development of seizures

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Viral infections of the central nervous system (CNS) trigger anti-viral host defense cells, initiating an inflammatory cascade to control viral replication and dissemination. The extent of the proinflammatory response in the CNS and the timing of the release of proinflammatory cytokines can lead to neuronal excitability. Both tumor necrosis factor (TNF)-α and interleukin (IL)-6 proinflammatory cytokines have been linked to the development of seizures in the Theiler's murine encephalomyelitis virus (TMEV)-induced seizure model. It is still unclear the extent to which the infiltrating macrophages, vs. resident CNS cells, contribute to acute seizures. Many cell-types can produce TNF-α and IL-6. In this study, we show that a significantly higher number of microglia produce TNF-α compared to infiltrating macrophages. In contrast, infiltrating macrophages produced significantly more IL-6. Mice treated with minocycline and wogonin, both of which are compounds that have neuroimmunomodulatory properties, have significantly fewer macrophages infiltrating into the brain and significantly fewer seizures. Therefore, we have identified infiltrating macrophages as an important immune cell contributing to the development of acute seizures.

P53
Characteristics of neurocognitive decline in virally-suppressed middle-aged HIV + individuals

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Background: The extent and characteristics of neurocognitive decline remain unclear in virally suppressed HIV + individuals. Methods: 58 HIV + individuals aged 45+ were enrolled with historically advanced disease (nadir CD4≤350 cells/μl; current stable cART≥6 months, screened for major neurological and psychiatric confounds, and undetectable plasma HIVRNA across the study period). All underwent a standard neuropsychological (NP) assessment, sensitive to HIV-related impairment that included 10 outcome measures at baseline and 18 months follow-up. Individual regression-based summary change scores were computed based on published normative change data. Four different classifications for neurocognitive decline were used to assess their associations with disease factors. Three involved increasingly stringent overall decline definitions (90% confidence interval [CI], 2-tailed, around the normative regression-based summary change score; 80% and 70%) and one definition was based on≥-1SD in 4/10 outcome measures to capture decline in specific NP measures.
Results: The 90% CI cut-off yielded 7% overall decline; the 80% CI, 10%, and the 70% CI, 14%. The minus 1SD in 4/10 measures cut-off yielded 20% of decline. Among the systemic HIV factors, a lower baseline CD8 was associated with all decline definitions ($p<.05$), but the minus 1SD in 4/10 measures cut-off definition. Past CVD events including abnormal lipid values and hypertension were associated with decline only when using the 7% decline ($p=.05$). Older age (60+) was associated with decline only when using the 7% decline. Baseline depression was not associated with any definition of decline.
Conclusion: Despite viral suppression neurocognitive decline occurs and at significant rates. Even milder forms of decline appear to be associated with long-term immune dysregulation. Longer longitudinal follow-up is warranted as older age and the long-term effect of CVD are already associated with decline after 18 months. Further, these decline data can be used as efficacy tools in clinical trials.

P54
Analysis of Pur-gamma expression and interaction with HIV-1 Tat partner Pur-alpha

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Analysis of Pur-gamma Expression and Interaction With HIV-1 Tat Partner Pur-alpha Ayuna V. Dagdanova, Brian C. Temple, Edward M. Johnson and Dianne C. Daniel. Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, VA 23507 Pur-alpha is a demonstrated activator of HIV-1 Tat-dependent transcription. Little is known about a related Pur family member Pur-gamma. Pur-gamma has three nucleic acid-binding amino acid domains, as does Pur-alpha, but Pur-gamma has different intervening amino acids, pointing to differences between the Pur proteins in cell function. Pur-gamma has been thought to be an embryonic form that is replaced by Pur-alpha in the postnatal mouse. Previous studies indicate relatively high expression of Pur-gamma during mouse embryogenesis between day E14 and birth, after which its levels decline, and those of family member Pur-alpha increase. After our recent finding that Pur-gamma is expressed in oligodendroglioma cells, we hypothesized that this protein might be expressed under special circumstances in adult tissues. Coimmunoprecipitation experiments reveal interaction of Pur-alpha and Pur-gamma in KG-1 oligodendroglioma cells. There is also coimmunoprecipitation of Pur-alpha and Pur-gamma in an astroglioma cell line, U-87 MG. Localization is cell-type specific, however, since Pur-gamma is mainly cytoplasmic in the U-87 MG cells as compared to a mainly nuclear localization in the oligodendroglioma cells. In summary, the non-embryonic, neoplastic cells display expression of Pur-gamma, at least a fraction of which interacts with Pur-alpha. We are currently investigating the interaction of Pur-alpha and Pur-gamma in the context of HIV-1 infection, in which Pur-alpha is known to function with Tat and to influence replication and transcription of HIV as well as the replication of JCV in PML in AIDS. Mutations in Pur-gamma and Pur-alpha allow us to examine the interaction of the Pur proteins with each other and with HIV-1 Tat.

P55
HIV-1 transcriptional regulation by C/EBP and NFAT via a novel downstream element in the LTR in cells of the monocyte-macrophage lineage

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Cellular and viral factors regulate HIV-1 transcription by binding to DNA elements present both upstream and downstream of the transcriptional start site in the long terminal repeat (LTR). TRANSFAC analysis of the HIV-1 subtype B LAI LTR revealed three potential downstream CCAAT enhancer binding protein (C/EBP) binding sites (DS1-3). The DS3 element is present immediately downstream of nucleosome-1 in the HIV-1 LTR, which suggested that it may have a functional role in regulating production of HIV-1 transcripts. Analysis across subtypes of HIV-1 indicated that the DS3 element has a high degree of conservation both in terms of nucleotide sequence and physical location in the LTR. Interestingly, this element overlaps with the previously identified AP3-like element, which has been shown to bind members of the nuclear factor of activated T-cells (NFAT) family of proteins. Results indicated that NFAT has a higher relative affinity for this element as compared to members of the C/EBP family. It was also observed that this element was able to compete with a low affinity upstream C/EBP binding site I (US1) with respect to C/EBP binding, suggesting utilization of both NFAT and C/EBP. In addition, an LTR containing single point mutation (T to C change at position 9) in the DS3 element that compromise binding of its cognate factors shows reduced transcriptional activity suggesting that DS3 serves as a positive regulator of LTR-directed transcription. The relative importance of NFAT and C/EBP with respect to DS3-mediated transcription and identification of relevant isoforms of C/EBP and NFAT is currently under investigation. Moreover, extracellular signals like cytokines (for example, IL-6) regulate this process by modulating the expression of regulatory transcription factors including C/EBP, especially in cells of the monocyte-macrophage lineage.

P56
JC virus encephalopathy is associated with a novel Agnoprotein-deletion JCV variant

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JC virus encephalopathy (JCVE) is a newly described gray matter disease of the brain caused by productive infection of cortical pyramidal neurons. We characterized the full length sequence of JCV isolated from the brain of a JCVE patient, analyzed its distribution in various compartments by PCR, and determined viral gene expression in the brain by immunohistochemistry(IHC). We identified a novel JCV variant, JCVCPN1, with a unique 143 bp deletion in the Agno gene encoding a truncated 10 amino acid peptide, and harboring an archetype-like regulatory region. This variant lacked one of three nuclear protein binding regions in the Agno gene. It was predominant in the brain, where it coexisted with an Agno-intact wild-type strain. Double immunostaining with anti-Agno and anti- VP1 antibodies demonstrated that the truncated JCVCPN1 Agno peptide was present in the majority of cortical cells productively infected with JCV. We then screened 68 DNA samples from 8 brain, 30 CSF and 30 PBMC samples of PML patients, HIV + and HIV- control subjects. Another JCVCPN strain with a different pattern of Agno-deletion was found in the CSF of an HIV+/PML patient, where it also coexisted with wild-type, Agno-intact JCV. These findings suggest that the novel tropism for cortical pyramidal neurons of JCVCPN1, may be associated with the Agno deletion. Productive and lytic infection of these cells, resulting in fulminant JCV encephalopathy and death may have been facilitated by the co-infection with a wild- type strain of JCV.

P57
IRS-1 interacts with ADAM10 to prevent TNFalpha-induced structural alterations of neuronal processes

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We have previously reported that IGF-1 counteracts the retraction of neuronal processess which is mediated by TNF-alpha. We have also demonstrated that IGF-I exerts these effects via interference with TNFalpha-mediated interaction of membrane associated beta1-integrin with insulin receptor substrate 1 (IRS-1), which is a key molecule in transmitting signals from the IGF-1R to intracellular pathways. Here we show that IGF-1 prevents the negative effects of TNF-alpha on stability of neuronal processes through interaction with disintegrin and metalloproteinase

ADAM10. Immunohistochemical analysis demonstrated an increased expression of ADAM10, in the cytoplasm of neurons and enlarged reactive astrocytes and oligodendrocytes in brain tissue from patients with HIV Encephalitis (HIVE). ADAM10 was also co-localized with TNFR1 in HIVE brain tissue samples. Long-term treatment of neuronal cells with TNF-alpha results in the loss of dendritic arborization and accumulation of ROS. These effects were prevented by IGF-1 stimulation. In the presence of IRS-1 the treatment of primary cortical neurons with IGF-1 or TNF-alpha leads to an increased expression of ADAM10 and beta1-integrin in cells. Silencing of IRS-1 abrogates this induction. Interestingly, IGF-1 and TNF-alpha combined treatment has negative effect on ADAM10 and beta1-integrin expression. Silencing of IRS-1 induced activation of caspase 3, although less in cells treated with IGF-1, suggesting that even moderate amounts of IRS-1 can inhibit apoptotic signaling in neurons. We have found that ADAM10 is able to form protein complexes with IRS-1 in immunoprecipitation and GST pull down assays. While IGF-1 promotes interaction of ADAM10 with IRS-1 and beta1-integrin in plasma membranes of neuronal cells, TNF-alpha inhibits these interactions. Presented results suggest new mechanism to sustain the stability of neuronal processes mediated by IGF1/IRS-1 signaling.

This work was made possible by a grant awarded by NIH to SA.

P58

Polyomavirus JC infection of human oligodendrocyte progenitor cells dysregulates production of chemokines and alters differentiation of oligodendrocytes

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Reactivation of the human neurotropic polyomavirus, in the CNS results in a fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). The lytic destruction of oligodendrocytes, which occurs at the terminal stage of the viral infection cycle, is considered a critical factor in the development of demyelination and the pathogenesis of PML. However, there is limited knowledge about the interaction of JCV with oligodendrocytes and its impact on denudation of axons at the early stage of viral reactivation and prior to the destruction of the infected cells. Using an in vitro system that recapitulates the differentiation and oligodendrocyte specification of human neural progenitor cells derived from fetal brain we demonstrate that JC virus delays progression of oligodendroglial lineage. JCV infection suppressed expression of mRNAs for oligodendrocyte-specific proteins at all stages of differentiation, but more prominently mRNAs specific for more matured oligodendrocytes, in particular, platelet-derived growth factor alpah (PDGFRalpha), myelin basic protein (MBP) and PLP. Concomitant with the observed changes in oligodendrocyte maturation during JCV infection was a significant dysregulation of the expression of several chemokines, including RANTES and CXC ligands GRO, IL-8, IP-10, CXCL16, ENA-78, GCP-2. Our finding of JC virus-induced delay in progression of the oligodendroglial lineage provides evidence that a differentiation block of oligodendrocyte progenitor cells infected with JCV may be a critical factor in failure of remyelination in PML. This work was made possible by grants awarded by NIH to KK.

P59

Essential function of the tyrosine-based vesicle sorting signal in agnoprotein of polyomavirus JC

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Infection of glial cells in the central nervous system with the polyomavirus, JCV, is responsible for the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). In addition to the early transforming proteins and the late capsid proteins, JCV also encodes a small regulatory protein, agnoprotein, which has many functions in the viral life cycle. Agnoprotein has a distinctive subcellular distribution with a large concentration in the perinulear region and a small but significant fraction in the nucleus. We now report that agnoprotein is present in the endoplasmic reticulum, golgi apparatus and plasma membrane of cells. Expression of agnoprotein inhibited the secretion of a VSVG-EGFP reporter by blocking

its transit from the Golgi to the plasma membrane. We noted that the sequence YSAL, which corresponds to the YXXL consensus sequence for a tyrosine-based vesicle sorting signal, is found near the C-terminus (amino acids 62-65) of the 71 amino acid agnoprotein. It is known that the release of polyomaviruses such as SV40 and JCV from cells can occur without cell lysis and this likely involves vesicular transport. To investigate a role for agnoprotein in this process, we mutated the YSAL motif to ASAV. We found that this mutation had a severe effect on the viral life cycle and inhibited viral protein synthesis and DNA replication when introduced into T-antigen-expressing SVGA cells or primary astrocytes. We conclude that this motif performs an essential function in the viral life cycle. This work was made possible by grants awarded by NIH to KK.

P60

Long-acting nanoformulated anti-retroviral drugs are neuroprotective in HIV-1 infected humanized mice brains

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Background: Compliance, escape from immune surveillance and cumulative toxicities underlie treatment failures for antiretroviral therapies (ART) for HIV disease. Towards this end our laboratories developed atazanvir (ATV) and ritonavir (RTV) nanoformulations (referred as nanoART). Methods: Newborn NOD/SCID-IL-2γcnull mice were transplanted with human CD34+ hematopoietic stem cells and infected at 22-weeks of age with HIV-1ADA. Eight weeks after infection 5 mice were injected subcutaneously (sc) at weekly intervals with nanoART at 250 mg/kg ATV/RTV (1:1) for six dosages, 2 animals with 2 dosages and six animals remain as untreated control. Another set of 5 uninfected animals treated by same schedule used for the drug safety profile evaluations. Animals were sacrificed 17 weeks (16 mice) and 11 weeks (2 mice) post-infection, for analysis of tissue drug levels, viral loads and histopathology. Quantitative immunofluorescence analysis was conducted on different regions of 5-μ thick brain sections to look for the inherent expression of neuronal markers by staining for MAP2, synaptophysin, neurofilament and astrocyte specific marker GFAP. Results: All HIV-1 infected and treated animals survived the study and retained human hematopoiesis. 8 weeks after viral infection all animals showed sustained viral load with a median value of 1.63 x 105 HIV-1 RNA copies/ml. viral loads were below the level of detection after 2 injections (2 mice) and 4 injections (5 mice) and viral rebound was observed 3 weeks after nanoART cessation in 4 mice. Histopathology of nanoART treated mice brain showed significant increase in expression of MAP2 in the cerebral cortex and whisker barrels as compared to untreated animals. NanoART treatment preserved damage of human lymphoid tissue by HIV-1 and prevented reduction of CD4+ T-cells. Conclusion: The development of nanoART and its current testing in HIV-1 infected humanized mice demonstrate robust neuroprotective outcomes.

P61

Upregulation of neuronal microRNA-142 in hive/sive leads to decrease in monoamine oxidase A expression and activity by inhibiting SIRT1

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MicroRNAs (miRs) have been implicated in pathogenesis of many neurodegenerative diseases. We investigated the role of altered miR expression in HIV encephalitis (HIVE) and determined that miR-142-3p and -5p are up-regulated in HIVE and its animal model Simian Immunodeficiency Virus Encephalitis (SIVE). We hypothesized that miR-142 expression is induced within neurons as a neuroprotective response in HIVE/ SIVE. We first validated miR-142 up-regulation in the SIVE brain through hybridization combined with immunofluorescent labeling. This revealed that in SIVE miR-142-3p and -5p are expressed within neurons. Second, to elucidate the downstream effects of miR-142 over-expression, we created stable clones of the BE(2)M17 cell line expressing miR-142 or a scrambled miR (miR-null), and compared the differences in global gene expression patterns using microarray followed by validation using quantitative real time PCR. We focused on one of the genes found to be down-regulated in miR-142 expressing clones, monoamine oxidase A (MAOA). Western blot analysis confirmed decrease in MAOA protein level in miR-142 clones, as well as in human neurons transduced with miR-142. The miR-142 clones also had lower MAOA enzyme activity.
Since, MAOA 3'-UTR does not have miR-142 binding site, we predicted that MAOA must be downstream of a direct miR-142

target. SIRT1, a MAOA transcription activator, is a predicted miR-142-5p target. Western blot analysis revealed a reduction of SIRT1 in the miR-142 clones. Down-regulation of SIRT1 by miR-142 could therefore lead to the observed decrease in MAOA.
MAOA, while necessary for catabolism of neurotransmitters, generates reactive oxygen species that can contribute to cell death. We performed cell viability assay on the BE(2)M17 clones three days after serum starvation. The miR-142 clones had higher cell viability, implicating a neuroprotective role for chronic increase in miR-142 expression. Thus we have uncovered a unique mechanism for neuroprotection in a viral infection of the brain.

P62
Increased levels of a specific chemiokines profile in cerebrospinal fluid from HIV-1 patients affected with leukoencephalopathies

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Cytokines and chemochines presented a different profiles in AIDS related opportunistic infections, according to the different patterns of neuroinflammatory responses displayed by the neurological infections.
We defined the profile of 48 cytokines/chemokines in cerebrospinal fluid (CSF) and serum samples periodically collected from 8 HIV-1 positive, cART-treated patients affected with two forms of leukoencephalopathies, and 6 HIV-1 negative patients with Acute Disseminated Encephalo Myelitis (ADEM), using a magnetic bead based multiplex immunoassays (Bio-Plex®). A Q-PCR were performed for HIV-1 gag (Delbue et al., 2011) and JCV TAg (Euro-RT Polyoma Panel kit, Eurospital Spa, Trieste, Italy) detection. Of the 48 cytokine measured, a pattern of proteins including MIG, IP-10, MIP1β, GM-CSF, M-CSF, MCP-1 and IL-16 was constantly over-expressed in HIV-1 positive patients as compared with HIV- 1 negative patients. Of note, among HIV-1 positive patients, the cytokines pattern was significant different in patients suffering by PML in respect to patients with NDLE. In particular, the chemokines IL-16 and MIG were over-expressed in the PML group while the chemokines M-CSF, GM-CSF, MCP-1 and MIP-1β showed significantly higher levels in NDLE group. Focusing the attention to the cytokines/chemokines profile in PML patients with respect to NDLE patients and excluding from the analysis the patients affected by ADEM, two cytokines, MIG and IL-16, were under-expressed in NDLE compared to PML patients.
Despite of the small size of analyzed samples, results from this pilot study suggests that probably co-existing signaling pathways may be involved in the pathogenesis of AIDS-related leukoencephalopathies, and may be regulated by a different cascade of cytokines. This pilot study showed that the complex interplay between chemokines and viruses infection in microglial cells emphasized once more the role of immunoregulation in AIDS-related neurological disorders, also during cART treatment.

P63
JCV and BKV urinary excretion increases during treatment with Natalizumab

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The efficacy of Natalizumab treatment in Multiple Sclerosis (MS) patients is very high, but 201 cases of Progressive Multifocal Leukoencephalopathy (PML) have been reported worldwide among treated patients.
Forty Natalizumab-treated MS patients (10 males/30 females) and 25 MS patients (9 males/16 females), treated with conventional therapies, as controls, were enrolled in this study. Urine, serum, and peripheral blood were collected monthly to monitor JCV, BKV and herpesviruses reactivation.
Viral loads were assayed by Quantitative Real-Time PCR and viral strains molecularly characterized. The mean number of follow-up for every patient was 15 (range: 1-25); a total of

2046 clinical specimens were analyzed. On the whole, JCV was found at least once in the urine of 52.5% (21/40) patients and 16% (4/25) controls ($p<0.001$), whereas BKV was found at least once in the urine of 57.5% (23/40) patients and 8% (2/25) controls ($p<0.0001$). During the follow up, JCV urinary excretion increased in 10 patients, decreased in 3 patients and remained stable in 8 patients; BKV urinary excretion increased in 16 patients, decreased in 5 patients and remained stable in 2 patients. JCV genome was sporadically detected in serum and peripheral blood, while no herpesviruses genomes were amplified. Median viral load of JCV during all the follow up (log 7.4 copies/ml) was statistically higher ($p<0,01$) than the median viral load of BKV (log 4.6 copies/ml). All the JCV strains amplified from the urine were archetype and most (13/21) were genotype 1. As for BKV, all the amplified strains were archetype (WW), with an high prevalence of genotypes I (14/23).

The results showed that JCV and BKV replication increased during Natalizumab treatment. Even if such aspect is still difficult to explain, it add information on the issue of PML development in Natalizumab treated patients, proving the relevance of polyomaviruses monitoring in urine.

P64

Latent viral infections in young patients with inflammatory diseases treated with biological agents: prevalence of JC virus genotype 2

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Background: Biological therapy is associated with increased susceptibility to viral infections. Reactivation of JC virus (JCV) and cytomegalovirus (CMV) in adults after biological therapy has been documented.

Objectives: To determine the long-term effects of biological therapy on human herpesvirus and polyomavirus infections in young patients.

Study Design: One hundred and eighty-six samples [urine, serum, and peripheral blood mononuclear cells (PBMCs)] from 62 patients (15.8±6.2 years old) with Crohn's disease, ulcerative colitis or juvenile rheumatoid arthritis treated with immunotherapy or conventional therapy for over 12 months were tested by real time PCR. One hundred and twenty-four samples (urine and PBMCs) from 62 matched healthy volunteers (13.8±8.6 years old) were included as controls. Sequencing of the JCV viral protein 1 (VP1) and transcriptional control region (TCR) was performed.

Results: Herpes simplex virus 1/2 and varicella zoster virus genomes were not detected in any patients, whereas Epstein–Barr virus, human CMV, and human herpesvirus-6 genomes were detected in 4.8%, 3.2% and 1.6% of the patients, respectively. JCV was detected in 22.6% (14/62) of urine samples from patients and in 8% (5/62) from controls, in 50% (7/14) of sera from patients shedding JCV, and in 71.4% (5/7) of matched PBMCs. There was a significant association between infliximab treatment and excretion of JCV genotype 2.

Discussion: We demonstrated subclinical infection/reactivation of JCV genotype 2 in young patients during infliximab therapy. Conversely, we did not show increased susceptibility to herpesvirus infection. Future studies are warranted to investigate the effects of JCV reactivation on the health of young patients treated with infliximab.

P65

Effects of methamphetamine on the epigenetic regulation of HIV-1 in the human brain

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Methamphetamine (METH) is a highly addictive psychostimulant abused worldwide; its use is particularly high among persons with HIV infection.

The interactions between METH and HIV are yet not completely understood. HIV + METH abusers present more cognitive abnormalities, higher plasma viral loads and more neurological damage than non-abusers. The potentiation of HIV neurodegeneration by METH may be mediated by different molecular mechanisms, including calcium dysregulation, oxidative stress and inflammation. Moreover, METH exposure alters DNA

methylation, histone acetylation and gene expression in animal models. DNA methylation is directly linked to regulation of HIV-1 latency, while histone deacetylation is needed to sustain silencing of integrated provirus.

The goal of this study was to investigate the effects of METH on the epigenetic regulation of HIV-1 expression in the human brain. We studied 30 postmortem frontal cortex samples from persons dying with HIV of whom n=15 had histories of METH abuse (HIV + METH+) and n=15 did not (HIV + METH-). Analysis of DNMT1 expression by qPCR showed increased mRNA levels in HIV + METH + cases. Profiling of DNMT3B, an enzyme that has been reported to have redundant functions to DNMT1 in the adult brain, showed that METH abuse did not alter its expression. Analysis of the status of global methylation in the brain showed an increase in methylation in the HIV + METH + group.

Taken together our results suggest that METH-induced epigenetic changes in the human brain might be mediated by increase expression of DNMT1. Better understanding of the molecular mechanisms that govern HIV1 infection in the brain in the context of drug abuse are crucial in the design of therapeutic interventions.

This work was supported by Pilot Project Grant PST3TP1 (to PD) from the Translational Methamphetamine AIDS Research Center (P50 DA26306 to IG) and NIH Grants MH62962 (to EM) and California NeuroAIDS Tissue Network U01 MH83506 (to IG).

P66

The activation of latent herpes simplex virus and suppression of LAT and mi-RNAs in trigeminal ganglia within the time-frame of a single cycle of viral replication

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Herpes simplex virus 1(HSV-1) replicates in oral and genital mucosa but establishes a latent infection in peripheral neurons. Much of the pain and misery associated with HSV-1 infection is due to lesions caused by viruses that reactivate from latently infected neurons. The virus requires VP16, a late protein introduced into cells during infection and at least ICP0, an α protein, to initiate transcription and overcome checkpoints during productive infection. During latent infection, the neurons accumulate only a long viral transcript (LAT) and viral mi-RNAs. Since neither VP16 nor ICP0 are present in neurons, a fundamental question is what enables the virus to reactivate. To answer this question we devised a model in which viral reactivation takes place within the time frame of a single viral replicative cycle. Briefly, mice are infected by corneal route. After 30 days, when no infectious virus can be detected in trigeminal ganglia, the ganglia are removed and incubated in medium containing antibody to NGF. In this system viral mRNA can be detected as early as 5 to 9 h after excision and the amounts increase during the 24 h interval. Using this system we report the following: (i) anti-NGF antibody accelerated the reactivation of latent virus possibly by inducing a strong stress response. In contrast, NGF plus EGF inhibited accumulation of transcript during that period. (ii) mRNAs transcribed from all classes of RNAs (α, β, γ1, γ2) increase at the same time even in the presence of cycloheximide indicating that transcription is totally disordered presumably by derepression of all viral genes. (iii) LATs and mi-RNAs decreased in amounts concurrent with increases in viral gene expression. (iv) The decrease in LATs and mi-RNA levels required de-novo transcription. These findings challenge the current models that the exit from latency required prior expression of either VP16 or ICP0.

P67

Osteopontin motifs required for enhancement of HIV-1 replication

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Osteopontin Motifs Required for Enhancement of HIV-1 Replication Osteopontin (OPN), a cytokine-like protein, is increased in SIV- and HIV-infected monocyte-derived macrophages and in the cerebrospinal fluid and brain tissue of HIV-infected individuals with and without neurocognitive disorder. At this time, little is known regarding the direct molecular mechanisms by which OPN enhances HIV-1 replication. Ectopic expression of OPN in a surrogate cell line that does not produce this protein endogenously, OPN was shown to enhance HIV-1 infectivity and replication through a pathway involving the activation of NF-κB binding sites within the HIV-1 promoter. OPN is a multifunctional protein that can be secreted from cells or as a variant expressed exclusively in an intracellular fashion. The protein is subject to post-translational modifications including glycosylation, phosphorylation and proteolytic cleavage. OPN has an aspartate-

rich domain, as well as integrin- and putative calcium- and CD44-binding domains. In this study we have used a series of OPN deletion mutants as well as OPN variants containing site-specific mutations targeting discrete motifs, to identify the regions of the protein that are required for enhancement of HIV-1 replication. The model system is based on the transfection of OPN mutant expression plasmids into TZM-bl cells that do not express endogenous OPN, followed by infection with a HIV-GFP tagged reporter virus and quantification of infection by FACS and anti-HIVp24 capsid ELISA. Cotransfection of the OPN mutant expression plasmids together with an HIV-CAT-LTR reporter promoter vector was used to quantify the ability of the OPN variants to activate the HIV promoter. Our preliminary data suggests that while motifs in the carboxyl-terminal portion of OPN are required for enhancement of HIV-1 replication, other non-contiguous regions may also contribute to the phenotype.

P68

Impact of morphine on human immunodeficiency virus type 1 and hepatitis C virus in human microglia cells is dependent on the autophagy lysosome pathway

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Injection drug users (IDUs) remain an important population at risk for blood-borne infections such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). Opiates have been suggested to have a cofactor role in the immunopathogenesis of viral diseases, as they have the potential to compromise host immune responses and enhance microbial infections. To determine the mechanisms by which opioids alter the neuropathogenesis of HIV-1 and HCV, we aimed to identify potential convergence points of opioid and HIV-1 / HCV signaling. The autophagic pathway may be one such convergence point. Levels of autophagy were analyzed in primary human microglia infected with the neurotropic HIV-1 strain R5 SF162, cell culture-derived HCV, or exposed to morphine alone or in combination with each virus. Autophagic proteins were detected by Western blot, and autophagosomes were identified using immunofluorescent microscopy and flow cytometry. To inhibit autophagy, microglia were treated with the phosphoinositide-3 kinase inhibitor 3-methyladenine or were transfected with autophagy-related protein 6 (Beclin1) small interfering RNA, and pro-inflammatory cytokines were quantified by ELISA. Increased levels of autophagosome formation as well as the autophagic proteins Beclin 1, Atg5 and LC3II were detected following 24 and 48-hour incubation with infectious HIV-1 and HCV, and increased levels of IL-1β and IL-6 were detected in the culture supernatant. Exposure to morphine alone or in combination with HIV-1 or HCV caused a decrease in the autophagic pathway and cytokine secretion. Downregulation of autophagy inhibited the early stages of HIV-1 replication, and Beclin1-knockdown microglia, when exposed to HIV-1 and HCV significantly reduced the induction of IL-1β and IL-6, suggesting that IL-1β and IL-6 production by microglia occurs through induction of autophagy. In conclusion, infection of microglia with HIV-1 and HCV induces the autophagy machinery and microglial activation, while exposure to morphine decreases the autophagy pathway and disrupts the host immune response.

P69

Role of agnogene deletion and archetype-like regulatory region in a JCV strain isolated from the brain of a patient with JCV Encephalopathy (JCVE)

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Background: JC Virus Cortical Pyramidal Neurons (JCVCPN) was isolated from the brain of an individual with JCV encephalopathy (JCVE). Post mortem analysis showed productive infection of cortical pyramidal neurons. JCVCPN contains an agnogene deletion from nucleotide 300 to 442 and an archetype-like regulatory region (RR).
Objectives: Our goal was to understand the effect of the JCVCPN agnogene deletion and RR type on replication in different cell lines. JCV infection of neurons is a newly observed phenomenon. JCVCPN is the first patient-isolated strain with a large agnogene deletion.
Methods: We compared the replication of JCVCPN with the prototype JCVMad-1 in Cos-7 (monkey kidney), IMR-32 (human neuroblastoma) and SVG (human fetal glial) cell lines. Chimeric viruses of JCVCPN and JCVMad-1 were generated by exchanging the RR and agnogenes. Levels of DNA

replication and mRNA expression were determined using qPCR and qRT-PCR, respectively.
Results: JCVCPN replicated viral DNA in Cos-7, IMR-32 and SVG cells, but to a lesser degree than JCVMad-1 in Cos-7 and IMR-32 cells. JCVCPN early and late mRNA expression was decreased compared to JCVMad-1 in Cos-7 and IMR-32 cells. Introduction of JCVCPN RR or deleted agnogene into JCVMad-1 resulted in decreased levels of DNA replication and mRNA expression. Introduction of JCVMad-1 RR or full length agnogene into JCVCPN partially rescues the decreased levels of DNA replication and mRNA expression. Deletion of the complete agnogene caused decreased DNA replication, while prevention of agnoprotein expression by addition of a stop codon had no effect.
Conclusions: Both the agnogene deletion and the archetype-like RR of JCVCPN contributed to its lower replication compared to JCVMad-1 in kidney cells. JCVCPN had no replication advantage in IMR-32 neuronal cells. Interestingly, the agnogene deletion phenotype was caused by the loss of the agnogene DNA, rather than the absence of agnoprotein.

P70
Focused beam microwave irradiation for measures of neural metabolites in a murine model of neuroAIDS

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Introduction: Biomarker discovery will provide mechanistic links between immune activation and the neuropathogenesis of HIV infection. Proton magnetic resonance spectroscopy (1H-MRS) has previously identified N-acetyl aspartate (NAA), glutamate/glutamine and creatine as such biomarkers for early and chronic stages of HIV-associated neurocognitive disordoers (HAND) in infected humans. Studies of SIV infection in non-human primates have replicated these 1H-MRS signatures. Our laboratories have shown that, in a murine model of HAND, NAA reductions and Cho elevations are found in the cerebral cortex. To expand upon such observations we used liquid chromatography mass spectrometry (LC-MS/MS) to detect a broad array of metabolites. LC-MS/MS metabolomic studies of brain regions would expand the significance of 1H-MRS findings but are restricted due to post mortem tissue metabolism. Focused beam microwave irradiation (FBMI) preserves brain tissue and metabolites by quenching brain enzymatic activities. Brain tissue prepared with FBMI allows metabolomic studies of subregions and parallel histological analysis to describe neural biochemical responses associated with HIV induced neuropathology.
Methods: Brains were bisected for parallel immunohistochemistry (IHC) and LC-MS/MS analyses. Nine amino acids and myo-inositol were quantified by LC-MS/MS on a ultra-performance liquid chromatography (UPLC) system coupled with a 4000 Q TRAP® hybrid quadrupole linear ion trap mass spectrometer. Results: An FBMI protocol was optimized for mouse brain preservation and its effectiveness validated by comparing pre- and post-FBMI levels of metabolites such as lactate and NAA by 1H-MRS. Additional metabolites were then measured from dissected brain tissue by LC-MS/MS. A degradation assay of metabolites in homogenized brain tissue demonstrated stability in FBMI treated tissue of all tested amino acids except glutamine up to a half hour. LC-MS/MS analysis of frontal and middle cortical regions was combined, demonstrating myoinostitol reduction with HIV infection.

P71
HIV infection of human astrocytes disrupts blood–brain barrier integrity by a gap junction dependent mechanism

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HIV infection of human astrocytes disrupts blood brain barrier integrity by a gap junction dependent mechanism. Eugenin A. Eliseo1, 3 and Berman J.W.1,2. Departments of Pathology1, Microbiology and Immunology2, Albert Einstein College of Medicine, Bronx, NY, 3 Public Health Research Institute (PHRI) and Department of Microbiology and Molecular Genetics, Newark, NJ, USA. HIV infection of the central nervous system (CNS) is an early event after primary infection, resulting in neurological complications in a significant number of individuals despite antiretroviral therapy. The main cells infected with HIV within the CNS are macrophages/microglia and a small fraction of astrocytes. The role of these few infected astrocytes in the

pathogenesis of NeuroAIDS has not been examined extensively. We demonstrated that, despite the fact that only a few astrocytes become infected with HIV, gap junction channels allows toxic intracellular signals to spread to neighboring uninfected astrocytes. Now we demonstrate that these few HIV infected astrocytes compromise blood brain barrier (BBB) integrity by misguided astrocyte end feet and dysregulated astrocyte signaling, suggesting an important role for these few infected astrocytes in NeuroAIDS. Alterations in BBB structure and integrity induced by HIV infected astrocytes were gap junction and perhaps hemichannels of connexin/pannexin and ATP receptors dependent, because blocking these channels was protective against BBB compromise. This disruption was dependent on arachidonic acid, activation of lipoxigenase/cycloxigenase as well as on BK channel activity. Our findings describe a novel mechanism of BBB toxicity within the brain triggered by low numbers of HIV-infected astrocytes and amplified by gap junctions that contribute to the pathogenesis of NeuroAIDS

P72

Identification of intracellular toxic signals required for bystander killing through gap junctions from HIV infected astrocytes to uninfected astrocytes

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HIV entry into the central nervous system (CNS) is an early event after infection, resulting in neurological dysfunction in a significant number of individuals. As people with AIDS live longer, the prevalence of cognitive impairment is increasing, despite antiretroviral therapy. The mechanisms that mediate CNS dysfunction are still not well understood, but are postulated to be a combination of inflammation, and viral infection and/or replication. In addition to those mechanisms, we recently demonstrated that HIV infection of astrocytes mediated survival of HIV infected cells and bystander killing of surrounding uninfected cells by a mechanism that is gap junction dependent. We now characterize the mechanisms of HIV mediated protection of infected astrocytes with an emphasis on mitochondrial dysregulation and identification of the intracellular factors that mediate bystander killing of uninfected cells. Our findings describe a novel mechanism by which HIV maintains survival of HIV infected astrocytes and we identify IP3, calcium and Cytochrome C as key signals involved in bystander killing of uninfected cells in contact with HIV infected astrocytes by a gap junction dependent mechanism. Thus, our data provide novel mechanisms of HIV survival and toxicity in the current NeuroAIDS era, where viral replication is not a major component due to effective antiretroviral treatments. Our findings identify new potential therapeutic targets to reduce the devastating consequences of NeuroAIDS.

P73

Potential Utility of Resting-State Magnetoencephalography as a Biomarker of CNS Abnormality in HIV Disease

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There is a lack of a neuroimaging biomarker for HIV Associated Neurocognitive Disorder (HAND). The purpose of this study was to analyze magnetoencephalography (MEG) data from patients with HIV disease and risk-group appropriate controls to determine the MEG frequency profile during resting state, and its stability over 24 weeks. We also set out to access neuronal functional connectivity measures to identify HIV-associated changes in brain function. 18 individuals (10 HIV+, 8 HIV-) completed neurobehavioral evaluations and 10 minutes of resting-state MEG acquisition, which were repeated 24 weeks later (1 HIV- control was lost to follow-up). Relative MEG power in the delta (0-4 Hz), theta (4-7 Hz), alpha (8-12 Hz), beta (12-30 Hz) and low gamma (30-50 Hz) bands was computed for 8 predefined sensor groups. Baseline eyes closed data was analyzed using mutual information (MI) between all pairs of MEG sensors. The median stability of resting-state relative power was 0.80 with eyes closed, and 0.72 with eyes open. The relative gamma power in the right occipital ($t(15)=1.99$, $p<.06$, $r=-.46$) and right frontal ($t(15\ t)=2.15$, $p<.05$, $r=-.48$) regions was associated with serostatus. The effect of age on delta power was greater in the seropositive subjects ($r2=.51$) than in the seronegative subjects ($r2=.11$).

Individuals with high theta-to-gamma ratios tended to have lower cognitive test performance, regardless of serostatus. After permutation testing (at $p<.005$) only one network of planar gradiometers MEG sensors above the right anterior region connecting to the left posterior region related to HIV serostatus was significant. A mean MI value distinguished between the serostatus groups with only one error (sensitivity=1.00, specificity=.88 ($X2=15.4$, $df=1$, $p<.01$, Relative Risk=.11). The stability of the wide-band MEG frequency profiles over 24 weeks supports the utility of MEG as a biomarker and through a measure of functional connectivity, it may be possible to distinguish between HIV-infected and uninfected individuals.

P74

Quiescent JCV in human neural progenitor cells is activated during lineage differentiation to astrocytes

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JC Virus (JCV), the etiological agent of the human brain demyelinating disease progressive multifocal leukoencephalopathy, can non-productively infect multipotential human neural progenitor cells. Upon serum-induced differentiation of infected progenitor cells to astrocytes, JCV commenced early gene expression, followed by DNA replication and late gene expression. Thus, measuring changes in gene expression during the course of astrocyte differentiation provides insight into factors required in the transcription and replication of JCV. We have used Affymetrix microarrays to determine the expression of over 25,000 genes at multiple times during astrocyte differentiation from neural progenitor cells. Of these, the expression of approximately 5000 genes changed during 30 days of differentiation. RNA for the astrocyte markers glial fibrillary acidic protein (GFAP), secreted protein, acidic and rich in cysteines-like 1 (SPARC-L1), and the astrocyte specific glutamate transporter SLC1A2 were upregulated within 24 hours of differentiation and remained upregulated, confirming the identity of these cells as astrocytes. More specifically, increased expression of orthodenticle homeobox 2 (OTX2) identified these glia as patterned for a mid/forebrain phenotype. Therefore, this system of astrocyte differentiation and JCV infection is useful for the investigation of glial differentiation and cellular factors involved in JCV replication. Ten genes were upregulated by one hour after serum addition and remained upregulated throughout differentiation, including SMAD7 and inhibitor of DNA binding (ID) family members. During astrocyte differentiation, the transcription factors nuclear factor I-A (NFI-A) was downregulated, and NFI-X was upregulated, as previously shown, demonstrating the reproducibility of the differentiation. These changes coincide with increasing JCV susceptibility during differentiation, adding further evidence to the observation that NFI-A represses JCV, while NFI-X enhances infection. This model of astrocyte differentiation and JCV infection may lead to the identification of a number of proteins that are important for JCV pathogenesis.

P75

Clonal immortalized human glial cell lines support varying levels of JCV infection

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JC Virus (JCV) is a ubiquitous human polyomavirus that causes the often-fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML), but replicates in limited cell types in culture, predominantly in human glial cells . Previously, a cell culture system was established from human fetal brain containing a heterogeneous population of neural-derived cells. Following introduction of an origin of replication-defective mutant of SV40 that expressed the large T protein, multiple phenotypes of cells became immortalized; some of which could support JCV replication. This SVG cell line has been used to propagate stocks of JCV and investigate viral-cell interactions. In the current study,

clonal cell lines were selected from the original SVG cell culture that exhibit variable levels of permissiveness to JCV replication. The SVG-5F4 clone showed reduced viral growth and expressed low levels of nerve growth factor (NGF) and high levels of glial cell line-derived neurotrophic factor (GDNF), while the SVG-10B1 clone was highly permissive to JCV expression and DNA replication and expressed high levels of NGF and low levels of GDNF. Additionally, levels of nuclear factor I-A, a transcription factor known to reduce JCV gene expression, were lower in SVG-10B1 cells than in SVG-5F4. Furthermore, SVG-10B1 cells supported persistent and stable JCV infection over months in culture. Thus, these SVG-derived cell lines can provide a system to investigate cell type differences in JCV pathogenicity. Further, the SVG-10B1 cells provide a reproducible and quantitative viral biology platform for drug discovery.

P76
Extracellular HIV-1 Vpr decreases astrocytic glutathione and ATP levels impacting neuronal survival

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Studies have demonstrated the accumulation of extracellular human immunodeficiency virus type 1 (HIV-1) Vpr in the serum and cerebrospinal fluid of HIV-1-infected patients in direct correlation with viral loads and disease progression. Late stage HIV-1-infected patients also suffer from different inflammatory conditions and manifest excessive intracellular oxidation, a condition that may potentially escalate and lead to irreversible fatal effects. While several other HIV-1 proteins (gp120, Tat and Nef) were found in different bodily fluids, very little is known about the downstream effects of Vpr. As one approach to examine the role of extracellular Vpr in HIV-1 disease progression, we have demonstrated that recombinant Vpr decreased the levels of glutathione (GSH) and ATP pools in cultured astrocytes, causing oxidative stress. Based on these results, and the aforementioned clinical manifestations, we hypothesized a correlation between enhanced extracellular Vpr in the CNS and a decline in astrocytic levels of GSH and ATP with disease progression. Results demonstrated declines in the levels of ATP and GSH were unrelated and due to a Vpr-induced decrease in GAPDH activity, thereby affecting the glycolytic pathway. In addition, exposure to extracellular Vpr promoted caspase-dependent apoptosis in astrocytes along with secretion of pro-inflammatory cytokines (IL-6 and IL-8) and chemoattractants (MCP-1 and MIF). Furthermore, excessive astrocytic GSH oxidation lowered extracellular levels of GSH and the supply of cysteine for neurons, which affected GSH synthesis within the neuronal compartment. This cascade of events within the astrocytic compartment thus led to oxidative stress within the neuronal population, impairing survival. Partial rescue of these effects was obtained upon supplementation with the anti-oxidant compound N-acetyl-cysteine. These results support a role for HIV-1 extracellular Vpr in deregulating the neuronal-astrocytic network, which possibly accelerates disease progression, but also offers a therapeutic approach aimed at targeting one of the causative agents of the astrocytic-neuronal network disruption.

P77
Alterations in neuronal ferritin heavy chain expression by HIV envelope protein gp120 and inflammatory cytokines

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Ferritin is a ubiquitous protein involved in iron sequestration and storage, composed of two different subunits, Heavy chain (FHC) and Light chain (FLC). Recent evidence has identified a novel role for FHC as a negative regulator of the chemokine receptor CXCR4. Our lab has previously demonstrated that μ-opioid receptor agonists, including morphine, upregulate FHC protein levels in neurons, which results in inhibition of prosurvival signaling mediated by CXCR4. Further, in vivo data indicate greater expression of FHC and inhibition of CXCR4 (Ser 339) phosphorylation, an index of CXCR4 activation, in brain tissue of HIV-positive drug abusers compared to HIV+/- controls. The aim of this study was to investigate potential components of HIV infection that could contribute to the upregulation of FHC in neurons. Our data indicate that the inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), as well as the HIV envelope glycoprotein (gp120IIIB), preferentially upregulate FHC protein expression, but not FLC, in neurons. Furthermore, although a trend towards upregulation of FHC was seen in neurons cultured without glia and treated with gp120IIIB, IL-1β, or TNF-α, studies performed in the presence of glia suggest a potential role of these cells in the effects of the

cytokines. On the contrary, our previous studies suggest that other mechanisms may be involved in morphine-induced ferritin regulation. Thus, different and potentially additive mechanisms may be responsible for FHC changes caused by opiates/HIV infection, a hypothesis also supported by preliminary studies in morphine-treated, SIV-infected non-human primates. Overall, these studies suggest that opiates and HIV can synergistically act on FHC and deprive neurons of important neuroprotective actions driven by CXCR4.

P78

Antiretroviral drugs activate the neuronal unfolded protein response and promote amyloidogenic processing of APP in primary neurons

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The advent of antiretroviral therapy (ART) as the mainstay for HIV treatment has led to a significant reduction in the incidence of HIV-associated dementia (HAD), a severe motor/cognitive disorder afflicting AIDS patients. However, the prevalence of HIV-associated neurocognitive disorders (HAND), a spectrum of HIV-related CNS dysfunctions ranging from mild cognitive deficits to HAD, has continued to escalate in the post-ART era. In addition, HIV-associated neuropathology has evolved from a subacute, subcortical, encephalitic condition to a prolonged, cortical, neurodegenerative disease. While increased longevity of HIV-infected populations is thought to contribute to alterations in HAND pathology, other risk factors, such as peripheral toxicities of ART and potential central effects linked to enhanced amyloidogenesis and aging, remain unexplored. Previous work has shown that ART is toxic to both the peripheral nervous system in vivo as well as dorsal root ganglion neurons in vitro. To explore ART-mediated neuronal toxicity in vitro we treated primary rat cortical cultures with commonly prescribed ART compounds from two classes, nucleoside reverse transcriptase inhibitors (NRTI), and HIV protease inhibitors (PI). Our studies suggest that PIs lead to a significant MAP2 loss over the course of 8 days of treatment, whereas NRTIs did not induce MAP2 loss. Given the known role of PIs in activating the unfolded protein response (UPR) in macrophages and hepatocytes we explored this cellular stress pathway in primary neurons as a potential mechanism of toxicity. We found that concentrations of PIs and NRTIs which approximate plasma Cmax levels, induce ER stress leading to phosphorylation of eIF2α, and enhanced translation of the β-secretase enzyme, BACE1. Additionally, we observed ritonavir-mediated amyloidogenic APP processing and increased Ab secretion in CHO cells stably expressing human APP. Furthermore, antiretrovirals administered to rats or SIV-infected macaques resulted in neuronal damage in the CNS, implicating these drugs as potential mediators of neurodegeneration.

P79

Drug-induced increases in CNS dopamine may exacerbate HIV-associated neurological disorders by altering HIV infection and cytokine secretion in human macrophages

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The neurological consequences of HIV infection are a major health issue, especially among HIV-positive drug abusers, despite effective antiretroviral therapy. HIV infected individuals who use drugs show changes in the development of HIV-associated neurological disorders (HAND). These may be due to elevated CNS dopamine levels, which mediate the addictive and reinforcing effects of many drugs of abuse. We hypothesize that dopamine exacerbates the development of HAND by increasing HIV infection and altering other functions in macrophages, the primary target for HIV in the CNS. We showed previously that high levels of dopamine significantly increase viral replication in macrophages by increasing the number of macrophages infected with HIV. We have also shown that macrophages express all subtypes of dopamine receptors and other dopamine related proteins. Our current data demonstrate that dopamine enhances HIV entry into these cells through activation of different sub-types of macrophage dopamine receptors. We also examined the effects of dopamine on macrophage cytokine production as these cells are important in regulating neuroinflammation. Dopamine significantly alters the secretion of the cytokines IL-6 and CCL2 in macrophages. In macrophages treated with LPS to model an inflammatory environment, dopamine modulates secretion of TNF-α, IL-10 and CXCL8 as well as CCL2 and IL-6. The dopamine mediated modulation of CCL2 and IL-6 is potentiated by HIV infection. We are defining the mechanisms by which dopamine effects viral entry and

cytokine production, and specifically examining how HIV infection alters the mechanisms regulating CCL2 production. These data suggest that dopamine mediated changes in macrophages increase CNS viral infection and exacerbate neuroinflammation, altering the development of HAND. Dopamine may be a common mechanism by which drugs of abuse exacerbate HAND in HIV infected drug-abusers. Supported by NIDA.

P80
HIV-1 RNA but not DNA in NNTC brain specimens is correlated with neurocognitive impairment

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Relationships between HIV replication in the brain and HIV-associated neurocognitive disorders (HAND) remain to be elucidated. HIV gag/pol RNA and DNA were measured in 119 brain specimens from the National NeuroAIDS Tissue Consortium (NNTC) archive. A correlation analysis with neuropsychological test performance within six months before autopsy was undertaken. DNA and RNA were extracted from fresh-frozen dorsolateral prefrontal cortex. Viral RNA and DNA were measured using reverse transcriptase qPCR and standard qPCR. The NNTC neurocognitive test battery was given and performance T scores were normalized for education, age, race and gender. The overall Spearman correlation between high HIV RNA concentration and a low composite T score was significant ($n=119$; $rho=-0.290$; $p<0.001$). The same analysis using brain HIV DNA was not statistically significant overall ($rho=-0.071$, $p<0.444$). Conclusion: A high level of replicating HIV in the brain was related to worse neuropsychological test performance. The clinico-neurovirological correlation was relatively specific for replicating HIV, because HIV DNA was not correlated with brain function significantly.

P81
HIV latency and viral DNA cache's in the human brain: post-integration DNA is highest in specimens obtained prior to HAART and is enriched in white matter

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To eradicate latent HIV reservoirs in the CNS, the amount of post-integration HIV DNA (PI-DNA), neuroanatomical cache's, and the effect of highly active antiretroviral therapy (HAART) all must be elucidated. The two-step Alu-gag assay was used to measure the proportion of PI-HIV DNA in 140 human brain specimens with at least 10E+3.8 copies of total HIV DNA per gram wet weight of frontal neocortex (TOT-DNA). The sampling contained specimens collected prior to and during the HAART era. PI-DNA was detected in 93 specimens using a single replicate and specimens with less than 10E+4.5 TOT-DNA usually required multiple replicates. The proportion of PI-DNA relative to TOT-HIV DNA in 27 decedents who died before the era of HAART was at least twice that of 27 post-HAART decedents paired demographically with equivalent concentrations of TOT-DNA (paired Wilcoxon signed rank test, $p<0.05$). The proportion of PI-DNA was significantly highest in white matter compared to either neostriatum or neocortex.
Conclusion: Post-integration HIV DNA in the brain is most concentrated in white matter and forms the largest pool size in the human brain by volume. The fact that specimens obtained prior to the era of HAART had a significantly higher proportion of PI-DNA resembles clinical and in vitro data using HIV-infected lymphocytes treated with HAART. The brain contains a relatively large pool of PI-DNA and must be addressed when attempting to eradicate latent HIV DNA from the body.

P82
Whole-body mapping of post-integration HIV DNA at autopsy: caches of latent DNA in the central and peripheral nervous systems and other non-lymphoid type tissue

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To eradicate latent HIV reservoirs in the CNS, systemic and cellular caches of post-integration HIV DNA in the body must be accounted for. We constructed a whole-body organ map of latent HIV DNA in three subjects with active HIV replication at the time of death, including one subject with HIV encephalitis. Total DNA was extracted from varied anatomical sites and post-integration HIV DNA (PI-DNA) was assayed using the two-step Alu-gag assay. Total HIV DNA (TOT-DNA) was measured using qPCR for HIV gag/pol. The ranking of TOT-DNA concentration, from highest to lowest was spleen = plasma > intestine > lung > liver = kidney > heart = skeletal muscle = deep peroneal nerve = dorsal root ganglion. Tissues containing a high concentration of TOT-DNA generally had the most abundant PI-DNA, but the ranking was not always congruous with the TOT-DNA. In a subject with HIV encephalitis the pool size in the central nervous system was relatively large and included components in the pituitary gland, choroid plexus, and leptomeninges. One patient had a relatively large cache in the colon, and two patients had relatively large caches in lung tissue. Conclusion: Post-integration HIV DNA is present in virtually all organs to some extent in subjects with active HIV replication. Patients can harbor specific caches in non-lymphoid tissues. Further tissue mapping will guide efforts to find and eradicate caches of latent HIV DNA. Studies in patients with low virus replication rates are ongoing to determine the extent to which residual pools of latent HIV DNA are replication competent.

P83

Secretion of soluble insulin receptor from human neuronal cells exposed to the cerebrospinal fluid from HIV-seropositive women correlates with cognitive performance

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Purpose: Insulin resistance (IR) and abnormal glucose metabolism are associated with cognitive impairment (HAND) among HIV-infected individuals. Previously, we showed an association between high CSF soluble insulin receptors (sIR) levels and HAND. However, the possibility that high glucose and factors present in the CSF of HIV-infected individuals may influence the secretion of the sIR from human neuronal cells remains to be elucidated.
Methods: CSF was collected from 24 HIV-infected women of which 16 had HAND (asymptomatic=7, symptomatic=9), 8 had normal cognition and 5 HIV(-) controls without a history of diabetes. A neuronal cells line (SH-SY5Y cells; 5×105 cells/well) were cultured in EMEM medium at 37°C and 5%CO2 in the presence of patients CSF or high glucose (25 mM) concentration for 24 hours. Secreted sIR full-length was quantified using a specific fluorescent-IR-ELISA. Membrane and intracellular IR were determined by flow cytometry. Results: A significant increase was observed in the sIR levels secreted from neuronal cells exposed to the CSF of HIV-infected patients ($p<0.001$) compared to HIV(-) controls. Increased sIR secretion from the cells was associated with the severity ($p<0.001$) of HAND. No significant differences were observed on secreted sIR full-length levels between cells exposed to high and normal glucose levels. High glucose decreased significantly ($p=0.04$) membrane-bound IR levels and increased significantly ($p<0.001$) the intracellular IR levels when compared to normal glucose exposed cells.
Conclusion: Secretion of the sIR from neuronal cells is influenced by CSF components from HIV-infected women and correlates to the severity of HAND. The effects of high glucose on neuronal cells are related to down-regulation of the membrane-bound IR and its increased internalization. R21MH 095524, S11NS046278, U54NS043011, 8U54MD007587, 2G12RR003051, 8G12MD007600, R25MH080661.

P84

CD135 (flt3) up-regulation in the CNS in SIV infection and encephalitis (SIVE) and by M-CSF in vitro: Support for CD135 and M-CSF as potential therapeutic targets for HIV infection and CNS disease

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Macrophage-colony stimulating factor (M-CSF) is believed to play a prominent role in AIDS progression and the development of HIV-associated neurocognitive disorders (HAND). In addition to increasing HIV infection and virus production in monocytes, M-CSF increases CD16+ monocyte frequency in circulation and CD163 expression. These cells are

phenotypically similar to macrophages that accumulate in the CNS in HIV encephalitis (HIVE) and are believed to contribute to the pathogenesis of HAND. Therefore, M-CSF may be a viable therapeutic target for treating or preventing HAND. Here, we report several tyrosine kinase inhibitors (TKI) with M-CSF receptor (CD115) activity, reduce the frequency and/or expression of CD16, CD163, CCR5, M-CSF and CD115 by monocytes in M-CSF treated peripheral blood mononuclear cells (PBMC). Interestingly, these data suggest that a proprietary TKI reported to be exclusive to CD115, decreases the frequency of CD135 [fms-like tyrosine kinase 3 (flt3)], a receptor tyrosine kinase expressed by monocytes and believed to interact exclusively with flt3 ligand (FL). M-CSF-treated PBMC showed increased frequency of CD135 by monocytes without changing CD135 expression level. Because M-CSF is elevated in cerebral spinal fluid (CSF) of patients with HIVE, it may alter expression of CD135 in the CNS. We examined CNS tissue from a relevant animal model for HIV-related CNS disease, SIV infected rhesus macaques, for CD135 expression. These studies showed greater expression of CD135 in CNS of SIV + macaques with even greater expression seen in those with encephalitis (SIVE), as compared to seronegative animals. This was observed as an increase in the number of CD135+ cells, as well as the degree of expression. These findings suggest that M-CSF/CD115 signaling may be involved in the regulation of CD135 and/or its ligand. Modulation of CD135 may be a potential adjunctive therapy, in addition to targeting CD115, as a therapeutic strategy for treating or preventing HAND.

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Metabolic profile of Progressive Multifocal Leukoencephalopathy lesions in patients with and without Immune Reconstitution Inflammatory Syndrome

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Background: Progressive multifocal leukoencephalopathy (PML) is an opportunistic disease in AIDS patients and other immunosuppressed patients, caused by the reactivation of JC virus. Since the advent of combined antiretroviral therapy, we are more often confronted with patients who develop PML in the setting of immune reconstitution inflammatory syndrome (IRIS). In this study, we characterized progressive multifocal leukoencephalopathy (PML) lesions by contrast-enhanced MRI and evaluated their metabolism using proton magnetic resonance spectroscopy (1H-MRS) in the setting of IRIS.
Methods: 42 PML patients underwent a clinical evaluation as well as a brain MRI and 1H-MRS at baseline and 3, 6 and 12 months later. The presence of IRIS was determined based on clinical and laboratory criteria. Ratios of N- acetylaspartate (NAA), choline (Cho), myo-inositol (mI), and lipid/lactate (LIP1 and LIP2) to creatine (Cr) were measured and correlated with the presence of contrast enhancement (CE) in PML lesions.
Results: IRIS occurred in 16/28 (57.1%) PML survivors (PML-S) and 1/14 (7.1%) PML progressors (PML-P). Lesions of PML-IRIS patients showed significantly higher Cho/Cr ($p=0.0001$), mI/Cr ($p=0.02$), LIP1/Cr ($p<0.0001$) and LIP2/Cr ($p=0.002$) and lower NAA/Cr ($p=0.02$) ratios compared to PML patients who did not have IRIS. Elevated Cho/Cr ratio was associated with CE within the 1H-MRS voxel, while lipids/Cr ratios were elevated in PML-IRIS lesions independently of CE. Follow up until 33 months from PML onset showed persistent elevation of mI/Cr ratio in lesions of PML-IRIS patients. A LIP1/Cr ratio above 1.5 combined with the presence of CE yielded a 79% probability of IRIS compared to 13% in absence of these criteria. Conclusion: 1H-MRS is a valuable tool to recognize and track IRIS in PML and may prove useful in the clinical management of these patients.

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Pharmacologic inducers of the macrophage antioxidant response inhibit HIV infection and neurotoxin production

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Background: HIV CNS infection causes a syndrome of cognitive, motor, and behavioral abnormalities collectively known as HIV-associated neurocognitive disorders (HAND). HIV-infected monocyte-derived-macrophages (HIV/MDM), a primary CNS reservoir, promote neurodegeneration through induction of neuroinflammation and neurotoxin release. These neurotoxins include small (<3 kDa), heat-stable, protease-resistant molecules which excite N-methyl-D-aspartate (NMDA) receptors. The excitotoxins glutamate and quinolinic acid (QUIN) both correlate with degree of neurologic impairments in HIV-infected individuals. Although antiretroviral therapy (ART) improves clinical outcomes, the

prevalence and associated morbidity of HAND remain high (~50%). Thus adjunctive neuroprotective therapies that address the pathological processes persisting in ART-treated individuals are needed. We have shown that HIV infection alters the macrophage antioxidant response (AR) and that pharmacological induction of the AR attenuates HIV replication and neurotoxicity. We hypothesized that HIV infection of MDM alters components of glutamate and QUIN biosynthesis and that inducers of the AR attenuate HIV neurtoxicity by modulating these neurotoxin pathways.
Methods: Monocytes and MDM were obtained from PBMCs of human volunteers. HIV/MDM were infected with cell-free HIV-1. RT activity assays determined viral replication in supernatants. AR activation was determined by western blot. Cell-based MAP2 ELISAs determined neuronal survival. Significance was defined as $p < 0.05$ using ANOVA or student's t-test.
Results: We have demonstrated that HIV replication reduces expression of heme oxygenase 1 (HO-1), a key component of the AR, and also alters components of the glutamate and QUIN biosynthesis pathways. Inducers of the AR decrease neurotoxin production, even without altering HIV replication, in HIV/MDM.
Conclusions: Our results suggest that inducers of the antioxidant response have therapeutic potential for HAND through suppression of HIV replication and neurotoxin production in MDM. This reduction of neurotoxin production may work through alteration of the biosynthetic pathways of glutamate and QUIN. Studies are ongoing to define the mechanism by which HO-1 modulates neurotoxin production.

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The impact of HIV-1 Tat on Pur-alpha mediated regulation of dendritic RhoA signaling

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Pur-alpha is an essential protein for brain development which localizes specifically to dendrites where it plays a role in the translation of neuronal RNA. Pur-alpha is also a binding partner of the HIV-1 Tat protein where it has been shown to upregulate TAR-mediated viral transcription. Here we examined two Rho GTPases, Rac1 and RhoA, which play opposing roles in neurite outgrowth and are critical for dendritic maturation during brain development, in the presence and absence of Pur-alpha. Pur-alpha is developmentally regulated in the mouse brain with expression increasing during the third week of postnatal development. RhoA levels analyzed by Western blotting rapidly fluctuate in the wild-type mouse brain, however, in the absence of Pur-alpha, a delay in the cycling of RhoA regulation was observed leading to reduced basal levels of RhoA after day 10 postnatal. Immunohistochemistry of brain tissues from Pur-alpha knockout mice displayed reduced RhoA levels and loss of perinuclear cytoplasmic labeling. While Rac1 levels remained relatively stable, changes in subcellular localization of Rac1 were seen in the absence of Pur-alpha. The absence of Pur-alpha did not appear to affect activation of RhoA, however, expression of HIV-1 Tat increased the active fraction of RhoA in neuronal cells, while at the same time decreasing the activity of Rac1. A similar effect was seen on RhoA and Rac1 basal levels. These findings suggest that Pur-alpha can regulate RhoA at multiple levels including basal protein levels, subcellular compartmentalization, as well as turnover of active RhoA in order to promote dendritic maturation. HIV-1 Tat dysregulates neuronal homeostasis by simultaneously inducing RhoA and inactivating Rac1 which leads to retraction. Thus, the neurotoxic effects of HIV-1 Tat may work through RhoA by targeting Pur-alpha to induce neuronal loss.
This work was supported by grants from NIH to JG and SA.

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Reduced Effectiveness of the NRTIs d4T and AZT in Astrocytes: Implications for Neuro-cART

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Background: HIV-1 penetrates the central nervous system (CNS) and can lead to HIV-associated dementia (HAD). While macrophages and microglia are the major sites of productive HIV-1 infection in the CNS, astrocytes undergo restricted infection. Up to 20% of astrocytes can become infected in vivo, often leading to astrocyte dysfunction, loss of neuronal support and the onset of HAD. Infected astrocytes represent a viral reservoir of long-lived cells, presumably not targeted by antiretrovirals (ARVs). Preventing the establishment of the infected astrocyte pool may be beneficial in delaying and/or preventing HAD and in virus eradication strategies. Here we sought to determine the effectiveness of ARVs used in Neuro-cART on inhibiting HIV-1 infection in astrocytes.
Methods: ARVs, including those used in Neuro-cART (ABC, 3TC, d4T, AZT, EFV, NVP, LPV, RAL), were assessed for their ability to inhibit infection in astrocytes. We generated single round HIV luciferase reporter viruses pseudotyped with VSVg envelope to facilitate efficient virus entry into astrocytes. Virus was added to the SVG astrocyte cell line, primary fetal astrocytes (PFA, n=5) or JC53 cells in the presence of titrating amounts of ARVs and luciferase assays were performed. Data were used to generate inhibition curves and to calculate EC50/EC90 values.
Results: With the exception of d4T/AZT, all ARVs tested inhibited viral infection in SVG, PFA and JC53 cells in a dose dependent manner. However, AZT and d4T had reduced anti-HIV-1 potency in PFAs, with EC90 values 234- and 675-fold greater than known CSF drug concentrations, respectively.
Conclusions: The reduced effectiveness of d4T and AZT in PFA suggests that Neuro-cART regimens containing these drugs may achieve suboptimal viral inhibition in astrocytes. These data have potentially important implications for the use of d4T/AZT in Neuro-cART, and suggest that astrocyte infection may remain untargeted by these regimens, potentially leading to poorer neurological outcomes for patients.

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Chronic Infection of Mice by Chimeric HIV-1 with Establishment of Integrated Provirus Reservoirs in Peripheral Tissues and Brain

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Currently available antiretroviral treatments (ART) can be highly successful in controlling HIV replication, progression to immunodeficiency, and development of most forms of frank AIDS. However, immune restoration under ART is not always complete and some nervous system diseases such as HIV-neurocognitive disorders are largely unresponsive to treatment. Moreover, even under clinically effective treatments infection-competent HIV persists as integrated DNA within cellular reservoirs. Here we describe a system for the establishment and study of HIV reservoirs in immunocompetent hosts, to model HIV-infected persons under successful ART. Inoculation of chimeric, mouse-tropic HIV, EcoHIV, into conventional mice causes acute productive infection that peaks at 2-4 days with chronic infection lasting up to 15 months. The HIV genome integrates in macrophages within 6 hours of virus inoculation and integrated virus persisted in macrophages and spleen cells for more than a year. Levels of viral 2LTR circular DNA, spliced Vif RNA, and virally encoded proteins declined over several months of infection, but levels of genomic RNA in macrophages and anti-Gag antibodies fluctuated throughout the follow up period, suggesting stochastic virus re-activation. The forms of the HIV genome in infected mice were compared to similar forms in peripheral blood cells from HIV-infected persons. Infection of athymic mice resulted in extended, efficient EcoHIV expression, confirming the role of adaptive immunity in controlling chimeric HIV infection in mice. Reservoirs of integrated proviral DNA were found in lymphoid tissue, macrophages, bone marrow, and the brain. These reservoirs contain replication-competent virus as shown the spread of EcoHIV into macrophages in athymic recipients of CD4+ cells from EcoHIV-infected mice. Our results establish an animal model for the study of reservoirs of infectious HIV in vivo. Supported by grants DA017618 and MH083627 from the National Institutes of Health, US Public Health Services.

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HIV-1 Vpr induced neuronal apoptosis is mediated in part through differential regulation of inflammatory molecular networks in infected target cells

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HIV-1 induces neuronal dysfunction through secreted host cellular factors, cytokines/chemokine and viral proteins including Vpr released from infected macrophages/microglia. To determine the factors and mechanisms involved in HIV-1 Vpr induced neuronal dysfunction, we utilized monocyte-derived macrophages (MDM) infected with either HIV-1 wild type (HIV-1wt) or Vpr deleted mutant (HIV-1ΔVpr) as models. Presence of Vpr differentially regulated several pro-inflammatory molecules including significant upregulation of IL-1β, IL-8 and TNF-α in MDMs at the transcriptional and/or protein levels. Upregulation of inflammatory factors is mediated through Vpr induced phosphorylation of SAPK/JNK and p38. Blocking of SAPK/JNK and p38 prevented the Vpr mediated elevation of IL-1β, IL-8 and TNF-α levels in MDMs confirming the involvement of SAPK/JNK and p38 in pro-inflammatory cytokine production. Further Vpr induced indirect effect on neuronal apoptosis through pro-inflammatory cytokines (IL-1β, IL-8 and TNF-α) was examined by exposing primary neurons to culture supernatants of HIV-1wt, HIV-1ΔVpr or mock infected MDMs. Supernatants from HIV-1wt infected MDMs containing elevated levels of IL-1β, IL-8 and TNF-α as well as viral proteins showed an enhanced neurotoxicity compared to mock or HIV-1ΔVpr infected MDM supernatants. Reduction of neuronal death in the presence of anti-IL-1β and anti-IL-8 antibodies implies that Vpr mediated neuronal cell death is in part mediated through released inflammatory factors. Collectively, these results demonstrate the ability of Vpr to impair neuronal survival indirectly by dysregulating multiple cytokines in the infected target cells.

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Primary Monocyte-Derived Microvesicles Are Internalized By Human Neural Cells: Novel Mechanism for HIV/HCV associated neurotoxicity?

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Coinfection with HIV and hepatitis C virus (HCV) is associated with mild cognitive impairment despite HIV viral suppression by ART. We propose that CD14+ monocytes are activated by exposure to HIV/HCV in the periphery and cross the blood brain barrier shedding microvesicles (MVs) that are taken up by neural cells with neuropathogenic consequences. Microvesicles (MVs) are small membrane enclosed sacs that are released from a variety of cell types, during normal as well as pathological states. MVs contain selectively packaged cellular proteins and nucleic acids including both mRNA and micro-interfering (mi) RNAs of the host cell. Interestingly, MVs are capable of transferring cellular information to other cells of different lineages and influencing their physiological environment.In support of this hypothesis, we show that human monocyte-derived MVs are internalized by primary neurons and astrocytes. Additionally, MVs released from HCV core protein-treated monocytes induce abnormal neuron aggregation suggesting that the MV 'cargo' derived from monocytes exposed to viral products is associated with neurotoxicity. We examined the RNA contents of monocyte-derived MVs from HCV and HIV/HCV-infected subjects by RT-PCR. This analysis revealed numerous, differentially expressed miRNAs that potentially could dysregulate neural cell function. Also detected in these MVs was positive strand HCV indicating MVs may be a vehicle for transport of HCV into the nervous system. These results suggest a monocyte MV-mediated mechanism for HCV entry into the CNS as well as modulation of neural cell function that may result in cognitive impairment.

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Malnutrition and Energy Wasting as the Intersection of drug abuse and HIV-infection in the CNS: A Novel Preventive Approach

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Substance abuse and HIV-1 infection severely impact the global health crises. Although, drug abuse/HIV is known to cause neurological disorders independently, drug abuse accelerates the progression of Neuro-AIDS by elusive multi-facet mechanisms. Here, we describe the underlying mechanisms that malnutrition by drug and energy wasting by HIV-1 infection as the intersection point of Neuro-AIDS. Daily i.p injection of methamphetamine (METH, 15.0 mg/kg) in mice causes inflammation and hemorrhagic stroke at 5-6 weeks. Brain capillary of these animals showed massive oxidative damage, impaired angiogenesis, inflammation, and glucose transport dysfunction. Activation of MMP-2/9 via VEGF-signaling was responsible for degradation of BBB basement membrane/VEGFR2 proteins and this neurological disease. Impairment of angiogenesis due to VEGFR2 reduction promotes infiltration of HIV-1 infected cells into the brain that is demonstrated by infusion of fluo-3 labeled macrophages via the common carotid artery catheter implantation. We observed that HIV-1 infected microglia amasses the limited supply of glucose during METH-induced malnutrition; an energy wasting process, which promotes oxidative stress, inflammation, neurotoxicity and progression of Neuro-AIDS. A combined therapeutic prevention of METH-induced malnutrition and energy wasting by HIV-infection is novel approach for mitigation of Neuro-AIDS among drug abusers. Sadly, the unique anatomical feature of the BBB and the restricted skull cavity hinders the cure of Neuro-AIDS. Our newly discovered neurotoxin drug S that traverses the BBB effectively eradicates HIV-1 infection in neuroimmune cells. We have evidence to show that treatment of acetyl-L-carnitine (ALC, antioxidant and stabilizer of energy oxidation) and drug S is able to mitigate malnutrition, HIV-infection and energy wasting. Thus, induction of hemorrhagic stroke is triggered by activation of MMPs, degradation of BBB components, and impairment of vascular wound healing. This Vascular oxidative injury and glucose related malnutrition is prevented by ALC, while drug S effectively eradicated HIV-infection in macrophage/microglia that protect neurons and astrocyte.

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Modification of Inhibitory Synapses Following Exposure to HIV-1 Tat

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Human immunodeficiency virus (HIV-1) infection of the CNS is associated with neuronal injury, including dendritic pruning, synapse loss, and cell death. Cognitive decline in patients with HIV-1-associated dementia (HAD) correlates with dendritic damage. We hypothesize that dendritic pruning and excitatory synapse loss are coping mechanisms that enable neurons to decrease excessive excitatory input. Cells infected by HIV-1 shed viral proteins, including the transactivator of transcription (Tat). HIV-1 Tat binds the low-density lipoprotein receptor-related protein (LRP) and is internalized, inducing over-activation of N-methyl-D-aspartate receptors (NMDARs) and subsequent loss of excitatory synapses. Overall network excitability is determined by the balance between excitatory and inhibitory synaptic connections; increasing excitatory synaptic activity will result in a reciprocal increase in inhibitory synaptic activity to maintain network stability. Using live cell confocal imaging of transfected neurons, we find that exposure to HIV-1 Tat (50 ng/mL) causes a marked up-regulation of inhibitory synapse number by 24 hours. The NMDAR antagonist dizocilpine and the LRP antagonist RAP blocked the Tat-induced increase in inhibitory synapse number, indicating that NMDAR activation was necessary for the Tat-induced gain in inhibitory synapses. Nutlin-3 is an E3-ligase inhibitor that prevents Tat-induced loss of excitatory synapses by blocking ubiquitination of PSD95. Nutlin-3 failed to prevent Tat-induced up-regulation of inhibitory synapses, indicating that changes in inhibitory synaptic transmission induced by Tat are secondary to changes in excitatory drive. These results suggest that inhibitory synapses might increase in HAD to maintain network equilibrium. We speculate that increases in inhibitory synapses are part of a neuroprotective mechanism gone awry and that pharmacological manipulation of inhibitory synaptic transmission could have pronounced effects on the synaptic networks altered by exposure to HIV-1 proteins. These findings could provide a viable target for designing new therapies to treat patients with HAD.

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Reduced Control of HIV Infection and Viral Neuropathogenesis in Knock-out Mice Lacking Type I Interferon Responses

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Host innate immune responses, particularly the type I interferon (IFN) system, serve as a first line of defenses against pathogens including HIV. Exogenous type I IFN can be effective in blocking HIV infection in vitro, particularly in macrophages. Other studies in culture indicate that HIV has evolved mechanisms to disrupt induction of cellular IFN responses, as also suggested by the observed dysregulation of IFN-responsive genes in lymph nodes, peripheral T cells, and brain tissues of HIV-infected patients. However, direct demonstration of the role of type I IFN in control of HIV infection in vivo is lacking. To this end, we compared HIV infection and neuropathogenesis in paired sets of wild-type (WT) and type I IFN receptor knockout (IFNRKO) mice on the same genetic backgrounds. Mice were infected with modified mouse-tropic HIV, EcoHIV. Following intraperitoneal (IP) inoculation of EcoHIV, IFNRKO mice showed significantly higher HIV expression in spleen and peritoneal macrophages and greater virus infiltration into the brain than WT mice. Although both IP-infected WT and IFNRKO mice had asymptomatic brain pathology, the IFNRKO mice showed greater global gene dysregulation in the brain. To facilitate study of HIV neuropathogenesis in this system, EcoHIV was inoculated directly into the brain by intracerebral injection, resulting in uniformly high HIV burdens in the brain and causing gross neuropathological changes including lesions of infiltrating macrophages and T cells, microglial activation, and astrocytosis. Extensive gene dysregulation, including upregulation of inflammatory cytokines and chemokines was detected by qPCR analysis of affected brain tissues. All the parameters of HIV neuropathogenesis, including HIV expression in microglia/macrophages, were significantly more pronounced in IFNRKO than in WT mice. Our results show directly that functional type I IFN responses are important but insufficient for control of HIV infection and pathogenesis in the brain. Supported by DA017618 & MH083627 from NIH.

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Aging amplifies HIV neurocognitive impairment: the effects may be related to vascular and metabolic factors

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Background: Modern antiretroviral therapy (ART) has substantially extended the survival of HIV infected persons into their later years, raising the possibility that age-related organ changes, including neurodegenerative and cerebrovascular changes, might amplify HIV effects on the brain. This study investigated the effects of age, HIV, and vascular and metabolic markers on neurocognitive (NC) function.
Methods: 1521 HIV + participants from the CHARTER study and 273 HIV- participants from the HNRP were selected for analysis. Subjects received comprehensive neuromedical, neurocognitive, and laboratory assessments. Logistic regression was used to examine the effects of age, HIV, and vascular metabolic markers on global and domain specific cognitive function. Interactions between age and HIV disease and vascular markers were also modeled in analyses of HIV + participants.
Results: HIV + cases performed worse than HIV- in all domains and globally (all $p<.01$). There were significant age x HIV interactions with older HIV + performing incrementally worse on working memory, learning and on global NC function captured by a global deficit score (GDS; all $p<.05$). Exploratory analyses of HIV disease and vascular and metabolic markers showed age interactions with AIDS diagnosis, systolic blood pressure (SBP), BMI, and cholesterol in predicting worse GDS. Multivariate modeling (predictors=age, AIDS, SBP, BMI, and cholesterol; individually and an age interaction term to predict GDS) showed that AIDS ($p=0.005$); the interaction of age and AIDS ($p=0.18$); and the interaction of age and cholesterol ($p=.05$) related to worse GDS. Current and nadir CD4, ART status, and plasma viral load were not related to GDS.
Conclusions: Our results show disproportionate reduction in neurocognitive performance in HIV + persons as they age, and that both severity of HIV disease and indicators of vascular metabolic changes that have been linked to vascular diseases of aging may play a role in amplifying these effects.

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TCF4 binds directly to the HIV LTR and associates with nuclear matrix protein SMAR1 to repress HIV transcription in astrocytes

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Astrocytes are generally restricted in HIV replication but under certain triggers can support productive HIV infection. Given that astrocytes make up 40-70% of brain cells, even low-level infection may contribute significantly to HIV-associated neuropathogenesis. Furthermore, although only 1-3% of astrocytes harbor HIV under resting conditions, this level of infection is sufficient to transmit HIV to infiltrating lymphocytes. We previously established that inhibiting β-catenin signaling via knockdown or treatment with IFNγ triggers productive HIV replication in astrocytes. We also determined that knockdown of β-catenin or its transcriptional partner TCF4 enhances docking of elongation-competent RNA Polymerase II, suggesting that β-catenin/TCF4 repress HIV at the level of transcription. To directly assess the impact of β-catenin/TCF4 on HIV transcription, we evaluated whether β-catenin/TCF4 associate directly with the HIV LTR. We identified four novel TCF4 binding sites on the LTR located at -336, -143, +66 and +186 from the +1 transcription initiation site. Strongest binding was observed at the -143nt site. By chromatin immunoprecipitation we confirmed that TCF4 and β-catenin are docked on the LTR at -143nt but are removed when this site is deleted. Furthermore, deletion or mutation of the -143nt TCF4 binding site in combination with knockdown of β-catenin or TCF4 enhanced basal HIV LTR activity by 4-fold in astrocytes that stably express an LTR-luciferase construct but had no effect on Tat-mediated transactivation. We further show that the nuclear matrix binding protein SMAR1 is tethered on the LTR at the -143nt region. TCF4 and β-catenin co-precipitate with SMAR1, which suggests that β-catenin/TCF4 interact with SMAR1 at -143nt and may render this region inaccessible to transcriptional machinery. Disrupting this complex in astrocytes may account for a more permissible state for HIV productive replication. This work is supported by F31 NS071999 (LJH) and R01NS 060632 (LA).

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A Tale of Mice and Men: Translational cross-species assessment of inhibitory deficits in HIV and comorbid methamphetamine dependence using a novel human open-field paradigm

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Inhibition, the process of withholding or attenuating an action or thought, is of central importance in the regulation of behavior and the ability to function in daily life. Deficits in inhibition are key features of both HIV and methamphetamine (METH) dependence and play a critical role in high-risk behaviors associated with drug use and disease transmission. Although HIV infection and METH exposure are both associated with cognitive dysfunction and neurotoxicity, the combined effects of these factors have not been examined comprehensively. While various domains of inhibition such as hyperactivity, novelty-seeking, and perseveration have been assessed in rodents by quantifying activity in open-field tests, similar measures have not been applied in human subjects with neurological infections. Our group recently established a human open-field paradigm (Human Behavior Pattern Monitor: hBPM), where motor activity and behavior are quantified in a room containing furniture and engaging novel objects, a task directly based on models of rodent exploration. This project assessed inhibition in human participants with HIV infection and METH dependence as compared to a mouse model of HIV, gp120 transgenic mice exposed to chronic METH treatment. We hypothesized that the combined effects of HIV infection/gp120 expression and METH exposure in both species would induce greater inhibitory deficits in human/rodent open-field tests than either factor alone. Human subjects with comorbid HIV infection and METH dependence exhibited the greatest motor activity during the first several minutes in the hBPM relative to comparison groups, with a trend towards elevated multiple object interactions. Expression of the gp120 protein did not significantly impact locomotion in mouse open-field, but chronic METH administration increased novelty-seeking, including time spent with a novel object. These studies demonstrate the utility of cross-species paradigms that can be used to elucidate HIV neuropathology and assess inhibitory dysfunction associated with comorbid substance use.

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Recombinant La Crosse viruses with mutations in the fusion peptide region are less neuroinvasive, but remain neurotoxic

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La Crosse virus (LACV), a member of the California serogroup of orthobunyaviruses, is a major cause of pediatric encephalitis and aseptic meningitis in the Midwestern and Southern United States where it is considered an emerging human pathogen. Our laboratory recently identified the LACV fusion peptide and we hypothesize that this region is a critical determinant of neuroinvasion. To test this hypothesis, we generated a panel of recombinant LACVs (rLACVs) containing mutations in the fusion peptide. In vitro testing of these recombinant viruses confirmed that rLACVs with targeted changes in the fusion peptide region have decreased replication and fusion phenotypes, yet remain neurotoxic in a primary rat neuronal culture system. These data suggest that the LACV fusion peptide is associated with properties of neuroinvasion (growth to a high titer in muscle cells and a robust fusion phenotype), but not necessarily with neurovirulence. Here, we extend these studies to our established murine model of LACV encephalitis, which mirrors many features of the human disease, including age-dependent susceptibility, to determine the neuroinvasive and/or neuropathogenic phenotypes of the mutant rLACVs. When suckling mice (susceptible to peripheral challenge of LACV) are inoculated subcutaneously with rLACVs, the fusion peptide domain mutants have a decelerated rate of disease progression. However, these rLACVs retained their ability to mediate severe neurologic disease without significantly slowing the survival kinetics when inoculated directly into the central nervous system of adult mice. Tissue titrations and histological studies are in progress to further characterize the in vivo consequences of changes in the LACV fusion peptide. Importantly, the high conservation of the fusion peptide domain among the Bunyaviridae makes these findings applicable to other bunyaviruses, including those that also cause neurologic disease.

P99

Induction of Non-Canonical Signaling Pathways in Primary Neurons Results in Tailored Responses to Interferon Gamma

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Interferons mediate their effects through activation of STAT1, though we have shown that STAT1 levels in primary mouse neurons are drastically reduced as compared to control fibroblasts, despite the ability of IFN gamma (IFNg) to protect neurons from viral infection. Moreover, in both MV-permissive STAT1 deficient mice and in primary hippocampal neurons explanted from these mice, the absence of STAT1 did not restrict the ability of the host response to mediate viral clearance. To identify the principal signal transduction pathways through which IFNg signals in neurons, we assessed the activation of two alternative signaling molecules that have been associated with IFNg signaling: Akt and Erk. Following IFNg stimulation, there was a rapid activation of Akt and Erk signaling pathways, distinct from the delayed and weaker response made in fibroblasts. This response, as expected, influenced expression of genes typically associated with these pathways, despite the fact that STAT1 responsive genes remain quiescent following IFNg stimulation. Since many of these molecules were pro-survival genes, we next asked whether IFNg conferred a protective advantage to treated neurons: indeed, the apoptotic effects of staurosporine were significantly blunted by the addition of IFNg, though pharmacological blocking the Akt and Erk pathways negated this protective effect. Interestingly, the paucity of available STAT1 in the cytoplasm may change the access of other signaling molecules to the IFNg receptors, since STAT1 KO MEFs show a similarly enhanced Akt and Erk response profile following IFNg stimulation. We propose a receptor occupancy model in which the natural paucity of cytoplasmic STAT1 in neurons allows for other signal transducers to access the IFNg receptors and trigger alternative pathways; these pathways result in induction of genes that afford protection of these nonrenewable cells under conditions of viral infection.

P100

Hippocampal dysgenesis in a model of gestational virus infection

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Epidemiologic evidence supports a role for maternal infection as a risk factor for autism and schizophrenia. Alterations in brain morphology and function in these conditions are proposed to result from direct influences of infectious agents, or the immune activation they induce, on neuronal proliferation and migration. Behavioral, histopathologic and imaging studies in autism and schizophrenia suggest involvement of hippocampal structures. We used a mouse model wherein maternal inflammation is induced by the viral RNA mimic, polyinosinic-polycytidylic acid (poly I:C), to test whether prenatal immune activation alters neurogenesis in dentate gyrus of offspring hippocampus. Previous work in this model using in vitro assays indicated dramatic reductions in intermediate progenitor cell proliferation and an increase in neuronal apoptosis. Offspring also showed deficits in prepulse inhibition and abnormalities in locomotor, learning and memory function. Effects were not observed in TLR3-/- mice or in wildtype C57BL/6 J mice pretreated with a nonsteroidal anti-inflammatory drug. To assess the role of hippocampal neurogenesis in these abnormalities, poly I:C and BrdU were administered to pregnant wildtype mice between gestational days 14 and 17. Prenatal poly I:C exposure was associated with markedly reduced numbers of BrdU-positive granule cells in dentate gyrus of offspring at postnatal day 8 ($p=0.007$). Exposure to poly I:C alone at gestational day 16 also showed reduced density of granule cells in dentate gyrus at postnatal week 4 and 11 ($p=0.002$); poly I:C effects on granule cell density were diminished by week 20 ($p=$NS). These findings indicate that late prenatal inflammation affects genesis and migration of neurons to the dentate gyrus, and may help explain the pathogenesis of behavioral deficits in this mouse model of infection-related neurodevelopmental disorder.

P101
Mechanisms of rabies virus-induced oxidative stress

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Despite severe clinical illness, the neuropathology of rabies is relatively bland with mild inflammatory changes and few degenerative neuronal changes. This has led to the concept that the underlying abnormality is neuronal dysfunction, but the precise mechanisms remain uncertain. We have evaluated CVS-infected transgenic mice that express the yellow fluorescent protein and found evidence of neuronal process degeneration involving dendrites and axons. We have used adult rodent dorsal root ganglion (DRG) cultures to evaluate structural changes in axons of CVS-infected cultures. In vitro infection of DRG neurons (permissive to infection) showed prominent axonal swellings associated with 4-hydroxy-2-nonenal (4-HNE) immunostaining, which is a marker of lipid peroxidation with oxidative stress, and good viability of the neurons. Ciliary neurotrophic factor, an activator of NF-κB, was highly neuroprotective of CVS-infected neurons in reducing the number of 4-HNE-labeled puncta. SN50, a peptide inhibitor of NF-κB, and CVS infection had additive effects in producing axonal swellings, indicating that NF-κB is neuroprotective. Western immunoblotting showed increased expression of NF-κB in infected DRG neurons. The fluorescent signal for p50 subunit of NF-κB was quantitatively evaluated in the nucleus and cytoplasm of DRG neurons. At 24 hrs there was a significant increase in the nucleus:cytoplasm ratio, whereas at 48 and 72 hrs there was significantly reduced nuclear localization of NF-κB in CVS infection. In CVS infection Krebs cycle enzyme (citrate synthase and malate dehydrogenase) activities were normal, whereas activities of electron transport chain Complex I and IV were significantly increased vs. mock infection. Neuronal dysfunction in rabies is associated with structural changes involving neuronal processes that are mediated by oxidative stress. Rabies virus inhibits nuclear activation of NF-κB. Mitochondrial dysfunction likely plays a role in the increased production of reactive oxygen species (ROS). Increased activity of Complex I may lead to ROS overproduction due to reverse electron transfer.

P102
Polymorphisms of DA receptors are associated with substance dependence and cognitive functioning in HIV + individuals

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It has been postulated that drugs of abuse may act in combination with HIV, leading to increased neurotoxicity and neurocognitive impairment. Mechanistically, HIV and drug abuse converge on the mesocorticolimbic dopamine (DA) system, which contains two main receptor subtypes. Dopamine receptor 1 (DRD1) has a strong cortical bias in its expression in the human brain and is widely implicated in cognitive function while dopamine receptor 2 (DRD2) is

most strongly linked with reward. Using a Manhattan HIV Brain Bank population of HIV + individuals (N=264), we evaluated polymorphisms in these genes in relation to opioid and cocaine dependence and found that three DRD1 and two DRD2 polymorphisms were significantly associated ($P<0.05$). Using regression analysis, we next examined these polymorphisms for their association with neuropsychological performance amongst seven cognitive domains (Motor, Speed of Information Processing, Verbal Fluency, Learning, Memory, Abstraction/Executive Functioning, and Working Memory) while factoring in opioid and cocaine dependency. In the Motor domain, we observed trend level associations for a single DRD1 polymorphism and two DRD2 polymorphisms ($P<0.10$). Similar observations were also seen for the Working Memory domain with a significant association found for a single DRD1 polymorphism ($P=0.05$) and a trend level association for a single DRD2 polymorphism ($P<0.10$). In both domains, the direction of the effect differed for substance use groups: in opioid and cocaine dependent individuals, the direction of the correlations with DRD1 and DRD2 were opposite to what was seen in subjects without substance dependence. We conclude that polymorphisms in DRD1 and DRD2 are strongly correlated with substance dependency; when this trait is accounted for in analysis, weaker associations emerge for a restricted number of cognitive domains. These results highlight the importance of the dopaminergic system in both substance dependence and cognitive performance in HIV + individuals.

P103

Plasma Gelsolin and HIV-1 Infected Macrophage: Implications for Chronic Neuroinflammation

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Introduction: Plasma gelsolin (pGSN, secretory isoform1) has been postulated to modulated inflammatory responses. Its expression is independently regulated in the central nervous system and the periphery. Based on experiments using GSN-/- mouse model, it has been postulated that modulation of pGELS expression might be beneficial when it is inhibited during acute inflammation and increased during chronic inflammation. Our previously work revealed reduced levels of pGSN in the CSF of individuals with advanced neurocognitive impairment due to HIV infection of the brain, but its expression in the brain parenchyma during infection is unknown.
Results: In vitro experiments showed that HIV-1 infected macrophage secrete pGSN. This is further supported by immunohistochemistry studies on sections of SIV infected monkey brains showing accumulation of pGSN in macrophage nodules. This indicates that increased levels of pGSN are present only in a local milieu, thus it will not have impact on the overall net decreased concentration of pGSN in the CSF of HIV-1 infected subjects. Accumulation or clearance of pGSN within macrophage nodules and elsewhere can be regulated by MMPs for which pGSN is a substrate. Moreover, our studies did not show accumulation of pGSN around neurons which are postulated to be the major producer of pGSN in the brain. Our preliminary data show that exposure of uninfected macrophages to pGSN leads to secretion of pro-inflammatory cytokines such as TNF-a and IL1b. We find that exposure of control or HIV-1 infected macrophages to pGSN leads to transient inhibition of phosphorylation of p38MAPK (no change in levels of p38MAPK itself), providing mechanistic clues.
Perspective: Our working hypothesis is that pGSN contributes to persistent and low levels of neuroinflammation within and around macrophage nodules.
Support: This work was supported, in part, by NIH Grant P20DA026146; 5R01DA030962; P01 DA12065; P30 MH062261.

P104

VZV infection of human brain vascular adventitial fibroblasts downregulates STAT mRNA expression and inhibits translocation pSTAT to the nucleus

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Primary varicella zoster virus (VZV) infection causes varicella (chickenpox), after which virus becomes latent in ganglionic neurons. With advancing age, a declining cell-mediated immunity to VZV leads to virus reactivation and manifests as zoster (shingles). Zoster may be complicated by VZV vasculopathy (TIAs or stroke) caused by productive virus infection in arteries. Within the first year after zoster, there is a 30% increased risk of stroke. In VZV vasculopathy from 4 to 10 months after zoster, both viral

antigen and abundant leukocyte subsets are found. To understand the mechanism(s) by which VZV persists despite a robust immune response, we focused on the Jak-STAT pathway that is commonly activated after viral infection. In uninfected cells, type I interferons (specifically IFNα) bind to interferon receptors, resulting in auto-phosphorylation of Jak proteins, which in turn phosphorylates one of the STAT proteins (pSTAT). pSTAT then complexes with interferon regulatory factor (IRF) 9, and the complex is translocated to the nucleus. After translocation, pSTAT upregulates transcription of multiple antiviral proteins (Mx1, RNAse L and protein kinase R). In SCID-hu xenograft models, VZV-infected skin cells have decreased amounts of IFNα, and pSTAT does not translocate to the nucleus, as in uninfected bystander cells. Herein, we showed that in VZV-infected primary human cerebrovascular adventitial fibroblasts, expression of STAT1α mRNA was downregulated by ~50% compared to mock-infected fibroblasts. In contrast, Jak mRNA was unaffected by VZV infection. Immunostaining revealed that pSTAT did not translocate to the nucleus in VZV-infected cells, but was seen in the nucleus of uninfected bystander cells as well in mock-infected cells. Overall, VZV infection of cerebral adventitial fibroblasts blocked translocation of pSTAT to the nucleus. Our findings provide a potential mechanism by which transcription of antiviral proteins are downregulated by VZV, which in turn leads to viral persistence, vascular remodeling and potential stroke.

P105
Involvement of the antioxidant response pathway in neuroglial, neuronal, and astrocytic cultures exposed to antiretroviral compounds

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Despite restoration of immune function due to effective viral suppression by antiretroviral (ARV) therapy, 30-60% of HIV-positive patients suffer from neurological impairment of varying severity, collectively termed HIV-Associated Neurocognitive Disorder (HAND). Persistent inflammation and viral reservoirs are touted as sources for continuous oxidative damage in the central nervous system of individuals with HAND on ARV. ARV drugs induce ER and oxidative stress, reactive oxygen species (ROS) and cell death in several non-neural cell types. Furthermore, we have shown ARV drugs induce ROS, subsequent mitochondrial depolarization and eventual neuronal toxicity in cortical neuroglial cultures. In non-neural cells, oxidative stress induces the endogenous antioxidant response (EAR), activating transcription factor Nrf2 which upregulates expression of protective enzymes. Thus, we hypothesized that EAR is activated in neuroglial cultures in response to ARV compounds, altering cellular function/survival. Primary rat cortical neuroglial, enriched astrocytic, and enriched neuronal cultures were treated with ARV drugs: zidovudine, ritonavir, saquinavir, or EAR inducer tert-butyl hydroperoxide (tBHP) for 1,2, 6, 12, or 24 hours and assayed for ROS by dihydroethidium staining, as well as Nrf2 stabilization and expression of Nrf2 targets, HO-1 and NQO1, by qPCR and immunoblot. Our findings show rapid ROS accumulation in neuroglial and enriched neuronal culture, resulting in Nrf2 stabilization, followed by effector protein induction. Compared to neuroglial cultures, ROS accumulation in enriched neuronal culture begins earlier, leading to damage/death by 12-24 hours. No ROS accumulation or EAR activation was seen in astrocytic cultures. Rather, beginning at 12 hours, ARV compounds and tBHP show a reduction of ROS. Astrocytes buffer ROS via constitutively high glutathione levels, which may account for the ROS reduction observed. Our data suggest the EAR is transiently activated in response to ARV, and targeted, sustained increases in EAR activity may promote survival in neurons exposed to ARV-, and possibly HIV-, induced oxidative stress.

P106
CNS-IRIS is mediated by HIV-Tat activated T cells via a novel antigen independent mechanism involving histone acetylation

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Background: Immune Reconstitution Inflammatory Syndrome (IRIS) is characterized by deterioration in clinical status after the initiation of anti-retroviral therapy. It may involve the CNS, even in the absence of opportunistic infections, resulting in a severe T-cell mediated encephalitis and death.
Methods and Results: Immunohistochemical analysis of brain sections from a patient with CNS-IRIS demonstrated a presence of T-cells, IL-17+ cells and HIV-Tat but an absence of p24 antigen. Hence to determine if extracellular Tat could activate T-cells, isolates of T-cells from normal human donors (n=5) were exposed to Tat protein and gene expression was determined by microarray analysis and cytokine arrays. Select

cytokines were confirmed by ELISA. Tat-treated T-cells released IL-17 and granzyme B in a dose-dependent manner. T-cell activation was not blocked by inhibition of T-cell receptor (TCR) signaling and Tat was able to activate T-cells from ova-specific mice, indicating a novel TCR independent mechanism of T-cell activation.. Tat cellular uptake was visualized by ImageStream analysis and shown to be dependent on clatharin-mediated endocytosis. Interestingly, Tat did not cause proliferation of T-cells as determined by tritated thymidine incorporation. Tat-mediated T-cell activation was dependent upon upregulation of VEGF-C resulting in autocrine VEGFR2 activation. Using pharmacological inhibitors, the cascade was found to involve activation of NF-κB, PI3 kinase, mTOR and CDKs but was independent of PKC. HIV is only capable of infecting activated T-cells therefore we examined if extracellular Tat activation of T-cells enhanced HIV infection. Tat stimulation enabled HIV by increasing histone acetylation which modified chromatin structure allowing for viral integration. Conclusions: HIV Tat protein activates T-cells in a novel antigen independent manner leading to an alteration of chromatin state, increased gene transcription, release of cytokines and cytoxic granules and enhanced viral infection. This mechanism of T-cell activation opens new avenues for therapeutic intervention for HIV-associated IRIS and chronic inflammation.

P107

HIV-1 Tat Neuropathological Effects Mediated by Cellular IRF3 and 7 Transcription Factors Involving a Zinc-coordinating C Residue in Tat Partners

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The IRF proteins are becoming recognized as an independent class of transcription factors regulating critical cellular gene expression pathways in response to external threats. Little is known about IRF activities in neurons and glial cells of the CNS, especially in neuroAIDS, in which these cells are pathologically affected but are not infected with HIV-1. Data presented here indicate that HIV-1 infection in the CNS elicits an IRF response in non-infected neurons and glial cells and that this response is subverted by the HIV-1 protein, Tat. Tat contains a region of 20 amino acids, highly conserved among HIV-1 clades, with 6 Cys (C) residues. Recent X-ray crystallographic data from Tat bound to Cyclin T1/Cdk9 and from Purα show that Tat requires C261 from Cyclin T1 to coordinate two Zn atoms in a manner that stabilizes Tat structure, enabling it to bind RNA. Purα, another well-characterized Tat-binding partner, contains a domain with a C residue and several aa's homologous to those of Cyclin T1, and mutations in the Tat C-rich region inhibit binding to Purα, as they do to Cyclin T1. The interferon regulatory factors 3 and 7 (IRFs 3,7) are redirected in cellular localization by Tat and colocalize with Tat. Both IRFs contain a C domain homologous to those of Cyclin T1 and Purα, and GST-Tat pulldowns from lysates of U-87 MG glial cells show that Tat binds to both. Confocal laser microscopy, together with GST-Tat pulldowns, reveal that Tat binds to phosphorylated (i.e., activated) and non-phosphorylated forms of IRF3. Tat targets cytoplasmic, non-phosphorylated IRF3 for proteolytic degradation. The remaining, phosphorylated IRF3 is dimerized and colocalized with Tat in nuclear structures. Results reveal a novel, IRF-mediated pathway for potential intervention in Tat activities. Supported by NIH R01 NS35000.

P108

SV40 neuronal infection in SIV-infected rhesus

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SV40 NEURONAL INFECTION IN SIV-INFECTED RHESUS S. Kaliyaperumal1, X. Dang2, C. Wuthrich2, H.L. Knight1, C. Pearson1, K.G. Mansfield3, I.J. Koralnik2, S.V.Westmoreland[1] [1]New England Primate Research Center, Southborough, MA. 2 Beth Israel Deaconess Medical Center,

Division of NeuroVirology, Department of Neurology, Boston, MA. 3 Novartis, Cambridge, MA. Simian virus 40 (SV40), family Polyomaviridae, can cause fatal demyelinating CNS disease analogous to progressive multifocal leukoencephalopathy (PML) caused by JC virus (JCV) in immunosuppressed humans. Recently, we have demonstrated that JCV may infect cerebellar granule cell neurons and cortical pyramidal neurons in immunosuppressed people. In addition, passaged neurotropic isolate, SV40CNS1, infected neurons when inoculated intravenously in two SHIV-infected rhesus. To examine whether SV40 neuronal infection occurs spontaneously in immunosuppressed macaques, we analyzed archival brain specimens from 15 SIV-infected rhesus with AIDS and SV40 brain infection from 1997 to 2011. In addition to white matter SV40 distribution in classical demyelinating PML, some of the 15 monkeys exhibited meningeal, subpial neocortex, and periventricular virus. This distribution pattern of corresponded to broader viral tropism with neuronal infection in 9/15 cases (60.0%). In all 9 cases, MAP2+ neurons were positive for SV40 large T-antigen (T-ag), but only 2/9 cases exhibited neurons positive for VP1, suggesting that 7/9 cases had restrictive neuronal infection (VP1-, T-ag+) while 2/9 had productive neuronal infection (VP1+, T-ag+). SV40-infected neurons were detected in parietal, occipital, and temporal cortices, hippocampus, thalamus, and brain stem. The presence of SV40 DNA in cortical samples was verified by PCR and sequencing. A mutation in the agnoprotein was detected in 1/9 cases with SV40 neuronal infection, which may impact T-ag transcription. These observations confirm that spontaneous SV40 neuronal infection occurs in immunosuppressed macaques, which parallels JCV-neuronal infection in immunosuppressed people. Neuronal infection appears to be an important aspect of both SV40 and JCV pathogenesis in their respective hosts.

P109
To prevent or repair HIV-associated brain injury

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HIV-1 infection often devastates the immune and the nervous system. Combination anti-retroviral therapy (cART) extends the life expectancy of HIV patients, but fails to prevent HIV-associated neurocognitive disorders (HAND). Therefore, additional neuroprotective strategies are urgently needed. Some HIV-infected individuals do not progress to AIDS or neuroAIDS, suggesting that protective host factors and mechanism exist that may be harnessed to prevent or repair HIV-associated brain injury and HAND. CCR5 and CXCR4 are chemokine and major HIV co-receptors. Virus-associated or soluble HIV-1 envelope glycoprotein gp120 interacts with CD4 in conjunction with CCR5 and CXCR4 leading to infection and/or cellular signaling. However, natural CCR5 ligands are important components of the anti-viral immune response that possess neuroprotective properties. Therefore, our group studies the potential role of CCR5 in promotion and prevention of HIV-associated brain injury using in vitro and in vivo approaches. AIDS patients and mice expressing HIV-1 gp120 as transgene in the brain (gp120tg) share distinct neuropathological features, such as a decreased number of neurites and synapses, pronounced astrocytosis and activated microglia. Recently, we generated and characterized crosses of HIVgp120tg with CCR5KO mice. Microarray analysis of brain tissue shows that HIV-1 gp120 affects the expression of about 800 genes in the presence, but of only about 50 genes in the absence of CCR5. Of the CCR5 ligands, only the expression of CCL5 is increased in gp120-transgenic brain, independently of the presence or absence of CCR5. However, quantitative fluorescence and deconvolution microscopy indicates that the knockout of CCR5 largely abrogates neuronal injury and microglial activation triggered by viral gp120, but not astrocytosis. Altogether, CCR5 appears to be a host factor that contributes to HIV-associated microglial activation and neuronal injury, and thus provides a therapeutic target not only to limit HIV infection but also to protect and possibly repair the brain.

P110
Replication of Chimeric HIV in Mouse Brain induces HAND-like Pathology and Cognitive Disease Independent of HIV Envelope Glycoprotein

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Inoculation of mice with mouse-tropic, gp120-negative HIV, EcoHIV, establishes chronic systemic infection that remains mostly under host immune control similar to some HIV-infected individuals. Despite this control, low levels of virus and mild neuropathogenic changes were found in the brain of infected mice. To further explore the pathologic and cognitive consequences of this infection, we inoculated EcoHIV directly into mouse striatum and examined virus replication, brain tissue pathology, cellular gene changes, and mouse behavior in radial arm water maze. Because EcoHIV uses MLV envelope in place of gp120 for virus entry, infectious MLV was used as a control mouse pathogen. EcoHIV but not MLV induced extensive brain lesions containing infiltrating macrophages and T and B cells, and caused pronounced astrocytic and macrophage/microglia activation in brain parenchyma. Both EcoHIV and MLV were expressed in the brain, with HIV replication documented by detection of viral genomic DNA and RNA, 2LTR circles, presence of spliced viral RNA species, and synthesis of viral p24. HIV p24 was found mostly in macrophage/microglia but some neurons and infiltrating T lymphocytes were also positive. EcoHIV but not MLV-infected mouse brains showed significant changes in expression of selected cellular genes involved in HIV neuropathogenes in HIV-infected people including TNF-alpha, MCP-1, MIP-1alpha, and IP-10. Most importantly, EcoHIV but not MLV-infected mice exhibited significant learning and memory deficits in radial arm water maze tests. Thus intracerebral infection of immunocompetent mice with chimeric HIV lacking HIV gp120 recapitulates many features of advanced HAND. Our results indicate that while HIV replication is essential for these pathologies, expression of HIV gp120 is dispensable. The animal model described here permits experimental analysis of the link between HIV replication in the brain, expression of individual viral proteins, and behavioral and neurocognitive deficits. Supported by DA017618, MH083627 and DA026311 from NIH.

P111

Varicella-Zoster virus isolates obtained from patients with zoster complicated by post-herpetic neuralgia induce significant increases in sodium current density in the ND7/23- Nav1.8 neuronal cell line

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Varicella-Zoster virus (VZV) causes chickenpox (varicella) as a primary infection following which it becomes latent in sensory ganglia and reactivates to cause herpes zoster (shingles). The most important complication of zoster is post-herpetic neuralgia (PHN) which causes severe, chronic dermatomal pain which is extremely refractory to treatment. While host factors, such as age and immunosuppression, are important in the development of PHN, viral factors may also be important. We therefore hypothesized that PHN-associated, but not non-PHN-associated, VZV isolates could induce alterations in neuronal sodium ion channel activity since the latter are known to be associated with neuropathic pain. Twenty VZV isolates were studied blind from both PHN (11 samples) and non-PHN (9 samples) subjects. Viruses were propagated in the MeWo cell line from which and cell-free virus was harvested at 72 hrs and plated onto the ND7/23-Nav1.8 neuroblastoma cell line The latter showed constitutive expression of the exogenous Nav 1.8 and the endogenous Nav 1.6 genes encoding sodium channels both of which are known to be associated with neuropathic pain. Single cell sodium ion channel recording in the infected and control cells was performed after 72 hr by voltage-clamping. We found that PHN-associated VZV isolates significantly increased sodium current amplitude in the cell line when compared with non-PHN VZV, wild-type (Dumas) or vaccine VZV strains ((POka, Merck and GSK). This sodium current increase was unaffected by acyclovir pre-treatment but was abolished by exposing the infected cell lines to Tetrodotoxin (TTX) which blocks the TTX-sensitive fast channel Na v 1.6 but not the TTX-resistant slow Na v1.8 channel. Therefore, PHN-associated VZV sodium current increases were mediated in part by the Nav 1.6 sodium channel which has been reported to play a role in neuropathic pain. To our knowledge this is the first report of a pain-associated in vitro effect induced by VZV.

P112

Tropism of Chimeric HIV in Mouse Brain Cells

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Inoculation of mice with mouse-tropic, gp120-negative HIV, EcoHIV, establishes chronic systemic infection in peripheral T lymphocytes and macrophages with low levels of virus and mild neuropathogenesis in the brain. To begin to investigate the basis for EcoHIV neurotropism in mice, we examined EcoHIV infection in mouse mixed brain cell cultures and enriched populations of neurons, astrocytes, and bone marrow-derived macrophages (BMM) in vitro. We used cells from C57BL/NJ and 129X1/SVJ mice and tested infection with a Clade B EcoHIV/NL4-3 and Clade D EcoHIV/NDK viruses. HIV infection was scored by measurement of HIV DNA and RNA by qPCR, detection of green fluorescence protein (GFP) from EcoHIV indicator viruses, detection of viral p24 by immunohistochemistry, and p24 ELISA. The identity of infected cells was determined by double-staining for cell lineage markers. In mixed brain cell cultures, EcoHIV infected in a virus dose-dependent manner up to 90% of microglial cells, 0.01%-0.08% of neurons, and negligible fraction of astrocytes. EcoHIV/NDK was more infectious than EcoHIV/NL4-3 and cells from 129X1/SVJ were more susceptible than C57BL/NJ cells, attributed to higher expression of EcoHIV receptor in 129X1/SVJ mice. Similar to mouse microglia, murine BMM were highly susceptible to EcoHIV; the progeny virus produced in these cells could be passaged to uninfected BMM. Interestingly, exposure of enriched neuronal cultures to EcoHIV resulted in neurotoxicity without expression of viral proteins. We conclude that EcoHIV, which uses the ecotropic MLV receptor for cellular entry, reproduces HIV tropism to human CNS-derived cells by preferential infection of mouse microglia and macrophages. The molecular basis of the observed resistance of mouse neurons and astrocytes to EcoHIV expression is under investigation. The similarity of the HIV infection phenotypes in human and mouse CNS cells suggests that EcoHIV is suitable for study of HIV neuropathogenesis in mice. Supported by DA017618 & MH083627 from NIH.

P113

Proline rich fusion domain of the mouse hepatitis virus spike protein is involved in the neuronal cell fusion and induction of axonal loss concurrent with demyelination

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Neurotropic mouse hepatitis virus infection in mice causes meningoencephalitis, myelitis, demyelination and axonal loss. Infection of mice with RSA59, a demyelinating strain containing its wild-type A59 spike gene, causes axonal loss concurrent with macrophage-mediated demyelination, which mimics certain pathology of human demyelinating disease multiple sclerosis. In contrast, infection with RSMHV2, a non-demyelinating strain containing the MHV2 spike gene in the MHV-A59 genome, shows only rare early axonal degeneration. Axonal loss and demyelination can occur due to direct viral attack. The mechanisms mediating demyelination and axonal loss are dependent on spike-mediated axonal transport of viral particles from gray matter to white matter. MHV spike protein is synthesized as an 180 kDa glycosylated precursor, posttranslationally cleaved into two 90 kDa subunits, S1 and S2, with a receptor binding domain in the S1 that is responsible for the initial attachment of MHV to cell surface receptors. This binding event triggers a conformational change in spike that allows cleavage and S2 to initiate fusion of the host membranes. A candidate fusion peptide domain has been identified within S2. Homology modeling demonstrates that despite of the high sequence identity of these two fusion peptides, MHV-A59 fusion peptide is unique as it contains two consecutive proline residues in comparison to one in MHV-2. Because of insertion of an extra proline residue in the sequence, the central loop region of MHV-A59 fusion peptide has unique structural conformation which could be responsible for the fusion of the virus with the neural cell membrane and pathogenicity. To evaluate the role of these two consecutive proline residues in membrane fusion we have compared the putative domain of RSA59 spike protein with RSMHV-2 spike protein and design targeted RNA recombination to swap the domain between RSA59 and RSMHV-2 to confirm the putative domain is responsible for membrane fusion and subsequent demyelination.

P114

Vpr as a regulator of NF-κB in HIV-1: Selective inhibition of the TNF-α pathway

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Numerous studies have reported that Vpr alters NF-κB signaling in various cells, however, the findings have been largely conflicting with reports of both stimulatory and inhibitory effects of Vpr. Our aim was to investigate the role of Vpr signaling in myeloid cells and address controversies that have developed in the field. Our results show that Vpr expressed intracellularly is inhibitory to NF-κB, while extracelluar Vpr may have opposite stimulatory effects. Consistent with this notion, we report that Vpr has inhibitory effects that are specific to the TNF-α pathway, but not affecting the LPS pathway, suggesting that differential targets of Vpr may exist for NF-κB regulation. Further, we identify VprBP as one possible cellular component of Vpr's regulation of IκBα in response to TNF-α stimulation. We did not identify such a role for HSP27, which instead seems to inhibit Vpr functions. Finally, our findings suggest that NF-κB regulation by Vpr is further influenced by the presence of other HIV-1 components within infected cells, as chronically HIV-1 infected U1 cells with knockdown constructs for Vpr were unexpectedly less responsive to TNF-α. This data suggests that Vpr may serve an important role in vivo by selectively inhibiting immune activation while stimulating NF-κB mediated viral production in HIV-1 infected T-cells and myeloid cells.

P115
M-CSF receptor: An important oncogene in AML and potential therapeutic target?

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The oncogenic potential of M-CSF receptor (cFMS) has been noted for over thirty years, however, few current studies have focused on the role of the receptor in AML. In a clinical trial for AML, Sunitinib was found to hold some efficacy for treating the disease. The authors hypothesized that the primary therapeutic target of Sunitinib in AML is FLT3 kinase. However, FLT3 inhibition alone has not been shown to recapitulate all the effects of Sunitinib in vitro and the drug is known to have cross reactivity to other potential oncogenic receptors as well. In this study, we treated three myeloid cell lines, Mono-Mac 1, THP-1 and U937 with Sunitinib or a small molecule (cFMS-I) optimized for cFMS inhibitory activity to test the anti-cancer effect in of such treatment. We observed that only Mono-Mac 1 cells had diminished proliferation in vitro. Mono-Mac 1 cells had inhibited ERK as a result of cFMS inhibition and showed a dose dependent increase in cFMS expression with both Sunitinib and cFMS-I. Our results suggest potential for cFMS as an important target of Sunitinib or other similar drugs AML, either independently or in combination with other therapeutics. Alternatively, cFMS may be a marker for differentiation of AML and may be linked with responsiveness to certain therapeutics. In both cases, the future study of cFMS may produce more targeted therapeutic approaches and may be a suitable tool for the development of personalized medicine for AML.

P116
Cocaine-induced loss of white matter proteins in the adult mouse nucleus accumbens is inhibited by administration of a β-lactam antibiotic during withdrawal

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Cocaine remains among the most commonly abused drugs worldwide and poses a substantial health and economic burden. Imaging studies and post-mortem microarray data from human cocaine abusers reveal significant deficits in white matter (WM) integrity and myelin-related genes in

discrete brain regions, including the nucleus accumbens (NAc). While underlying mechanisms remain largely unknown, WM abnormalities likely contribute to the cognitive, motor, and psychological deficits commonly afflicting cocaine abusers. Furthermore, human studies indicate that abstinence from cocaine allows for recovery of some cognitive function and gray matter density. Interestingly, WM density does not appear to recover in these patients, indicating long-term and potentially irreversible damage as a result of repeated cocaine exposure. As human studies are often confounded by poly-drug abuse and co-morbid conditions, in vivo animal models are needed to delineate pathways contributing to WM loss and highlight potential treatment avenues. In this context, we have uncovered a new paradigm by which chronic cocaine treatment significantly decreases the expression of WM proteins in the NAc of adult mice. Furthermore, we demonstrate that this WM loss can be inhibited and/or reversed by administration of a β-lactam antibiotic, ceftriaxone, during a period of cocaine withdrawal. Mice exposed to a chronic cocaine paradigm (15 mg/kg daily for 14 days) followed by 30 days of cocaine withdrawal and a challenge dose displayed significant decreases in myelin basic protein, proteolipid protein, myelin-oligodendrocyte glycoprotein, and myelin-associated glycoprotein in the NAc. Moreover, these changes were inhibited by administration of ceftriaxone (200 mg/kg daily) during withdrawal. No effect on WM loss was observed in mice treated with ceftriaxone during cocaine treatment but vehicle only during the 30 day withdrawal prior to challenge. Our observations identify cocaine-mediated myelin loss in an adult mouse model and highlight a potential pharmacological intervention to reverse and/or prevent cocaine-induced WM loss.

P117

Cocaine Decreases Expression of Neurogranin via Alterations in Thyroid Receptor/Retinoid X Receptor Signaling

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Mounting evidence suggests a potential link between cocaine abuse, disruptions in hypothalamic-pituitary-thyroid (HPT) axis signaling, and neuroplasticity, but molecular mechanisms remain unknown. Neurogranin (Ng) is a gene containing a thyroid hormone responsive element (TRE) within its first intron that is involved in synaptic plasticity. Transcriptional activation requires heterodimerization of thyroid hormone receptor (TR) and retinoid X receptor (RXR) bound by respective ligands triiodothryonine (T3) and 9-cis-retinoic acid (9-cis RA), and subsequent binding of this complex to the TRE of the Ng gene. In this study, we have characterized the effects of chronic cocaine abuse on Ng expression in euthyroid and hypothyroid mice. In cocaine-treated mice, decreased Ng expression was observed in the absence of changes in levels of TH or other HPT signaling factors. Therefore, we hypothesized that cocaine decreases Ng expression via alterations in 9-cis-RA availability and TR/RXR signaling. In support of this hypothesis, RXR-γ was significantly decreased in brains of cocaine-treated mice while Cyp26a1, the main enzyme responsible for neuronal RA degradation, was significantly increased. Results from this study provide the first evidence for a direct effect of cocaine abuse on TR/RXR signaling, RA metabolism, and transcriptional regulation of Ng, a gene essential for adult synaptic plasticity.

P118

Neural crest lineage cells isolated from the bone marrow express JCV T-antigen: A model for JCV latency and reactivation

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JC virus (JCV) is a ubiquitous human polyomavirus and the etiological agent of progressive multifocal leukoencephalopathy (PML). In addition to its role in PML, numerous studies have demonstrated the transforming ability of JCV early protein, T-antigen, and its association with human cancers. JCV infection most likely occurs in childhood and it has been hypothesized that latent virus is maintained within the bone marrow, which harbors cells of many lineages in addition to the hematopoietic component. We have

recently shown that nonhematopoetic bone marrow-derived mesenchymal stem cells, also known as marrow stromal cells (MSCs), can be infected with JC virus and can undergo oncogenic transformation mediated by JC virus T-antigen to induce neural-like tumors in animals. MSCs are heterogenous cells that partially originate from the neural crest and have been shown to be capable of trafficking and have the potential for multilineage differentiation. MSCs isolated from the bone marrow of JCV T-antigen transgenic mice, when cultured in conditions typical for mesenchymal cells selected, for a population of T-antigen negative cells, which did not express neural crest markers, while JCV T-antigen positive cells arose in cells cultured under neural conditions and expressed p75, i.e. had neural crest characteristics. The JCV T-antigen positive neural crest-derived bone marrow cells exhibited SOX-10 and nestin positivity and could be successfully cultured long-term while maintaining their neural crest characteristics. We conclude that JCV T-antigen can be stably expressed within the neural crest population of the bone marrow. These findings suggest an appropriate population within the bone marrow for JCV to establish latency and these cells may be critical for understanding of JCV reactivation and distribution. Further, our data provides an excellent experimental model system for studding the cell-type specificity of JCV T-antigen expression and the role of bone marrow-derived stem cells in the pathogenesis of JCV-related diseases.

P119

Central role of cytochromes p450 2a6 in tobacco-mediated oxidative stress in maccrophages and astrocytes: implications with hiv-1 pathogenesis and neuroaids

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Tobacco consumption/smoking, which is highly prevalent in HIV + persons, is known to increase HIV-1 replication and perhaps AIDS/neuroAIDS development and progression. We hypothesize that cytochrome P450 2A6 (CYP2A6) plays a central role in nicotine (tobacco's major constituent) mediated oxidative stress, leading to increased HIV-1 replication in monocytes/macrophages and astrocytes. CYP2A6 is known to metabolize nicotine and activates other nicotine-derive nitrosamine ketone (NNK), which causes oxidative stress mediated liver toxicity and lung cancer. However, there is nothing known about the role of CYP2A6 in the HIV-1 setting. Monocytes/macrophages are the secondary target of viral replication and are the major viral reservoir, which also infiltrate into the brain and cause neuroAIDS. Astrocytes, a minor target of HIV-1 replication, upon activation is known to cause neuronal damage. To test our hypothesis we used in-vitro HIV-1 model U937 monocyte/macrophage and SVGA astrocyte cell lines and human primary macrophages. The results showed that CYP2A6 is predominantly expressed in monocytes/macrophages (36 % of total CYPs) and astrocytes (56 % of total CYPs). Subsequently, using a newly developed highly sensitive LC-MS/MS method, we showed that nicotine is metabolized into cotinine and NNK by CYP2A6 and produces reactive oxygen species. These results suggest that CYP2A6-mediated nicotine metabolism increases oxidative stress, which may be responsible for HIV-1 replication. We are now in the process of testing this hypothesis by measuring HIV-1 replication in nicotine-treated primary human monocytes/macrophages and astrocytes. Finally, we recruited healthy, smokers, HIV+, and HIV + smokers from Cameroon, Africa. We isolated monocytes/macrophages and lymphocytes from these subjects. Our preliminary results showed that there is a positive correlation between smoking and increased CD4, HIV-1 replication (p24), and CYP2A6 level, and negative correlation between smoking and expression levels of antioxidant enzymes. These studies open a new CYP pathway that may be responsible for smoking-mediated HIV-1 pathogenesis and neuroAIDS. Support: NIH DA031616-01

P120

Dopamine Depletion in Different Brain Regions of HIV-infected Individuals & Impact on Neurocognitive Performance

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Background: The central nervous system (CNS) is an early target of HIV-1 infection. The virus enters the CNS within days of primary systemic infection, and once in the brain, HIV-1 infects other cells and distributes in different regions, particularly in the fronto-striatal pathways, including the basal ganglia and causing progressive neurodegeneration in the regions of high dopaminergic activity. Although HIV-associated impairment in neuromotor and

neurocognitive functions is a hallmark of HIV-1 infection in the CNS, there remains a paucity of evidence for the direct relationship between changes in dopamine (DA) availability, as well as HIV-1 RNA levels in these regions and impairment in performance of neurocognitive functions. Objectives: We investigated the impact of HIV-1 on the status of DA in the postmortem brain tissues from fronto-cortical (FC) and basal ganglia (BG) regions of individuals who died of HIV/AIDS and determined the relationship between DA and its metabolite, homovanillic acid (HVA) availability, as well as regionally localized HIV-1 RNA levels and performance in neuropsychological (NP) functions evaluated during life. Results: Among the brain regions investigated, a decrease in DA availability was found in putamen (53%), caudate (47%) and substantia nigra (45%), and 2-28% in other brain regions of HIV/AIDS cases (N=38), compared to that in HIV-negatives (N=11). Decrease of DA in putamen and SN correlated with performance in memory, learning, information processing and verbal fluency. Changes in HVA levels correlated with motor functions, information processing, and attention/working memory, and HIV-1 RNA in the FC, caudate and GP inversely influenced motor functions, learning and attention/working memory. Conclusion: Findings of this study suggest that HIV invasion of the CNS profoundly disrupts the regional DA availability in the FC and BG regions in individuals with HIV/AIDS, and may be an important contributing factor for impairment in performance of different NP functions.

P121
Recurrent meningitis in a patient with AIDS

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Objective: Report a case of recurrent meningitis in a patient with AIDS, initially Cryptococcus infection and subsequently aseptic. Background: Even in patients with HIV/AIDS, recurrent meningitis is unusual. There is evidence that recurrence of cryptococcal meningitis (CM) results from persistence of the original infecting strain. Recurrence of meningitis symptoms following HAART therapy may be related to immune reconstitution rather than reinfection. Methods: Clinical presentation, laboratory and radiologic studies of a 54-year-old man over 11 years were reviewed. Review of literature was performed. Results: Patient presented in 2000, 2002 and twice in 2001, with headache, neck stiffness, and fever; CD4 counts were 30, 109, 88 and 38 respectively (viral load 150,000-400,000 copies/mL). Each time CSF grew Cryptococcus neoformans. He was treated with amphotericin-flucytosine, followed by fluconazole. CSF after treatment was negative for cryptococcus. HAART was maintained; however, compliance to fluconazole and HAART was unreliable. In 2004, CD4 count was 464 and has remained >200 to date. Since 2002, he presented several times with meningitic symptoms; CSF revealed lymphocytic pleocytosis, elevated protein (300-500 mg/dl) and low glucose levels (<30 mg/dl) without evidence for viral (HSV, CMV, VZV), toxoplasmosis, fungal, bacterial infection or atypical cells. Cryptococcal antigen (CA) was always positive in blood except for his last admission in 2012 (viral load 354 copies/mL). He was treated for presumed cryptococcus or tuberculous meningitis, or did not receive treatment. Brain MRIs persistently showed meningeal enhancement especially around the brainstem; MRI in 2012 showed progressive hydrocephalus and ventriculoperitoneal shunt was placed. Conclusions: The number of recurrences of CM in this patient makes his case unusual, as well as the later persistence of CSF and MRI abnormalities punctuated by episodes of clinical worsening without an identifiable agent. It is possible that immune reconstitution played a role in his later presentations, yet the exact etiology remains elusive.

P122
Aberrant neurite outgrowth by HIV-gp120 is mediated via activation of Cdk5

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Cyclin-dependent kinase 5 (Cdk5) is a serine/threonine protein kinase that activated by its neuron-specific activator, p35, or its truncated form, p25. Activation of Cdk5 is essential for many post-mitotic processes such as neuronal activity, neuronal migration during development and neurite outgrowth. However, deregulation of Cdk5 by association with p25 has been linked to the pathology of neurodegenerative diseases, such as Alzheimer's disease and amyotrophic lateral sclerosis. For in vivo studies, we utilized hippocampus of wild type and gp120 tg mice. In gp120 tg mice, there was a 1.5-fold increase in Cdk5 activity compared to wild type mice. p35 expression in gp120 tg mice was significantly lower and p25 was higher. To mark a population of newly born dentate granule neurons, retrovirus

expressing green florescent protein (GFP) was stereotaxically injected into the dentate gyrus of mice. For dendritic analysis, three-dimensional reconstruction of entire dendritic processes of each neuron was made using confocal images. Two-dimensional projection images were traced with NIH ImageJ. Elaborated dendritic trees in gp120 tg mice were seen in comparison to wild type mice at 2 weeks post injection of retrovirus. The dendritic length and branch number were increased in gp120 tg mice. Furthermore, Sholl analysis demonstrated greater dendritic complexity in neurons of gp120 tg mice. Thus, initial dendritic development was aberrantly accelerated in newly generated adult hippocampal neurons in gp120 tg mice. Cultured NPC-derived neural progeny were exposed to recombinant gp120 protein for 24 hrs. Immunolabeling with antibody against the neuronal marker β-III Tubulin revealed that gp120 treatment resulted in aberrant outgrowth of neurites. Taken together, these studies demonstrate that abnormal activation of Cdk5 mediated by HIV-gp120 result in abnormal dendritic development and that Cdk5 may be a therapeutic target.

P123

HHV-6A infection enhances EAE severity in the common marmoset

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A range of studies has associated the ubiquitous human herpesvirus HHV-6A with neurologic diseases, including multiple sclerosis (MS). The biology surrounding HHV-6A infection is largely unknown, though it is considered neurotropic. We investigated HHV-6A infection in common marmosets (C.jacchus), which unlike rodents express the receptor for viral entry. Marmosets inoculated with HHV-6A intravenously (n=4) exhibited neurologic symptoms and generated robust, virus-specific serum IgM and IgG responses. In light of work from our lab suggesting the olfactory pathway as a route of HHV-6 entry, we also inoculated marmosets intranasally with HHV-6A (n=4). Interestingly, marmosets inoculated intranasally failed to generate virus-specific serum IgM or IgG. Experimental autoimmune encephalomyelitis (EAE) is a common animal model in MS research for its recapitulation of T-cell mediated CNS inflammatory demyelination. As viruses may act as triggers in MS, we asked if marmosets previously inoculated with HHV-6A intranasally exhibited an altered EAE disease course compared to naïve marmosets. We observed that HHV-6A+EAE marmosets (n=2) exhibited clinical symptoms including weight loss and physical disability earlier and more aggressively than EAE alone marmosets (n=2). HHV-6A+EAE marmosets also exhibited more severe radiologic disease by MRI, with increased white matter lesion load and leukocortical lesions in the brain. Post mortem MRI of spinal cords is ongoing. Previous marmoset EAE studies have correlated seropositivity for an immunogenic myelin protein, MOG, with overt clinical EAE. We detected increased levels of serum anti-MOG IgG in HHV-6A+EAE marmosets compared to EAE alone. Moreover, following EAE induction, we detected virus-specific serum IgM in one HHV-6A intranasal marmoset, suggesting a synergy between viral infections and this autoimmune trigger. Immunohistochemical analyses of CNS tissues and flow cytometry-based analyses of cellular immune responses to HHV-6 and MOG are ongoing. Such an animal model may help elucidate the role of viruses in a process of autoimmune pathogenesis such as MS.

P124

Longitudinal analysis of interactive effects of host genotype, stimulant use, and HIV status upon neurocognitive functioning

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HIV-associated neurocognitive impairment is highly prevalent among HIV-1 infected individuals in the United States. Putative causes include chronic neuroinflammation mediated largely by cytokine activity and the loss or dysfunction of dopaminergic neurons. To investigate this, we examined genetic susceptibility loci within cytokine and dopamine-related genes that may increase risk for neurocognitive impairment, especially among users of drugs that affect the dopaminergic system. METHODS: This longitudinal study utilized general linear modeling to examine the interactive effects of HIV status, stimulant use, and host genotype upon neurocognitive functioning. Data were obtained for 602 HIV + and 508 uninfected individuals enrolled in the Multicenter AIDS Cohort Study. Approximately 20% were stimulant users (cocaine and/or methamphetamine). Only data obtained prior to 1996 were used to avoid confounding effects of highly active antiretroviral therapies. The statistical model controlled for marijuana use, alcohol use, depression, hepatitis C, nadir CD4, and practice effects. Neurocognitive domains examined were working memory, learning, memory, motor, executive, and processing speed. The False Discovery Rate was used to correct for multiple comparisons. RESULTS: No 4-way interactions were found. After refitting, a significant 3-way interaction was found for the COMT gene, indicating that the influence of COMT genotype on learning ability over time is affected by HIV status and stimulant use. A 3-way interaction for the CCL3 gene indicated that executive functioning over time is influenced by CCL3 genotype and HIV status. Finally, 2-way interactions across many of the models indicated that HIV results in decline in motor functioning, and processing speed to a lesser extent, and that stimulant use improved motor but worsened working memory functioning over time. CONCLUSIONS: The findings suggest that genetic susceptibility loci within both dopamine and cytokine-related genes interact with HIV status and/or stimulant use to impact the course of neurocognitive functioning in a domain-specific manner.

P125
TLR3 Signaling of Human Cerebral Endothelial Cells Inhibits HIV-1 Replication in Macrophages

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Emerging evidence show that human cerebral ECs that are part of the blood-brain barrier (BBB) also play an important role in immunological regulations. To gain insights into innate immunity of cerebral ECs against viral infections, we studied the immune activation of D3 human cerebral microvascular endothelial cells (hCMEC/D3). It was found that PolyI:C treatment had no effect on IFN expression while transfection significantly upregulated the expression of IFN-beta and IFN-lambda, at both mRNA and protein levels. RIG-I activation also moderately increased IFN-beta and IFN-lambda expression of D3 cells. Immunofluorescence study showed that only transfection can induce PolyI:C into D3 cells. IFN- beta and IFN-lambda induction by PolyI:C stimulation could be inhibited by disruption of TLR3 function as well as RIG-I siRNA. Investigation of the mechanism revealed that PolyI:C stimulation induced nuclear translocation of IRF3 and IRF7. To study the functional significance of brain ECs activation, we applied the supernatant (SN) from PolyI:C-stimulated D3 cultures to HIV-infected macrophages and observed significant inhibition of HIV replication. The SN from PolyI:C-stimulated D3 cultures induced the expression of ISGF-3gp48, STAT3 phosphorylation, JNK and Erk MAPK kinase activation, through which triggered the expression of ISGs. The inhibitory activity of the SN could be neutralized by antibodies against IFN-beta and IL-10Rbeta, confirming that IFN-beta and IFN-lambda are the key factors in the SN responsible for the anti-HIV activity. Our results thus demonstrated that TLR3 signaling activates human cerebral ECs in terms of IFN induction and for the first time showed that the newly identified IFN-lambda was involved in this activation. These observations indicate that hCMECs may be a key regulatory bystander, playing a crucial role in brain innate immunity against HIV infection using IFN-beta and IFN-lambda-dependent mechanism.

P126
Activation of HERV-K expression by HIV-1 infection

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Human endogenous retroviruses (HERVs) are genomic sequences of retroviral origin which are believed to be integrated into the germline chromosomes millions of years ago. Although mostly defective and inactive, some of the HERVs may be activated under certain physiological and pathological conditions. One of the HERVs, HERV-K has an intact genomic structure and may have preserved the capability to make viral particles. HERV-K activation has been detected in in the plasma and brains of patients with HIV-1 infection, suggesting that exogenous retroviruses may regulate HERV-K expression. We hypothesized that HIV-1 protein Tat and Rev may directly up-regulate the expression of HERV-K. We examined the brain tissues from patients with HIV-1 infection for HERV-K reverse transcriptase (RT) expression. We found that immunostaining density of HERV-K RT was significantly higher in HIV-1 infected patients. HERV-K RT was higher in HIV-1 p24 positive brains compared to that of p24 negative brains of HIV-1 infected patients. To study the HERV-K activation by HIV-1 in vitro, we constructed a plasmid encoding the HERV-K consensus genome and confirmed its expression and viral particle production in HeLa cells. Co-transfection of the HERV-K and HIV-1 plasmids significantly increased the HERV-K env and pol mRNA levels. Unlike HIV-1, there is no transactivator protein identified from HERV-K genome. However, we found that HIV-1 Tat significantly up-regulated HERV-K expression. HIV-1 Rev also increased HERV-K expression, and had an additive effect together with Tat. HERV-K Rec, the HIV-1 Rev equivalent, significantly activated HERV-K expression, while Np9, a deletion mutant of Rec, only had a marginal effect. Both of them were less effective than HIV-1 Rev, but also had additive effects together with Tat. In conclusion, HIV-1 Tat and Rev can significantly up-regulate HERV-K expression, which could be the mechanism of HERV-K activation in HIV-1 infected patients.

P127
Persistent HIV replication in human astrocytes following lysosomal escape

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Astrocytes are a critical reservoir of HIV-1 in the human brain. Human astrocytes do not express HIV-1 receptor CD4, but express its coreceptors CXCR4, CCR5 and DC-sign. Endocytosis has been proposed as a pathway for HIV-1 entry into astrocytes. However, the infected astrocytes are considered to be non-productive. Here, we challenge some of these current dogmas. Although we found that cell-free HIV infected only rare human astrocytes, there was no obstacle for HIV-1 replication in these cells because viral particles were continuously released into the extracellular medium over 60 days following either infection with VSV-G pseudotyped HIV-1 or transfection with HIV-1 proviral DNA. HIV-1 could also replicate continuously in astrocytes infected with both X4 and R5-tropic strains of cell-free viruses following treatment with either the lysosomotropic agent, chloroquine, or Tat basic-HA2 peptide which destabilizes the membrane of endosomes/lysosomes. The virus released from the astrocytes chronically infected with HIV-1 for >140 days could be successfully transmitted to lymphocytic cell lines. Electron microscopy confirmed the process of endocytosis by which HIV gained entry into compartments of endosomes/lysosomes in astrocytes. Additionally, the persistent infection of astrocytes was probably species-specific because SIV strains, which share the receptor and coreceptors with HIV, could not establish a persistent infection in astrocytes despite treatment with chloroquine. These observations indicate that endocytosis is a major pathway for HIV entry into astrocytes and the infectivity is limited by the trapping of HIV particles in the endosomes/lysosomes. Once the virus escapes the lysosomes, it forms a persistent infection in these cells. Thus chloroquine and other lysosomotropic agents should be used with caution in patients with HIV infection.

P128
Virological synapses and bridges mediate lymphocyte-to-astrocyte transmission of HIV

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HIV infection in human astrocytes and its role in HIV latency in the brain have been strengthened by cumulative in vivo evidence and have been shown to occur predominantly in the perivascular regions of the brain. While infection of astrocytes by cell-free HIV is inefficient in vitro, we found that significant infection of HIV-1 could be easily established by co-cultivating human astrocytes with lymphocytes that were infected by HIV-1 NL4-3-based reporter virus, NLENG1. This was confirmed using several HIV-infected lymphocytic cell lines (MT4, CEM-ss, Jurkat-Tat) and with HIV-infected peripheral blood mononuclear cells. Multiple modes of interaction between astrocytes and the infected lymphocytes (especially Jurkat-Tat) were observed by video microscopy and 2D/3D electron microscopy. These included partial invagination of the infected lymphocyte into astrocytes, formation of virological synapses, formation of large interface between the cell membranes of two cells, and interdigitation of long filopodia arising from both cell types forming bridge-like structures. Viral particles were concentrated at the interface of these cells and along the bridges. We also observed villus-like or synaptic structures stretching onto astrocytes and HIV particles appeared in the space within these structures. When the infected lymphocytes were placed in the upper chamber of transwells, a decrease in infection of astrocytes was observed. Infection of astrocytes by the infected lymphocytes could be significantly inhibited by anti-CXCR4 monoclonal antibody and pharmacological inhibitor of CXCR4 but not by anti-CCR5, anti-DC sign or $\alpha 4\beta 7$ integrin antibodies. In conclusion, infection of astrocytes can be easily established by cell-cell contact with HIV-infected lymphocytes and CXCR4 is a critical receptor that mediates this transmission. This study indicates that the cell-cell transmission might play a critical role in HIV infection of astrocytes in the brain.

P129
Theranostic development of magnetite antiretroviral nanoparticles

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Our laboratories developed a novel drug delivery system to carry antiretroviral therapies (ART) to cell and tissue sites of HIV-1 infection. Although nanoformulated ART (nanoART) were tested for uptake, release and antiretroviral efficacy, more complex approaches are needed to determine drug biodistribution and therapeutic efficacy. To address this, small magnetite-containing ART (SMART) polymer nanoparticle systems were developed. Here we combined ART and superparamagnetic iron oxide particles into hydrophobic cores of polymer particles utilizing its hydrophilic shells to ensure stability. We prepared SMART by flash nanoprecipitation of hydrophobic magnetite nanoparticles and amphiphilic poly(ethylene oxide-b-lactide) copolymers with ART to effect high supersaturations and kinetically controlled aggregates. The resulting SMART particles had narrow size distributions with polydispersity indices between 0.1-0.15. We administered SMART to macrophages and observed, as a result, changes in the transverse relaxivities. Importantly, SMART was ingested within eight hours by human monocyte-derived macrophages (MDM). Finally, SMART were injected I.P. at a ritonavir (RTV) concentration of 100 ng/kg in the reticuloendothelial system of mice. T2* weighted and T2 mapping MRI were acquired preinjection and 24 hours later. Results indicate that the liver and spleen contained the greatest concentrations of SMART particles which were commensurate with post-mortem tissue RTV concentrations as determined by ultra performance liquid chromatography. We posit that with flash precipitation, the development of SMART particles permit simultaneous studies of drug biodistribution with measurements of therapeutic efficacy of antiretroviral drugs. We posit that SMART will serve as a platform to screen nanoART biodistribution and therapeutic endpoints. The screening capabilities would permit analyses of multiple different formulation systems that would speed translation of the

work from the laboratory bench to the patient and provide theranostic development.

P130
Theranostic development of small magnetite antiretroviral therapy (SMART)

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Our laboratories developed a novel drug delivery system to carry antiretroviral therapies (ART) to cell and tissue sites of HIV-1 infection. Although nanoformulated ART (nanoART) were tested for uptake, release and antiretroviral efficacy, more complex approaches are needed to determine drug biodistribution and therapeutic efficacy. To address this, small magnetite-containing ART (SMART) polymer nanoparticle systems were developed. Here we combined ART and superparamagnetic iron oxide particles into hydrophobic cores of polymer particles utilizing its hydrophilic shells to ensure stability. We prepared SMART by flash nanoprecipitation of hydrophobic magnetite nanoparticles and amphiphilic poly(ethylene oxide-b-lactide) copolymers with ART to effect high supersaturations and kinetically controlled aggregates. The resulting SMART particles had narrow size distributions with polydispersity indices between 0.1-0.15. We administered SMART to macrophages and observed, as a result, changes in the transverse relaxivities. Importantly, SMART was ingested within eight hours by human monocyte-derived macrophages (MDM). Finally, SMART were injected I.P. at a ritonavir (RTV) concentration of 100 mg/kg resulting in > 100 ng/kg in the mouse reticuloendothelial system. T2* weighted and T2 mapping MRI were acquired preinjection and 24 hours later. Results indicate that the liver and spleen contained the greatest concentrations of SMART particles that were commensurate with tissue RTV concentrations as determined by ultra performance liquid chromatography. We posit that with flash precipitation, the development of SMART particles permit simultaneous studies of drug biodistribution with measurements of therapeutic efficacy of antiretroviral drugs. We posit that SMART will serve as a platform to screen nanoART biodistribution and therapeutic endpoints. The screening capabilities would permit analyses of multiple different formulation systems that would speed translation of the work from the laboratory bench to the patient bedside in the new field of theranostics.

P131
HIV-1 Tat induces microglial neurotoxicity via voltage-gated Kv 1.3 channels

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Microglia activation and resultant pro-inflammatory responses play a crucial role in the pathogenesis of HIV-1-associated neurocognitive disorders. As such, studies on identification of specific targets to control microglia activation and resultant neurotoxic activity are imperative. Increasing evidence indicates that voltage-gated K + (Kv) channels are involved in the regulation of microglia functionality. We hypothesize HIV-1 Tat activates microglial Kv1.3 channel resulting in neurotoxic activity. To test this hypothesis, we investigated role of KV 1.3 channel in Tat-induced neurotoxicity in primary rat microglia cultures and brain slides. Our results revealed incubation microglia with Tat (200 ng/ml), markedly increased the levels of KV1.3 mRNA and protein expression, which were blocked by Margtoxin (MgTx), a specific KV1.3 channel blocker. Heat inactivated Tat did not alter the levels of KV1.3 mRNA and protein expression. Whole-cell patch recordings showed Tat enhanced microglia KV1.3 currents in a dose-dependent manner. To examine the association of Tat enhancement of KV1.3 current with microglia-induced neurotoxicity, we detected microglia cytokine and nitric oxide (NO) production by Elisa and microglia-induced neuronal injury via TUNEL. Elisa analysis showed that Tat-treated microglia produce higher amount of IL-1β, IL-6 and NO than untreated microglia. TUNEL staining revealed that K + channel blocks, MgTx, PAP or 4-AP attenuated apoptotic neurons induced by Tat-activated microglia from 30.68±4.37 % to 9.56±2.78 %, 11.72± 2.42 % or 7.29±3.84 %, respectively. Knockdown of Kv1.3 gene by transfection of Kv1.3-siRNA abrogated neurotoxicity mediated by Tat-treated microglia, suggesting the involvement

of Kv1.3 in Tat -induced microglial neurotoxic activity. Similar results were obtained from brain slides. Blockade of KV1.3 channel expression by MgTx, PAP, and 4-AP on brain slides, significantly inhibited microglia activation and resultant neuronal injury. Taken together, our data suggest that brain infection of HIV-1 induces microglial cytotoxic activity via Kv1.3 and that microglial Kv1.3 could be a potential target for the development of therapeutic strategies.

P132

Integrating role of T-antigen, Rb2/p130, CTCF and Boris in mediating non-canonical endoplasmic reticulum-dependent death pathways triggered by ER-chronic stress in mouse medulloblastoma

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Distinct molecular pathways could be constitutively active in mouse T-Antigen positive and T-Antigen negative medulloblastoma cell lines, and contribute to their phenotypic differences as well as to cellular responses, cell cycle progression, cell death and survival. The diversity of these responses might be due to distinct activities of Rb2/p130, CTCF and BORIS proteins in response to an altered network of signaling evoked by the T-Ag presence. Here, we provided evidence supporting a role for the T-Antigen in causing chronic endoplasmic reticulum (ER) stress and aberrant Caspase-12 expression and activation, subsequently driving to both massive cell death, and likely selection of cells with a higher malignant phenotype. Also, we observed that endoplasmic stress, either chronically caused by the T-Ag or transiently induced by glucose deprivation, is accompanied by the formation of complexes between the retinoblastoma related protein Rb2/p130 and the chromatin insulator CCCTC-binding factor CTCF, or the CTCF-paralogue Boris. In this abstract, we present the first evidence supporting a role of the T-Antigen in inducing/maintaining chronic ER-stress, as well as, indicating a role of Rb2/p130, CTCF and Boris as potential mediators of non-canonical ER-dependent death pathway in mouse medulloblastoma.

P133

HIV Tat upregulates VEGF receptors 1 and 2 on astrocytes and brain endothelial cells in a focal adhesion kinase-dependent manner

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HIV infection has been reported to include increased VEGF in serum, although curiously, not in CSF. The HIV protein Tat binds to receptors for VEGF (both VEGFR1 & 2), inducing angiogenesis in Kaposi's sarcoma spindle cells. We hypothesized that HIV Tat may increase or induce VEGFR expression on brain endothelial cell (ECs) and astrocytes. As VEGF has been associated with decreased tight junction protein expression, we further hypothesized that VEGFR expression would correlate with activation of ECs by Tat both temporally and spatially. To test our hypothesis, we examined brain tissues from macaques infected with neuropathogenic strains of SIV. Expression of VEGFR1 was increased on astrocytes in macaques with SIV encephalitis; VEGFR2 upregulation was restricted to ECs. Mechanisms of upregulation were confirmed using primary cultures of ECs and astrocytes derived from control macaques. Our results demonstrated that increased VEGFR1 and 2 expression was dependent on focal adhesion kinase activation using the highly selective inhibitor, PF-573,228. VEGFR1 was expressed on astrocytes only in those brains of macaques with detectable virus by in situ hybridization. These results provide proof-of-concept for our

hypothesis that BBB disruption in HIV is mediated, at least in part, through VEGF receptor expression.

P134

Neurosteroid-mediated regulation of brain innate immunity in HIV/AIDS: DHEA-S suppresses neurovirulence

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Neurosteroids represent a network of cholesterol-derived molecules synthesized within the brain, which exert trophic and protective actions. Infection by human (HIV) and feline (FIV) immunodeficiency viruses causes neuroinflammation and neurodegeneration. Secretion of neuroinflammatory host and viral factors by activated glia and infiltrating leukocytes comprise the principal neuropathogenic mechanisms although the impact of neurosteroids on neurological infections is unknown. Herein, we investigated the interactions between neurosteroid-mediated effects and lentivirus infection outcomes. Analyses of HIV-infected and uninfected persons' brains disclosed a reduction in 5α-reductase, P450scc and 3βHSD expression in neurons of HIV-infected persons. Neurons exposed to supernatants from HIV-infected macrophages exhibited suppressed 5α-reductase and 3β-HSD expression without reduced cellular viability ($p<0.05$). HIV-infected macrophages treated with sulphated DHEA (DHEA-S) showed suppression of inflammatory gene (IL-1β, IL-6, TNFα) expression ($p<0.05$). FIV-infected animals treated with DHEA-S demonstrated a reduction of inflammatory gene transcripts (IL-1β, TNF-α, CD3ε, GFAP) in brain ($p<0.05$). Blood CD4+ T-cell levels were increased in DHEA-S treated FIV-infected animals ($p<0.05$). DHEA-S treatment also prevented neurobehavioral deficits and neuronal loss among FIV-infected animals, compared to vehicle-treated animals ($p<0.05$). Reduced neuronal neurosteroid expression accompanied lentivirus infections but treatment with DHEA-S limited inflammation and neurobehavioral deficits. Neurosteroid-derived therapies might be effective in the treatment of virus- or inflammation-mediated neurodegeneration.

P135

Uneven expression and response to stimuli of multiple sclerosis- (MS)-associated retrovirus (MSRV)env and syncytin-1 by cell subpopulations in vivo and in vitro: inference for multiple sclerosis

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Two members of the -W family of human endogenous retroviruses (HERV-W) were related to multiple sclerosis (MS): MSRV (MS-associated retrovirus), released by cells of MS patients as virus-like particles, and ERVW-1, an element producing only the syncytin-1 env protein. In vitro and in animal models MSRVenv and syncytin-1 cause neuroinflammation, neurodegeneration, alterations of immune and stress responses. Also EBV (Epstein Barr virus) was proposed as MS co-factor, by unknown mechanisms. In the past we monitored MS patients in various temporal and clinical stages: in all cases, striking parallelisms between MS behaviour and MSRV/HERV-W presence/load were found. The expression of MSRVenv and syncytin-1 by peripheral blood mononuclear cells (PBMC) from MSRV(+) volunteers and MS patients, and U87-MG astrocytes was studied, by discriminatory env-specific RT-PCR and flow cytometry. To clarify whether MSRV and syncytin-1 are differentially expressed, T, B, NK and monocyte subsets were separated by immuno-specific adsorption to magnetic beads. Monocytes were also differentiated in macrophages (MDM). Alternatively, HERV-Wenv-specific immunostaining was monitored by flow cytometry in PBMC

sorted for markers of cell subsets. Similar studied were carried out on astrocytes. Results showed that basal expression of HERV-W/MSRV/syncytin-1 occurs in monocytes, NK, and B, but not in T cells; one third of the astrocytes have surface HERV-Wenv. The uneven expression in PBMC is amplified in cells from naïve MS patients, and dramatically reduced during therapy. If cells are exposed to the EBVgp350 protein, MSRVenv and syncytin-1 are activated in B cells and monocytes/MDM, but not in T cells, nor in the highly expressing NK cells. The latter cells, but not the T cells, are activated by proinflammatory cytokines. We concluded that in vitro interactions among the two proposed MS-cofactors, HERV-W and EBV, occur in cells from blood and from brain. It is likely that it might occur also in vivo.

P136

Src homology-2 domain containing protein tyrosine phosphatase (shp)-2 and p38 regulate chemokine cxcl8 expression in human astrocytes: implications for hiv neuropathogenesis

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Chronic inflammation initiated by HIV-1 infection of the brain via release of cytokines/chemokines is an important factor in neuropathogenesis of HIV-associated dementia (HAD). CXCL8, one of the first chemokines found in the brain, is upregulated in brain and cerebrospinal fluid (CSF) of HIV-1 infected individuals suggesting its potential role in HIV-associated neuroinflammation. The major contributors to the CXCL8 pool are known to be astrocytes. Interleukin (IL)-1β activated astrocytes exhibit significant upregulation of CXCL8, and the mechanisms involved are being elucidated. In the present study, we investigated the underlying intracellular signaling pathways that lead to production of CXCL8 in astrocytes activated with pro-inflammatory stimuli. Primary human astrocytes showed decreased CXCL8 mRNA and protein levels when pre-treated with pharmacological inhibitors of Src-homology 2 domain containing protein tyrosine phosphatase (SHP)-2 and p38 mitogen-activated protein kinase (MAPK), before activation with IL-1β. Transfecting astrocytes with SHP2 and p38 overexpression plasmids led to increased CXCL8 RNA and protein levels, whereas corresponding dominant negative mutants (SHP2 CS and p38agf) had no effect on basal CXCL8 levels. However, treatment with p38 inhibitor in SHP2 overexpressing astrocytes abrogated overexpression and showed no increase in CXCL8 levels suggesting SHP2 to act upstream of p38 MAPK. Transfection efficiency was confirmed by SHP2 phosphatase and p38 in vitro kinase assays. We also show p38 phosphorylation and activation in SHP2 transfected astrocytes. These results show that non-receptor tyrosine phosphatase SHP2 has an important role in expression of CXCL8 in astrocytes during inflammation. Also, SHP2 directly or indirectly modulates p38 MAPK in signaling cascade leading to production of CXCL8. The study provides insight for detailed understanding of mechanisms involved in CXCL8 production during neuroinflammation.

P137

Mechanistic aspects of DC:T cell interaction during HTLV-1 associated oncogenesis and neuroinflammation

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of two immunologically distinct diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the HTLV-1-specific CD8+ cytotoxic T cell response is seen in both pathogenic states, its actual significance in preventing viral load and controlling disease progression remains questionable. Utilizing a newly standardized dendritic cell and a T cell polychromatic antibody cocktail, we investigated the immune activation of these cells in a patients' cohort from Jamaica including HTLV-1 seronegative controls, asymptomatic carriers (ACs), ATL, and HAM/TSP. Extensive immune profiling revealed that CD8+ T cells from both HAM/TSP and ATL patient samples demonstrated some functional responses, albeit to a much lesser extent than those responses seen in ACs. Furthermore, DCs from HTLV-1-diseased individuals exhibited an altered maturation and adhesion phenotype as compared to ACs. The expression of an inhibitory molecule

PD-1 and its ligand, PD-L1 was upregulated in CTLs and DCs, respectively in both diseased groups. While comparing the matched proviral loads to the flow cytometry results, we identified unique immune signatures distinguishing ACs from ATL and HAM/TSP patients. Collectively, these results suggest that modulation of both DCs and CD8+ T cells and/or blockade of the PD-1/PD-L1 pathway may be useful in therapeutic interventions of ATL and/or HAM/TSP.

P138

The triple-edged sword of prion protein (PrP): Agent receptor, innate protector, and amyloid escape artist

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Human CJD, sheep scrapie and other TSE agents represent the tip of the iceberg of undiscovered and hidden viruses in the environment that can initiate and contribute to late-onset neurodegeneration. Recent studies demonstrate brain PrP can be destroyed without loss of infectious titer (1). This underscores PrP as a host receptor for a foreign environmental agent. Transmissions to wt mice of the geographic BSE and kuru (kCJD) agents, in addition to the cloned 263 K scrapie agent, also revealed vastly different agent doubling times (ti), ranging from 4-33 days. In GT cells, 5 unique TSE agents all showed a ti of ~1 day, a remarkable change strongly indicating complex animal recognition and control of TSE agent replication and clearance. Innate immune molecules have been documented at early (pre PrP-res) stages of brain infection. GT cells also display innate protective mechanisms that limit maximal agent titers in a strain dependent fashion; kCJD rapidly induces high PrP-res but produces 1,000 fold lower titers than FU-CJD, an agent that slowly induces less total PrP-res (2). Thus PrP can have a protective role against TSE agents, as found for HIV and other infectious agents. However, once a TSE agent induces a PrP-res cascade, amyloid production can continue, even after the infectious agent is eliminated. It is likely that other neurodegenerative diseases, including a subset of Alzheimer's Disease, are initiated by environmental infectious agents that are suppressed or no longer present in the host. 1) Miyazawa K, Emmerling K, & Manuelidis L (2011) High CJD infectivity remains after prion protein is destroyed. J Cell Biochem 112:3630-7; PMID: 21793041 2) Miyazawa K, Emmerling K, & Manuelidis L (2011) Replication and spread of CJD, kuru and scrapie in vivo and in cell culture. Virulence 2:188-99; PMID: 21527829

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Lipopolysaccharide induced expression of the key genes involved in inflammasomes in the spleen of HIV-1 transgenic rat

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Inflammasomes are protein complexes that activate caspase-1 to up-regulate pro-inflammatory cytokines, such as IL-1β. Of the four well-characterized inflammasomes, AIM2, IPAF, NLRP1 and NLRP3, NLRP3 can be induced by the endotoxin, lipopolysaccharide (LPS). We previously showed that LPS-induced serum levels of interleukin-1β (IL-1β) are significantly greater in the HIV-1 transgenic (HIV-1Tg) rat than in control F344 rats. In this study, we compared the expression of 84 key genes involved in inflammasome function in the spleen of HIV-1Tg rats and F344 control animals with and without LPS treatment. Adult HIV-1Tg and F344 male rats were randomly assigned to receive either LPS (250 μg/kg) or saline (i.p.). Two hours following treatment, the animals were sacrificed, and their spleens were collected. Total spleen RNA was isolated for real time PCR using the Rat Inflammasomes RT² Profiler™ PCR Array. Expression of the 84 genes examined was comparable in the spleen of the HIV-1Tg and F344 rats given saline, indicating that gene expression of the four known inflammasomes was not altered in the presence of HIV-1 viral proteins. While gene expression for the NLRP3 inflammasome, the IL-1β pro-inflammatory cytokine, the Cxcl1, Cxcl3, Ccl2, and Ccl7 chemokines, and other downstream signaling molecules, including NF-κB, was up-regulated in both the HIV-1Tg and F344 rats in response to LPS, the magnitude of the up-regulation was much greater in the HIV-1Tg rats, except for NLRP3. LPS-induced up-regulation of NLRP3 was 4.5-fold greater in the spleen of F344 rats, but only 2.7-fold greater in HIV-1Tg rats. Conversely, LPS-induced up-regulation of IL-1β was 16.8-fold greater in the spleen of HIV-1Tg rats, but only 10.9-fold greater in the F344 rats. Our data suggest that there may be additional mechanisms other than NLRP3 inflammasome production underlying LPS-induced up-regulation of IL-1β in the spleen of HIV-1Tg rats (NIH DA007058 and DA016149 to SLC).

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Novel Spi-B binding sites in PML-associated JC virus sequence: Insights into molecular regulation of viral gene expression

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Progressive Multifocal Leukoencephalopathy (PML) is a fatal demyelinating disease caused by multiplication of JC virus (JCV) in oligodendrocytes. PML is a serious complication of natalizumab therapy for multiple sclerosis (MS). JCV persists in the kidney and B cell precursors in the bone marrow. Persistence and neurovirulence are controlled by regulation of gene expression from variable sequences in the viral noncoding control region (NCCR). Promoter/enhancer sequences within the NCCR can differ among sites of persistence and disease within a single individual. Importantly, the NCCR sequence found in virus in the kidney (archetype) is conserved while viral sequences isolated from the brains of PML patients contain unique variations that usually contain tandem repeats. Sequence variations include addition/ deletion of promoter elements, inhibitory sequences, and transcription factor binding sites. Spi-B is a transcription factor that binds to cellular promoters to affect transcriptional activity in developing B cells, which can harbor persistent JCV infection, and to representative JCV NCCRs in glial cells that supports early viral gene expression. Novel Spi-B binding sites were identified in NCCR sequence obtained from a variety of PML patient tissues. The PML-derived NCCRs were inserted in frame with the early and late gene start sites in a plasmid containing the entire Mad-1 (PML) genome. The PML-derived NCCRs permitted increased early viral gene expression compared with the kidney-associated archetype. Mutational analysis showed that eliminating Spi-B binding to a subset of these sites significantly impaired early viral gene expression. Preliminary studies demonstrate that Spi-B is upregulated in developing B cells in response to natalizumab therapy in MS patients, a known risk factor for PML. Therefore in the case of Spi-B, we propose that naturally occurring JCV sequence variation, and cellular changes induced by drug treatment, may synergize to create an environment suited for increased viral multiplication and devel-opment of PML.

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Human brain slices exposed to HIV show impaired neural progenitor cell renewal and accelerated differentiation

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HIV-1 invades the brain early during infection, leading to a spectrum of neurological abnormalities from mild cognitive dysfunction to dementia. These effects may be attributed to the direct interaction of viral proteins with neural cells, or could result from inflammatory processes that accompany viral infection. To investigate HIV-1 infection of the CNS, we developed an in vitro two culture system using human fetal brain slices exposed to H9T-cells that chronically produce HIV-1 IIIB (J Neurosci Meth, 2011). Brain slices incubated for up to 21 days in neural progenitor culture medium, or exposed to uninfected H9 or H9/IIIB, showed midline nestin, the neural progenitor antigen, and mid-line or para-midline glial fibrillary acidic acid protein (GFAP). In slices cultured only in medium, there was extensive co-localization of GFAP and nestin antigens, suggesting renewal of neural progenitor cells. In HIV-1-exposed slices, GFAP expression was more dense and did not co-localize with nestin, suggesting differentiation of progenitors into astrocytes. There was no co-localization of the signaling protein phosphorylated Stat3 with GFAP. To further investigate progenitor maintenance and differentiation, slices were exposed to H9/IIIB, then analyzed for mRNA expression patterns. By seven days of exposure, H9/IIIB-exposed slices expressed 2-3 fold higher levels of mRNA for genes associated with neuronal structure, as compared with slices cultured in medium alone, or slices exposed to uninfected H9 cells. GFAP mRNA level was approximately ten-fold higher by day 7 in H9/IIIB-exposed slices compared to H9-exposed slices. Thus GFAP mRNA expression at day 7 predicted the increase in GFAP antigen at day 21. mRNA for transcripts associated with progenitor cell renewal (Hes1, Hes5, nestin) were 2-5 fold lower in H9- and H9/IIIB-exposed slices as compared to slices cultured in medium alone. These data suggest that HIV-1 infection and associated inflammation impair neuroepithelial progenitor maintenance and potentiate accelerated astrocyte differentiation.

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Apolipoprotein E genotype influences neurofilament-light expression in differentiating neuroepithelial cells exposed to HIV-1

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Apolipoprotein E (apoE) has been identified as a genetic factor influencing susceptibility to neuronal injury in Alzheimer's disease, cerebrovascular disease, and neurodegenerative disease. In addition, the apoE4 allele has been associated with an increased risk of dementia or peripheral neuropathy in HIV-1 infected individuals, and faster disease progression for those who are homozygous for the ε4 allele. In vitro, when neural progenitor cells (NEP) are allowed to differentiate in the presence of HIV-1 supernatants, differentiation is not inhibited, but neurons "fail to thrive". This is characterized by a relative decrease in neurofilament-light (NF-L) expression that is dependent on the apolipoprotein E (apoE) genotype of the culture: apoE3/E4 cells showed relatively reduced NF-L expression upon HIV-1 exposure, but differentiating apoE3/E3 or apoE4/E4 cells did not (Martinez et. al., J Neurovirology, 2012). Addition of 1 μg recombinant apoE4 to differentiating apoE3/E3 cultures resulted in a 35% decrease of NF-L expression upon HIV-1 exposure as compared to mock-treated cultures and 13% decrease as compared to cultures where apoE4 was not added; thus mimicking the phenotype of an apoE3/E4 culture. This underscores the detrimental effect of apoE4 on neural antigen expression, and by extension, on neurite elaboration and complexity. Blocking the ApoE receptor with lactoferrin led to decreased expression of NF-L with HIV-1 exposure in a pattern dependent on lactoferrin concentration. mRNA expression of NF-L by 16 days of viral exposure is predictive of the protein expression pattern observed on day 21-22 of incubation. These data suggest that apoE phenotype may be a factor influencing susceptibility to neurological abnormalities observed in some individuals infected with HIV-1.

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Functional proteomic analyses support a role for macrophage depots of long-acting antiretroviral drug nanoformulations

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Access to medicines, malabsorbtion, drug toxicities, poor pharmacokinetics and pharmacodynamics, and compliance can limit the benefits of antiretroviral therapy (ART) in treating human immunodeficiency virus (HIV) infected people. To combat such limitations our laboratory developed long-acting nanoformulations of commonly used crystalline ART using Food and Drug Administration approved excipients. Here, ART drugs including ritonavir (RTV), atazanavir (ATV), and efavirenz (EFV) were packaged into nanoparticles that target macrophages for cell carriage and delivery. This cell-based delivery system results in cell and tissue depots that release drug slowly over days to weeks resulting in reductions in viral replication with limited systemic toxicities. Despite these benefits little is known about particle-macrophage interactions and the effects of carriage on the functional and migratory capacities, which could facilitate drug administration and optimize ART bioavailability. To this end, proteomic approaches and systems biology were used to evaluate the effects of ART nanoparticle carriage on human monocyte-derived macrophages (MDM). Pulse stable isotope labeling of amino acids in cell culture (pSILAC) were used to evaluate global proteomic changes after MDM nanoparticle exposure and resulted in a broad range of activation-linked proteins including those with free radical scavenging, antigen presentation, cell mobility, phagocytosis, and cellular differentiation and development as well as those affecting lipid metabolism. These data indicate an enhanced ability of macrophages to establish nanoparticle ART depots. Moreover, these data also coincided with electrophysiological changes in macrophage outward K + currents indicative of cell activation. Taken together, these data demonstrate how nanoparticle ART-macrophage interactions promote drug loading and enhance functional and migratory capacities of the macrophage facilitating its role as cell drug depots for future clinical developments.

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BNZ-gamma peptide, a potential therapeutic agent in HTLV-1 associated myelopathy

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HTLV-1 was the first exogenous retrovirus identified as pathogenic to humans. While the majority of infected subjects remain lifelong asymptomatic carriers, 3-5% develop a relentlessly progressive inflammatory myelopathy termed HTLV-1 associated myelopathy/ tropical spastic paraparesis (HAM/TSP). The precise mechanism of HAM/TSP pathogenesis remains unclear however many findings suggest that HTLV-1 can activate the infected lymphocytes (CD4+ and CD8+) in the peripheral circulation leading to enhanced migration into the CNS. Once in this compartment, there seems to be a preferential expansion of the infected cells and a compartmentalized interaction between virus-specific CD8+ T-cells and virus- infected CD4+ lymphocytes leading to bystander damage of neural tissues. Spontaneous proliferation (SP) is the ability of HTLV-1 infected lymphocytes to proliferate in vitro without IL-2 or antigenic stimulation. It is thought to be the surrogate marker of lymphocytic activation in this condition. Functionally, it is particularly dependent on the induction of the stimulatory cytokine loops IL-2/IL-2Rα, IL-15/IL-15Rα and IL-9/IL-9Rγ. All these cytokines share a common chain receptor, the gamma chain (γC), offering a potential therapeutic target in HAM/TSP. BNZ- γ is a peptide that can bind the γC receptor and selectively block the binding and downstream signaling of Il-2, IL-9 and IL-15. We are at the early stages of evaluating the effects of this peptide on in vitro immunologic markers of HAM/TSP. We have demonstrated that it is able to suppress SP as quantified by radioactive Thymidine incorporation (5 of 7 patients showed significant suppression). We also showed that BNZ- γ can suppress the expression of lymphocyte activation markers (CD25 expression and STAT5 phosphorylation) both in CD4+ and CD8+ T-cells. We are now studying the effects of BNZ- γ on other phenotypic and functional markers of effector / memory T-cells that might be of clinical , translational interest.

P145
Immune Markers of Neuropsychiatric Outcomes in HIV Infected Adolescents

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Background: HIV associated neurocognitive disorders (HAND) are a common manifestation of HIV infection that confer a worsened morbidity and mortality to affected individuals, despite adequate antiretroviral therapy (ART), undetectable viral loads, and optimal CD4+ T-lymphocytes. In adults, a general immune dysregulation independent of CD4+ T-lymphocyte suppression may contribute to neuropathogenesis. However, a similar mechanism has not been examined in adolescents in the context of their still developing neurologic and immune systems and physiologic pubertal neuro-endocrine fluctuations. Objective: To determine the relationship of activated CD8+ T-lymphocytes and NK cell count/function with functional neuropsychiatric outcome in behaviorally-infected HIV + adolescents. Methods: This secondary analysis of prospectively collected data examined behaviorally-infected HIV + adolescents enrolled in the Reaching for Excellence in Adolescent Care and Health (REACH) Cohort of the NICDH/NIH at 15 sites in the Unites States. Flow cytometry measurements of activated T cells (CD8+/CD38+) and NK cell count (CD3-/CD16+/CD56+), and NK cell functional activity (lytic units per NK cell and per PBMC) were examined in comparison to functional outcomes (depression, anxiety, grades in school) using a multivariable logistic regression model with generalized estimating equations for repeated measures intra-individual. Results: 326 adolescents (mean age 17.5± 1.0 years for boys and 17.4±1.2 years for girls) were included in this study, 81% of which were followed for ≥2 years (range 1-4.8 years). 75% of subjects were female, and 74% were African American. Subjects who were pregnant or immediately post-partum were excluded. At enrollment, 8% of the cohort had an absolute CD4+ count <200, 51% had a viral load < 10,000, 17% qualified for an AIDS diagnosis, and 41% were antiretroviral therapy (ART) naïve. Further data to follow with poster. Conclusion: This project will provide preliminary data in understanding the role of immune dysregulation in the development of HAND in HIV + adolescents.

P146
Antiretroviral Neurotoxicity

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While improved penetration of antiretroviral compounds into the central nervous system is needed to control HIV infection, it also carries the risk of toxicity. Efforts to target antiretroviral compounds to the CNS balance these risks against the potential gain but are hampered by incomplete information on potential neurotoxicity. To begin to assess potential neurotoxicity we performed in vitro studies in which primary neurons were exposed to relevant concentrations of each major class of antiretroviral compounds. Dendritic beading, pruning of processes and calcium destabilization were used as sensitive indices of neural damage. We found a wide range of toxicities, with median toxic concentrations ranging from 2 to 10,000 ng/ml. Some toxic concentrations overlapped concentrations currently seen in the CSF but the level of toxicity was typically modest when compared to challenges that mimic HIV-associated neuropathogenesis. The greatest neural damage was associated with abacavir, efavarenz, etravirine, nevarapine, and atazanavir, while the lowest was seen with darunavir, emtracitabine, tenofovir, and maraviroc. To provide an index of potential risk the log of the minimum in vitro toxic concentration was divided by the log plasma or CSF concentration previously reported in patients undergoing therapy. Eleven compounds showed tangible risk and five compounds minimal risk based on this criterion. Antiretroviral combinations used clinically did not show additive damage and in some cases showed less damage than would have been predicted based on individual toxicities. Toxicity was not predicted by depolarization of mitochondria or by acute toxic effects on neuronal calcium regulation. These data provide initial evidence to identify potential risks of antiretroviral compounds in the CNS. They further illustrate the need to more closely examine the mechanisms of toxicity to help guide the rational selection of antiretroviral regimens that minimize the risk of neurotoxicity. Supported by the AIDS Research and Reference Reagent Program

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Antiretroviral therapy (ART) donwregulates Neural Cell Adhesion Molecule (NCAM) and CX3CL1/MCP-1 levels in HIV-1 infected patients monitored through 48 weeks of treatment: (CXCL12/CX3CL1) chemokines regulate NCAM and prevent gp120 neurotoxicity

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HAD is still prevalent in ART treated patients with Minor Cognitive impairment. The glycoprotein gp120 from HIV-1 uses the chemokine receptors CCR5 and CXCR4 as coreceptors for entry into target cells. Some chemokine receptors promotes neurotoxicity by gp120 glycoprotein binding, whereas others can have neuro-protective effects. Chemokines (CXCR4/CCR5/CX3CR1) are expressed on neurons/glia and their aberrant activation by gp120 neurotoxine affects synaptogenesis and promotes apoptosis and inflammation. Chemokines colocalize with NCAM (Merino et al., 2008, 2011), a cell adhesion involved in neurogenesis and memory formation. Understanding the mechanism by which CXCR4 and other chemokines regulates NCAM levels in the CNS would lead to novel therapeutic strategies in NeuroAIDS. Aim, Material and methods: pro/antinflammatory cytokines (IL-1 Beta, IL-4, TGF Beta), chemokines (CX3CL1 = Fractalkine) and cell adhesion molecules (NCAM soluble) were quantified by ELISA in a subset of naïve HIV-1 infected patients monitored through 24 and 48 weeks of ART treatment (n=55) as compared to their controls. Base line characteristic were: (ART: 65% of NNRTI –Non Analog Nucleoside Reverse Transcriptase Inhibitor, HIV-1 Duration: 2 years; CD4 Nadir: 249; CD4: 260; Viral Load: log 4.5+-0.7; Mean CD4 Increment at 48 week is 193 cells/mm3 and all patients (100%) show undetectable viral load (<50 copies/ml; 1.7 Log). In addition, we studied whether chemokines (CXCL12 = SDF1 -20 nM o/n or CX3CL1 = Fractalkine -20 nM o/n) might prevent gp120 neurotoxicity (200 pM, o/n) by promoting dendritic branching PSA-NCAM dependent levels in cortical neurons at 7 DIV. Results and conclusions HIV-1 infection increases MCP-1, fractalkine soluble and PSA-NCAM levels, which were reduced by ART for 48 weeks of treatment; suggesting their usefulness to predict ART efficacy in HIV-1 infected patients. In addition, NCAM acts a target of gp120 III Beta neurotoxicity in cortical neurons at 7 DIV. Interestingly, CX3CL1 prevented gp120 III Beta cell death by increasing PSA-NCAM levels in vitro.

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CD34+ hematopoietic progenitor cells are a reservoir for JC Virus in natalizumab-treated MS patients

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Natalizumab is a monoclonal antibody therapy to treat relapsing remitting forms of multiple sclerosis (MS) and Crohn's disease by blocking the migration of inflammatory cells into the brain and gut. Natalizumab, however, is associated with a risk of JC Virus (JCV) induced progressive multifocal leukoencephalopathy (PML) at an incidence of 1/500. The emergence of JCV pathogenesis in this setting has changed PML from being a rare disease, mostly seen in HIV infected individuals, to a significant complication in patients receiving immunomodulatory treatments. Before treatment (baseline), and at regular intervals over 9 months during the course of administration, a total of eighty-eight blood samples from twenty-six natalizumab-treated patients were drawn. In comparison with plasma, progenitor-cells (CD34+) and B-lymphocytes (CD19+) were sorted from each blood sample via flow cytometry for quantitation of JCV DNA by qPCR.. Of patients in this longitudinal study, at some time-point 11/26 (42%) were viremic in at least one compartment: 15% in plasma, 23% in CD34+, and 19% in CD19+. Fifty-eight percent had no detactable viral DNA in any of the three compartments. Single blood samples were also collected from 24 patients with greater than 24 natalizumab infusions. Twenty-nine percent of these single time-point patients showed viremia in at least one compartment: 8% in plasma, 13% in CD34+, and 13% in CD19+. Four of the single time-point patients who tested sero-negative were viremic in CD34+, or CD 19+. Because bone marrow is a site of JCV latency, and natalizumab augments migration of CD34+ from bone marrow 3 to 10 fold. These results demonstrating JCV DNA detection in CD34+ and CD19+ provide a potential mechanism for the increased incidence of PML in MS patients treated with natalizumab.

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Translational Spatial Task and its Relationship to HIV-Associated Neurocognitive Disorders Diagnosis and Apolipoprotein E4 in HIV-Seropositive Women

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HIV-associated neurocognitive disorders (HAND) continue to be a neurological complication of HIV infection, with increased morbidity and mortality in the era of combined antiretroviral therapy (cART). Hippocampal function alterations associated with HIV-1 infection have traditionally been assessed using the Morris Water Maze (MWM) in rodents and neuropsychological (NP) tests in humans. However, these methods may not be comparable. Memory Island (MI) Test is a virtual reality-based computer program that tests hippocampus-dependent spatial learning and memory resembling the MWM. MI may better bridge the gap between rodent models of HAND and human HAND. We used the MI test to understand the effects of HIV-1 infection, apolipoprotein E (ApoE) allele status, and CSF ApoE protein levels on spatial learning and memory. During a single visit, HIV-seropositive women (n=20) and controls (n=16) were evaluated with neurological exams, neuropsychological (NP) tests, the MI test, and CSF ApoE assays. CSF total ApoE protein levels were lower than non-HIV controls, however levels did not correlated to any biological or neurocognitive performance measures. We observed significantly reduced learning and delayed memory performance on the MI test in HIV-seropositive group compared with controls. When the HIV-seropositive group was stratified by cognitive performance, the impaired group performed worse in ability to learn the task and in the delayed memory trial. In addition, there was a significant difference were found in NP test and MI between ε4 carriers and non-carriers suggesting it is a confounding factor that should be considered for future studies. Our findings suggest that the MI test is sensitive in detecting spatial deficits in HIV-seropositive women with HAND. Since these results for human HAND patients on the MI test resemble findings previously reported for rodent HIV models on the MWM, we propose that the MI test can serve as a translational instrument for the study of HAND.

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Alterations in prepulse inhibition of the auditory startle response in two different animal models of HIV-1 infection

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HIV-1-associated neurocognitive disorders (HAND) are commonly observed in HIV-positive individuals, despite

the success of combination antiretrovirals in diminishing the most severe forms of HAND. One of the earliest neurological alterations found in the progression of HIV-1 infection prior to obvious cognitive deficits is the disruption of brainstem auditory evoked potentials, which indicates a deficit in sensory gating. We have studied prepulse inhibition (PPI) of the auditory startle response (ASR) as an operational measure of sensorimotor gating in two animal models of HIV-1, either via injection of HIV-1 protein Tat or gp120, or with the use of the HIV-1 transgenic rat, which expresses 7 of the 9 HIV-1 genes. Intra-hippocampal injection of Tat or gp120 disrupted PPI, evidenced by a leftward peak shift in the inhibition function compared to control animals. The use of an apomorphine challenge suggested an enhanced inhibition across ISIs (8–120 ms) on the peak ASR amplitude as the neonatal gp120 dose increased, implicating alterations in dopamine (DA) system function. Preliminary data suggest that a similar leftward peak shift effect occurs in the HIV-1 transgenic rat and that this effect persists through 6 months of age. The observed sensorimotor gating deficits in these animal models of HIV-1 occurred in the absence of wasting or alterations in growth rate. The use of behavioral measures such as the ASR and PPI that can detect early neurological alterations may be instrumental in predicting the development of later more obvious cognitive deficits of HAND and thus determining an appropriate course of treatment. In addition, our findings add to the growing body of evidence that the HIV-1 transgenic rat model may have great utility in the study of HAND and DA system alterations implicated in HAND. [Supported by NIH grants: DA013137, HD043680]

P151
Hepatitis C Virus is Associated with Arteriolar Thickening in a Predominantly HIV + Cohort

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Cerebral small vessel disease has been examined in clinical HIV cohorts with variable implication of HIV-associated, demographic, and metabolic risks. No studies of human brain have examined the relationship between cerebral arteriolar wall thickness and HIV-associated as well as metabolic variables. Accordingly, we measured mean arteriolar thickness (sclerotic index (SI): 1 – inner diameter/outer diameter) in deep white matter of 126 brains (96 HIV+, 30 HIV-) from the Manhattan HIV Brain Bank. Bivariate correlations with SI were performed with general medical variables: age, gender, race, hypertension (HTN), hyperlipidemia, diabetes, obesity, cirrhosis, hepatitis C virus infection (HCV), herpes virus infection (CMV, HSV or VZV); and HIV-associated variables: HIV infection status, HIV risk, CD4 count, plasma HIV load, and antiretroviral therapy (ARV) at the time of death. Of the general variables, age, HTN, race, HCV, and cirrhosis were associated with SI; of the HIV-associated variables, only ARV at death. Addressing co-linearity prior to multiple regression, partial correlations were run with HCV and cirrhosis, HTN and race, and HTN and age. With HCV controlled, cirrhosis lost significance; and with HTN controlled, age lost significance. Accordingly, for the entire sample, HCV, black race, and HTN were entered into multivariate analysis, accounting for 15% of the variance in SI ($R2=.15$, $p<.001$). Each was independently associated with SI, and HCV had the largest effect (betas for HCV: 0.222, HTN: 0.179, black race: 0.172, all p's<.05). For the HIV sample, inclusion of ARV in the model increased R2 to 0.205, with only HCV, HTN, and ARV remaining significant or at trend level (betas for HCV: 0.273, ARV: 0.187, HTN: 0.177, p values .005, .055, and .082 respectively). We conclude that HCV infection constitutes a significant, independent risk factor for cerebrovascular thickening. With recent demonstration of cerebral endothelial infection by HCV, this implicates cerebral vasculature in HCV neuropathogenesis.

P152
HIV-1, miRNAs and neuronal deregulation

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Studies have shown that HIV-infected patients develop neurocognitive disorders characterized by neuronal dysfunction. The lack of productive infection of neurons by HIV suggests that viral and cellular proteins, with neurotoxic activities, released from HIV-1 infected target cells can cause this neuronal deregulation. The viral protein R, a protein encoded by HIV-1, has been shown to alter the expression of various important cytokines and inflammatory

proteins in infected and uninfected cells, however the mechanisms involved remain unclear. Using a human neuronal cell line, we found that Vpr can be taken up by neurons causing i- deregulation of calcium homeostasis, ii- endoplasmic reticulum-calcium release, iii- activation of the oxidative stress pathway, iv- mitochondrial dysfunction and v- synaptic retraction. In search for the cellular factors involved, we performed microRNAs and gene array assays using human neurons (primary cultures or cell line, SH-SY5Y) that we treated with recombinant Vpr proteins. Interestingly, Vpr deregulates the levels of several microRNAs (e.g. miR-34a) and their target genes (e.g. CREB), which could lead to neuronal dysfunctions. Therefore, we conclude that Vpr plays a major role in neuronal dysfunction through deregulating microRNAs and their target genes, a phenomenon that could lead to the development of neurocognitive disorders.

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Observations on the Presentation and Deterioration of Progressive Multifocal Leukoencephalopathy in a Patient with Congenital HIV Infection

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Objective: To describe the course of progressive multifocal leukoencephalopathy in a 20 year old female with congenital HIV infection. Background: A 20 year old female with congenital HIV presented to the Bellevue Hospital with left sided clumsiness. She admitted non-compliance with antiretrovirals. Examination disclosed left hemibody sensory loss, left hemiataxia and a left Babinski sign. Results: CD4 count was 43 and HIV viral load was 90,000 copies/mL. CSF was acellular, glucose and protein concentrations were normal, and JC virus DNA concentration was 750 copies/mL. HSV, VZV, EBV, and BK DNA were not detected by PCR. MR revealed multiple foci of T2WI abnormality compatible with the diagnosis of PML. The patient was hospitalized and treated with antiretrovirals. Three weeks later the patient's neurological examination had deteriorated. CD4 count and HIV viremia had improved, but CSF JC virus DNA concentration had increased to 7000 copies/mL and MR demonstrated expansion of the previously noted hyperintensities without contrast enhancement. At the time of writing, the patient remains hospitalized on the neurological service and severely debilitated. Conclusions: Patients with PML may deteriorate despite an adequate virologic response to HAART and inflammatory immunologic reconstitution is often the mechanism. PML-IRIS, therefore, is often defined as a neurological deterioration despite increasing CD4 count and decreasing viral load, but considerable clinical heterogeneity exists within such a broad definition and immunologic reconstitution may account for every such case. We take the absence of contrast enhancement and increasing CSF concentration of JC virus DNA as evidence that our patient deteriorated in spite, rather than because, of a reconstituted immune system. Such a clinical profile may suggest progressive JV virus-induced oligodendrocytic injury as opposed to maladaptive immune reconstitution.

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A Brief and Useful Method to Detect Neurocognitive Impairment in HIV Infection: The NEU Screening

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Background: Neurocognitive impairment (NCI) is frequently observed in people with HIV. This complication is connected to worse daily functioning and poor quality of life, but also to difficulties in clinical management. We developed a study to find a brief and useful method to

screen NCI in patients with HIV. Methods: Seven hospitals in Barcelona (Catalonia, Spain) participated in this cross-sectional study. A comprehensive neuropsychological battery including 15 computerized and paper-based tests was used covering 7 recommended neurocognitive areas (2-3 hours of duration). NCI was defined as performing at least 1 standard deviation below the mean in at least 2 areas, and this was considered as a gold standard for data comparisons. ROC curves and sensitivity and specificity calculations were performed comparing different combinations of tests. Results: A total of 114 patients with HIV were included although 106 completed all study assessments. Subjects were mostly middle-aged (44 years) men (86%) with undetectable plasma viral load (82%), a median of education level of 12 years, CD4 cell count of 601 cells/μL and nadir CD4 cell count of 204 cells/μL. NCI was seen in 51 patients (48%) and 26 (51%) out of them reported cognitive complaints. According the easiness and total time of duration, the combination of tests with better sensitivity and specificity to detect NCI comprised TMT-A, TMT-B and COWA tests (74% and 81% respectively). This combination was applied overall in less than 10 minutes, implied easy instructions for both application and correction, and was called NEU screening. Conclusions: The NEU screening can rapidly detect NCI in people with HIV, additionally showing high sensitivity and specificity properties. This method may help in the initial stage of the diagnosis of an HIV-associated neurocognitive disorder, considering further use of comprehensive neuropsychological batteries in next steps.

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Differences in North American and West European Study Populations When Detecting Neurocognitive Impairment in HIV Infection: A Comparison of Three Screening Methods

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Background: Several brief methods have been proposed to screen neurocognitive impairment (NCI) in people with HIV. In this study we compared the properties of 2 methods validated in the USA with a recent tool proposed in Europe: the NEU screening. Methods: The NEU screening is a suggested method to detect rapidly NCI in people infected with HIV. This tool includes 3 paper-based measures and is characterized for showing high statistical sensitivity and specificity properties. We compared the NEU with another 2 screenings methods, both validated in the USA, and with similar properties: the Brief NeuroCognitive Screen (BNCS, Ellis et al, 2005) and a combination of 2 measures offered by the HIV Neurobehavioral Research Center (HNRC, Carey et al, 2004). We used as gold standard existence of NCI determined by a comprehensive neuropsychological battery including 15 tests and covering 7 recommended neurocognitive areas. Results: In the original studies, the sensitivity and specificity showed by the BNCS and HNRC methods were 65% and 72%, and 78% and 85%, respectively. In the case of the NEU screening, this was 74% and 81%. However, when both American-population-based methods were replicated in our European sample of patients (106 subjects), the sensitivity and specificity detecting NCI were 66% and 85% for BNCS, and 47% and 91% for HNRC. Regarding easiness and time of duration, characteristics of the 3 methods were similar, although considering applicability, BNCS and NEU appeared to be more feasible than HNRC due to the paper-based use. Conclusions: Screening tools for NCI detection in HIV infection appear to present distinct statistical properties, probably due to cultural discrepancies in cohorts from different countries. In our West European population the NEU screening appears to offer optimal characteristics, although this should be confirmed in larger cohorts of people infected with HIV.

P156

Persistent humoral immune responses in the CNS limit recovery of reactivated murine cytomegalovirus

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BACKGROUND: Experimental infection of the mouse brain with murine CMV (MCMV) elicits neuroimmune responses that terminate acute infection while simultaneously preventing extensive bystander damage. Previous studies have determined that CD8+ T lymphocytes are required to restrict acute, productive MCMV infection within the central nervous system (CNS). In this study, we investigated the contribution of humoral immune responses in control of MCMV brain infection. METHODOLOGY/PRINCIPAL FINDINGS: Utilizing our MCMV brain infection model, we investigated B-lymphocyte-lineage cells and assessed their role in controlling the recovery of reactivated virus from latently-infected brain tissue. Brain infiltrating leukocytes were first phenotyped using markers indicative of B-lymphocytes and plasma cells. Results obtained during these studies showed a steady increase in the recruitment of B-lymphocyte-lineage cells into the brain throughout the time-course of viral infection. Further, MCMV-specific antibody secreting cells (ASC) were detected within the infiltrating leukocyte population using an ELISPOT assay. Immunohistochemical studies of brain sections revealed co-localization of CD138+ cells with either IgG or IgM. Additional immunohistochemical staining for MCMV early antigen 1 (E1, m112-113), a reported marker of viral latency in neurons, confirmed its expression in the brain during latent infection. Finally, using B-cell deficient (Jh-/-) mice we demonstrated that B lymphocytes control recovery of reactivated virus from latently-infected brain tissue. A significantly higher rate of reactivated virus was recovered from the brains of Jh-/- mice when compared to Wt animals. CONCLUSION: Taken together, these results demonstrate that MCMV infection triggers accumulation and persistence of B-lymphocyte-lineage cells within the brain which produce antibodies and play significant role in controlling reactivated virus.

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The immune repertoire in varicella zoster virus (VZV) vasculopathy and its association with VZV-induced cerebrovascular remodeling

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VZV is the only human virus proven to replicate in cerebral arteries and cause transient ischemic attacks and stroke (VZV vasculopathy). We analyzed cerebral arteries in early and late VZV vasculopathy to identify immunological features that help in diagnosis and point to mechanisms of VZV-induced cerebrovascular remodeling. Normal and VZV-infected temporal and cerebral arteries from two subjects with early (4 weeks) and late (10 months) VZV vasculopathy were examined histologically and by immunohistochemistry using antibodies directed against VZV, leukocytes (CD45), T-cell subsets (CD3, CD4 and CD8), B cells (CD20), activated macrophages (CD68), neutrophils (CD15), natural killer (CD57) and mast cells. In early VZV vasculopathy, VZV antigen was present in adventitia, corroborating previous reports that adventitia is the initial site of VZV infection. In late VZV vasculopathy, VZV antigen was found in media, also supporting spread of virus from "outside-in". In early and late VZV vasculopathy, T cells, macrophages and rare B cells were present in adventitia and thickened intima. Early VZV vasculpathy was distinguished by abundant neutrophils in adventitia, and inflammation was associated with intimal thickening. Late VZV vasculopathy was distinguished by the presence of viral antigen without inflammation in media, providing the first evidence of a human virus in an immunoprivileged site. Because neutrophils are abundant in adventitia of early VZV vasculopathy and play a significant role in cardiopulmonary vascular remodeling, we studied primary human brain vascular adventitial fibroblasts (BRAFs) infected with VZV in vitro. Compared to uninfected BRAFs, there was a 4-fold increase in IL8 mRNA and protein (a neutrophil chemoattractant) in VZV-infected BRAFs, and conditioned medium from VZV-infected BRAFs attracted neutrophils in transwell assays. Thus, an early step in VZV-induced cerebrovascular remodeling may involve secretion of IL-8 and recruitment of neutrophils which in turn secrete factors that contribute to vascular remodeling, persistent inflammation and stroke.

P158

Beta-catenin, a suppressor of HIV replication, positively regulates glutamate transporter-1 expression in astrocytes

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Canonical β-catenin signaling regulates the expression of hundreds of genes involved in cell differentiation, communication, apoptosis/survival and proliferation. β-catenin signaling is particularly robust in astrocytes and contributes to a restricted state of HIV replication in these cells. Signals that disrupt β-catenin signaling such as IFNγ alleviate restricted HIV replication in astrocytes. We evaluated the cellular consequences of disrupted β-catenin signaling in progenitor-derived astrocytes (PDAs). Knockdown (KD) of β-catenin expression by targeted siRNA impacted several genes involved in the glutamate transport network, including glutamine synthetase. This finding prompted us to evaluate the impact of β-catenin on the expression of glutamate transporter 1 (GLT-1), also known as Excitatory Amino Acid Transporter 2 (EAAT2). GLT-1 is the predominate glutamate transporter expressed in astrocytes and is responsible for the majority of glutamate transport in the adult brain. β-catenin KD of PDAs resulted in inhibition of GLT-1 mRNA and protein levels by approximately 3.4 and 3-folds, respectively. β-catenin KD also inhibited GLT-1 promoter activity as assessed by GLT-1 promoter linked to a luciferase reporter gene. Conversely, over expression of a constitutively active β-catenin construct led to a dramatic induction of GLT-1 mRNA, protein, and promoter activity. Collectively, these gain and loss of function studies demonstrate that β-catenin is a positive regulator of GLT-1 expression and for the first time identify GLT-1 as a target gene of β-catenin signaling. These findings are relevant to HIV neuropathogenesis because they suggest a model in which inflammatory mediators (e.g IFNs) repress β-catenin signaling in astrocytes. This repression of β-catenin leads to a dramatic reduction in GLT-1 and consequently glutamate accumulation that culminates in neuronal toxicity, which in turn can set the stage for further inflammatory processes leading to further pathology in the CNS.

P159
Methamphetamine and Inflammatory Effects on Neuronal Autophagy

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Neurodegenerative diseases are associated with decreased autophagy, and thus maintaining the normal or enhancing activity of this system has become a common theme in protection from neurodegenerative diseases. The activated immune response in the neuroinflammation induced by HIV infection of the brain creates a harsh environment for cellular homeostasis. Our lab has demonstrated decreased neuronal autophagy in neuroAIDS, using both in vivo and in vitro systems. Cytokines drive the inflammatory process, and have a myriad of effects on the brain and specifically neurons. Meanwhile drugs of abuse also affect the brain and neurons, creating a co-morbidity scenario. Therefore we hypothesize the autophagy process in neurons is hindered through signaling pathways of released cytokines from immune and glial cells in the brain during HIV infection and that autophagy is further modulated by a commonly used drug of abuse, methamphetamine. To examine this hypothesis we are studying the effects of cytokines on autophagy in a neuronal cell culture model, combining cytokine treatment with methamphetamine. This is performed in conjunction with the use of a number of chemical probes with defined effects on the autophagy pathway in order to step in the autophagy process affected by cytokines and methamphetamine. Our data reveals that interferons (both type I and II) decrease autophagy by over 30% at the level of autophagy induction. Methamphetamine (used at physiological concentrations) significantly alters this cytokine modulation of autophagy. Current work is aimed at examining the cytokine-receptor pathway and how it interacts with methamphetamine and the autophagy process. The biochemical process of cytokine signaling will unravel the mechanism of autophagy disruption and subsequent neurodegeneration in neuroAIDS, both in the presence and absence of the use of abused substances.

P160
JC virus T-antigen regulates glucose metabolic pathways in brain tumor cells

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Expression of the JCV early protein, T-antigen, which has transforming activity in cell culture and in transgenic mice, results in

the development of a broad range of tumors of neural crest and glial origin. Evidently, the association of T-antigen with a range of tumor-suppressor proteins, including p53 and pRb, and signaling molecules, such as β-catenin and IRS-1, plays a role in the oncogenic function of JCV T-antigen. We demonstrate that T-antigen expression is suppressed by glucose deprivation in medulloblastoma cells and in glioblastoma xenografts that both endogenously express T-antigen. Mechanistic studies indicate that glucose deprivation-mediated suppression of T-antigen is partly influenced by 5'-activated AMP kinase (AMPK), an important sensor of the AMP/ATP ratio in cells. In addition, glucose deprivation-induced cell cycle arrest in the G1 phase is blocked with AMPK inhibition, which also prevents T-antigen downregulation. Furthermore, T-antigen prevents G1 arrest and sustains cells in the G2 phase during glucose deprivation. On a functional level, T-antigen downregulation is partially dependent on reactive oxygen species (ROS) production during glucose deprivation, and T-antigen prevents ROS induction, loss of ATP production, and cytotoxicity induced by glucose deprivation. Additionally, we have found that T-antigen is downregulated by the glycolytic inhibitor, 2-deoxy-D-glucose (2-DG), and the pentose phosphate inhibitors, 6-aminonicotinamide and oxythiamine, and that T-antigen modulates expression of the glycolytic enzyme, hexokinase 2 (HK2), and the pentose phosphate enzyme, transaldolase-1 (TALDO1), indicating a potential link between T-antigen and metabolic regulation. These studies point to the possible involvement of JCV T-antigen in medulloblastoma proliferation and the metabolic phenotype and may enhance our understanding of the role of viral proteins in glycolytic tumor metabolism, thus providing useful targets for the treatment of virus-induced tumors. This work was made possible by grants awarded by NIH to KK.

P161
HIV-1 LTR single nucleotide polymorphisms (SNPs) correlate with disease parameters

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The long terminal repeat (LTR) regulates HIV-1 gene expression by interacting with multiple host and viral factors. Cross-sectional studies in the pre-HAART era demonstrated that single nucleotide polymorphisms (SNPs) in C/EBP site I and Sp site III from peripheral blood-derived LTRs increased in frequency as disease severity increased and correlated with HIV-1-associated dementia. Current studies focus on the identification of LTR signatures derived from peripheral blood virus that can be used as molecular markers to identify HIV-1-infected individuals more prone to developing advanced stage disease and/or neurologic disease. A prospective, longitudinal study was conducted on 458 HIV-1-seropositive patients currently enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T-cell count, and viral load were collected approximately every 6 months. SNP density within the entire HIV-1 LTR was determined by comparison to the conB reference sequence and showed areas of increased variability. The collection of extensive clinical parameters on these patients have allowed for cross-population and longitudinal analyses of the impact of these parameters on the development of SNPs during the course of disease. To date, multiple SNPs have been detected to be significantly associated with CD4 T-cell count and viral load, as well as with change in CD4 T-cell count and change in viral load. These SNPs were identified in areas of the LTR that have been previously reported to be important to viral promoter function and some in areas that have been less well functionally characterized. These results suggest that the HIV-1 genomic swarm may evolve during the course of disease in response to selective pressures that lead to changes in prevalence of LTR SNPs and that LTR SNPs may produce alterations in viral function and can be predictive of more advanced stage HIV disease.

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HIV infection of astrocytes increases release of Dickkopf-1 protein by a gap junction and hemichannel dependent mechanism

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Human immunodeficiency virus-1 (HIV) is a major public health issue, with a significant CNS complication of infection, NeuroAIDS. In vivo, microglia/macrophages are the main cells infected. However, a low but significant number of HIV infected astrocytes also has been detected, but their role in the pathogenesis of NeuroAIDS is not well understood. Our previous data indicated that HIV infection of astrocytes increased expression of the glycoprotein, dickkopf-1 protein (Dkk1), a soluble inhibitor of the wnt pathway. In HIV infected cultures of human astrocytes, secretion of Dkk1 was highly regulated by functional gap junction channels and connexin43 hemichannels. We also demonstrated that Dkk1 expression in astrocytes was increased in human brain tissue sections of individuals with HIV encephalitis as compared to tissue sections from uninfected individuals. We demonstrated that in primary cells, Dkk1 secretion did not participate in bystander killing of uninfected astrocytes or viral reactivation. However, its secretion regulates neuronal damage measured by collapse of neuronal processes. Thus, we demonstrated that HIV infection of astrocytes dysregulates secretion of Dkk1 by a mechanisms that involves both gap junctions as well as hemichannels that contributes to the neuropathogenesis observed in HIV infected individuals.

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Simian varicella virus infection of chinese rhesus macaques produces ganglionic infection in the absence of rash

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Varicella-zoster virus (VZV) causes varicella (chickenpox), becomes latent in ganglia along the entire neuraxis and may reactivate to cause herpes zoster (shingles). VZV may infect ganglia via retrograde axonal transport from infected skin or through hematogenous spread. Simian varicella virus (SVV) infection of rhesus macaques provides a useful model system to study the pathogenesis of human varicella zoster virus (VZV) infection. To dissect the virus and host immune factors during acute SVV infection, we analyzed four SVV-seronegative Chinese rhesus macaques infected intratracheally with cell-associated 5 X 103 plaque-forming units (pfu) of SVV-expressing green fluorescent protein (n=2) or 5 X 104 pfu of wild-type SVV (n=2). All monkeys developed viremia and SVV-specific adaptive B- and T-cell immune responses, but none developed skin rash. At necropsy 21 days postinfection, SVV DNA was found in ganglia along the entire neuraxis and in viscera, and SVV RNA was found in ganglia, but not in viscera. The amount of SVV inoculum correlated with the extent of viremia and the immune response to virus. Our findings demonstrate that acute SVV infection of Chinese rhesus macaques leads to ganglionic infection by the hematogenous route and the induction of a virus-specific adaptive memory response in the absence of skin rash.

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Performance of Older vs Younger Persons with HIV Infection on Measures of Executive Function

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Background: Executive functions include working memory, mental flexibility, and inhibition and are vulnerable to the

effects of both cognitive aging and HIV infection. This study assessed whether people with HIV infection 50 years or older performed more poorly on executive function measures compared to HIV-infected younger individuals. Methods: As part of a larger study of persons treated for HIV infection, participants completed measures of executive functions including the Trail Making Test, Tower of London, Purdue Pegboard, and Iowa Gambling Task. The performance of persons 50 years or older was compared to that of younger participants using ANCOVA models that corrected for education, race, and CD4 count. Results: Older participants performed more poorly on several measures of executive function after correction for possible confounders, including the Iowa Gambling Task ($F=5.30$, $df=1, 85$, $p=0.03$) and the Trail Making Test (Trails A, $F=5.67$, $df=1, 117$, $p=0.02$; Trails B, $F=4.76$, $df=1, 116$, $p=0.03$). Older participants performed equally well as younger participants on the Tower of London ($F=0.08$, $df=1, 114$, $p=0.77$) and a measure of simple motor speed (Purdue $F=1.77$, $df=1, 114$, $p=0.19$). Conclusions: Persons 50 years of age and older may perform more poorly on some measures of executive function than do younger persons. They showed less evidence of implicit learning on the Iowa Gambling Task and lower levels of psychomotor speed on Trails A and B. These differences may result from HIV infection, cognitive aging, or their interaction. Understanding the impact of executive function in the context of HIV plus aging is important given the aging of the HIV population and since these abilities may have a significant impact on everyday functioning.

P165
PINCH in the cellular stress response to Tau-hyperphosphorylation

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Hyperphosphorylated Tau (hp-Tau) detaches from microtubules and can form paired helical filaments and tangles leading to neuronal dysfunction. In healthy ageing, aberrant proteins such as hp-Tau are cleared from the cell by the heat shock response (HSR) machinery. In the case of hp-Tau, the HSR complex sorts aberrant Tau for either repair or degradation. However, if the cellular machinery fails to clear abnormal proteins, accumulation of aberrant proteins can occur. Accumulation of hp-Tau accounts for more than 20 neuropathological diseases including AD and HIVE. In this context, we have discovered that a protein called PINCH binds to hp-Tau and may function in its clearance from neurons. Likewise, PINCH is detectable in the CSF of HIV and AD patients, but is nearly undetectable in healthy controls. Briefly, PINCH is composed of 5 double zinc finger domains and has no catalytic activity. PINCH is highly conserved, plays a key role in multi-protein complex formation, and facilitates cell spreading, migration and survival. Growing evidence points to significant overlap between mechanisms involved in HIV-associated neurocognitive disorders (HAND) and age-related neurodegenerative diseases. HIV + individuals diagnosed decades ago are beginning to face age-associated CNS changes. Combined with infection and long-term exposure to cART, age-related neurodegeneration may be exacerbated. During neuronal stress, PINCH protein is required to maintain neurite extensions. Additionally, studies in cancer suggest that PINCH promotes cell survival, but no direct mechanism has been identified. We show that during the cellular stress response, PINCH binds to hp-Tau and may contribute to changes in intracellular levels of hpTau. These studies address a new mechanism by which aging and HAND may interact. We hypothesize that in diseases with a tauopathy component, PINCH is expressed by neurons to promote cell survival through its interactions with Tau and the heat shock protein response machinery.

P166
CSF microRNAs in HIV-Associated Neurological Disorders

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HIV-associated neurological disorders (HAND) comprise cognitive, motor and behavioral impairments of various entities which affect a substantial number of HIV-1 infected individuals. Understanding the biology of brain HIV-1 infection and identifying markers for its pathological manifestations, including HIV-Encephalitis, are of critical importance and it continues to be an active field of research. MicroRNAs are short non-coding RNAs that modulate gene expression by

translational repression. Because of their high stability in intracellular as well as extracellular environments, miRNAs have been recently emerged as important biomarkers in several human diseases, but they have not been tested in the CSF of HIV-positive individuals. Here, we present results of a pilot study aimed to investigate a putative miRNA signature in the CSF of HIV-positive individuals with neurological disorders. We utilized a high throughput approach of miRNA detection arrays and focused on the identification of differentially expressed miRNAs in the CSF of ten HIV-positive individuals compared to ten HIV-negative samples. The group of HIV + individuals contained nine cases of HAND and, among those, four had HIVE. All the HIV- samples had non-viral acute disseminate encephalomyelitis and no signs of cognitive impairment. Comparison analysis revealed 23 down-regulated miRNAs ($p<0.1$) in HAND compared to the HIV-negative group and, interestingly, no miRNA was found up-regulated in this group. Comparison between HIVE and HIV- groups showed 36 differentially regulated miRNAs ($p<0.1$), of which only three were upregulated. Although validation with an independent number of clinical samples is required before trying definite conclusion, this work offers the base for future investigation. Supported by MH 079751 and P60 AA009803-18

P167
The role of chemokines in the pathogenesis of viral encephalitis

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There is a plethora of viruses spread by mosquitoes, many of which cause debilitating, often fatal, neurological diseases such as acute encephalitis. Semliki Forest virus (SFV), a low risk neurotropic pathogen, provides an excellent model for studying neuroinvasion and immune responses in viral encephalitis. Chemokines are important for leukocyte entry into the CNS for virus clearance. However, influx of leukocytes may also promote neuropathology that includes neuronal damage and thus chemokines can play damaging pathogenic roles in viral encephalitis. Unfortunately, we have only limited insights into the precise nature of chemokine involvement in encephalitis and an improved understanding of this important axis is central to the development of novel therapies. In the SFV model system, we comprehensively investigated chemokine expression in the CNS parenchyma during the development of viral encephalitis with a view to associating this with coincident leukocyte entry. Therefore mice were infected intraperitoneally with SFV and brains harvested and analyzed by Taqman low-density arrays to examine and compare chemokine expression over time of infection. On post-infection day (PID) 7 expression of CCL2, 3, 5, 7, CXCL9 and CXCL10 were significantly upregulated (up to 520 fold). This coincided with leukocyte influx into the CNS starting PID5 and peaking on PID7. At PID7, immunohistochemical staining showed T-cell and macrophage accumulation in the meninges and, in association with isolated foci of virus, in the brain parenchyma. B-cells appeared in the brain by PID 10 and were localized primarily around the ventricles suggesting entry of B-cells into the CNS via the cerebrospinal fluid (CSF)-brain barrier. In summary, we provide the first comprehensive analysis of chemokine expression during SFV encephalitis and subsequently highlight novel potential therapeutic targets. Chemokine expression mediates leukocyte recruitment to inflamed tissues and is likely to be pivotal in viral clearance.

P168
Physical dependence to opioid medications: First preclinical comparative study in neuroAIDS

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Both drug abuse and HIV represent serious public health threats because they are interrelated and influence each other functions. Opioid dependence is common in HIV clinics and there is a need to find alternative, effective and safe medications for HIV-infected opioid-dependent patients. The HIV-1 envelope glycoprotein, gp120 (HIV-gp120) is implicated in the pathogenesis of neurological disorders associated with HIV and interferes with opioid system. The goal is to examine and compare the chronic effectiveness of the most common clinically used medications for opioid dependence (methadone and buprenorphine) in presence of HIV-gp120 in the brain. First we monitored rat weight during 4, 7 or 14 day induction periods with buprenorphine (0.5, 1.5 or 3 mg/kg) as well as during subsequent “withdrawal” periods in the absence and presence of HIV-gp120 in the brain. Parallel experiments with methadone (5 or 10 mg/kg) afforded positive control data. Male S.D. rats (175-200 g) were weighed and injected s.c. at 10 AM daily with buprenorphine, methadone or saline as indicated above. The mean weight of rats receiving buprenorphine during induction did not differ significantly from that of control (saline) animals. Sudden, weight loss (on day 5 of “withdrawal”) was associated with only the 3 mg/kg - 14 day buprenorphine dosing schedule. The infusion of HIV-gp120

into the brain did not precipitate or alter weight loss during the spontaneous withdrawal period after the buprenorphine cessation. Rats on 5-10 mg/kg methadone for 7 days show a significant body weight lost on day 1 of "withdrawal" and during the treatment period (even in the absence of HIV-gp120 in the brain). Based on these data it appears that in the presence of HIV-gp120 in the brain, the chronic use of buprenorphine for opioid dependence has advantages over methadone. Financial Support: DA 029414 and DA 031605.

P169

Differential effects of cocaine on cytokine profiles within patients in the DREXELMED HIV/AIDS Genetic Analysis Cohort

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HIV infection is prevalent among substance abusers. We evaluated the relationship between illicit drug use and HIV-1 disease progression in HIV-1-infected patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T cell count, and viral load were performed approximately every 6 months. Drug abuse is common in the cohort, with 87.6% of patients admitting past use; 29.7%, currently abusing drugs; and 36.2% testing positive for drug use at the time of visit. Cocaine use is heavily favored, with 80.5% of drug-using patients admitting to past or current cocaine use. Most patients use multiple drugs simultaneously. The cohort can be categorized into nonusers (PN), cocaine only (preferential, PC) users, cannabinoid only (preferential, PM) users, and multidrug users. Nonusers are more likely to remain on HAART (94.4%), whereas PC are less likely (83.4%). The overall health of the PN subcohort is better than that of those in the PC subcohort. Patients in PN are less likely to suffer from opportunistic infections and have higher current and nadir CD4 counts. Additionally, the peak and the current viral loads in PN are substantially lower than those in PC patients. Since, cocaine is known to have immunomodulatory effects, the cytokine profiles of PN and PC individuals were analyzed to understand the effects of cocaine on cytokine modulation and HIV-1 disease progression. Among the 30 cytokines investigated, interestingly, differential levels of the HIV-1 suppressive factors, MIP-1α, MIP-1β, and RANTES, were established within the PC subcohort. In conclusion, illicit drug use appears to facilitate HIV-1 disease progression based on these assessments.

P170

Primary HIV-1 infection is characterized by elevation of cerebrospinal fluid biomarkers indicating early neuronal damage

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Background The extent of neurologic damage during presymptomatic HIV-infection is incompletely understood. Cerebrospinal fluid (CSF) biomarkers such as neurofilament light chain (NFL) are elevated in subjects with advanced HIV-infection and HIV-associated dementia, and are associated with central nervous system impairment. We measured NFL and other CSF biomarkers of neuronal injury during primary HIV-infection (PHI). Methods In antiretroviral naïve subjects with PHI, CSF NFL was analyzed using a new, highly-sensitive, two-site enzymatic quantitative immunoassay with a lower limit of detection of 50 ng/L. Detection of t-tau, β-amyloid, and soluble amyloid precursor protein-alpha and -beta (sAPP-α and sAPP-β) used standard ELISAs. Analyses employed the Mann-Whitney test and Spearman correlations. Results 84 PHI subjects had a median age 36(18-61) years, CD4+ T cell count 546(111-1608) cells/uL, and log10 plasma HIV RNA level of 4.57(1.69-7.08) at 96.5(15-376) days post-infection. HIV-uninfected controls had a median age of 43(26-66) years. Median NFL in 81 PHI subjects was elevated at 565(120-2830) ng/L, compared with 364(193-793) ng/L in 20 controls ($p<0.01$). Median sAPP-α was 721(293-1285) ng/L in 21 PHI subjects compared with 435(324-783) ng/L in 23 controls ($p=0.02$). History of neurologically symptomatic seroconversion was not associated with higher NFL. No significant differences in t-tau, sAPP-β, and β-amyloid were detected between a subset of PHI subjects and controls. NFL correlated with CSF inflammatory markers including neopterin ($r=0.40$; $p=0.0002$), IP-10 ($r=0.42$;$p=0.001$), white blood cell count ($r=0.33$; $p=0.003$), and CSF:plasma albumin ratio ($r=0.59$;$p<0.0001$). NFL also correlated with plasma ($r=0.23$; $p=0.04$) and CSF ($r=0.23$;$p=0.04$) HIV RNA levels, CSF t-tau ($r=0.51$;$p=0.004$) and CSF β-amyloid ($r=0.5$; $p=0.02$),. Conclusion Biomarkers of neuronal damage are elevated in subjects with PHI compared to HIV-uninfected controls. NFL, a sensitive marker of neuronal injury, correlates with markers of CSF inflammation during PHI. These findings suggest that HIV-related neuronal damage starts during early HIV-infection and is mediated by neuroimmune activation during this period.

P171

PDGF-CC mediated neuroprotection against HIV Tat involves TRPC-mediated inactivation of GSK 3 beta

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Platelet-derived growth factor-CC (PDGF-CC) is the third member of the PDGF family, and has been implicated in embryogenesis as well as in the development of the CNS. The biological function of this isoform remains largely unexplored in the context of HIV-associated neurocognitive disorder (HAND). PDGF-CC is critical for embryonic development and has homology to VEGF. In the present study, we demonstrate that exposure of human neuroblastoma cells SH-SY5Y to HIV transactivator protein, Tat resulted in decreased intrinsic expression of PDGF-CC as evidenced by RT-PCR and western blot assays. Reciprocally, pretreatment of SH-SY5Y cells with PDGF-CC abrogated Tat-mediated neurotoxicity by mitigating Tat-induced apoptosis and neurite & MAP-2 loss. Using the pharmacological and loss of function approaches we identified the role of phosphatidylinositol 3-kinase (PI3K)/Akt signaling in PDGF-CC-mediated neuroprotection. We report here a novel finding that the involvement of the transient receptor potential canonical (TRPC) channels in PDGF-CC-mediated neuroprotection. We next examined the key signal downstream of PI3K-glycogen synthase kinase 3 beta (GSK-3β), which was inactivated (increased phosphorylation at ser-9) in response to PDGF-CC. This was further validated in cells transfected with dominant-negative GSK-3β which blocked PDGF-CC-mediated neuroprotection. In the presence of PI3K inhibitor or TRPC blocker, PDGF-CC lost its ability to inactivate GSK-3β, thereby suggesting the intersection of PI3K and TRPC signaling at GSK-3β. β-catenin, an important mediator downstream of GSK-3β was also demonstrated to accumulate in the nucleus in cells exposed to PDGF-CC. Taken together our findings lead to the suggestion that PDGF-CC could be developed as a therapeutic target to mitigate Tat-mediated neurotoxicity with implications for HAND.

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First specific therapeutic approach targeting a pathogenic Human Endogenous Retrovirus Protein in Multiple Sclerosis

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HERV-W family in human DNA retains copies expressing an envelope protein (Env), which activates a pro-inflammatory and autoimmune cascade through interaction with Toll-Like receptor 4 (TLR4) on antigen-presenting

cells. This Env protein was evidenced by several independent RT-PCR and immunohistological studies in MS brain lesions (reviewed in: Perron et al. J Neurol Sci. 2009). Env antigenaemia was detected in about 75% of MS sera ex-vivo, confirmed in parallel by quantitative RT-PCR as well as sequence analyses. Another confirmation of the presence of this endogenous retroviral protein in MS was obtained showing immunohistological detection in brain demyelinated lesions, mostly within perivascular macrophages and microgliocytess in the parenchyma. A similar detection pattern was obtained in lesions of 8/8 MS and 0/5 controls with three anti-Env monoclonals specific for three distant epitopes (Multiple Sclerosis Journal 2012, in press). A pre-clinical proof of concept based on "Experimental Allergic Encephalomyelitis" (EAE) induced with Env protein, in both C57/Bl6 mice with MOG and in Humanized SCID (Hu-SCID) mice with MBP was evidenced. Clinical symptoms were correlated with important inflammation evidenced by on MRI and associated with histological demyelination. T-lymphocyte anti-myelin autoimmunity was also shown in splenocytes. Moreover, in both MS models, a murine, chimaerized and further humanized anti-HERV-W/Env monoclonal antibody significantly inhibited clinical symptoms compared to untreated controls in both preventive and therapeutic i.v. injection protocols. Radiolableing of the antibody evidenced an accumulation clustering with Env antigen in mouse brain with stereotaxic injection of Env in cerebral white matter, but not in Sham controls injected with buffer only. The regulatory approval of the pre-clinical and toxicology package has been obtained with a humanized IgG4 construct and the Phase I clinical trial has been achieved in Europe. This humanized therapeutic antibody represents the first attempt to treat a human disease by targeting a Human Endogenous retroviral Protein.

P173

Inhibition of poly(ADP-ribose) polymerase (PARP)-1 protects blood brain barrier in HIV CNS infection

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Despite immune recovery in individuals on combination antiretroviral therapy, the frequency of HIV associated neurocognitive disorders (HAND) remains high for reasons that are not well understood. HIV-associated neurodegeneration is driven by chronic inflammatory responses in the brain secondary to a low level of HIV replication in CNS reservoir cells (macrophages, microglia) and injury to the blood brain barrier (BBB) mediated by pro-inflammatory factors in blood and migration of leukocytes across the BBB. Therapies targeting inflammation and HIV replication should be beneficial for amelioration of HAND. PARP-1 inhibitors emerged as potent anti-inflammatory and immunomodulatory compounds. Although the modulatory effects of PARP inhibitors on immune cells have been studied to some extent, nothing is known about their effects in the setting of HIV CNS infection. We propose that PARP inhibition will attenuate BBB injury caused by HIV-1 via effects on monocytes, brain endothelium, activated microglia and HIV-1 infected macrophages. We found increased expression of PARP in brain endothelium and macrophages in human brains with HIV encephalitis. PARP suppression in primary human brain microvascular endothelial cells (BMVEC) improved BBB integrity and augmented expression of tight junction proteins. PARP inhibitors prevented barrier disruption caused by inflammation. PARP inhibition in BMVEC diminished monocyte adhesion/migration across a BBB model, down-regulated adhesion molecules in the endothelium and decreased activity of RhoA/Rac1 (controlling BBB integrity and monocyte migration across the BBB). PARP inhibitors down regulated inflammatory genes increased by TNFα in BMVEC. In monocytes, PARP inhibitors down regulated the active form of β-integrin that paralleled RhoA/Rac1 suppression. PARP inhibitors decreased expression of pro-inflammatory molecules and diminished HIV replication in human macrophages. In vivo treatment with a PARP inhibitor decreased enhanced BBB permeability in mice with systemic inflammation. These results point to the relevance of PARP suppression in protection of the BBB in the setting of HIV-1 infection

P174

Targeted BDNF delivery across the blood-brain barrier for neuro-protection using liposome formulated magnetic nano carriers: an in-vitro study

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Parenteral use of drugs such as opiates exert immunomodulatory effects and serve as a cofactor in the progression of HIV-1 infection, thereby potentiating HIV related neurotoxicity ultimately leading to progression of NeuroAIDS. Morphine exposure is known to induce apoptosis and down

regulate cAMP response element-binding (CREB) expression in cultured cells. Use of a neuroprotective agent (BDNF; which protects neurons against apoptotic cell death) and a μ-opioid receptor antagonist (CTOP; which reduces opiate addiction and block the synergistic neurotoxicity caused by opiates) could be of therapeutic benefit in the treatment of opiate addiction. Current treatments are not effective due to impenetrability of therapeutic molecules across the blood brain barrier (BBB). Therefore development of a drug delivery system that can cross BBB may have significant therapeutic advantage. In the present study, we hypothesized that magnetically guided nano carrier may provide a viable approach for simultaneous targeting of multiple drugs across the BBB. We developed a magnetic based nanocarrier bound to BDNF and evaluated its efficacy and ability to transmigrate across the BBB using an in vitro BBB model. We investigated the induction of apoptosis by flow cytometry and CREB expression by RT-PCR. Results show that nano formulation is able to cross the BBB and effective in suppressing the apoptosis induced by morphine and inducing CREB expression, suggesting that the developed nanocarrier will provide a potential therapeutic approach to treat opiate addiction.

P175
Impact of age on markers of HIV-1 disease

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The introduction of HAART has resulted in significantly lower mortality rates, culminating in an HIV epidemic that increasingly affects older adults emphasizing the importance of understanding the risk factors associated with aging and HIV-1 infection. The frequency of dementia among HIV-positive patients 50 years and older is 2 to 3 times greater than that seen in HIV-positive patients between the ages of 20 and 39. Additionally, immune failure commonly occurs with both aging and HIV infection. Current studies focus on the impact of age on LTR signatures that correlate to HIV-1 clinical parameters (CD4 T cell count and viral load). A prospective, longitudinal study was conducted on 458 HIV-1 seropositive patients currently enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4 + and CD8+ T-cell count, and viral load, along with patient history including age, were collected approximately every 6 months. Using a linear mixed model for longitudinal data, we found age modified the SNP effect on CD4 counts and VL to different scales, however, due to the current sample size, only the modification of vSNP244 on CD4 was close to significant ($p=0.0686$). A variation at that locus increased CD4 counts by 36.851 cells/μL for all individuals. For the younger group, the individuals with the variation have much larger CD4 counts than those without the variation. Additionally, age is known to have immunomodulatory effects. The cytokine profiles of adult and aged individuals were analyzed to understand the effects of age and HIV-1 infection on cytokine modulation and HIV-1 disease progression. Among the 30 cytokines investigated, interestingly, cytokine levels appeared to vary between these two populations. In conclusion, age, in the context of HIV-1 infection, appears to impact viral gene expression and immune activation.

P176

Elevated Ferritin Heavy Chain and dysregulated CXCL12/CXCR4 signaling within cortical neurons of drug abusers and HIV + individuals

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HIV progression and HIV-associated neurocognitive disorders (HAND) are directly and indirectly exacerbated by substances of abuse, including opiates. The mechanisms by which opiates aggravate HIV and HAND are complex and not well defined. One critical factor in the neuropathogenesis of HIV relates to the interaction between opioid receptors and the HIV co-receptor, CXCR4, which is abundantly expressed in both the immune and nervous system. Importantly, CXCR4 and its natural ligand (the chemokine CXCL12/SDF-1) are involved in fundamental neuronal and glia functions, including neurotransmission, cell differentiation, and survival. Our previous studies in murine models have shown that prolonged morphine treatment down-regulates CXCR4 function due to increased levels of a novel regulator of CXCR4, the ubiquitous iron binding protein Ferritin Heavy Chain (FHC), and that this protein mediates the effect of morphine on CXCR4 both in vitro and in vivo. These data suggest that alterations of CXCR4 function induced by opiates may contribute to HIV neuropathology, via regulation of FHC. To further test this hypothesis and evaluate potential clinical implications, we measured protein expression of FHC, CXCR4 and pCXCR4 (Ser 339 - as indication of receptor status) within brain tissue samples from control subjects, illicit drug abusers (including opiate users), and HIV + patients by multispectral immunohistological image analysis. Our results demonstrate elevated FHC protein and decreased pCXCR4 expression within digitally isolated cortical neurons of patients with a history of drug use or HIV. Additionally, we studied non-human primates (NHPs) treated with morphine, SIV or both. Importantly, we found consistent results between the human and NHP cohorts suggesting that opiates or HIV/SIV alone are sufficient to induce neuronal FHC expression in vivo and that this is associated with disruption of CXCL12/CXCR4 signaling. Furthermore, preliminary analyses on both human and NHP cohorts suggest neuronal FHC expression is associated with disease progression and neurocognitive impairment.

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TNF-alpha stimulates outgrowth of neuronal processes upon injury via stimulation of NF-kappaB-induced EPHB2 signaling

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We utilized primary mouse neuronal culture in scratch assay to determine the impact of TNF-alpha and IGF-1 upon neural cell survival and growth after injury. Our results demonstrated that treatment of the scratched neuronal culture with TNF-alpha (100 ng/ml) promotes more neurite outgrowth, both in length and complexity, than IGF-1 (200 ng/ml) in response to injury, suggesting that the effects of TNF-alpha may allow for the remodeling of neuronal process to promote re-growth. In order to analyze a more dynamic condition, human neuronal stem cell culture treated with TNF-alpha, IGF-1, or both was subjected to a human synaptic plasticity array to show changes in expression of genes involved in synaptic plasticity, LTP, and LTD. Reduced expression of Arc and Homer1 with TNF-alpha treatment reflects a lack of dendritic stabilization, allowing for modification of processes for response to injury. Several genes were up-regulated with TNF-alpha treatment that are directly involved in the growth and plasticity of neuronal processes including BDNF, EPHB2, GRIP1, KIF17, MMP9, NF-kappaB1, NTF3, and RELN. It has been shown that TNF-alpha can induce neuroprotective effects through NF-kappaB, therefore, we propose that upon TNF-alpha treatment following induced injury, neurite outgrowth occurs primarily through EPHB2 receptor signaling via stimulation of NF-kappaB. Our observation provides a new avenue for the investigation on the impact of HIV-1 in neuronal cell damage and the involvement of TNF-alpha and IGF-1 and other cytokines in this event.

P178
Cell-Targeted Long-Acting Nanoformulated Antiretroviral Therapy

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Nanomedicine can revolutionize conventional antiretroviral therapy (ART) through improved access, adherence and toxicity reductions. A means to reach such goals is through cell-targeted drug delivery by mononuclear phagocytes (MP; monocytes, dendritic cells and macrophages) to target regions where nanoformulated ART (nanoART) could affect virus elimination. To this end, we developed crystalline nanoART of ritonavir, atazanavir and efavirenz coated with either folic acid, N-formyl methionine leucine phenylalanine (fMLP) or a mannose monomer conjugated polymer using click chemistry. Nanosuspensions were manufactured by high-pressure homogenization, and particle size, charge and polydispersity determined by dynamic light scattering and electron microscopy. Formulation composition and Integrity were determined by 1 H nuclear magnetic resonance. Uptake into macrophages was measured after stimulation with lipopolysaccharide, tumor necrosis factor alpha, interferon gamma, granulocyte macrophage colony-stimulating factor (GMCSF) or cytokine mixtures. Confocal microscopy and reverse transcriptase assay was used to determine compartmentalization and antiretroviral efficacy. NanoART sizes ranged from 137.9 nm to 221.7 nm, zeta potentials from -4 mv to -26 mv, and polydispersity indices from 0.11 to 0.30; drug loading was >70%. Macrophage uptake of 100 μM nanoATV was ~35-40 μg/million cells, with saturation at approximately 16 hrs. Folic acid and mannose-coated nanoART facilitated particle uptake in all cell cultures. However, fMLP tagged nanoART showed improved uptake only with GMCSF. NanoART was retained in macrophages for up to 15 days. We posit that accelerated particle uptake and immune modulatory functions of targeted nanoART make it attractive formulations for antiretroviral drug delivery.

P179
HTLV-1 Tax-miRNA-chromatin: a novel mechanism and concept to understand viral gene expression post-integration in a cell-type dependent manner

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RNA interference (RNAi) is a natural cellular mechanism to silence gene expression and is predominantly mediated by microRNAs (miRNAs) that target messenger RNA. Viruses can manipulate the cellular processes necessary for their replication by targeting the host RNAi machinery. This study explores the effect of human T-cell leukemia virus type 1 (HTLV-1) transactivating protein Tax on the RNAi pathway in the context of a chromosomally integrated viral long terminal repeat (LTR) using a CD4 + T-cell line, Jurkat. Transcription factor profiling of the HTLV-1 LTR stably integrated T-cell clone transfected with Tax demonstrates increased activation of substrates and factors associated with chromatin remodeling complexes. Using a miRNA microarray and bioinformatics experimental approach, Tax was also shown to downregulate the expression of miRNAs associated with the translational regulation of factors required for chromatin remodeling. These observations were validated with selected miRNAs and an HTLV-1 infected T cells line, MT-2. miR-149 and miR-873 were found to be capable of directly targeting p300 and p/CAF, chromatin remodeling factors known to play critical role in HTLV-1 pathogenesis. Overall, these results are first in line establishing HTLV-1/Tax-miRNA-chromatin concept and open new avenues toward understanding retroviral latency and/or replication in a given cell type.

P180
The tug-of-war between dendritic cells and HTLV-1: lessons from in vivo studies

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The human immune system is under constant challenge from many viruses, some of which the body is successfully able to clear. Other viruses have evolved to escape the host immune responses and thus persist, leading to the development of chronic diseases. Dendritic cells (DCs) are professional antigen presenting cells that play a major role in both innate and adaptive immunity against different pathogens.

For the past few years our efforts have been focused on exploring the participation of DCs in the pathogenesis of HTLV-1. We observed previously that depletion of DCs in CD11c-DTR transgenic mice enhanced the susceptibility to cell-free HTLV-1 infection. We further performed the host-pathogen interaction studies utilizing Flt3 ligand derived murine bone marrow DCs (FL-DCs). First, the kinetics of viral entry, proviral integration, and expression of the viral protein Tax was established and then effects of cell-free HTLV-1 was examined on these cells. Phenotypically, FL-DCs demonstrated activation and produced an array of proinflammatory cytokines as well as IFN-α. Virus-matured FL-DCs also stimulated proliferation of autologous CD3+ T cells and IFN-γ production. Gene expression studies revealed upregulation of interferon-stimulated genes, most cytokines, and transcription factors but a distinct downregulation of many chemokines. Overall, these results highlight the critical interaction of DCs with a human chronic virus important for the early immune responses.

P181
Regulation of alternative splicing of the mu-opioid receptor by acute morphine treatment and functional implications of MOR-1X up-regulation

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Intravenous drug abuse, including abuse of opioids, has contributed substantially to the HIV epidemic. Additionally, opioid abuse has been shown to exacerbate HIV pathology, particularly neurocognitive dysfunction. Despite numerous studies, the exact interaction between HIV infection and opioid signaling is still poorly characterized. This may be due, in part, to an incomplete understanding of the mu-opioid receptor (MOR), the primary receptor for opioids of abuse. Recently, multiple isoforms of the mu-opioid receptor have been identified. However, the functional significance of these isoforms as well as regulation of their expression is poorly characterized. Of the known MOR isoforms, we found that one particular isoform, designated MOR-1X, is significantly up-regulated by morphine treatment. Furthermore, this up-regulation is specific to cell lines of neuronal origin. There is also evidence that acute morphine treatment alters the levels of particular splicing factors. However, whether this is directly involved in the regulation of MOR alternative splicing and the up-regulation of the MOR-1X isoform is still to be determined. Comparison of MOR-1 and MOR-1X found that the unique C-terminal tail of MOR-1X contains 2 PKA phosphorylation sites as well as a second agonist-induced phosphorylation site, a region highly conserved among opioid receptors. Functional analysis of MOR-1X found that, upon stimulation by morphine, this receptor alters multiple GPCR-regulated pathways, including the GSK3beta/Bax apoptotic pathway. Specifically, morphine treatment of HEK293 cells overexpressing MOR-1X resulted in an increased level of phosphorylated GSK3beta and a decreased level of Bax. This data suggests that multiple factors, including opioid abuse, alters the expression of MOR isoforms and that this alteration may have functional consequences, particularly in HIV neuropathogenesis, by altering apoptotic pathways. This work was made possible by a grant awarded by NIH to KK.

P182
PAHs–induced ROS accumulation and oxidative DNA damage increase mutagenic potential of JC polyomavirus T-antigens

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Polycyclic Aromatic Hydrocarbons (PAHs) are the products of incomplete combustion of organic materials present in cigarette smoke, deep-fried food, and in natural crude oil. PAHs interact with genomic DNA by forming DNA adducts and by causing oxidative DNA damage. Here we ask if chronic exposure to PAHs can affect transforming potential of viral oncoprotein, large T-antigen of the human polyomavirus JC (JCV T-antigen). We have extracted and characterized DMSO soluble fraction of PAHs from the crude oil obtained from the 2010 Deepwater Horizon (DWH) oil spill

in the Gulf of Mexico. The PAHs detected in this fraction include Fluoranthene, Pyrene, Benzo-α-Pyrene, Phenanthanene and Chrysene. The obtained DMSO fraction (oil-PAHs) was tested in exponentially growing cultures of JCV T-antigen positive and negative mouse embryo fibroblasts, R508. The PAHs were cytotoxic only at relatively high doses (1:50 dilution). In this condition, tested cells responded to the oil-PAHs by cell cycle arrest followed by apoptotic cell death. However, cell growth and cell survival were practically unaffected when the oil-PAHs were used at higher dilutions ranging between 1:100 and 1:500. At these nontoxic doses oil-PAHs caused accumulation of the reactive oxygen species (ROS), oxidative DNA damage, and DNA double strand breaks (DSBs), and these DNA lesions were partially neutralized by the ROS scavenger, NAC. Importantly, R508/T cells exposed to the oil-PAHs demonstrated significantly lower involvement of homologous recombination directed DNA repair (HRR), and higher non-homologous end joining (NHEJ). This mutagenic shift between DNA repair mechanisms was accompanied by a significant increased of clonogenic potential of the affected cells. Our results indicate a potential synergistic action between viral and environmental factors, in which JCV T-antigen attenuates HRR and oil-PAHs trigger DNA damage and potentiate NHEJ.

P183

Priming the pump: Tat primes astrocytes for subsequent cytokine secretion, quantitative morphological changes and BBB disruption through TLR2

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Despite HAART therapy, one third of individuals infected with HIV continue to develop HIV-associated neurocognitive disorders (HAND) including loss of blood-brain barrier (BBB) function and activation of numerous cell types within brain, including astrocytes. Loss of astrocyte foot processes in contact with the BBB leads to BBB dysfunction. Astrocytes express toll-like receptors (TLRs) that recognize specific pathogen-associated molecular patterns. Tat has recently been shown to increase TLR2 expression. Increased TLR2 expression would have two downstream effects: the astrocytes would be more likely to respond to an inflammatory insult, either from a secondary/opportunistic infection or from a second round of virus entering brain. Secondly, there would be increased secretion of proinflammatory cytokines secreted by astrocytes. The objective was to quantify astrocyte activation following Tat priming. Our hypothesis was that altered Toll-Like Receptor (TLR) expression on astrocytes would diminish BBB integrity. Ex vivo brain slice cultures incubated with SIV-infected macrophages had increased TLR2 on astrocytes with shortened and thickened astrocyte foot processes. Parallel slices, were subsequently incubated with the TLR2 agonist PAM3Cys. Compared with controls, secretion of CCL2, IL-6 & IL8 was increased tenfold in the slices incubated with SIV and subsequently stimulated with TLR2 agonist. Priming also increased vimentin expression in astrocytes, but decreased nestin and focal adhesion kinase. Pam3Cys stimulation of Tat-primed astrocytes was also shown to diminish the integrity of our blood-brain barrier model as measured by electrical resistance. In summary, these data confirm that priming of astrocytes is important to downstream events. There are alterations in morphology and intermediate filament expression specific to primed astrocytes. Additionally, proinflammatory cytokines known to be important for lentiviral neuropathogenesis, are dramatically upregulated. Understanding mechanisms behind this inflammation will allow rational design of specific anti-inflammatories.

P184

Development of a biodegradable nanoparticle for co-delivery of mu opioid receptor siRNA and Saquinavir: therapeutic potential for HIV-1 positive drug users

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Introduction: Substance abuse is a world-wide public health concern. Opiates are widely abused addictive drugs that modulate immune function. Transmission of human immunodeficiency virus (HIV-1) infection is also a possible consequence of addictive drug use. The application of nanotechnology to diagnose and treat various diseases has attracted considerable interest. Nanoparticles can be used to deliver a wide range of payloads to targeted cells. Their surfaces can be modified to form stable electrostatic complexes with anionic nucleic acids such as small interfering RNA (siRNA), for targeted gene silencing. We proposed to develop a multifunctional biodegradable nanoparticle for potential therapeutic applications in HIV-1 positive substance abuse

users. Methods: We proposed to develop a drug/siRNA co-delivery biodegradable nanosystem. The FDA approved polylactic acid (PLA) polymer was used as the backbone for the biodegradable nanoparticle. Saquinavir, a protease inhibitor, was mixed with the PLA backbone and encapsulated in the hydrophobic core. Negatively charge mu opioid receptor siRNA was then conjugated to the cationic surface of the PLA-Saquinavir nanoparticle by electrostatic interactions to produce nanoplexes. Nanoplexes were incubated with normal human astrocytes (NHA) or with human monocyte derived macrophage (MDM). Uptake of nanoplexes was determined using confocal imaging. Gene expression was analyzed using quantitative PCR. Results and Conclusions: Herein, confocal microscopy demonstrates uptake of the PLA-siRNA-Saquinavir nanoplex by both NHA and MDM. In addition, we observed a decrease in gene expression for the mu opioid receptor in both NHA and MDM. Preliminary results further show a decrease in HIV-1 viral replication in MDM treated with morphine. These observations indicate that drug/siRNA loaded nanoparticles can deliver therapeutics to astrocytes and to macrophages. These results highlight the potential of this nanoformulation in the treatment of HIV-1 positive substance abuse users and the potential use in Neuro-AIDS and other neurological disorders.

P185
Identification and Survival of HIV Infected CD4+ expressing CD8+ T cells in the Brain

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CD8+ T cells infiltrate the brain early in HIV/SIV infection but their role, whether pathogenic or protective, is unclear. In the delicate environment of the CNS, highly activated CD8+ T cells may on one hand control HIV replication but on the other lead to bystander damage through inflammatory mediators. We and others have identified a unique subset of highly activated CD8+ T cells that dimly express CD4 on their surface (double positive (DP) T cells). DP T cells account for approximately 60% of HIV- specific T cell responses in HIV Long Term Non-Progressors. Using the NOD/SCID/IL-2rcg-/- (NSG) mice reconstituted with human peripheral blood lymphocytes, we identified DP T cells in the brain. DP T cells constituted 5-10% of the CD3+ T cell population in the brain. Both DP T cells and CD4 single positive T cells were permissive to HIV infection, at three weeks post-infection the majority of CD4 single positive T cells were depleted while the DP T cells remained. Interestingly, culturing of human progenitor derived astrocytes with supernatants from primary activated CD8+ T cells, that are enriched in DP T cells, caused a significant activation of astrocytes, as measured by expression of IFNg and HLA-DR. Inversely, supernatant from astrocytes lead to an increase in CD4 expression on CD8+ T cells, creating a feedcback loop of more activated CD8+ T cells that can alter astrocyte phenotype and function. Collectively These data demonstrate the presence of DP T cells in the CNS which persist depsite HIV infection, and highlight a dynamic interaction between astrocytes and lymphocytes that can mediate inflammatory responses in the CNS.

P186
The Prevalence of a Positive Screen for Neurcognitive Impairment in HIV-1 Infected Patients Across Western Europe and Canada, The CRANIum Study - Gender analysis

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Background The prevalence of neurocognitive impairment (NCI) in people living with HIV is reported between 20%-50%, with some data that women could be at greater risk of developing this complication. The primary objective of the CRANIum study was to describe the prevalence of a positive screen for NCI and depression/anxiety in an HIV-1 infected adult population. Here we present gender analysis results on the prevalence of a positive screen for NCI. Methods CRANIum was an epidemiologic, cross-sectional study that included HIV-1 infected patients >18 years old attending a routine clinic visit, with an a priori recruitment target of 40% females. One third of patients were ART-naive, one third on a PI/r- and one third on a NNRTI-

based regimen. Patients on ARV-therapy had to be on the same regimen for ≥9 months. The Brief Neurocognitive Screen (BNCS) was used to screen for NCI. Impairment level was additionally assessed by pre-defined categories utilizing average T-scores. A mental health summary score (MHSS) was calculated using the MOS-HIV questionnaire. Results 2863 evaluable patients (38.3% female) were included from 15 countries. Mean age was 42.9 years for both genders. Of the female subjects, 67.2% were Caucasian (males 85.9%, $p<0.001$), 25.8% were black (males 5.9%, $p<0.0001$) and 78.6% were ARV-experienced (males 62.7%, $p<0.0001$). 51.7% females had a positive screen for NCI (males 35.1%, $p<0.0001$). By average T-score, 55.5% of females had normal neurocognitive function (males 71.2%, $p<0.0001$). The MHSS was lower in females than in males (median 49.7 vs 51.8, $p=0.0027$). Conclusions Prevalence of a positive screen for NCI in women exceeded 50%, which was significantly higher when compared to male patients. These findings might confirm a greater vulnerability of female HIV-infected patients for NCI, however the differences in demographic constitution and disease characteristics between genders need to be considered when interpreting results.

P187
Methadone and Buprenorphine Modulation on HIV-1 infection, Inflammation and Neurotoxicity

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The frequent use of opiates among HIV-1 patients raises concern about how these drugs impact HIV-1 replication at the cellular and molecular level. Previous studies have indicated that methadone (MTD) enhances HIV-1 replication in microglia raising a major concern that MTD and perhaps buprenorphine (BUP) could also play a role in the pathogenesis of HIV-associated neurocognitive disorders (HAND). HIV-1 infection provokes immune activation of macrophages enhancing migration to the brain and secretion of neurotoxins affecting glial cells and neurons. In this study, we used an HIV-infected glia-neuron crosstalk system to investigate whether BUP and MTD modulate HIV-1 replication using quantitative RT-PCR, measuring pro-inflammatory cytokine production using a cytometric bead array (CBA), and neurotoxicity using MTT and caspase-3 assays. We show that both MTD and BUP enhanced HIV-1 replication in human glial cells in single culture and co-culture. This enhancement was associated with a significant increase in the production of inflammatory cytokines. Furthermore, supernatants from single culture and co-culture of HIV-infected human glial cells treated with MTD and BUP augmented neuronal toxicity and increased glutamate levels. In addition, we studied the effect of the HIV-Tat protein in our experimental model. We show that the combination of HIV-Tat and these opiates upregulate the expression of CXCR4 and CCR5 receptors and TNF-α production. We also show a diminution of glutamate uptake by astrocytes. These results suggest that addition of either MTD or BUP to an HIV-infected or Tat-treated human glia cells augment inflammation and neurotoxicity. The findings also provide further information about the cellular and molecular mechanisms through which opiates may modulate HAND pathogenesis. Supported in part by NIH-RCMI grant G12 RR03035 and in by Title V PPOHA grant number P031M105050 from the US Dept. of Education to UCC.

P188
Methamphetamine (METH) affects blood brain barrier (BBB) function via β-catenin down regulation

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Recent studies in animal models have indicated that METH can induce impairment of the BBB. We demonstrated that METH alters BBB function through direct effects on endothelial cells. To confirm these observations in vivo, we used brains from macaques infected with simian immunodeficiency virus (SIV) and exposed to METH in doses similar to ones in METH abusers. Results published from this cohort indicated significant increases in brain viral load and altered natural killer cell immune responses with no effect of METH on blood viral load. We performed immunostains for Iba-1 (microglia) and claudin-5 (tight junctions). We found enhanced microgliosis in white matter in SIV + METH macaques as compared to SIV+. Quantitative analysis showed a significant increase of Iba-1 staining (36%) in SIV + METH-exposed animals when compared

to SIV + macaques. We found a decrease in claudin-5 staining in SIV + METH vs. SIV + macaques paralleling enhanced microgliosis. β-Catenin plays a role in junctional regulation and was suggested as a target for METH in astrocytes. We detected β-catenin by immunostaining and found diminished amounts of β-catenin brain endothelium in SIV + METH compared to SIV + group. To confirm this intriguing observation, we treated primary human brain endothelial cells (BMVEC) with METH and found a 60% decrease of β-catenin. β-Catenin phosphorylation by glycogen synthase kinase (GSK) 3β leads to its degradation. If GSK3β is involved in the β-catenin decrease due to METH exposure, its inhibitor (LiCl) should prevent such an effect. Indeed, application of LiCl prevented β-catenin decrease in BMVEC. Functional significance of this observation was confirmed by trans-endothelial electrical resistance (measure of barrier integrity) in BMVEC treated with METH in presence of LiCl. METH exposure led to a 10% drop in resistance and GSK3β inhibition prevented this decrease. Further studies are needed to clarify pathways altering β-catenin signaling in METH exposure. Supported by DA025566

P189
Attenuation of HIV-1 replication by a novel cannabinoid receptor (CB) 2 agonist

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Monocytes and macrophages play a crucial role in the progression of HIV infection. These cells are of the initial targets for HIV-1 infection and provide a means for which the virus to persist sin the CNS. Here, we have explored the use of the endocannabinoid system to regulate HIV-1 replication. There are two well-characterized CB receptors with distinct physiological properties. The psychoactive effects of cannabinoids are associated with the CB1 receptor, while the CB2 receptor is known for anti-inflammatory actions. FACS revealed that CB2 expression was up regulated by 3-fold upon differentiation from monocytes to macrophages. A further CB2 increase (2-fold) was observed when macrophages were infected with HIV-1 for 5 days confirming previously by us observed CB2 increase in HIV-1 infected brain macrophages. We tested a commercially available CB2 agonist, JWH133 and a novel resorcinol-based compound, O-1966. The results from multiple donors using the reverse transcriptase assay (RT), and qPCR for gag and pol indicated that the addition of increasing concentrations (range of 1 μM -10 μM) of JWH133 or O-1966 markedly attenuated HIV-1 replication at 3, 5 and 7 days post infection. RT showed attenuation of HIV-1 replication by O-1966 that was greater than that of JWH133 and provided up to 50% inhibition when compared to control. The mechanism of action does interfere with infectivity since co-receptors CXCR4 or CCR5 were unaffected by CB2 activation. Our results suggest that CB2 signaling can affect HIV-1 replication. Single round infections along with the use of LTR-β-Gal-luciferase reporter assays showed a significant decrease in LTR activation following CB2 activation. The actions of the CB2 agonist were specific to the receptor since CB2 antagonist, SR144528, nullified the actions of JWH133 and O-1966. These results provide an indication of the usefulness of specifically targeting CB2 activation for controlling HIV-1 pathogenesis. Supported by AA015913

P190
Differential proliferation of neural stem/progenitor cells during Herpes Simplex encephalitis

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Endogenous neural stem/progenitor cells (NSCs) respond to inflammatory cues in the brain to proliferate, migrate, and differentiate into new neurons or glial cells. However, little is known about NSC response to inflammation during herpes simplex encephalitis (HSE). Quantitative and phenotypic analysis of neurogenic regions in Herpes Simplex Virus (HSV)-1 infected brain showed significant increase in the number of proliferating Ki-67(+) endogenous brain cells at 3 d.p.i. On further analysis, it was found that increase in numbers of Nestin(+) stem cells were delayed until 6 d.p.i., indicative of cell-type specific proliferative responses in infected brains. Interestingly, at 15 d.p.i. proliferation of brain cells was abrogated and both Ki-67(+) proliferating cells and Nestin(+) stem cells decreased significantly compared to uninfected animals (8.5±2.9% vs. 43.0±4.9% and 7.70±4.2% vs. 18.8±2.0% respectively). These data suggest temporal, cell type specific modulation of proliferative responses during HSE. The specific cellular phenotypes involved in the proliferative response are currently under investigation. To determine if modulation of NSC proliferation is

associated with expression of neurogenic factors, gene expression analysis was performed. Using a PCR array, expression of several neurogenic factors was found to be down-regulated at 15 d.p.i., including Noggin, Epidermal Growth Factor (EGF) and Fibroblast Growth Factor (FGF)-2. On further analysis of FGF-2 expression kinetics during HSE, it was found that this gene was significantly up-regulated at 3 d.p.i. and subsequently down-regulated at 15 d.p.i, suggesting an association between observed inhibition of brain cell proliferation and changes in growth factor expression. Studies are underway to identify mechanisms by which FGF-2 alters neurogenesis during HSE. These studies will help identify novel points of intervention to develop therapies for neurological deficits ensuing viral encephalitis.

P191
Oxidative Stress, Proinflammatory Responses and HIV Expression in the HIV-1 Transgenic Rat

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Objective: To examine associations for the presence of oxidative stress related to impaired nicotinamide adenine dinucleotide (NAD) metabolism and activation of the cellular oxidases nicotinamide adenine dinucleotide phosphate (NADPH) and the dual oxidases (DUOX) with proinflammatory responses and HIV-1 expression in the HIV transgenic (TG) rat. Design/Methods: Frozen sections of brain from 3-6 month old TG and wild-type (WT) Fisher 344/NHsd control rats were examined by immunofluorescence staining for numbers of GFAP + (astrocytes) and Iba1+ cells (macrophage/microglial (M/M) cells) and for co-labeling of these cells for TNF-α, gp160, tat, nef and vif. Tissue was also examined for expression of CD38, a NAD glycohydrolase that depletes intra- and extracellular NAD, and for expression of the NADPH and DUOX isoforms NOX4 and DUOX1, respectively, by PCR. Results: Numbers of GFAP + cells were similar for TG and WT rats whereas Iba1+ cell numbers were higher for TG rats. Expression of TNF-α was demonstrated for both astrocytes and M/M cells and was higher in brains from the TG than from the WT rats. Astrocytes and M/M cells expressed gp160 and tat, whereas nef and vif was expressed by primarily astrocytes. CD38 expression was higher in brains from TG rats and co-localized with staining for both GFAP and Iba1. NOX1 and DUOX1 gene expression was also increased in the TG rat brains relative to WT rats. Conclusions: Further studies of proinflammatory responses and effects of HIV protein expression, and activation of CD38 and cellular oxidases in the HIV TG model may provide clues for developing treatment approaches for cognitive impairment resulting from HIV infection in humans.

P192
Neurocognitive Impairment among Treatment-Naïve, HIV-Infected Individuals in Nigeria

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Objective: To examine outcomes on neuropsychological (NP) testing among individuals at risk for HIV-related neurocognitive impairment (NCI) in Nigeria, where, with 3.3 million infected with HIV, the burden of HIV/AIDS second highest of all countries worldwide. Design/Methods: ART-naïve seropositive (SP) and seronegative (SN) control subjects were recruited at two antiretroviral treatment clinics. NP test data obtained at the time of study enrollment were analyzed. Multiple regression analyses were performed with HIV serological status, education, and ethnicity included as covariates. Effect sizes were determined by estimation of the Cohen's d statistic. Results: To date 133 individuals have been recruited for the studies, 81 who have been administered the complete neuropsychological battery (31 SP and 50 SN). Among the SP group, 23 were WHO stage I, four were stage II, three were stage III and one was stage IV. The mean age and sex ratios were similar; however, the SP group had a lower level of education ($p=0.03$). Significant relationships were found for HIV serostatus with performance on the overall NP battery ($p=0.0096$) and on tests of learning ($p=0.0085$), speed of information processing ($p=0.01$); executive function ($p=0.01$); and memory ($p=0.02$). Effect sizes for these analyses ranged between 0.34-0.36. On the

full battery, 14 SP (46%) had scores >1 SD below the mean of the controls; of these, 7 (23%) had scores >2 SD below the mean. Ethnicity and education did not impact performance on the tests. Conclusions: HIV- related neurocognitive abnormalities were frequent in this treatment-naïve patient group, who overall presented during the earlier stages of infection.

P193
DigiSLab: a Virtual Reality Project in Medicine

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Sbarro Health Research Organization Inc. (S.H.R.O.) is a nonprofit organization dedicated to elucidating the molecular basis of different human diseases. S.H.R.O. eHealth program focuses on the intersection of medical informatics, public health, communication and eLearning/education, with the goal of improving health care through the latest information and communication technology. One important aspect of S.H.R.O.'s eHealth Program is Digi S Lab, a Virtual Reality (VR) project. VR is a computer simulated 3D graphical environment with and within which people can interact. With this technology, visitors, represented by "avatars" or computer representation of the user, can enter and fully interact with a virtual environment. DigiSLab contains virtual buildings, auditoriums, laboratories, offices, meeting rooms and a hospital. Typical applications of DigiSLab are: (1) a virtual laboratory, a space where it is possible to experience different molecular biology laboratory experiments; (2) a VR program for modeling weight loss skills; (3) virtual environments to deliver a relaxation protocol to patients undergoing chemotherapy; (4) developing real-time feedback between the virtual and physical worlds by using tools to track different information of the real user.

P194
Complete prevention of HTLV-1 infection in humanized mice by a neutralizing monoclonal antibody to envelope gp46

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Human T-cell leukemia virus type 1 (HTLV-1) causes both neoplastic and inflammatory diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Since these disabling and/or life threatening diseases are not yet curable, it is important to prevent new infections. In this study, we have established a simple humanized mouse model of HTLV-1 infection for evaluating therapeutic and immunomodulatory interventions. Using this model, we tested the effect of monoclonal antibodies specific to HTLV-1. HTLV-1-negative normal human peripheral blood mononuclear cells (PBMCs) were transplanted directly into the spleens of severely immunodeficient mice (NOD/SCID/γCnull:NOG) together with the mitomycin-treated HTLV-1 producing T cells (ILT-M1). Before (one hour) and after (24 hours) transplantation of human PBMCs, monoclonal antibodies against HTLV-1 as well as human IgG isolated from both HTLV-1 infected and non-infected individuals were inoculated intraperitonealy. On day 14, human PBMCs were isolated from mouse spleen, and tested for HTLV-1 infection by real time PCR and flow cytometry. Similar to the naturally HTLV-1 infected PBMCs, both CD4+ and CD8+ T cells isolated from untreated or isotype antibody treated mice were found to be HTLV-1 infected, and the CD8+ T cells harbored HTLV-1 to a lesser extent. Also, HTLV-1 Tax expression was negative in isolated human PBMCs but became positive after 16 hours of culture. Surprisingly, although non-neutralizing monoclonal antibodies to gp46, monoclonal antibody to gag p19, and normal human IgG did not block the infection, neutralizing monoclonal antibody to gp46 and human anti-HTLV-1 IgG completely blocked the infection. Our findings provide a new strategy for preventing initial HTLV-1 infection and blocking further spread in vivo. The potential mechanisms involved in the antibody effect will also be discussed.

P195
Age-dependent susceptibility of primary neurons to La Crosse virus infection

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A number of viral encephalitides disproportionately affect the very young and/or the very old and, accordingly, rates of encephalitis-associated hospitalization are highest for adults≥65 years old and children <1 year old. LACV is a common cause of pediatric encephalitis and aseptic meningitis in the Midwestern United States where its principal mosquito vector, Ochlerotatus triseriatus, resides. In most cases, LACV infection results in a nonspecific febrile illness, but in a small proportion of children – almost exclusively under 16 years of age – this is followed by acute encephalitis. Age-dependent susceptibility to LACV encephalitis is paralleled in a murine model of disease; newborn mice are sensitive to subcutaneous (s.q.) inoculation of <1 plaque-forming unit (PFU) of LACV whereas weanling and adult mice are resistant to much higher doses (>100,000 PFU) administered by the same route. However, the underlying biological basis for this age-dependent susceptibility is not fully understood. In this study, we describe a primary rat (embryonic day 18) neuronal culture model of LACV infection that also reflects the age-dependent susceptibility to disease. These primary neuronal cultures become resistant to LACV-mediated toxicity as they mature, following approximately 10 days in vitro. We have compared gene expression profiles of young, vulnerable primary rat neuronal cultures to mature cultures that are resistant to LACV-mediated toxicity to identify pathways involved in determining the age-dependent susceptibility and resistance to LACV-mediated toxicity.

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Effects of abused and therapeutic drugs in an in vitro model of cellular injury in NeuroAIDS

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Methamphetamine (METH) abuse is a serious public health concern because it can lead to irreversible damage in the brain, and seems to increase the risk of infection with HIV-1. In the last 15 years the use of highly-active antiretroviral therapy (HAART) has increased longevity of HIV patients. However, the prevalence of HIV-associated neurocognitive disorders (HAND) has continued to grow. Also, the combined effect and potential interaction of recreational drugs, therapeutic drugs and virus on the brain is poorly understood and was therefore addressed in the present study. First we analyzed the neuronal injury of METH, HAART and HIV-1 gp120 separately using mixed neuronal-glial cerebrocortical cell cultures derived from rat. Neuronal injury and death was analyzed in fixed cells using specific markers for neurons (MAP-2) and synapses (Synaptophysin) in combination with nuclear DNA (Hoechst) staining and subsequent fluorescence microscopy. We observed that treatment of neurons with METH at 100 μM, but not lower concentrations, increased neuronal damage. Surprisingly, while 100 μM METH increased neuronal damage induced by gp120, 50 and 10 μM METH partially ameliorated toxicity of the viral envelope. When we analyzed the effect of HAART compounds representing four different pharmacological categories (AZT, NVP, SQV and 118-D-24) in the presence or absence of gp120, none of the chemicals caused a significant neuronal injury during 24 h exposure. However, during 7-day incubation, neuronal synapses were compromised, while surprisingly MAP-2 positive dendrites were spared. Contrarily, HAART and gp120 separately caused significant neuronal injury and death in the same period of time. Paradoxically, the combination of therapeutic compounds and psychostimulant drug primarily compromised neuronal MAP-2-positive dendrites while sparing Synaptophysin-reactive pre-synaptic terminals. Altogether, our findings indicate that the overall positive effect of HAART in HIV infection is accompanied by some neurotoxicity which possibly is aggravated in the presence of abused drugs.

P197

Carboxy-terminus DNA binding domain of VP2/VP3 stimulates DNA binding of JC virus large T-antigen to Ori: Evidence for coupling of viral DNA replication to encapsidation

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JC virus (JCV) is the etiological agent of a fatal brain disease, known as progressive multifocal leukoencephalopathy (PML). JCV is a member of the polyomavirus family and latently infects majority of human population (70-80%). Once reactivated, it lytically infects the oligodentrocytes in the central nervous system (CNS) in patients with underlying immunosuppressive conditions, including Hodgkin's lymphoma, lymphoprolative diseases and AIDS. In recent years, however, PML, is also steadily increasing in a small percent of the patients with autoimmune disorders, including multiple sclerosis (MS) and Crohn's disease, who are treated with antibody-based regiments (natalizimab, efalizumab and rituximab). Infectious JCV virions are composed of the viral DNA, which is situated in the innermost core of a virion, and the capsid proteins (VP1, VP2 and VP3), which are synthesized in the cytosol and transported into the nucleus for virion assembly. However, the mechanism of virion assembly for JCV is completely unknown. Our initial studies demonstrated that capsid proteins (VP2/VP3) enhance the large T-antigen (LT-Ag) binding activity to the viral origin (Ori) through the interaction with their far C-terminal DNA binding domain, suggesting that the capsid proteins may be involved in the efficiency of viral DNA replication. In addition, our experimental data also suggest that the host chaperon protein, Hsp70, may play a critical role in tethering the viral capsid proteins into the viral replication centers in the nucleus and mediate the interaction between LT-Ag and capsid proteins, which supports an idea that the viral DNA replication is coupled to the encapsidation process. We will further characterize the mechanism(s) of the chain of events taking place during the JCV virion assembly, which will shed more light on our understanding of JCV virion biogenesis and this may provide opportunities to develop effective therapeutic strategies against JCV infections in affected individuals.

P198

Hydrophobic core region of JC virus agnoprotein is involved in stable dimer/oligomer formation and protein stability

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Agnoprotein is a multifunctional regulatory protein of polyomaviruses, including JC virus (JCV), BK virus (BKV) and simian virus 40 (SV40) and forms highly stable and SDS-resistant dimeric/oligomeric structures whose function functions are currently unknown. Our previous mapping studies with JCV agnoprotein have indicated that amino acids 17 to 42 are involved in dimer/oligomer formation. Interestingly, this region is also involved in formation of an amphipathic helix as evidenced by three dimensional modeling studies. The functional significance of this region was examined by deletion analysis in the viral background and results showed that the mutant virus is unable to sustain its replication cycle after first round of propagation, highlighting the importance of this region for agnoprotein function. In this study, we further defined the region responsible for the dimer and oligomer formation and examined the functional significance of this region by mutagenesis analysis. Amino- and carboxy- terminal deletion mutants of JCV agnoprotein demonstrated that the dimer/oligomer formation domain is confined to the hydrophobic core region, which spans amino acids from 28 to 39. Moreover, further internal deletion analysis of the region confirmed that amino acids spanning from 30 to 37 are sufficient to confer the dimer/oligomer formation property of agnoprotein, thus play a critical role in stability and function of agnoprotein. Indeed, functional studies demonstrate that a mutant virus lacking this region poorly expresses agnoprotein and replicates significantly less efficiently than WT. These findings, altogether, demonstrate that the hydrophobic core region of agnoprotein is critically important for stability and function of agnoprotein and thus represents a prime target for developing novel therapeutic agents for progressive multifocal leukoencephalopathy.

P199

JC virus agnoprotein enhances large T antigen binding to the origin of viral DNA replication and is involved in viral DNA replication

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Agnoprotein is a small regulatory protein of JC virus (JCV) and its expression is critically important for the successful completion of the JCV life cycle, which is evident from the extremely poor replication behavior of the agnoprotein null mutants. Agnoprotein mostly resides in cytoplasm with high concentrations accumulating around the perinuclear region of the infected cells, and a small amount of the protein is also consistently detected in the nucleus, indicating a possible nuclear function for agnoprotein. In this study, we investigated the regulatory roles of agnoprotein on viral DNA replication. DNA binding studies demonstrated that agnoprotein enhances the binding activity of large T-antigen (LT-Ag) to the viral origin (Ori) without directly interacting with DNA. The predicted amphipathic α-helical domain of agnoprotein plays a major role in this observed enhancement. Interestingly, agnoprotein has three Phe residues and all of which localize to this domain. It has been shown that Phe residues play critical roles in protein-protein interaction, protein folding and stability. The functional relevance of these Phe residues was investigated by site-directed mutagenesis. When mutated to alanine (Ala), all individual Phe mutants lost their ability to enhance LT-Ag binding but exhibited a differential behavior in viral DNA replication. However, a triple Phe mutant (F31AF35AF39A), showed a greater negative effect on viral DNA replication than the individual Phe mutants alone. Moreover, immunocytochemical and replication studies suggest that a strategic subcellular distribution pattern of agnoprotein is also important for execution of its full function, as evidenced by a substantial decrease observed in viral DNA replication when the protein is exclusively expressed in the nucleus. Collectively, these studies indicate a close involvement of agnoprotein in the JCV DNA replication cycle and that it represents a target for therapeutic intervention to inhibit the progression of the JCV-caused disease, progressive multifocal leukoencephalopathy (PML).

P200

Agnoprotein is released by infected cells: potential biomarker for the reactivation of JCV and the progression of PML

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Among the other human polyomaviruses, JCV is the most abundant polyomavirus which infects ~80% of the human population during childhood, and establishes a persistent life-long infection. JCV replicates in glial cells in the brain, and causes the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML is usually seen in patients with underlying immunocompromised conditions, notably among AIDS patients and those on chronic immunosuppressive regimens. Little is known about the progression of the disease since there is no biomarker for the detection of JCV reactivation and infection in affected individuals. Most PML cases are diagnosed in patients at a late stage of the disease after the onset of the neurological complications. There are no established diagnostic markers applicable in the clinic to predict which patients will develop the disease. Here, we discovered that the small viral protein, agnoprotein, may serve as a biomarker for the detection of JCV reactivation and replication in patients under risk of developing PML. We discovered that agnoprotein is released from cells infected with JCV, and can be detected in biological samples, e.g. serum and urine. We are in the process of developing a highly sensitive and specific ELISA-based test for agnoprotein detection in human serum and urine samples. We believe that the development of a detection system for agnoprotein in biological samples from patients who are at risk of developing PML will be of great interest to clinicians as well as to diagnostic testing laboratories and to pharmaceutical companies who develop and market immunosuppressive drugs which have been associated with polyomavirus-based diseases.

P201

Conditioned medium from PBMCs treated with the A3 adenosine receptor agonist CF102 inhibits JCV propagation in glial cells

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The fatal demyelinating disease progressive multifocal leukoencephalopathy (PML), for which there is no effective therapy, is caused by replication of human neurotropic polyomavirus JC (JCV) in glial cells of the brain. There is evidence that proinflammatory cytokines can promote reactivation of latent virus and onset of PML. The A(3) adenosine receptor (A(3)AR) mediates a variety of anti-inflammatory protective effects. Overexpression of A(3) AR is a feature of inflammatory cells and PBMCs of patients with inflammatory diseases. Here, we investigated effects of A(3)AR agonist CF102 on JCV transcription and replication. While direct treatment of astrocytes with CF102 had no effect, indirect treatment of astrocytes with conditioned medium from CF102-treated PBMCs inhibited JCV infection as measured by viral gene expression, viral DNA replication and virus particle release. Cytokine array analysis of PBMC-conditioned media showed CF102 induced marked changes in cytokine profile. These data suggest that CF102 may offer a novel avenue for the treatment of PML.

P202

Bag3-mediated autophagic degradation of large T antigen in JCV transformed cells

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Replication of the human neurotropic polyomavirus JC (JCV) in glial cells of the brain causes the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML). Infection of glial cells by JCV, and expression of viral regulatory and structural proteins leads to a broad range of changes in the gene expression profile of the host which mostly favors the replication of the virus. There is evidence that BAG3 protein, a member of the human Bcl-2-associated athanogene (BAG) family of proteins which regulates apoptosis, is downregulated upon JCV infection and that this effect is mediated by JCV T-Ag via repression of the BAG3 promoter. Bag3 functions as a molecular co-chaperone through its interaction with Hsc70/Hsp70 and functions in the regulation of the cellular stress response, proliferation and apoptosis. The functional consequence of large T- mediated downregulation of Bag3 during JCV infection is unknown. Here, we investigated the possible impact of Bag3 on large T expression in the absence of viral infection by utilizing JCV transformed cell lines which express JCV-early genes under the control of the viral early promoter. A series of molecular studies indicated that overexpression of Bag3 in JCV transformed cell lines inhibits the expression of large T antigen by inducing the autophagic degradation of the protein. Protein-protein interaction studies identified that large T and Bag3 interact through their Zn + and PXXP (proline rich) domains, respectively. Functional assessment of this binding revealed that Bag3 interaction with large T antigen was important for the autophagic degradation of the protein. These results open a new avenue of research to better understand the interplay between viral large T antigen and cellular Bag3 proteins in the development of the diseases caused by JCV. Supported by grants awarded by NIH to KK.

P203

Impact of p38SJ/DING on HIV- and ethanol-induced neuronal cell injury

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The neuropathogenesis of HAND is directly related to HIV replication in CNS and neuronal injury. Although neurons are not productively infected with HIV-1, they can be injured by neurotoxins, including HIV-Tat, released by activated microglia and macrophages. HIV-1 modulates intracellular signaling pathways, including Mitogen-Activated Protein Kinases (MAPK) cascades. MAPKs (ERK, JNK and p38) phosphorylate and direct Myocyte-specific enhancer factor (MEF2) to multiple diverse and

mutually exclusive pathways, such as cell proliferation, neuronal cell survival, differentiation, synapse development or inflammation and cell death. HIV-1 can alter neuronal integrity by controlling of phosphorylation of MAPKs and MEF2. Previous information suggests that HIV-1-induced apoptosis in brain can be potentiated by ethanol. Ethanol induces neuronal cell injury by dysregulating several signaling pathways that are also controlled, in part, by the MAPKs and MEF2. Our preliminary studies indicate that p38SJ/DING, a novel plant-derived phosphatase inhibits HIV-mediated toxicity in human neurons and stimulates neuronal outgrowth after injury. We also found that p38SJ/DING prevents ethanol-induced neuronal damage by regulating the phosphorylation of MAP kinases and their downstream target MEF2, a regulator of diverse cellular processes. Our data also suggest that ethanol potentiates HIV-1-induced neuronal loss, while p38SJ prevents Tat- and ethanol-mediated neuronal injury, and preserves impaired by Tat and ethanol neuronal integrity and synaptic plasticity in human neurons. Here we identified signaling pathways activated by p38SJ/DING that increase survival of neurons exposed to HIV-1 and ethanol. Findings from our proposed studies will provide p38SJ/DING-based tools to reduce deleterious effects of HIV-1 and ethanol on the brain and contribute to better understanding of neuropathogenesis of AIDS and alcoholism. This work was made possible by grants awarded by NIH to SA.

P204
Role of mitochondrial protein porin in Tat-induced neurotoxicity

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HIV-1 transactivator protein Tat is an important regulator of neuronal cell survival that is involved in the modulation of the cell cycle, differentiation, cytoskeleton dysfunction and apoptosis. We utilized a neuronal SK-N-MC cell line constitutively expressing Tat, to examine the molecular events that involve mitochondria in the regulation of cell survival. We demonstrate that the level of expression of mitochondrial outer membrane protein VDAC (porin) is inhibited in Tat-expressing cells. Inhibition of VDAC by Tat in neuronal cells, or treatment of Tat-negative cells with VDAC inhibitor, DIDS, resulted in the induction of caspase-3 activation and increased DNA damage. Progression of the cell cycle from G0/G1 to G2 in Tat-positive cells was delayed, number of apoptotic cells was increased, and mitochondrial bioenergetics was strongly affected by Tat. Importantly, the mitochondrial content of tubulin was decreased, suggesting that the interaction between mitochondria and cytoplasm was dysregulated in Tat-positive cells. The reduced level of VDAC was also observed in human neurons treated with recombinant Tat protein. These data demonstrate that Tat dysregulates mitochondria, which results in the inhibition of mitochondrial VDAC and activation of caspase-3, which in turn, leads to the neuronal cell injury. This work was made possible by grants awarded by NIH to SA.

P205
Role of γδ T cells in the regulation of HTLV-1 infection

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γδ T cells, a small subset of T lymphocytes, are involved in innate immunity. It has been demonstrated that γδ T cells have cytotoxic activities against cells infected with a variety of viruses. However, there is little evidence suggesting a cytotoxic activity of γδ T cells against HTLV-1-infected cells. Therefore, we investigated whether γδ T cells play a protective role in the defense against HTLV-1. Using PBMCs from asymptomatic carriers (ACs) and patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), we assayed the frequency of CD3 + Vγ9+ (γδ T) cells and the correlation between its frequency and the HTLV-1 load. The frequency of γδ T cells was significantly decreased in HAM/TSP patients compared with that in ACs. The frequency of γδ T cells was inversely correlated with the proviral load. These results suggest that γδ T cells have a protective effect on HTLV-1-infected individuals. Next, CD3 + Vγ9+

cells and CD3 + Vγ9- cells were separated from PBMCs of HTLV-1-infected individuals by FACS and the proviral load of each population was quantified by real-time PCR. The proviral load in γδ T cells was markedly lower than that in CD3+ lacking γδ T cells. Furthermore, we cultured PBMCs from HTLV-1-infected individuals in the presence of IL-2 and zoledronate. The majority of cells contained in these cultures became γδ T cells and the proviral load was markedly decreased, especially when CD4+ cells (a major reservoir of HTLV-1) are depleted from the PBMCs prior to culture. The cultured PBMCs showed strong cytotoxic activities against a HTLV-1-infected cell line as well as an ATL cell line. These results raise the possibility of γδ T cell immunotherapy in HTLV-1-infected individuals.

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Effects of SP / neurokinin-1 receptor binding in nestin + human fetal brain cells

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Children perinatally infected with HIV-1+ are at increased risk to develop neurocognitive deficits and behavioral /psychiatric disorders. Underlying cellular mechanisms may include the effects of HIV-associated neuroinflammation on cells critical for brain development, specifically nestin positive neural progenitors and astroglia with progenitor potential. Substance P (SP) binding to the neurokinin-1 receptor (NK-1R) is implicated in HIV/AIDS neuroinflammation and neuropsychiatric disorders. SP effects on neural progenitors have not been examined in HIV/AIDS. Human fetal brain cells (HFBC), grown in 10% fetal bovine serum and passaged (P) at 70% confluence, were tested for expression of nestin, GFAP, NK-1R isoforms, CXCR4 and CCR5 by fluorescence immunocytochemistry (ICC) and qPCR normalized to GAPDH. NK-1R responsiveness to SP (500nM) was determined by calcium mobilization and an impedance assay. The effect of SP (10-500nM) +/- gp120 (50 ng/mL) on nestin, NK-1R, CXCR4 and CCR5 expression was assessed. CCL2 production after SP +/- gp120 x24hr was measured by ELISA (pg/mL). Nestin positive HFBC express CXCR4 and full length NK-1R, with very low amounts of truncated NK-1R, and no CCR5. SP provoked calcium mobilization and cell contraction. Nestin + cells were present in P6-P21 cells. Treatment (x24hr) with SP +/- gp120 had <2-fold effect on nestin and none on CXCR4 or CCR5 transcripts, whereas preliminary ICC results after a 6-day SP incubation suggested that the percent of cells positive for GFAP and CCR5 increased (33% to 81%, and 0% to 26.5%, respectively), while nestin positive decreased (40% to 12%). SP exposure resulted in a 16% increase in CCL2 in P21 cells, but not in earlier passage HFBC; and gp120 had no effect. This suggests that prolonged SP exposure may hasten progenitor progression to a more restricted astroglial phenotype and upregulate CCR5, events that could contribute to CCL2-linked neuroinflammation and viral entry, respectively. (Supported by NIH-NIMH- 1U01-MH-090325).

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Progressive Multifocal Encephalopathy in a 15-year Old Girl with Missed Perinatal HIV Infection

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Background: Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by JC virus (JCV). While well-described among adults with HIV infection and AIDS, PML has rarely been reported in HIV-infected children. We describe a patient with missed perinatal HIV infection who presented with AIDS and PML at 15 years of age. Case Report: A 15 year-old girl with a history of recurrent HSV keratitis presented with a six –week history of fatigue, weight loss, right hand and leg clumsiness, dysarthria, and gait instability. Although the patient's biological mother was known to be HIV positive, the patient's HIV status was unknown. MRI on admission demonstrated T2 signal hyperintensities within the cerebellum, pons, and medulla. Her serum HIV-1 viral load was 82,000 copies/mm3 and CSF JCV PCR was 4,200 copies/mm3. The patient was diagnosed with AIDS and PML and started on nevirapine, tenofovir/emtricitabine, and raltegravir. Shortly afterwards, she developed clinical and radiographic features consistent with immune reconstitution inflammatory syndrome (IRIS). Despite high-dose intravenous corticosteroids, her condition deteriorated and she expired eight weeks later. Postmortem analysis showed classic histopathologic features of PML as well as JCV infection of numerous cerebellar granule cell neurons. While glial cells were productively infected, as demonstrated by expression of JCV VP1 capsid protein, most granule cell neurons expressed JCV T-antigen only, consistent with restrictive infection. Discussion: PML is a rare complication of HIV infection among pediatric patients, described in fewer than 20 published cases. Prior reports are limited and often do not include postmortem findings. Our patient's histopathology was notable for evidence of both glial and granule cell neuronal infection. Additionally, this case highlights the need to implement policies that optimize HIV counseling and testing in HIV-exposed infants and high-risk pediatric populations.

P208

Expression of HIV gp120 protein increases sensitivity to the rewarding properties of methamphetamine in mice

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Methamphetamine (METH) abuse and Human Immunodeficiency Virus (HIV) infection induce neuropathological changes in corticolimbic brain areas involved in reward and cognitive function. Little is known about the combined effects of METH and HIV infection on cognitive and reward processes. The HIV/gp120 protein induces neurodegeneration in mice similar to HIV-induced pathology in humans. We investigated the effects of gp120 expression on associative learning, preference for METH and non-drug reinforcers (saccharine and quinine), and sensitivity to the conditioned rewarding properties of METH in transgenic mice expressing HIV/gp120 protein (gp120-tg). The gp120-tg mice learned the operant response for food at the same rate as non-tg mice indicating no deficits in associative learning and unimpaired reward and motivational function for a natural reinforcer. In the two-bottle choice procedure with restricted access to drinking solutions, gp120-tg mice exhibited greater preference towards METH and saccharin compared to non-tg mice; while preference for quinine was similar between genotypes. Under conditions with unrestricted access to METH self-administration, all mice, independent of genotype and sex, decreased preference for METH indicative of METH-induced aversion. However, male gp120-tg mice decreased preference for METH at lower concentrations than non-tg male mice indicating higher sensitivity to the aversive effects of METH. The gp120-tg mice developed METH-induced conditioned place preference at lower METH doses compared to non-tg mice indicating higher sensitivity to the conditioned rewarding effects of METH. There were no differences in METH pharmacokinetics between genotypes. These results indicate that gp120 expression is associated with increased preference for METH and highly palatable non-drug reinforcer (saccharine), and increased sensitivity to METH-induced conditioned reward. Nevertheless, the sensitivity to the rewarding and motivational properties of a natural reinforcer was similar between genotypes. These data suggest that HIV positive individuals may have increased sensitivity to METH leading to high abuse potential of METH in this population.

P209

Differential regulation of glycolytic pathway by HIV-1 infection of its target cells

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Regulation of glucose metabolism has been shown to play an important role in the pathogenesis of many diseases, primarily because deregulation of this metabolic pathway can lead to either apoptosis or extended life span of the cells involved. The Human Immunodeficiency Virus Type-1 (HIV-1) infects both activated CD4 + T cells as well as terminally differentiated macrophages during the course of HIV-1 pathogenesis. Hence, we focused on the possible regulation of glycolytic pathways by HIV-1 infection in two different cell types by monitoring the effect of HIV-1 infection on the activities of important glycolytic enzymes, Hexokinase (HK), glucose-6-phophate dehydrogenase (G6PD) and Pyruvate kinase (Muscle) 2 (PKM2). We used a human CD4 + T cell line Jurkat latently infected with HIV-1 (J.Lat 6.3) and a monocyte cell line (U1) latently infected with HIV-1. Jurkat and U937 cell lines served as controls for T-cell lines and monocyte derived macrophages, respectively. We observed that the activity of HK, G6PD and PKM2 enzymes were markedly different under HIV-1 replication in the two cell lines. The T-cell line showed a five fold increase in HK activity, whereas the U1 cell line showed a six fold decrease in HK activity. However, in the case of G6PD activity the trend was reversed such that in U1 cells G6PD activity was increased about three fold. The G6PD activity of infected the T-cell line decreased about 1.3 fold. The PKM2 enzyme also showed cell line based difference in its activity; in U1 cells, the activity decreased about two fold with viral replication, but in the J.Lat cell line, PKM2 activity did not show any steady trend of variation. Thus, the glycolytic pathway in different HIV-1 target cells is differently affected by viral replication. This work was made possible by grants awarded by NIH to SA.

P210
Role of Neuron-Glia Crosstalk in HIV-1 Neuropathogenesis

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Human brain derived cell culture models have helped in understanding HIV-1 neuropathogenesis to a great extent especially as there are no reliable animal models for studying pathogenesis of HIV Associated Neurocognitive disorders (HAND). Recent studies have reported an increase in Connexin 43 expression upon exposure of primary human astrocytes with live HIV virus. However, the underlying signaling pathways and the consequences of the augmented gap junction communication remain unexplored. In an attempt, to gain better insights into the molecular mechanisms underlying glia mediated HIV neurotoxicity, we employed human neuron-astrocyte co-culture model using cells differentiated from human fetal brain derived neural precursor cells. We explored HIV-1 Tat mediated alterations at Gap Junction Channels as they are one of the major mode of intercellular communication and transfer of ions that may modulate signaling pathways. We observed significant increase in several connexins (Cx 40, 26, 36 and 43) following exposure to full length HIV-1 Tat protein. This increase promoted cell death of adjoining neurons as elucidated by the TUNEL assay. Concomitant with increased connexin expression, we noticed that cell proliferation was inhibited as evidenced by attenuated Ki67 positive cells. These observations were reversed by the use of α-glycyrrhetinic acid (a Gap Junction blocker) as its pretreatment restored cell viability and proliferation. Our experimental data paves way for a better understanding of neuron-glia interplay, glia mediated neuronal damage in HIV neuropathogenesis and suggests possible target for therapeutic management of neuroAIDS. This research work was supported by NBRC core funds and research grant from Department of Biotechnology, New Delhi. PG and MP are recipients of Research Fellowships from Council of Scientific and Industrial Research, New Delhi, India.

P211
Functional properties of the HIV-1 LTR containing naturally occurring SNPs within a chromatin-based microenvironment

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Human immunodeficiency virus type 1 (HIV-1) gene expression is driven by the long terminal repeat (LTR), which contains binding sites that interact with multiple host and viral factors. Selective pressures within the host as well as the low efficiency of reverse transcriptase lead to genetic alterations within the viral genome resulting in viral quasispecies that can be differentially regulated and can potentially form niches within specific cell types and tissues. Previous studies identified a single nucleotide polymorphism (SNP) within C/EBP site I (3T, C-to-T change at

position 3 of the site) that correlated with HIV-1-associated dementia. In addition, our current patient cohort shows a SNP within Sp site III (5T, C-to-T change at position 5 of the site) that occurs as frequently as the consensus subtype B sequence. Stably transfected cell lines were developed using bone marrow progenitor, T, and monocytic cell lines (TF-1, Jurkat, and U-937, respectively) to explore the LTR phenotype associated with these genotypic changes from an integrated microenvironment. The LAI LTR was coupled to the green fluorescent protein (GFP), and polyclonal HIV-1 LTR-GFP stable cell lines were developed. To examine the mechanism of LTR driven gene expression as well as epigenetic modifications that may control it, clones were derived from each population of cells. The clones were examined with respect to basal transcription, cytokine treatment, and Tat transactivation. Results suggest that nonexpressing clones within monocytic cell lines cannot be induced to express under all conditions examined. However, the nonexpressing LAI 3T and LAI 5T Jurkat clone genotypes could be induced into expression. Results demonstrate that the site of LTR integration, epigenetic modifications to viral and host DNA, cellular phenotype, and genetic variation may determine the overall level of LTR activity and potential to be activated from a quiescent state.

P212
Molecular Diagnosis of Central Nervous System Opportunistic Infections in Zambian HIV + Adults

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Background: Knowledge of central nervous system(CNS) opportunistic infections(OI) in the HIV-infected population of sub-Saharan Africa is limited. Objective: We sought to characterize the prevalence of CNS OI in an urban Zambian adult HIV + population using PCR technology on freshly collected CSF. Methods: Between 10/1/10–2/30/12, we analyzed CSF from 174 HIV + patients evaluated by a single neurologist(O.S.) because of symptoms concerning for CNS OI at the University Teaching Hospital in Lusaka, Zambia. We used pathogen-specific primers to detect DNA from JCV, VZV, CMV, EBV, HSV 1 and 2, mycobacterium tuberculosis(MTB), and toxoplasma gondii (TG)via real-time PCR on an Corbett Rotor-Gene 6000. Samples were run in duplicate. Genomic DNA from the respective organisms was used as control. Results: Of 174 CSF samples, 92(52.9%) were PCR + for at least one pathogen. DNA from EBV was found in 56(32.2%), MTB in 20(11.5%), CMV in 8(4.6%), JCV in 7(4.0%), VZV in 4(2.3%), HSV-1 in 1(0.6%), HSV-2 and TG in none. In addition, bacteriological diagnosis showed Cryptococcus in 33(19.0%) and pneumococcus in 4(2.3%). The average available CD4 T-cell count of patients with CSF infection ranged from 68/μl(VZV) to 266/μl(JCV). Pathogen-specific treatment was given only to patients with Cryptococcus, MTB and pneumococcus. The mortality was high, ranging from 42.9%(JCV, EBV) to 75%(CMV) during hospitalization. Interestingly, 37/92 (40.2%) CSF samples had two or three pathogens. Of 56 EBV + samples, 31 (55.3%) had co-infection with one or two other agents, including 14 with Cryptococcus, 10 with MTB, 3 with both MTB and CMV, 1 each with JCV, VZV, HSV-1, and Pneumococcus. Conclusions: CNS OI are frequent and potentially treatable complications of AIDS in Zambia. Multiple pathogens often coexist. EBV is the most prevalent CNS organism in isolation and in co-infection. Whether it is a true pathogen or merely a marker of CNS inflammation requires further investigation.

P213
Investigation of polyomaviruses replication in a nephropatic pediatric population receiving rituximab therapy

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Rituximab is a chimeric monoclonal antibody reacting with the CD20 antigen on B-cells. It has been proposed as treatment for idiopathic nephrotic syndrome (INS), recurrent idiopathic nephropathy (RIN) and focal segmental glomerulosclerosis (FSGS) refractory to steroids. Rituximab influences T-cell immunity and may predispose the patients to opportunistic infections, such as progressive multifocal leukoencephalopathy (PML) caused by the polyomavirus JC

(JCV). Little is known about the risk of latent viruses infections or reactivations in pediatric patients receiving biological therapies. In the longitudinal study reported herein, the effects of rituximab on JCV and BK Virus (BKV) replication were investigated. Blood, serum and urine samples were obtained from eleven pediatric patients (mean age: 11 years) with INS and RIN, when rituximab therapy was initiated, and then every month, for at least six months. JCV and BKV Real Time PCRs were performed, followed by sequencing of viral protein 1 (VP1) and non coding control region (NCCR). The same investigations were conducted on samples collected from eight pediatric patients (controls, mean age: 6 years), with INS or FSGS, treated with conventional chemotherapy. JCV was detected in the urine of one patient (9%), and one control (12.5%) whereas BKV was detected in the urine of 7/11 patients (63.6%) and 2/8 controls (25%) and sporadically in blood samples from four patients. No significant differences were observed in the mean viral loads and in the viral molecular characterizations between the two groups. In conclusion polyomaviruses replication seems not to be associated with rituximab therapy in pediatric populations.

P214

Brief Hypercapnia Reveals an Underlying Cerebrovascular Pathology in a Murine Model for HIV-1 Associated Neuroinflammation: Role of NO-cGMP Signaling and Normalization by Inhibition of Phosphodiesterase-5

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Cerebral blood flow (CBF) is disregulated in persons with human immunodeficiency virus 1 (HIV-1), for reasons that remain unclear. To test whether disregulation of CBF might be due to virally-induced neuroinflammation, we used a well-defined animal model (GFAP-driven, doxycycline-inducible HIV-1 Tat transgenic [Tat-tg] mice). Exposure to brief hypercapnia revealed an underlying cerebrovascular pathology in the Tat-tg mice. In control animals, brief hypercapnia induced a brisk increase in cortical flow and vascular dilation, as measured by laser Doppler flowmetry and in vivo two-photon microscopy. These responses were significantly attenuated in Tat-tg mice, but cortical microvascular morphology and capillary density were unaltered, suggesting that the functional pathology could not be attributed to vascular remodeling. To examine the mechanistic basis for the diminished cerebrovascular response to brief hypercapnia, Tat-tg mice were treated with (i) gisadenafil, a phosphodiesterase 5 inhibitor (PDE5) that prevents degradation of cGMP, and (ii) tetrahydrobiopterin (BH4), which is a limiting cofactor necessary for NO production. Gisadenafil largely restored normal vasoreactivity to brief hypercapnia in Tat-tg mice, whereas BH4 had little effect. These data show that HIV-associated neuroinflammation can cause cerebrovascular pathology, through effects on cGMP metabolism and possibly PDE5.

P215

Myocyte Enhancer Factor-2 (MEF-2) is involved in HTLV-1 Tax-mediated chromatin remodeling and plays critical role in viral infection

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The transactivator protein of human T cell leukemia virus type 1 (HTLV-1), Tax, is required for the activity of viral promoter LTR (long terminal repeat) and is capable of regulating both virus and host transcription. However, the clear molecular mechanism of Tax-mediated viral gene expression is not known. In the studies proposed herein, we have performed the extensive Protein/DNA transcription factor array analyses on the HTLV-1 infected cell lines (MT-2, and SLB-1), a latent cell line (MT-1) and on a Tax-expressing cell line (C8166) in comparison to normal CD4+ T cell line (Jurkat), the primary target infected cell poupulation. Interestingly, both MT-2 and SLB-1 cells demonstrated the upregulated transcriptome profile while MT-1 and C8166 cells showed either no expression or down-regulation of majority of the factors analyzed. Significant changes were observed in several new nuclear and cytoplasmic factors in case of HTLV-1 infected cells whose LTR-binding activity was confirmed by the promoter binding assays. Of these factors, Myocyte Enhancer Factor-2 (MEF-2), a chromatin-remodeling factor, was found to be of great interest, and therefore, its recruitment on HTLV-1 LTR was confirmed by the CHIP assay in both MT-2 and HTLV-1-infected primary CD4+ T cells. Furthermore, an incraese in MEF-2 expression was observed upon HTLV-1 infection. In order to understand nechanism of MEF-2 activity, we confirmed its binding with Tax by co-immunoprecipitation and also observed its

direct effect on HTLV-1 infection. Overall, these studies are first to elucidate the involvement of a novel chromatin remodeling factor, MEF-2, in HTLV-1 pathogenesis.

P216
Prospects for cannabinoid therapies in viral encephalitis

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Background: Cannabinoids are promising therapies to support neurogenesis and decelerate disease progression in neuroinflammatory and degenerative disorders. Objectives: The present study uses a rodent model of chronic viral encephalitis based on Borna Disease virus to compare effects of a general (CB1 and CB2) and a specific (CB2) cannabinoid receptor agonists on frontostriatal neurogenesis and gliogenesis Methods: 5-bromo-2'-deoxyuridine (BrdU) was used to label neural progenitors in the subventricular zone, striatum and frontal cortex of Borna Disease rats (adolescent male Lewis rats ic infected with BDV). Rats were then treated with the general cannabinoid agonist WIN 55,212-2 (1 mg/kg ip BID) for 1 or 2 weeks, or the more specific CB2 agonist HU-308 (5 mg/kg ip) for 2 weeks. Brains were processed for BrdU-immunoreactive cells and cell type specific markers. Results: One week treatment by WIN limits reactive gliogenesis and macrophage activity in favor of new cell, particularly oligodendroglia, development. However, WIN-treated rats develop tolerance to the anti-inflammatory effect by 2 weeks, showing no significant differences between WIN and vehicle treated groups at that time. On the other hand, 2 weeks treatment with HU-308, attenuates inflammatory disease progression by limiting microglia activation and proliferation and reducing BrdU + cell destruction by phagocytosis. Conclusion: Activation of CB2 receptors on microglia is a nontolerizing mechanism of controlling CNS inflammation during viral encephalitis and uses a nonpsychotropic cannabinoid agonist.

P217
Challenges to Neurotherapeutics Development for HIV-associated neurocognitive disorders (HAND)

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Despite adequate viral suppression in the periphery, neurological complications that arise from HIV infection, ranging from peripheral sensory neuropathy to cognitive decline and dementia continue to persist. This likely, because viral replication in the brain may not be completely controlled or the drugs may be toxic themselves. The HIV proteins secreted from infected macrophages and astrocytes are cytotoxic to the surrounding neuronal cells. Mechanistically, this neurotoxicity may be mediated via HIV protein-induced oxidative stress and neuroinflammation. However, all clinical trials using anti-inflammatory or neuroprotective agents have failed in HAND. Hence we have developed an approach to screen drugs that not only have neuroprotective and anti-inflammatory properties, but also protect adult neural progenitor cells, and facilitate their proliferation, differentiation and/or neuronal regeneration. For this purpose, we have developed techniques for generation of neurons from skin fibroblasts and hematogenous stem cells. While this can be used for moderate throughput screening, it has the distinct advantage of selecting patients who may best respond to these drugs. We have identified about 30 drugs that will now be screened using this approach. Candidate drugs will be tested for their ability to achieve target tissue concentrations in vivo. A candidate neuroprotective agent should have a plausible mechanism of action to elucidate its effects. The therapeutic agent should address the issues of viral reservoirs in the CNS. Finally an adjunctive neurotherapeutic may possess antiretroviral activities, but at a minimum, it should not promote HIV replication or infection. Individual compounds, or combination of agents with complementary mechanisms that fulfill these requirements may become potential therapeutics for HAND.

P218
The impact of chronic morphine exposure on the structure and function of an in vitro model of the blood-brain barrier

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About one-third of human immunodeficiency virus type 1 (HIV-1) cases leading to acquired immunodeficiency

syndrome (AIDS) in the United States have been attributed to injection drug use, frequently involving the abuse of opioids. Opioid abuse by HIV-1-infected individuals leads to more rapid disease progression, increased viral replication and peripheral viral load, and increased incidence and severity of neurocognitive abnormalities compared to non-drug abusers. The blood-brain barrier (BBB) is an obstacle that must be overcome during neuroinvasion and HIV-associated neurocognitive disorders (HAND) development. HIV-1 proteins can directly impact BBB permeability, and drugs of abuse including cocaine and methamphetamine have been shown to increase BBB leakiness and cellular transmigration. Previous in vitro and in vivo studies addressing the role of mu-opioids in altering BBB suggest that exposure increases cellular transmigration and overall barrier leakiness. In this study, a human brain microvascular endothelial cell (hBMEC) line, hCMEC/D3, was used to establish an in vitro transwell model of the BBB to investigate the effects of chronic (24, 48, or 72 h) morphine treatment on barrier structure and function. We observed that hCMEC/D3 cells form a confluent monolayer with a basal rate of passage of a 70 kDa tracer molecule comparable to primary hBMECs. While chronic morphine treatment does not induce overall barrier leakiness, mRNA levels of tight junction proteins were observed to change throughout the course of chronic treatment. Future experiments will investigate the impact of chronic morphine treatment on transcellular migration of mononuclear cells, tight junction protein expression, adhesion molecule surface expression, and cytokine/chemokine secretion.

P219

Regulation of progranulin expression in human microglia and proteolysis of progranulin by matrix metalloproteinase-12 (MMP-12)

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Background: The essential role of progranulin (PGRN) as a neurotrophic factor has been demonstrated by the discovery that haploinsufficiency due to GRN gene mutations causes frontotemporal lobar dementia. In addition to neurons, microglia in vivo express PGRN, but little is known about the regulation of PGRN expression by microglia. Goal: In the current study, we examined the regulation of expression and function of PGRN, its proteolytic enzyme macrophage elastase (MMP-12), as well as the inhibitor of PGRN proteolysis, secretory leukocyte protease inhibitor (SLPI), in human CNS cells. Methods: Cultures of primary human microglia and astrocytes were stimulated with the TLR ligands (LPS or poly IC), Th1 cytokines (IL-1/IFNγ), or Th2 cytokines (IL-4, IL-13). Results were analyzed by Q-PCR, immunoblotting or ELISA. The roles of MMP-12 and SLPI in PGRN cleavage were also examined. Results: Unstimulated microglia produced nanogram levels of PGRN, and PGRN release from microglia was suppressed by the TLR ligands or IL-1/IFNγ, but increased by IL-4 or IL-13. Unexpectedly, while astrocytes stimulated with proinflammatory factors released large amounts of SLPI, none were detected in microglial cultures. We also identified MMP-12 as a PGRN proteolytic enzyme, and SLPI as an inhibitor of MMP-12-induced PGRN proteolysis. Experiments employing PGRN siRNA demonstrated that microglial PGRN was involved in the cytokine and chemokine production following TLR3/4 activation, with its effect on TNFα being the most conspicuous. Conclusions: Our study is the first detailed examination of PGRN in human microglia. Our results establish microglia as a significant source of PGRN, and MMP-12 and SLPI as modulators of PGRN proteolysis. Negative and positive regulation of microglial PGRN release by the proinflammatory/Th1 and the Th2 stimuli, respectively, suggests a fundamentally different aspect of PGRN regulation compared to other known microglial activation products. Microglial PGRN appears to function as an endogenous modulator of innate immune responses.

P220

Targeted neuropsychological deficits in HIV/HCV coinfection

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The extent of neuropsychological (NP) impairment in HIV/HCV coinfected individuals remains unresolved. Depending on the cohort and testing measures, multiple studies have demonstrated a range of outcomes from mild to no impairment. To identify NP impairment in coinfected patients, we evaluated NP performances in HCV monoinfected and HIV/HCV

coinfected subjects and healthy controls who had no detectable liver damage nor recent illicit drug use. Nineteen HCV monoinfected and 17 HIV/HCV coinfected and 17 controls, all males, were evaluated for non-clinical depression and in 7 NP domains addressing attention, executive function, information processing, fine motor speed, and verbal/visual learning/memory. NP assessments were summarized as a Global Deficit Score (GDS), which integrates and normalizes relevant NP test results into a unitary global score. To better reflect the effects on cognitive impairment and account for the background intelligence variations, cognitive reserve score (CRS) was also evaluated. CRS is a composite of education, occupation and general intelligent quotient. CRS reflects the baseline or starting point for the cognitive evaluation. Higher CRS means more potential to compensate the loss of cognitive abilities. We found HIV/HCV had a higher Beck Depression Inventory II (BDI) score (11.1±7.5) than HCV monoinfection (6.7±6.0, p=0.065) and controls (5.4±4.1, p=0.010). HIV/HCV also had a higher GDS score 0.77±0.74) than HCV monoinfection (0.46±0.34, p=0.036) and controls (0.42±0.33, p=0.021). No correlation of BDI and GDS was found. Controls had a higher CRS score (177±36.4) than HCV mono (144±26.4, p=0.005) and coinfection (146±26.9, p=0.008). Coinfection also demonstrated lower performance than controls in verbal learning (p=0.014) and visual memory (p=0.043). HCV monoinfection showed no difference in 7 domains tested compared to controls. We conclude that, firstly, cognitive reserve plays an important role in evaluating neurological performance; secondly, even suppressed HIV in HCV-infected patients impacts neurological impairment.

P221

Cellular immune exhaustion in progressive multifocal leukoencephalopathy (PML)

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Background Progressive multifocal leukocencephalopathy (PML) is a deadly brain disease caused by the reactivation of the human polyomavirus JC (JCV). While asymptomatic JCV primary infection occurs in up to 80% of the general population, reactivation resulting in PML is observed mainly in immunosuppressed individuals. With no currently available treatment, more than half of the PML patients have a rapid fatal outcome. We have shown that JCV-specific CD8+ cytotoxic T lymphocytes (CTL) play a crucial role in the containment of PML progression and improved survival. Host CTL dysfunction may be associated with T cell exhaustion, involving the inhibitory receptor, programmed cell death 1 (PD-1). While, exhausted CD8+ T cells expressed high levels of PD-1 in chronic viral infections, the blockade of this pathway resulted in increased expansion of activated viral specific T cells. We examined the contribution of PD-1 to host cellular immune dysfunction in PML. Methods We enrolled 30 subjects, including 10 PML/HIV-positive and 10 PML/HIV-negative patients, 5 HIV-positive patients, and 5 healthy controls. We characterized CD4+ and CD8+ T cells, as well as JCV-specific CD8+ T cells for PD-1 expression by flow cytometry. Furthermore, PD-1 blockade was performed to detect augmentation of JCV-specific CTL. Results PD-1 expression was elevated on total CD4+ and CD8+ T-cells (medians 36% and 24%) in PML patients compared to healthy control subjects (medians 14% and 18%; p=0.0015 and p=0.033). In PML patients, JCV-specific CD8+ cytotoxic T-lymphocytes (CTL) expressed PD-1 more frequently than total CD8+ T-lymphocytes (means 39% and 78%, p=0.0004). Blocking the PD-1 receptor increased interferon gamma release in a subgroup of PML patients. Conclusions PD-1 expression on JCV-specific CTL indicates that immune exhaustion plays a role in PML. Augmentation of JCV CTL immune response with PD-1 blockade suggests that PD-1 receptors may be a valid therapeutic target for this deadly disease.

P222

JC Virus Infection in a Humanized Mouse Model

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Background Progressive Multifocal Leukoencephalopathy (PML) is caused by reactivation of JC virus(JCV). There is no animal model of PML since JCV only infect humans. Humanized mice provide an ideal model for studying JCV primary infection, latency, and reactivation. Methods Immunodeficient mice, NOD/SCID/IL-2R, were engrafted with human hematopoietic cells and thymus. JCV(Mad-4 or CY archetype) strains were injected intraperitoneally. Mice were sacrificed between 47-103 days after initial infection and lymphocyte were phenotyped by flow cytometry. Detection of anti-JCV IgM and IgG was performed by ELISA. JCV DNA was extracted from whole blood, plasma, and urine and detection performed by quantitative PCR(qPCR). JCV-specific T-cell responses were measured in splenocytes by ELISPOT and ICS assays. JCV PCR was performed in urine 27 and 90 days post infection. Hematopoietic organs, kidneys, and brains were tested for presence of JCV nucleic acids by qPCR and protein by immunohistochemical staining. Results All mice tolerated intraperitoneal injection and infection of JCV without developing any illness, similar to primary infection in humans. JCV DNA was detected in the plasma and whole blood of JCVMad-4 mice between day 14-67. Two JCVMad-4 mice also had detectable JCV DNA in the urine. Anti-JCV IgM was detected in 7/13 JCVMad-4 and 4/14 JCVCY infected mice between days 47 and 90 after infection. On post-infection day 108, 3/7 JCVMad-4 infected mice had detectable JCV specific T-cell response in both ICS and ELISPOT assays. Conclusions The humanized mice provide an animal model of early events surrounding JCV infection. Primary infection is asymptomatic and virus is rarely detected in whole blood and plasma, but is occasionally excreted in the urine. JCVMad-4-infected mice can develop both humoral and cellular immune responses to JCV. This model may prove very valuable for studying JCV primary infection, host immune response, and determinants of viral latency and reactivation.

P223
JC virus reactivation and immune responses in Hematopoietic stem cell transplantation (HSCT)

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Background: JCV causes progressive multifocal leukoencephalopathy(PML) in immunocompromised patients. Up to 80% of the general population is seropositive for JCV and JCV-specific cellular immune response is necessary for containment of viral proliferation. The mechanism of JCV reactivation and immunity in a transplanted immune system remains unclear. Methods: We prospectively enrolled 30 patients undergoing allogeneic HSCT and collected blood and urine samples pre HSCT, 3, 6, and 12 months after HSCT. We detected JCV DNA by PCR in blood and urine samples, performed ELISA for detection of JCV IgM and IgG, as well as Elispot and ICS for measurement of T-cells responses to JCV. We detected JCV-epitope specific T-cells by tetramers, VP1p100/p36, staining in HLAA0201-positive patients. Multivariate analysis accounted for conditioning regimens, cancer diagnosis, concurrent viremia, age, and transplant type. Results: Pre HSCT, JCV DNA was detected in 7/30 urine, 5/30 PBMC, 6/30 plasma samples. While viruria remained stable throughout, viremia was detected in only 1/22 plasma and none of 22 PBMC samples 12 months after HSCT. Prevalence of anti-JCV IgG remained stable between 83% pre HSCT to 72% at 12 months. Anti-JCV IgM was rarely detected. ELISPOT, ICS, and tetramer staining results correlated with each other. A significant increase in T cell responses 12 months after HSCT was observed in CD4 and CD8 T-cells. While JC viruria correlated with detection of anti-JCV antibodies, the cellular immune response to JCV measured by ELISPOT was inversely correlated with anti-JCV IgG response. Conclusions: JC viruria triggers an antibody response in HSCT. However, an increase in JCV-specific cellular immune response 12 months after HSCT leads to suppression of JC viremia, and decrease in JCV humoral immune response. This prospective study in HSCT patients, provides a model of provides a model of interactions between the host immune response and viral activation in multiple compartments.

P224
Wnt signaling in NeuroAIDS

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HIV-1/AIDS patients often develop neurological complications, such as neurocognitive deficits and neuropathic pain. We are interested in elucidating the host mechanisms in CNS that interact with HIV toxins and contribute to NeuroAIDS-related neurodegeneration. Our current studies focus on the Wnt signaling, which plays important roles in neurogenesis, axon outgrowth, dendritic arborization, and synapse differentiation. Our previous studies demonstrated that Wnt proteins in central neurons are synthesized and secreted in response to synaptic activation and that the synaptic activity-gated Wnt signaling regulates long-term potentiation (LTP). More recently, we found that Wnt signaling was specifically up-regulated in the spinal cord dorsal horn (SDH) from the HIV-1 patients who had developed chronic pain, which indicates that the dysregulated Wnt signaling probably is an important host factor in the SDH that facilitates the development of HIV-1-associated chronic pain. We further found that HIV-1-related chronic pain was also associated with the activation of astrocytes in the SDH. In addition, our studies on animal models revealed that HIV-1 gp120 rapidly induced Wnt expression in the SDH and that Wnt signaling controlled astroglial activation and cytokine expression. The results collectively suggest that gp120 may contribute to the SDH neuropathogenesis of HIV-1-associated chronic pain by aberrantly activating Wnt signaling and neuroinflammation.

P225
MicroRNA-9 is Increased in CNS Neurons in the HIV-infected Brain and in Methamphetamine Exposure

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MicroRNAs (miR) regulate phenotype and function of neurons by binding to miR-response elements (MRE) in the 3′ untranslated regions (3′UTR) of various messenger RNAs to inhibit translation. MiR expression can be induced or inhibited by environmental factors like drug exposure and viral infection, leading to changes in cellular physiology. We hypothesized that the combined effects of methamphetamine (Meth) and human immunodeficiency virus (HIV)- infection in the brain will induce changes in miR expression, and have downstream regulatory consequences in neurons. We first used a PCR-based array to screen for differential expression of 380 miRs in the frontal cortex of HIV-positive Meth abusers and matched controls using frozen autopsy tissues. The screening results showed significantly increased expression of the neuron- specific miR-9. In vitro, we used SH-SY5Y cells, an experimental system for dopaminergic studies, to determine miR expression by quantitative PCR after exposure to Meth in the presence or absence of conditioned media from HIV-infected macrophages. Again, we found that miR-9 was three-fold increased compared to controls. We also examined the inwardly rectifying potassium channel, KCNMA1, which has alternative splice variants that contain an MRE to miR-9. We employed the Rapid Extension of cDNA Ends technique to identify alternate 3′ UTRs of KCNMA1 both in vitro and in the autopsy specimens. We are currently testing whether differential splice variant expression of KCNMA1 operates via the increased miR-9, by pre- treating the neurons with anti-miR-9 locked nucleic acid. Our preliminary results suggest that HIV and Meth -induced elevated miR-9 leads to suppression of MRE-containing splice variants of the inwardly rectifying potassium channel, KCNMA1, which may affect neurotransmitter release in dopaminergic neurons. MiR-9 may be a therapeutic target for Meth abuse in HIV-positive individuals.

P226
Relationship of HIV status and immunosuppression to brain response during a lexical retrieval task among HIV-infected individuals: An fMRI study

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Observed lexical retrieval impairments among HIV patients are consistent with disruptions to frontal-striatal functions, which may become further compromised during severe immunosuppression. However, not all patients will demonstrate overt neurobehavioral impairments. The purpose of the current study was to examine whether HIV infection confers brain functional changes during phonemic access in light of normal neurobehavioral performance. Twenty-eight participants (16 HIV positive; 12 HIV seronegative) were recruited to undergo fMRI while engaged in word retrieval tasks. Neuroimaging data was analyzed using FSL. Immunosuppression was measured using current CD4 count. Results: There were no significant differences between HIV + (M=48.3 SD=10.5) and HIV- (M=47.5, SD=8.9) groups on overt phonemic retrieval performance. However, functional activation differences were found as a function of HIV status, with HIV + individuals demonstrating greater activation in basal ganglia structures (caudate, putamen, globus pallidus) structures compared to HIV – individuals (corrected with Z value >2.0; p<.05). Within the HIV + group, lower CD4 count was associated with increases in activation in frontal-striatal regions. These findings are consistent with recent fMRI studies of HIV-associated neurologic impairment and suggest that HIV patients hyperactivate frontal-striatal networks as a compensatory mechanism for neurological compromise.

P227

Pathways to Neurodegeneration: The Effects of HIV and Aging on Resting State Functional Connectivity

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Background: HIV + patients are now living longer as a consequence of highly active antiretroviral therapy (HAART). However, many HIV + individuals continue to have cognitive impairment despite these medications. Older HIV + patients may be at increased risk for developing neurodegenerative disorders. Methods: This study used resting state functional connectivity magnetic resonance imaging (rs-fcMRI) to non-invasively investigate the neurobiology of five functionally defined brain networks in 52 HIV + (44% on HAART) and 52 HIV- controls. Network composite scores were computed for five networks: default-mode network (DMN), control network (CON), salience network (SAL), dorsal attention network (DAN) and sensorimotor network (SMN). Independent changes in rs-fcMRI due to HIV and aging were assessed by ANCOVA. Results: A reduction in rs-fcMRI correlations was seen within the DMN (p=.003), CON (p=.006), and SAL (p=.03) networks while the DAN and SMN remained relatively spared. Compensatory increases in correlations were not seen within any networks. Neither markers of HIV (HIV viral load or CD4+ cell count) nor degree of cognitive impairment associated with HIV correlated with rs-fcMRI measures. Aging led to a decrease in correlations within the DMN (p=.03) and SAL (p=.006) networks for both HIV + and HIV- subjects, however no interaction was present between HIV and aging. Conclusions: These results suggest that HIV and aging cause similar decreases in rs-fcMRI. HIV could serve as a model for accelerated neurodegeneration within brain network connections. Further longitudinal studies of older HIV + patients are needed.

P228

HIV-Vpr in the hippocampus impairs novel recognition learning

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Human Immunodeficiency Virus (HIV) infected individuals may be at an increased risk of developing neurological abnormalities. Viral proteins, such as Vpr, are considered to cause neuronal damage and may play a role in hippocampal loss of function. Some of these viral proteins promote activation of cellular pathways including apoptosis and immune activation. Vpr itself is known to be pro-apoptotic and neurotoxic. We propose that HIV Vpr is involved in the dysfunction of hippocampal-dependent learning by disrupting neuronal integrity. Live rats were tested by the novel object and novel location tasks to assess HIV-Vpr effects in hippocampal-dependent learning and memory. Control rats treated with GFP show an increase in object exploration, while rats treated with Vpr show an impairment in object exploration. We expect the Vpr-treated animals to show learning deficiencies that will correlate with histopathological signs of neuron disruption. We expect that Vpr will be neurotoxic in our studies as well, and will help clarify the role of Vpr in the

brains of HIV infected humans. Research supported by R03DA026722 and G12RR003050.

P229

Adenine nucleotides in supernatants from HIV-infected macrophages induce excitoxic damage through activation of purinergic receptors on neurons

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Products released from HIV-infected monocyte-derived macrophages (HIV/MDM) are thought to contribute to neurological injury by promoting excitotoxic damage to synapses. Identifying the toxic products released from HIV/MDM and their neuronal targets could lead to the development of new therapies to combat cognitive dysfunction in HIV-infected individuals. In these studies we exposed neurons to supernatants from HIV/MDM or mock-infected MDM (MDM) and determined effects on calcium flux, dendrite morphology and survival. Supernatants were heat inactivated and thus contained primarily small heat-stable molecules. Although supernatants from both HIV/MDM and MDM induced rapid calcium influx in primary neurons, those from HIV/MDM were several orders of magnitude more potent that supernatants from MDM. Calcium influx induced by HIV/MDM was inhibited with general antagonists of purinergic receptors and was partially reduced by a specific antagonist of P2X7 receptors or L-type voltage operated calcium channels (VOCC). We determined that (HIV/MDM) supernatants contain low μM amounts of ATP and lesser amounts of ADP, AMP and adenosine, suggesting that adenine nucleotides in the supernatant directly activated purinergic receptors on neurons. Indeed, pre-incubation of HIV/MDM supernatant with apyrase to degrate ATP prevented it from inducing a rapid calcium flux in neurons. Inhibiting NMDA or AMPA receptors had no effect on supernatant-induced calcium flux in neurons. However, inhibition of NMDA receptors in addition to P2X7 and L-type VOCCs protected neurons from HIV/MDM-induced dendritic damage. These data suggest that ATP contained in HIV/MDM supernatants rapidly activated calcium permeable purinergic receptors on neurons. Thus, NMDA receptor activation and excitotoxicity in response to HIV/MDM may occur through a secondary release of glutamate that is stimulated by ATP ligation of purinergic receptors. Modulators of P2 receptors may be neuroprotective in HIV-infected individuals.

P230

A novel high-throughput screening assay to identify inhibitors of HIV-1 gp120 protein interaction with DC-SIGN

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The 2010 UNAIDS report states that approximately 34 million people are living with human immunodeficiency virus type 1 (HIV-1), despite highly active antiretroviral therapy (HAART). The current ARV therapy has many disadvantages including a cost trajectory unsustainable for economically challenged countries, serious side effects, and the development of drug-resistant strains. Several measures are under way to develop alternatives for ARV therapy, particularly for the control of early HIV-1 infection, but lack of efficient drug targets and assays hinders the search of potential ARV molecules. The dendritic cells present in the mucosal tissue, together with CD4+ T lymphocytes and macrophages, are among the first cells to encounter HIV-1. The dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN) molecule plays a crucial role in binding HIV-1 through high affinity interaction with viral envelope glycoprotein gp120. DC-SIGN, a C-type lectin, is expressed high on cells in the mucosal tissue of the rectum, uterus and cervix, facilitates early HIV-1 infection after sexual transmission. In this study we report a novel target-specific high-throughput screening (HTS) assay capable of quantifying the binding as well as the inhibition of DC-SIGN and gp120. The specificity of the assay was determined through competitive inhibition while optimization occurred for DMSO tolerance (0.5%), Z' factor (0.51), signal-to-noise ratio (3.26), and coefficient of variation (5.1%). For assay validation previously recognized antagonists of DC-SIGN/gp120 binding were tested to detect inhibition demonstrating the suitability of the assay for future HTS screen of potential inhibitors that block the binding

between DC-SIGN and gp120, which may prevent early HIV-1 infection.

P231
Extinction of Tumor Antigen Expression by SF2/ASF in JCV-transformed Cells

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The human neurotropic polyomavirus JC, (JCV), induces a broad range of neural-origin tumors in experimental animals, and has been repeatedly detected in several human cancers, most notably neural-crest origin tumors including medulloblastomas and glioblastomas. The oncogenic activity of JCV is attributed to the viral early gene products, large T and small t antigens, as evident by results from in vitro cell culture and in vivo animal studies. Recently, we have shown that alternative splicing factor, SF2/ASF, has the capacity to exert a negative effect on transcription and splicing of JCV genes in glial cells through direct association with a specific DNA motif within the viral promoter region. Here we demonstrate that SF2/ASF suppresses large T antigen expression in JCV-transformed tumor cell lines, and the expression of SF2/ASF in such tumor cells thereby inhibits the transforming capacity of the viral tumor antigens. Moreover, down-regulation of SF2/ASF in viral-transformed tumor cell lines induces growth and proliferation of the tumor cells. Mapping analysis of the minimal peptide domain of SF2/ASF responsible for JCV promoter silencing and tumor suppressor activity suggests that 25 amino acid residues 76 to 100 of SF2/ASF are functionally sufficient to suppress the growth of the tumor cells. Further employment of a genetic approach to identify functional domains of SF2/ASF-25mer that are involved in suppression of JCV transcription and a chemical approach for design of cyclic peptides based on the NMR solution structure of SF2/ASF-25mer peptide platform may offer a novel strategy for the treatment of PML. Supported by grants awarded by NIH to KK.

P232
CV-NCCR is targeted by negative transcription factors

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Patients undergoing immune modulatory therapies for the treatment of autoimmune diseases such as multiple sclerosis, and individuals with an impaired-immune system, most notably AIDS patients, are in the high risk group of developing progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the white matter caused by human neurotropic polyomavirus, JCV. It reactivates under immunosuppressive conditions and replicates in oligodendrocytes leading to multifocal demyelinated lesions in the brain. We have previously demonstrated that the cellular alternative splicing factor SF2/ASF is a negative regulator of JCV in glial cells. SF2/ASF only inhibited splicing of JCV genes but also showed a strong suppression of basal transcription of the viral early and late promoters. This negative impact of SF2/ASF was dependent on its

ability to bind a specific region within viral promoter mapped to the 98 bp repeated region of the virus. In order to investigate the importance of the second 98 bp repeated region in regulation of JCV gene expression and replication, we created a mutant JCV strain with only one 98 bp repeated domain which also served only one binding site for SF2/ASF [JCV-Mad1-(1X98)]. Surprisingly, this large deletion within viral NCCR increased the rate of viral early transcription assessed by reporter gene constructs. However, the replication efficiency of this mutant virus was similar to the wild type virus. We also created a mutant strain with no SF2/ASF binding site [JCV-Mad1-ΔCR3 (1X73)]. Transcription and replication efficiency of the mutant virus was analyzed in PHFA cells. Interestingly, JCV-Mad1-ΔCR3 (1X73) showed three and two fold higher early promoter activity than JCV-Mad1-WT and JCV-Mad1-(1X98), respectively. However, this mutant virus was unable to propagate in PHFA cells. Further analyses of the transcription mediated by mutant promoter sequences revealed that JCV-Mad1-ΔCR3 (1X73) was defective in late gene transcription. Supported by grants awarded by NIH to KK.

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Coexistance of HIV and MS; From clinical relevance to a transgenic rat model

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Multiple Sclerosis is an autoimmune disease characterized by acute inflammation and demyelination. A viral etiology has been suggested as a possible contributor to the pathogenesis of MS either by methods linked to molecular mimicry or resulting from direct effects from the viral entity. In this study we initiated the development of an animal model of experimental allergic encephalomyelitis (EAE) utilizing the HIV-1 transgenic (TG) rat, which incorporates a non-infectious HIV genome. Methods: For induction of EAE, 2 HIV TG rats and 1 wild-type (WT) rat were inoculated with 100ug of guinea pig myelin basic protein (MBP) + 2ug of Pertussis toxin (PT) and 2 TG and 2 WT rats were inoculated with MBP alone. The rats were evaluated for 28 days for signs of weakness, levels of disability was measured using EAE scores (0=no weakness; 1=tail weakness; 2=mild limb weakness; 3=hind legs weakness; 4=quadriparesis; 5=moribund). Results: At day 15 post-immunization, TG rats immunized with MBP + PT developed mild tail paralysis (score=1), hind limb paralysis (score=3) at day 16 with subsequent complete recovery over the next two days. TG rats immunized with MBP alone developed mild tail paralysis at day 4 (score=1), hind limb paralysis by day 12 (score=3), and quadriparesis (score=4) on day 28. In contrast, WT rat immunization with MBP + PT resulted in quadriparesis at day 14 and death at day 15, whereas WT rats immunized with MBP alone developed tail weakness (score=1) at day 6 and was moribund by day 15. Conclusions: HIV TG rats develop less severe clinical EAE than WT rats, and disease severity was further attenuated by the use of PT in the immunization regimen. The improvement observed in rats immunized with MBP + PT may be analogous to clinical remissions that can be observed in patients with multiple sclerosis.

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Astrocyte-elevated gene-1 modulates astrocyte responses to injury and stress: A possible role in Reactive astrogliosis

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Astrocyte elevated gene-1 (AEG-1), a novel human immunodeficiency virus (HIV-1)- and tumor necrosis factor (TNF)-α-inducible oncogene, has engendered tremendous interest in the field of cancer research as a therapeutic target for many metastatic aggressive tumors. However, little is known of its role in regulating astrocyte behavior and function during non-cancerous conditions and HIV-1 infection in the brain. Based on its oncogenic role in cancer, here we investigate whether AEG-1 induction in astrocytes alters their response to reactive astrogliosis, a hallmark of CNS pathologies like infection, trauma, ischemia and neurodegeneration. Preliminary studies with in vivo mouse model of reactive astrogliosis revealed a significant induction of AEG-1 expression following injury-induced astrogliosis, which was further supported by subsequent in vitro studies. A dramatic increase in the nucleolar localization of AEG-1 protein was noted in cultured human astrocytes following injury and/or treatment with hydrogen peroxide (H2O2), an

inducer of ROS, which hinted towards its plausible role in the nucleolus. Here, we show that AEG-1 protects the astrocytes from oxidative stress-induced DNA damage and plays a role in astrocyte recruitment to injury by increasing their migratory and proliferative potential. We have performed DNA fragmentation ELISA, MTT and in-vitro cell migration assays to address these questions. To further supplement our findings, we also studied the correlation between AEG-1 expression and brain aging, one of the many factors responsible for astrogliosis under disease-free conditions. For this we analyzed AEG-1 mRNA expression in a cohort of 45 HIV- human brain mRNA samples within the age group of 20-90 years. In conclusion, this study suggest that AEG-1 may play a role in protecting human astrocytes from oxidative damage induced during reactive astrogliosis and increase their migratory and proliferative potential following injury.

P235
CSF Hypocretin-1 Levels and HIV infection – No Association with Addictive Behavior but Do Correlate with Cognitive Impairment

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Hypocretin/orexin (hcrt/ox) system is composed of hypothalamic peptides that have been related to narcolepsy/insomnia, obesity, and motivational behaviors. The relationship of Hcrt/ox levels to dementia is unclear. Recently hypothalamic dysfunction was shown to be associated with mood disorders in drug abusing HIV-seropositive patients. We thus studied the association of CSF hcrt/ox levels in HIV-seropositive women with history of dependence and cognitive function. Retrospective study of 70 HIV-seropositive women and 5 CSF controls, patients were stratified by smoking habits using the Fagestrom Test for Nicotine Dependence (FTND) and urine toxicology into Never Smokers (n=18), Prior Smokers (n=20), Smokers (n=17), Smokers + to Marijuana (n=6), and Smokers + to Cocaine (n=10). HAND was determined using modified AAN HIV dementia criteria. No differences in age, education, viral load (plasma and CSF), CD4 (current and nadir), hepatitis C virus, BMI, BDI, and HAND were observed between groups. CSF hcrt/ox levels were determined using the fluorescent immunoassay kit, (Phoenix Pharmaceuticals) with an intra- and inter-assay validity of 10% and 15% respectively. Parametric and non parametric statistics were used for analysis. No associations were observed between CSF hcrt/ox levels and HIV-seropositive women stratified by smoking and drug abuse. No correlations were observed between CSF hcrt/ox levels and age, BDI, BMI, viral immune profile, and FTND score. Controlling for drug abuse, a correlation was observed between CSF hcrt/ox levels and HAND ($p=0.03$), where the HIV normal group had lower levels than the impaired groups. A negative correlation was observed between CSF hcrt/ox levels and NPZ and psychomotor speed and verbal memory domains ($p<0.05$). Our findings suggest that CSF hcrt/ox levels are not useful in the evaluation of HIV-seropositive women with drug abuse dependence. However, may have a role in HAND. Further studies should be performed to support current findings. R21MH095524, S11NS046278, U54NS043011, U54RR026139, 8U54MD007587, 2G12RR003051, 8G12MD007600, R25MH080661

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Cocaine and Marijuana alter lipidomics in HAND

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Regardless of the great advances in antiretroviral therapy, HIV-associated neurocognitive disorders (HAND) persists as a major clinical problem in the HIV infected population. Several biochemical mechanisms of the neuropathogenesis of HAND have been implied, but still the identification of

biomarkers is currently needed. A proper balance of sphingolipids is necessary for normal neuronal function. Levels of ceramides are altered in several neurodegenerative conditions including Alzheimer's disease and HAND. Ceramides Ce18:0, Ce16:0, and Ce24:1 are consistently related to neuronal dysfunction, neuronal death, and HAND progression. However, ceramides might also be altered by the use of neurotoxic drugs, such as cocaine and marijuana. In this study, we used a lipidomic approach to examine levels of Ce18:0, Ce16:0, Ce24:1, and its respective precursors, sphingomyelin (Sc18:0, Sc16:0, and Sc24:1) in the CSF of 54 HIV-seropositive women. Patients were tested for marijuana and cocaine the same day the CSF was collected (negative [n=40], marijuana [n=6], and cocaine [n=9]). Cognitive performance was determined using the modified AAN criteria and stratified into normal cognition, asymptomatic impairment, MCMD, and HIV-associated dementia (HAD). Interestingly, we did not find differences in the sphingomyelin/ceramide (Sc/Ce) ratio when analyzing all patients by HAND. Analysis of patients with negative toxicology (n=40), revealed all three Sc/Ce ratios lowered with HAD, but significant for Sc18:0/Ce18:0 ($p<0.05$). Regardless of HAND, patients who tested positive to marijuana and cocaine had consistently lower levels of all three Sc/Ce ratios and higher levels of Ce than those with negative toxicology ($p<0.05$, cocaine only). Altered Sc/Ce ratios are present in HAD and use of drugs of abuse. However, the subacute effects of drugs of abuse on lipid metabolism may occur earlier than HAND. We propose performing toxicology tests when studying lipidomics as biomarkers of HAND, since the proper balance sphingolipids might be altered by the use of these specific drugs.

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Systemic reactivation of Epstein Bar Virus (EBV) correlates with the magnitude of parenchymal brain abnormalities in AIDS-associated CNS opportunistic infections

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Background: Opportunistic infections of the CNS (CNS-OIs) by viruses are rare, albeit lethal, at the onset of HIV-associated immunosuppression. Epstein Bar Virus (EBV),JC polyomavirus (JCV) or cytomegalovirus (CMV) are likely to reactivate during HIV-induced immunosuppression and play critical roles in neuroAIDS. The relevance of viral reactivation in HIV-infected patients at risk of neuroAIDS was assessed by viral load of JCV, EBV and CMV in blood and cerebrospinal (CSF) in a cohort of patients in a University Hospital in Cali-Colombia. Material and Methods: 65 patients with AIDS-related CNS-OIs (Toxoplasma encephalitis [TE], Cryptococcal meningitis [CM], Neurosyphilis [NS] and TB meningitis [TBM]), 10 with CNS viral OIs or neoplasms, 10 with other neuroAIDS diseases and 10 HIV + immunocompromised controls without neuroAIDS were studied. Viral load was performed by qPCR assays. Results: Viral detection in CSF was low (6.3%) and corresponded to cases with CNS viral OIs or neoplasm. Viral reactivation in blood was only 13.7% for JCV and 20% for CMV. Systemic reactivation of CMV seems to occur only with CMV-associated neuroAIDS. In contrast, the rate of EBV reactivation in blood was higher in the group with neurological complications (48.2%) compared with the AIDS control group without neuroAIDS (10%) ($p<0,015$). All 95 patients were EBV infected as judged by plasma IgG. Reactivation of EBV was more frequent in patients with neuroinfection (TE and TBM or CM) and parenchymal brain lesions (71%) defined by neuroimaging whereas EBV load was low in meningitis or neuroAIDS without parenchymal disease (30%); ($p=0.001$). We found inter-individual variability of EBV loads in blood and no correlation with the degree of immunosuppression (CD4+ counts or CD4/CD8 ratio). Conclusion: Reactivation of EBV in AIDS may be a surrogate marker of impaired immune surveillance to control CNS-OIs. The association of EBV with CNS-OIs may play a role in pathogenesis.

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Evidence for humoral regulation of HHV-6 reactivation in multiple sclerosis and association with MRI activity

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Human herpesvirus 6 (HHV-6), a neurotropic betaherpesvirus, has been associated with central nervous system diseases such as encephalitis, epilepsy and multiple sclerosis (MS). HHV-6 specific intrathecal antibodies presenting as virus-specific oligoclonal bands (OCBs), as well as presence of HHV-6 DNA in CSF, have been described in a subset of

patients with MS. We investigated the presence of HHV-6 specific OCBs by isoelectric focusing and affinity immunoblotting, and of CSF antibodies by a chemiluminescence-based immunoassay in 37 patients with MS. These findings were correlated with viral DNA detection in CSF by nested PCR and disease activity assessed by contrast enhancing MRI lesions (CELs). Nine of 37 (24.3%) MS patients had HHV-6 specific OCBs, and five (13.5%) had HHV-6 DNA in CSF. HHV-6 DNA in CSF was associated with low levels of HHV-6 antibodies in CSF. Patients with HHV-6 DNA in CSF had significantly more CELs than patients with HHV-6 specific OCBs or those who did not have HHV-6 DNA in CSF. In addition, CELs were found more often in MRIs from patients with positive HHV-6 DNA in CSF. The severity of lesion burden was also inversely associated with HHV-6 antibodies in CSF. Our results suggest that intrathecally produced HHV-6 antibodies, some, of which present as virus-specific OCBs, might play a role in maintaining latency or limiting dissemination of HHV-6 reactivation in CNS. Patients with undetectable or low levels of HHV-6 antibodies might therefore be more prone to reactivation of latent virus in CNS, seen as viral DNA in CSF. This might lead to, or be a consequence of, inflammation, which is a hallmark of active plaques in MS brain tissue.

P239

Methamphetamine Use Exacerbates HIV-associated Neurocognitive Impairment in Acute and Early HIV Infection

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Methamphetamine (MA) use is a risk factor for HIV infection, and its comorbid presentation is associated with additive structural and functional injury to frontostriatal neural circuitry, including neurocognitive impairment. Yet little is known about the role of MA in the expression of neurocognitive compromise during the acute and early stages of HIV infection (AEH), which is associated with rapid viral replication, increased immune activation, and alterations in brain metabolism. The present study examined the impact of MA use on HIV-associated neurocognitive impairment (HNCI) among 46 antiretroviral-naïve adults with a median duration of infection of 75 [IQR=25,87] days and current CD4 count of 609 (IQR= 431,717.5). Participants were administered a brief neurocognitive battery adjusted for demographics and assessed executive functions, memory, psychomotor speed, and verbal fluency. Sixty-one percent of the participants evidenced HNCI as determined by global deficit scores (GDS) >0.5. Participants were also administered the Drug Abuse Screening Test (DAST-20) for MA use and the Alcohol Use Disorders Identification Test (AUDIT). Spearman's rho correlations revealed a moderate relationship (rho=0.38; p= 0.011) between DAST total and GDS, whereby individuals with greater risk of MA use disorders exhibited lower neurocognitive functioning, particularly in fine motor coordination. Within a small subset of participants who underwent lumbar puncture (n=14), DAST total was correlated at a trend level with viral load in CSF (rho=0.50; p=0.067), but not plasma (rho=0.04; p>0.10). Alcohol use was not associated with neurocognitive functioning or viral loads (ps>0.10). These results suggest that MA use may play an important role in the risk of neurocognitive impairment and increased HIV RNA in CSF during AEH. The neural mechanisms of MA's apparent exacerbation of CNS injury in AEH remain to be determined, but may involve dopaminergic systems vulnerable to effects of neuroinflammation, vasculopathy, and/or oxidative stress that have been previously reported in chronically infected MA users.

P240

Giant cell encephalitis in R5 SHIV infected rhesus macaques

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Division of Comparative Pathology, Southborough, MA, USA. 2 Aaron Diamond AIDS Research Center, New York, New York, USA. 3Tulane National Primate Research Center, Tulane University Medical Center, Covington, Louisiana, USA. Multinucleated giant cell encephalitis has not been reported in R5 SHIV-infected rhesus macaques. Fifty rhesus macaques were inoculated with R5 SHIVSF162P3N by one of three routes: intravenously (n=9), intrarectally (n=17), or intravaginally (n=24). Forty-three monkeys became viremic and 25 developed AIDS. Of the monkeys with AIDS, 7 (7/25, 28%) developed giant cell encephalitis (SIVE). Rapid progressor phenotype was evident in 5/7 (71.4%), and adaption to utilize the CXCR4 co-receptor (X4 co-receptor switch) was observed in 4/7 (57%) macaques with SIVE. Previous studies demonstrated high levels of viral replication sustained in macrophages of some of these animals that underwent co-receptor switch late in disease as a consequence of the ability of adapted virus to bind CD4 more efficiently. SIVE lesions were present in gray and white matter in the cerebrum, cerebellum, and brainstem of affected animals. Lesions were comprised of virally infected CD68+ and CD163+ macrophages accompanied by robust astroglial and microglial activation characterized by upregulation of GFAP and Iba-1, respectively. Of interest, the four macaques with the greatest amount of virus and macrophage/microglial activation in the brain were inoculated mucosally (either intrarectally or intravaginally). In summary, the R5-SHIV-infected rhesus macaque provides a new and robust model of HIV encephalitis that will contribute to a better understanding of viral tropism to the brain. Additionally, X4 co-receptor adaptation may play an important role in lentiviral neuropathogenesis.

P241
Neuropathogenesis of Japanese encephalitis virus

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Japanese Encephalitis (JE) virus is a mosquito-borne positive-sense RNA virus and a member of the genus Flavivirus, family Flaviviridae. The JE serocomplex includes West Nile (WN), St. Louis encephalitis and Murray Valley encephalitis viruses, among others. JE is the leading cause of viral encephalitis worldwide causing significant morbidity and mortality with more than 50,000 cases reported annually. To study the neuropathogenesis of JE virus, the P3 strain was inoculated into mice via the intraperitoneal route. In this mouse model, brief viremia is followed by invasion of the central nervous system, crossing the blood-brain barrier by an undetermined mechanism. At various time points, brains were harvested, formalin fixed and paraffin embedded for slide preparation. JE virus antigen was found throughout the brain, including the neurons, endothelial cells and astrocytes, and neuropathological alterations, including hemorrhage and perivascular infiltrates, were observed. Previous in vivo studies with JE and WN viruses have shown mortality following neuroinvasive disease correlates with high viremia titers, whereas mice with low or undetectable viremias did not show clinical signs of disease or detectable virus in the brain. Examination of mouse brains harvested at early and late time points of JE infection shed light on the neuropathogenesis of this disease.

P242
Junctional Proteins are Critical to the Transmigration of Maturing and HIV Infected Monocytes Across the BBB: Implications for NeuroAIDS

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Human immunodeficiency virus (HIV) enters the central nervous system (CNS) early after primary infection and results in deficits, known as HAND, or HIV associated neurocognitive disorders, in 40-60% of HIV infected individuals despite successful combined antiretroviral therapy. Monocytes are critical cells involved in HAND as they bring virus into the CNS upon transmigration across the blood brain barrier (BBB). The mechanisms by which monocytes transmigrate across the BBB are not fully understood, but are proposed to be facilitated by the junctional proteins ALCAM, JAM-A, PECAM-1, and CD99. A subset of circulating mature monocytes with surface CD14 and CD16 is increased in HIV infected individuals and accumulates in the brain of those with HAND. This CD14+CD16+ monocyte subset is highly susceptible to HIV infection and has been found to harbor viral DNA. We developed a culture system to enrich for this subpopulation and showed that CD14+ CD16+ monocytes preferentially transmigrate across our in vitro model of the human BBB in response to CCL2, a potent monocyte chemoattractant elevated in the CNS of HIV infected individuals with HAND. We demonstrated that CD14+CD16+ monocytes have higher surface expression of ALCAM, JAM-A, PECAM-1, and CD99 relative to

CD14+CD16- cells which may promote their preferential entry into the CNS. Upon HIV infection, there is increased transmigration of the CD14+CD16+ subpopulation across the BBB in response to CCL2, compared to uninfected cells, which, with high levels of infection, disrupts BBB integrity. Blocking antibodies to ALCAM and JAM-A inhibited the transmigration of both uninfected and HIV infected CD14+CD16+ monocytes to at or below baseline levels, suggesting their importance in facilitating monocyte transmigration across the BBB. Targeting the increased junctional proteins present on mature, HIV infected CD14+CD16+ monocytes, which preferentially infiltrate the CNS, represents a novel therapeutic strategy which may reduce the ongoing chronic neuroinflammation which occurs during HAND.

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Suppression of HIV Associated Macrophage Neurotoxic Activity by Neurotrophin Receptor Stimulation

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Macrophages and microglia are thought to play a prominent role in the development of HIV-associated neuronal damage. While toxic actions have received considerable attention, there is also a growing appreciation for the potential beneficial effects of macrophages to change phenotypes. The ability to control macrophage actions has important therapeutic implications but little is known about the conditions that support the development of the widely varying phenotypes. Recent work from our lab has documented expression of the p75 neurotrophin receptor (p75NTR) and the nerve growth factor (NGF) receptor, Tropomyosin-Receptor Kinase A (TrkA) on human monocyte-derived macrophages and human monocytes. Exposure of the macrophages to HIV virions induced the secretion of neurotoxins and suppressed the secretion of IL-10 and several growth factors. Addition of the natural ligand, NGF, to macrophages resulted in widespread changes in the profile of secreted proteins and a decrease in HIV-induced neurotoxin production. These effects were mimicked by an experimental neurotrophin ligand, LM11A-31, which selectively targets the p75NTR. LM11A-31 promoted widespread changes in protein expression including TGF-associated proteins, IP-10, TNFa, IL-17, MMPs, IL-13 and various growth factors. A suppression of toxin production was also seen after blockade of PI3K with wortmannin or endosome acidification with chloroquine, whereas a block of M1 activation with gadolinium resulted in only a small suppression. NGF increased phosphoAkt in the macrophages but the increase could not account for the protective effects. Blockade of TrkA activation enhanced toxic activity of the medium suggesting that neurotrophin signaling may play an active role in the toxic outcome. Together these data show that macrophages express functional neurotrophin receptors that control activation and may offer new therapeutic targets for the control of inflammatory damage. Supported by NIMH Grant MH085606

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Single nucleotide polymorphisms within the HIV-1 LTR correlate with use of drugs in the DREXELMED HIV/AIDS Genetic Analysis Cohort

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HIV infection is prevalent among substance abusers. However, the effects of illicit drugs on HIV-1 disease progression are not well established. We evaluated the relationship between illicit drug use and HIV-1 disease progression in patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort in Philadelphia, PA. History of illicit drug, alcohol, and medication use, CD4+ and CD8+ T cell count, and viral load were performed approximately every 6 months. Drug abuse is common in the cohort, with 87.6% of patients admitting past use; 29.7%, currently abusing drugs; and 36.2% testing positive for drug use at the time of visit. Cocaine and marijuana use are heavily favored, with 80.5% of drug-using patients admitting to past or current cocaine use and 72% admitting to marijuana use. Most patients use multiple drugs simultaneously. The cohort can be categorized into non-users (PN), cocaine only (preferential, PC) users, cannabinoid only (preferential, PM) users, and multidrug users. Nonusers are more likely to remain on HAART (94.4%), whereas PC and PM are less likely (83.4% and 78%, respectively). Drug users exhibited lower current CD4+ T cell count, lower nadir CD4+ T cell, higher current viral loads, and higher peak viral loads than non-users. In addition, single nucleotide polymorphisms (SNPs) that are unique to cocaine, marijuana, or non-users were identified. In conclusion, illicit drug use appears to facilitate HIV-1 disease progression and selects for genetic variations unique to mono- and multi-using HIV/AIDS patient cohorts.

P245
Cooperative roles of NF-κB p65 and NFAT4 in regulation of JCV

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ABSTRACT The human polyomavirus JC (JCV) infects glial cells and causes the fatal central nervous system demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML is the direct result of a productive and lytic infection of oligodendrocytes and is mainly associated with severe immunosuppression. Infection by JCV is extremely common and after primary infection, JCV persists in a latent state. The rarity of PML suggests that the virus is tightly regulated. In our previous studies, we showed that NF-κB and C/EBPβ regulate JCV early and late promoter activities via a DNA control element, KB, which also mediates the stimulatory effects of proinflammatory cytokines such as TNF-α on JCV gene expression. Here, we show that NFAT4 and NF-κB (p65) interact at the KB element to co-operactively activates both JCV early and late transcription. This interplay is inhibited by C/EBPβ LIP and by agents that block the calcineurin/NFAT signaling pathway. Taken together, these observations indicate a regulatory role of the three transcriptional factors on JCV. The interplay between these three transcriptional factors may be important in controlling the balance between latency and reactivation of JCV infection, which leads to PML.

P246
Epigenetic regulation of JC virus

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ABSTRACT The human polyomavirus JC (JCV) is the etiologic agent of the rare devastating neurologic disease Progressive multifocal leukoencephalopathy. Globally, the prevalence of infection with JCV is high and JCV is believed to be persists in a latent state after primary infection. Even though JCV is common in the population, PML is extremely rare which suggest that the virus is very tightly regulated by cell-mediated immunity. Previously our laboratory and others have shown that the JCV promoter and its transcriptional activity are regulated by multiple transcriptional factors. Here we provide evidence that JC virus promoter activity under goes another level of regulation by epigenetic events involving protein acetylation. We have found that histone deacetylase inhibitors profoundly stimulate the transcription of both the early and late JC virus promoters in transient transfection experiments and in stable early and late reporter cell lines. This suggests that the acetylation status of histones may be an

important mechanism controlling the latency/ reactivation status of JCV.

P247

HIV-1 induces cytoskeletal alteration and reorganization during endothelial-monocyte interactions: modulatory role of CCR5

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Most HIV strains that enter the brain are macrophage (M)-tropic, thus use the C-C chemokine receptor 5 (CCR5) to bind and infect target cells. CCR5 is expressed in both monocytes / macrophages and human brain microvascular endothelial cells (HBMEC), and could be involved in endothelial-monocyte interactions and migration of infected cells into the central nervous system (CNS). Because the cytoskeleton is a network of protein filaments involved in cellular movement and migration, we investigated whether CCR5 and the cytoskeleton are involved in endothelial-monocyte interactions and migration of HIV-infected cells into the central nervous system (CNS). Using a cytoskeleton phospho-antibody array, we investigated the expression and activation of cytoskeletal proteins in infected and non-infected monocytes following interaction with HBMEC, in the presence or absence of CCR5 antagonists. Compared to non-infected monocytes, co-culture of infected monocytes with HBMEC increased the expression and phosphorylation of cytoskeletal proteins, with expression of thirteen proteins upregulated by 2-fold or more. Fourteen cytoskeletal proteins showed over 2-fold increased in phosphorylation, including merlin, cortactin, Vasodilator-stimulated phosphoprotein VASP, Extracellular signal-regulated kinases (ERK)1/2, Rac1/cdc42, and calcium/calmodulin-dependent protein kinase I CaMK1-a. The CCR5 antagonists TAK-799 and maraviroc prevented HIV-1-induced upregulation and phosphorylation of cytoskeletal proteins, prevented HIV-1 infection of monocyte-derived macrophages (MDM), and diminished viral-induced adhesion of monocytes to HBMEC. Analysis of molecular networks and canonical pathways associated with differentially expressed proteins in monocytes suggests that during monocytes-endothelial interactions, HIV-1 alter the expression and phosphorylation of proteins associated with cellular assembly and reorganization, cell signaling, cell morphology, and cellular movement, and CCR5 antagonists prevented these HIV-1-induced alterations. Data suggest that in addition to preventing MDM infection, CCR5 antagonists could prevent viral-induced cytoskeletal alterations, reduce HIV-induced adhesion and migration of monocytes across the blood-brain barrier and viral entry into the CNS.

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Biomarkers of disease activity in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)

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Human T-lymphotropic virus type-1 (HTLV-1) causes a chronic neuroinflammatory disorder termed HTLV-1 associated myelopathy / tropical spastic paraparesis (HAM/TSP). The clinical course and disease activity of patients with HAM/TSP are different among individuals. Therefore, the treatment plan should be designed based on these backgrounds of patients. However, there is little information about the natural history of HAM/TSP and biomarkers of disease activity that is associated with prognosis. As the candidate for biomarkers to evaluate the disease activity of HAM/TSP, HTLV-1 proviral load in PBMC, several cytokines and chemokines in serum or cerebrospinal fluid (CSF) are known to be increased in HAM/TSP patients. However, little is known which parameter of these candidates is most associated with disease activity. Therefore, we investigated the clinical course of 28 HAM/TSP patients without any history of treatment. Furthermore, we measured quantitatively the concentration of a series of cytokines and chemokines in serum and CSF, and HTLV-1 proviral DNA load in PBMC. Then, the level of these markers was evaluated for the correlation with disease progression. In HAM/TSP patients, the cell count, the level of neopterin, CXCL9 and CXCL10 in CSF was correlated with disease activity with statistical significance, while the proviral DNA load and serum markers were not. Importantly, the patients with increased levels of these biomarkers showed clinical progression, and the patients with low levels of these markers showed less or no progression. Therefore, based on the clinical course and laboratory findings, HAM/TSP can be

classified into 2 major groups; (1) active (with inflammation) and (2) inactive (less inflammation). Furthermore, 'active' patients presented two different clinical course; (1-A) rapidly progressive and (1-B) slowly progressive. And 'inactive' patients were clinically less- or non-progressive. This classification might be useful to determine the therapeutic strategy for patients with HAM/TSP.

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Cocaine-mediated upregulation of Glial Fibillary Acidic Protein: Role of Early Growth Response Gene 1

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Astrocytes, the specialized glial cells which makes up more than 60% of CNS cellular population, can response to all neural injuries ,viral infection and substance of abuse though a process referred to as reactive astrogliosis. This process is characterized by enhanced glial fibrillary acidic protein (GFAP) expression, cellular hypertrophy, and astrocyte proliferation. However, there have been relatively few studies designed to specifically access the effects of cocaine on astrocyte activation and dysfunction ,also the cellular and molecular mechanism underlying this process are still not completely defined. Here we investigated the role of cocaine in triggering quiescent astrocytes into reactive astrocytes by detecting the expression level change of GFAP both in vitro and in vivo. In cultured astrocytes, the exposure of rat and human astrocytes to cocaine results in the induction of GFAP both the mRNA and protein levels. In the some cells, by using sigma-1 receptor antagonist BD1047, we demonstrate that cocaine induce GFAP expression in astrocytes through the binding to its cognate sigma-1 receptor .we also provide evidence that cocaine-induced GFAP expression is through activation of ERK1/2 ,p38 and JNK signaling pathways by using the selective pharmacological inhibitors. Furthermore, this effect was regulated by activation of downstream transcription factor early-growth response 1(Egr-1). In In vivo study, we show that the GFAP expression are increased within 1 weeks after cocaine injection in mouse, together with enhanced asctrocyte proliferation, and Egr-1 up-regulation. Our results indicate that cocaine could activate astrocytes by induce GFAP expression and this effect is mediated through its cognate sigma-1 receptor and activation of MAPK dependent pathways and downstream transcription factor Egr-1. Understanding the regulation of GFAP expression may provide insights into the development of potential therapeutic targets for neuroinflammation associated with drug abuse.

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An in vitro model of non-productive VZV infection in human neurons

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Primary varicella-zoster virus (VZV) infection in humans produces varicella (chickenpox), after which virus becomes latent in ganglionic neurons. Analysis of the physical state of viral nucleic acid and virus gene expression during latency requires postmortem acquisition of fresh human ganglia. To develop an in vitro model of VZV infection of human neurons, we obtained highly pure (over 95%) terminally differentiated human neurons derived from induced pluripotent stem cells from Cellular Dynamics International and infected them with cell-free VZV (Zostavax vaccine). Fourteen days after infection, the cells appeared normal compared to human fetal lung fibroblasts (HFL) that developed a cytopathic effect (CPE) less than a week after VZV infection. Neither the tissue culture medium nor a homogenate prepared from VZV-infected neurons produced a CPE in HFL. The presence of the VZV genome in infected neurons was confirmed by qPCR using VZV primers for ORFs 4, 11, 28, 62 and 68. About 500,000 to 1 million copies of VZV genome per 100 ng of nucleic acid were detected in infected neurons. Non-productively infected neurons were also examined for the presence of transcripts corresponding to VZV ORFs 63, 9, 29 and 64. All four transcripts were found. The copy number of ORF 63 (the most abundant VZV transcript in latently infected human ganglia) was 60-100 times higher than that of ORF 9 (the most abundant VZV transcript in productively infected cells). This model provides a unique in vitro system to study the VZV-neuronal relationship and the potential to investigate mechanisms of VZV reactivation.

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Regulatory T cells (Tregs) in the Cerebrospinal Fluid from AIDS-associated Cryptococcal meningitis share a focused-T cell receptor (TCR) repertoire with classical CD4+ T cells and exert a profound inhibitory effect on the CD8+ T cell proliferation

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Background: Tregs, CD4 + CD25 + FoxP3+, accumulate within the CNS opportunistic infections (CNS-OIs). It is thought Tregs induced in OIs are cytokine and antigen-driven cells from classical effector CD4+ cells (induced Tregs) and share similar TCR repertoire with other CD4+ cells. To determine if Tregs in CSF are part of specific immune response to CNS-OIs, we assessed their TCR repertoire and modulatory function. Material and methods: Tregs TCR Vb chain repertoire was phenotyped by flow cytometry (FC) in CSF and blood samples from six patients with AIDS cryptococcal meningitis (CM). 24 Variable beta (Vb) chain families, covering over 70% of the TCR repertoire was assessed. The CSF Tregs inhibitory effect on T cell proliferation was tested using co-cultures of purified Tregs and anti-CD3/CD28 stimulated CD4+ or CD8+ T cells labeled with carboxyfluorescein succinimdyl ester to quantify cellular division by FC. Results: TCR profile of Tregs (CD4 + Foxp3 +) in CSF mirrored the Vb pattern of CD4 + T cells (CD4+ FoxP3-). Compared with the TCR repertoire in blood, some Vb chains were more frequently utilized by T cells in the CSF, suggesting specific recruitment or expansion within the CNS. In contrast, the TCR repertoire of the CD4-Foxp3- T cells (judged as CD8+ T cells) was different from Tregs and CD4 + T cells in CSF. At least one Vb chain was of common use by all T cells subpopulations in the CSF. The regulatory effect of Tregs from CSF on the T cell proliferation was higher on cerebral CD8+ T cells (60-80%) as compared with CD4+ T cells (20-40%). In contrast, both T cell subsets from blood were inhibited by Tregs isolated from the CSF compartment. Conclusion: This study provides evidence of an antigen-induced origin of Tregs trafficking into the CSF from AIDS-associated CM patients and reveals their immune inhibitory capability on LT CD8+ critical for controlling CM.

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Cathepsin B in HIV-infected CHME-5 microglia mediates neuronal apoptosis

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Human immunodeficiency virus type one (HIV-1) infects mononuclear phagocytes (monocytes, perivascular macrophages, dendritic cells, and microglia). These infected cells secrete soluble factors, cytokines and viral components that affect the tissue homeostasis. Prior investigations found that HIV-1 promotes the expression and secretion of the cysteine protease cathepsin B in monocytes derived macrophages (MDM), contributing to apoptosis in neurons. In this study, we asked if microglia cell line CHME-5 respond in the same way to HIV-1 infection and secrete cathepsin B that induce neuronal apoptosis. To test this hypothesis CHME-5 human microglia cell line was inoculated with HIV-1ADA at a MOI of 0.1 for up to 12 days. Serum-free supernatant of infected CHME-5 microglia was added to Human Neuroblastoma cell line (HTB-11) at 3, 6, and 12 days post-infection (dpi). Neuronal apoptosis was detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay at these three time points. Percentage of apoptotic neurons was assayed by Image-based Tool for Counting Nuclei (ITNC) from Image J software (NIH). Cathepsin B concentration and activity was measured from the CHME-5 supernatants. Parallel studies were conducted with primary human microglia. Our results show a low but productive HIV infection in CHME-5 microglia. A significant increase of percentage apoptotic neurons at 6dpi and 12dpi was obtained that was reverted with the specific cathepsin B inhibitor (CA-074) and by pre-treated medium with cathepsin B antibody. No differences in cathepsin B concentration or activity were found between uninfected and HIV-infected CHME-5 and primary microglia. Our results demonstrated that CHME-5 microglia secreted cathepsin B induces neuronal apoptosis in HIV-infected cells through an indirect mechanism. R01 MH083516-01, SNRP-NINDS-1-U54NS431, and CRR-2G12-RR003051/ NIMHHD 8G12-MD007600 Translational Proteomics Center.

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HIV viral protein Tat impairs early differentiation of neural stem cells through inhibiting NFκB signaling

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In the era of highly active antiretroviral therapy, a milder form of HIV-associated neurocognitive disorders (HAND) has become prevalent among the HIV-infected population. Both clinical and animal studies have demonstrated the impairment of neurogenesis induced by HIV infection or viral proteins. Neurogenesis is initiated from the self-renewing neural stem cells (NSCs) that produce committed daughter neural progenitor cells through asymmetric division. Previous studies demonatrated the inhibition of neuronal differentiation and promotion of astrogliosis by HIV viral proteins. However, the underlying molecular mechanisms remain poorly understood. Our recent studies demonstrated that NFκB signaling is required for NSC initial differentiation. To gain insight into the potential role of NFκB in mediating HIV viral protein-induced inhibition of neuronogenesis, we examined the effects of Tat on the NFκB activity, self-renewal and tripotential differentiation of NSCs. Primary neurospheres were cultured from neonatal mouse brain. Dissociated single cells were cultured as monolayer in matrigel-coated plate under proliferation or differentiation condition and treated with or without viral protein Tat for 24 hours. The activity of NFκB signaling was determined using adenovirus-mediated NFκB-luciferase reporter assay. The stemness frequency was determined by assessing the self-renewing and multipotent capability of NSCs using NeuroCult® neural colony-forming cell assay. We found that Tat treatment for 24 h had no effect on the constitutive NFκB activity and the stemness frequency of NSCs under proliferation conditions. However, the activation of NFκB signaling under differentiation condition (withdrawal of EGF/FGF2) was significantly inhibited by Tat exposure. Differentiation-induced reduction of the stemness frequency was significantly reversed by Tat treatment. These data suggest that Tat inhibited the initial differentiation of NSCs and such inhibition might result from the blockade of NFκB signaling. Impediment in the first rate-limiting step of neuronogenesis by HIV infection may lead to abberent neuronal lineage differenitation and consequent neurocognitive disorders in the milder HAND.

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Heroin Enhances HIV Infection by Inhibiting Anti-HIV miRNAs in Macrophages

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Opioids have a cofactor role in the immunopathogenesis of HIV disease. However, the mechanism(s) of their actions remains to be clarified. We thus investigated whether heroin, one of the most widely abused drugs, inhibits intracellular innate immunity in human blood monocyte-derived macrophages and facilitates HIV infection/replication. Heroin treatment was found to suppress the expression of endogenous IFN-α/β in macrophages. In addition, heroin treatment of macrophages impairs the expression of anti-HIV miRNAs and APOBEC3G, the newly identified intracellular restriction factors of HIV replication. These in vitro findings were supported by the in vivo studies showing that the heroin-dependant subjects had significantly lower levels of anti-HIV miRNAs in macrophages than the normal subjects. These data were in parallel with the observation that heroin treatment enhanced HIV infection of macrophages. Thus, heroin of abuse may serve as a facilitator in impairing intracellular innate anti-HIV immunity and promoting HIV infection and replication. Keywords: Heroin, HIV, miRNAs, IFN-α, macrophage

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Inhibition of long-term potentiation by HIV-1gp120 in rat hippocampal slices

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Human immunodeficiency virus type one (HIV-1) envelope glycoprotein 120 (gp120) plays an important role in the pathogenesis of HIV-1-associated neurocognitive disorders (HAND). It is shed off from virions and/or secreted from virus-infected mononuclear phagocytes (MP) and has the potential to diffuse and interact directly with surrounding and distant neural cells. Alternatively, it may act indirectly on local and distant neural cells by stimulating uninfected MP to release cellular neurotoxins. Previous studies from our laboratory and others have demonstrated that gp120 inhibited long-term potentiation (LTP) in the CA1 area of rat hippocampus. However, it is not clear whether gp120 inhibits LTP via acting on presynaptic terminals or

postsynaptic membrane, or both. To further investigate the site(s) of action, we studied effects of gp120 on both spontaneous and electrically evoked mini excitatory postsynaptic currents (mEPSCs) in the CA1 region of rat hippocampal slices. Bath perfusion of gp120 (200pM) significantly reduced spontaneous mEPSC frequency without change on mEPSC amplitude. Further analysis revealed that gp120 reduced the mean quantal content. Taken together, these results indicate that gp120 inhibits LTP through presynatic mechanisms. As LTP is widely considered a synaptic mechanism for learning and memory, the inhibition of LTP by gp120 may contribute to HAND pathogenesis.

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Anti-JCV Neutralizing Antibodies as a Potential Therapy for the Treatment of PML

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JC polyomavirus (JCV) is the causative agent of a demyelinating disease of the central nervous system, progressive multifocal leukoencephalopathy (PML). The incidence of PML is related to prior exposure to JC virus and an altered immune system as a consequence of HIV/AIDS, certain hematologic malignancy's, or the use of immunomodulatory drugs. Currently, there is no specific antiviral therapy that has been proven effective for the treatment of PML. JCV is a common pathogen in humans with roughly 55% of the population being seropositive for anti-JCV antibodies and 30% actively shedding virus in urine suggesting an asymptomatic infection of the urogenital track. To date, approximately 85% of virus isolated from the CNS of PML patients contains a mutation in the VP1 protein, which has never been observed in urine isolates of healthy or PML afflicted patients. The exact role of VP1 mutations is currently unknown but these distinct changes may alter receptor tropism or may represent a mechanism of immune escape by loss of recognition by neutralizing antibodies. Here we describe the binding and neutralization properties of monoclonal antibodies to the JC virus capsid protein VP1. We determined the affinity and specificity of these antibodies using virus like particles of wild type and various VP1 mutants isolated from PML patients. Furthermore, we established the neutralization capacity of these antibodies to inhibit infection by wild type viruses and viruses containing PML-associated VP1 mutants in an in-vitro assay. Modeling of the serum and parenchymal antibody concentration of a humanized neutralizing antibody was performed based on the pharmacokinetic profile in mice. The modeling suggests that in humans, dosing of a the antibody at the time of PML diagnosis may offer a protective window by limiting viral spread until immune reconstitution occurs by removal of the underlying immunosuppression. The discovery of an antibody that is successfully able to neutralize a wide range of JCV genotypes may provide a potential treatment for PML.

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