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ABSTRACTS

P1
The Effect of Rhinovirus Infection on Cough Receptors on Airway Sensory Nerves

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Studies of the interaction of viruses with peripheral nerves in the airways are lacking despite the association of virus infection with exacerbations in asthma, associated with debilitating chronic cough and lung congestion. Human rhinovirus (HRV), a member of the picornaviridae family, represents a major cause of the common cold worldwide. In non-asthmatics HRV infection rarely causes serious problems. However infection contributes to more than 60% of asthma exacerbations where the cough reflex is hyper-reactive and provokes severe coughing and wheezing. We investigated the effect of HRV infection on the cough reflex by determining the expression of receptors implicated in the cough process in sensory nerves. Transient receptor potential vanilloid 1 (TRPV1), TRPA1 and TRP melastatin 8 (TRPM8) have been shown to be involved in physiological and pathological aspects of cough. We hypothesised that HRV may directly and/or indirectly interact with these receptors on sensory nerves in the airways. Human neuroblastoma cells (IMR-32) undergo differentiation to acquire characteristics of peripheral nerve cells with positive neuronal markers. They exhibit a wide variety of voltage gated calcium channels representative of sensory nerve cells of the PNS and are positive for the three TRP receptors. The level of receptor mRNA was measured by quantitative real-time PCR at different time points post-infection with HRV or treatment with UV inactivated virus. Receptor expression at the protein level was determined by immunofluorescence and FACS. Up-regulation of TRPA1 and TRPM8 occurred at low multiplicity of infection (MOI) while at high MOI both live and UV-inactivated HRV down-regulated receptor expression. These results suggest that virus may both induce cough and interfere with cough related airway clearance which may depend on the level of infection at different time points *in vivo*. Understanding the interaction between HRV and "cough" receptors may indicate potential therapeutic targets and strategies to block these interactions.

P2
Proteinase-Activated Receptor-1 activation causes dorsal root ganglion neuronal injury in lentivirus distal sensory polyneuropathy

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Distal sensory polyneuropathy (DSP) is a frequent complication of lentivirus infections including both human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) infections of the peripheral nervous system. Proteinase-activated receptors (PARs) are G protein-coupled receptors that are implicated in the pathogenesis of neuroinflammation and neurodegeneration. Although PAR-1 is expressed on different cell types within the nervous system including neurons and glia, little is known about its role in the pathogenesis of inflammatory peripheral nerve diseases, particularly lentivirus-related DSP. Herein, we investigated the expression and functions of PAR-1 in the peripheral nervous system during lentivirus DSP. PAR-1 expression was increased in autopsied human dorsal root ganglia (DRGs) of HIV-infected patients. HIV infection induced PAR-1and IL-1β transcript expression in DRGs and monocyte-derived macrophages, which was associated with neurite retraction ($p < 0.05$). Activation of PAR-1 with a selective PAR-1-activating peptide also resulted in neurite retraction in conjunction with calcium activation in neurons ($p < 0.05$). PAR-1 mediated neurite retraction was reversed by nerve growth factor (NGF) in rat DRG cultures. FIV infection of cultured feline DRGs also resulted in neurite retraction together with the induction of PAR-1 and IL-1β. Exposure of FIV-infected DRGs to IL-β receptor antagonist prevented neuronal injury and IL-1β induction. *In vivo*, FIV infection was associated with PAR-1 induction and hyposensivity

to mechanical stimulation. These data indicated that activation and upregulation of PAR-1 in neurons likely contributes to DRG neuronal damage during lentivirus infections but might also provide new targets for future therapeutic interventions in HIV-related DSP.

P3
The aging brain and haart

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HIV-1 infected (HIV+) adults who benefit from highly active antiretroviral therapy (HAART) have plasma viral loads below detectable levels, maintain immune function without significant opportunistic diseases, and live to old age. Chronic comorbid conditions unrelated to HIV are more common in older HIV+ adults than their younger counterparts which may affect the clinical outcome of HIV infection. In the current era of HAART, HIV-associated neurocognitive disorders (HAND) continue to impact the clinical outcome of HIV infection, even in the context of systemic viral suppression. Asymptomatic neurocognitive impairment affects 21-30% of asymptomatic HIV+ individuals and mild neurocognitive disorder comprises 5–20% of the HIV+ population overall. Increasing evidence suggests that HAND in older adults in the HAART era represents "deficits of multiple etiologies including brain HIV variants and loads, aging-related cerebrovascular and neurodegenerative changes, chronic adverse effects of antiretroviral agents, and other comorbid factors. Also, methamphetamine dependence was shown to increase the risk of neuropsychological impairment in HIV+ patients. In summary, it will be important to: 1) understand the interactions among potential factors contributing to HIV-associated neural injury in old age; 2) identify a subset of HIV+ patients who are more susceptible to the development of HAND; and 3) identify accurate and practical biomarkers for detecting HIV-associated neural injury at stages earlier than the onset of clinical manifestation or poor neuropsychological performance, in order to allow disease-modifying therapeutic interventions, as well as evaluation of the response to treatment.

P4
The Role of Osteopontin in HIV Pathogenesis: *In vitro* and *Ex vivo* Analyses

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Osteopontin (OPN, protein; SPP1, gene) an early T-cell and macrophage activation marker is induced in several pathological conditions including HIV infection. Indeed, a previous study showed that OPN was increased in the cerebral spinal fluid (CSF) and plasma of SIV-infected macaques with encephalitis and in patients with HIV-associated dementia. To determine whether there is a correlation between OPN protein levels and HIV-associated neurocognitive impairment (HAND), we developed an ELISA assay to quantify OPN in plasma and CSF samples of HIV-infected patients from the Northeast AIDS Dementia Cohort (NEAD) and in HIV-negative controls. To determine whether there was a relationship between OPN levels and HIV replication we quantified the SPP1 transcript in HIV-infected human macrophages. SPP1 transcript was significantly increased in HIV-infected macrophages. Knockdown of OPN expression in primary macrophages significantly reduced HIV-1 replication. To determine the impact of OPN on discrete steps of the HIV life cycle, a heterologous cell culture system using TZM-bl cells that did not express OPN was developed. Ectopic expression of OPN in TZM-bl cells enhanced HIV infectivity, syncytium formation, viral transcription and particle production. Infection of TZM-bl cells with HIV through an endocytic pathway did not abolish OPN-mediated enhancement of viral replication suggesting that OPN affects a step (s) in the HIV life cycle occurring after viral entry. In HIV-infected TZM-bl-OPN expressing cells, increased degradation of the NF-κB inhibitor, IκBα, was observed compared to cells infected only with HIV. In addition, the nuclear-to-cytoplasmic ratio of NF-κB was significantly increased in HIV-infected cells over-expressing OPN compared to uninfected or cells infected only with HIV. These findings suggest that HIV-1 has evolved molecular mechanisms to exploit for its own advantage OPN-mediated signaling pathways known to play a role in cellular immunity.

P5
CSF Monocyte Reservoirs in HAND Development and the Possible Role of Insertional Mutagenesis

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Background: HIV-associated neurocognitive disorders (HAND) remain a concern despite antiretroviral therapy (ARTs). Identifying biomarkers has been challenging but detection of HIV DNA in T-cell and monocyte reservoirs provides some promise

that may also contribute to understanding mechanisms leading to HAND. Furthermore, individuals with HAND have shown to contain higher amounts of peripheral activated monocytes, CD14+/16+, with higher HIV DNA levels in comparison to other cell types. Additionally, HIV may preferentially integrate near or within particular genes and cause insertional mutagenesis where gene expression becomes dysregulated. The current study focuses on CNS compartments in HAND pathogenesis by measuring HIV DNA within various CSF subsets and identifying their integration sites. Materials: Six CSF samples were obtained from the Hispanic-Latino Longitudinal Cohort of HIV-seropositive women from the University of Puerto Rico who presented with HAND (n = 3) and normal cognition, NC (n = 3). The CSF cells were sorted into CD14-, CD14+/16+, and CD14+/16- fractions through fluorescent activated cell sorting; DNA was extracted and a quantitative multiplex PCR assay was used to determine HIV DNA copies per cell. The DNA was also used for a DNA walking assay to identify integration sites. Results: For majority of the samples, HIV DNA was lowest in CD14- cells and highest in CD14+/16- cells ($p = 0.03$); however, subjects with HAND had higher percentages of CD14+/16+ compared to those with NC, 12.7% versus 0.2% respectively. Common integration sites found across subsets included genes involved in apoptosis, macrophage behavior, and transcriptional regulation. Conclusion: The detection of high HIV DNA in CD14+/16- could indicate latent viral reservoirs where HIV evolution and compartmentalization may be occurring and the higher percentages of CD14+/16+ cells in HAND individuals may suggest ongoing damage occurring though inflammation. Identification of common integration sites suggests that insertional mutagenesis may be involved in HAND neuropathogenesis. (Support by AI081450, NS053345, P20RR011091, S11NS046278, U54NS43011, P20RR11126).

P6
Determination of HIV-1 envelope and long terminal repeat sequence variation within the DREXELMED HIV/AIDS Genetic Analysis Cohort as a predictive tool for disease severity

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Throughout human immunodeficiency virus type 1 (HIV-1) infection, the error prone nature of viral reverse transcriptase results in extensive alteration of the viral genomic architecture that may facilitate immune escape and possibly enhance the ability of HIV-1 to infect and persist in certain cell types. It is known that HIV-1 exhibits different biological properties at different stages of infection. Analysis of isolated virus from individual patients throughout the course of their disease progression reveals that the prevalence shifts from being predominantly macrophage-tropic during the stable asymptomatic stage to being non-macrophage-tropic during progression towards acquired immunodeficiency syndrome (AIDS). Previous studies of the HIV-1 long terminal repeat (LTR) have shown that specific variations within the C/EBP site I and Sp site III (C-to-T change as positions 3 and 5 of the binding sites, respectively) isolated from the peripheral blood (PB) correlate with disease progression and HIV-associated dementia in the pre-HAART era. The 3T/5T-containing LTRs may be representative of LTR genotypes that are preferentially retained because of specific functional properties that may be involved in disease pathogenesis in the PB, CNS, and perhaps other compartments. We propose that selected LTR sequences may be co-selected with specific envelop sequences and play an important role in determining the extent of disease progression and cell types infected within the PB and brain. Herein, the cloning of a 4.4 Kb fragment of the HIV-1 provirus is described, including sequences extending from Vpr through the 3' LTR, from patients enrolled in the DREXELMED HIV/AIDS Genetic Analysis Cohort. Presented here is the genomic sequence analysis of specific configurations of HIV-1 LTR in association with specific envelope genotypes, disease severity, and HIV-1-associated neurologic disease.

P7
The use of a luciferase immunoprecipitation systems (LIPS) assay in the detection of anti-HTLV-I antibody responses in HAM/TSP, ATL, and asymptomatic HTLV-I carriers

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A number of diseases including adult T cell leukemia/lymphoma (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), are caused by human T-cell lymphotropic virus type I (HTLV-I) infection. In patients with ATL or HAM/TSP, the antibody titer and provirus load are elevated compared to levels in asymptomatic carriers (AC). HTLV-I positive asymptomatic carriers are also distinguished by a low expression of Tax-specific antibody in comparison to HAM/TSP patients. Although there are methods to determine if a patient is infected with HTLV-I, these tests do not differentiate between asymptomatic carriers, HAM/TSP patients, or ATL patients. Moreover, immunoassays, which detect anti-HTLV-I antibodies are typically non-quantitative. Recently, the Luciferase Immunoprecipitation System (LIPS) was developed as a highly sensitive technology that can efficiently detect antibody responses utilizing mammalian cell-produced, recombinant fusion protein antigens for efficiently evaluating antibody responses to multiple viral proteins. LIPS was used to profile antibody responses to the three major immunodominant regions of HTLV-I (env, gag, tax) to evaluate the anti-HTLV-I antibody responses. Interestingly, HAM/TSP patients showed significantly higher titers of anti-Env and anti-Tax antibodies, compared to AC and ATL patients. As an extension of our preliminary LIPS analysis of small groups of HTLV-I infected patients and controls, we utilized the LIPS assay to detect anti-HTLV-I antibodies in samples from 576 persons from Jamaica. These included normal donors, AC, ATL patients, HAM/TSP patients, and HTLV-I/II western blot seroindeterminate donors. The assay's high throughput, as well as its ability to quantify antibody titers to three of the proteins of HTLV-I, prove its potential as an extremely useful tool in clinical investigations of HTLV-I related diseases. The assay may also be used to track disease progression in correlation with increases in quantitative values of anti-HTLV-1 antibodies, as well as to distinguish between HAM/TSP patients and asymptomatic carriers.

P8
The Contribution of Antiretroviral Therapy to Neuronal Damage and Death as a Mediator of Cognitive Decline in HIV-Associated Neurocognitive Disorder

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The use of combined antiretroviral therapy (cART) has led to a significant reduction in the incidence of HIV-associated dementia (HAD), the most severe form of HIV-associated neurocognitive disorders (HAND) afflicting AIDS patients. However, the prevalence of HAD and more subtle CNS dysfunctions remains high. While increased longevity of HIV-infected populations is thought to contribute to alterations in HAND pathology, other risk factors, such as cART toxicity in the CNS, as has been previously observed in peripheral nerves, has not been investigated. Initial studies in our laboratory indicate therapeutic concentrations of ART drugs are toxic to primary cortical neurons inducing mitochondrial depolarization and neuronal endoplasmic reticulum (ER) stress response, the latter of which has been associated with altered APP processing and beta-amyloid generation in Alzheimer disease (AD). Thus, we hypothesized that in conjunction with HIV, chronic cART contributes to neurodegeneration in HAND by altering APP processing, and contributing to AD-like pathology. We treated primary rat cortical neurons individually or in combination, with commonly prescribed HAART compounds from two classes, nucleoside reverse transcriptase inhibitors (NRTI), and HIV protease inhibitors (PI). Our studies demonstrate that PIs lead to increased expression of APP and beta secretase protein levels in addition to oxidative and ER stress markers. Furthermore, increased beta-amyloid (1-40) and (1-42) secretion into the media were observed in cultures exposed to therapeutically relevant drug combinations. Importantly, ER stress activation alone increases APP expression in primary neurons. Finally, immunofluorescent staining of postmortem hippocampal tissue from HIV-infected patients showed the presence of oligomeric forms of beta-amyloid in addition to increased APP expression, while amyloid plaques were not detected. These findings suggest ER stress activation as a mechanism to alter APP metabolism through increased beta secretase activity, contributing to the shift in the clinical picture seen among the HIV-positive population.

P9
Simian Immunodeficiency Virus Infection in the Brain Leads to Differential Type I Interferon Signaling During Acute Infection

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Using an accelerated and consistent simian immunodeficiency virus (SIV) macaque model of HIV associated neurological disorders, we have demonstrated that virus enters the brain during acute infection. However, neurological symptoms do not manifest themselves until late stage of infection, indicating that immunological mechanisms exist within the central nervous system (CNS) that control viral replication and associated inflammation. We have shown that interferon beta, a type I interferon central to viral innate immunity, is one of the major cytokines present in the brain that is responsible for establishing SIV in a latent state. We have now examined interferon alpha, another type I interferon. In classic type I interferon signaling, interferon beta signals through the interferon α/β receptor in an autocrine and paracrine manner, and signaling leads to expression of interferon alpha. Surprisingly, we have found that when interferon beta is up regulated during acute SIV infection in brain, interferon alpha is down regulated as compared to uninfected controls. We demonstrate that down regulation of interferon alpha is coupled with suppression of signaling molecules downstream of the interferon receptor, namely Tyk2, STAT1 and IRF7, as indicated by either lack of protein phosphorylation, lack of nuclear accumulation, or transcriptional and/or translational repression. These data provide a novel observation that during acute SIV infection in the brain, there is a branch in the interferon signaling pathway that results in the down regulation of interferon alpha. It is possible that this differential regulation of type I interferons is a mechanism intrinsic to the CNS. These results indicate that the type I interferon signaling pathway is tightly regulated in the brain, where certain arms of the antiviral responses are induced while others are suppressed.

P10
Elevated CSF Neurofilament H levels in HIV-associated neurocognitive disorder

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Background: Recent studies suggest that >50% of HIV infected patients virologically controlled on antiretrovirals may have HIV-associated neurocognitive disorder(Heaton R.,2010). Development of sensitive surrogate markers of neuronal injury are critical for clinical trials with neuroprotective agents for this population. Methods: Western blots were done on CSF samples of HIV infected patients (n = 76) with varying levels of neurocognitive impairment and compared to patients with Multiple Sclerosis (MS;n = 9),and patients with "other" neurological diseases(OND; n = 9). Neurofilament heavy chain was visualized using anti-neurofilament heavy chain (H) antisera (Millipore) and anti-rabbit680 (Licor) and imaged on the Odyssey Infrared Imaging system. Using Quantity One Software levels of neurofilament H were semi-quantitatively determined. Results: CSF levels of neurofilament H were significantly higher in all HIV-infected patients compared to patients with MS or OND. There was no significant difference between the neurofilament levels of individuals who were neurocognitively normal, had asymptomatic neurocognitive impairment(ANI)or had mild neurocognitive dysfunction(MND). Surprisingly however, patients with HIV associated dementia (HAD)had lower levels of neurofilament although still significantly higher than that of individuals with MS or OND. Conclusions: Surprisingly high levels of neurofilament H were found in the CSF of HIV- infected individuals who were neurocognitively normal suggesting that neuronal injury occurs early in the course of infection and by the time neurocognitive impairment becomes clinically obvious, significant injury may already have occurred resulting in a "burn out" effect. Thus neuroprotective treatments need to be initated early to minimize the advancement of neurological symptoms. Supported by NIMH Center and GCRC.

P11
Neuro-AIDS- due to infection or immunmodulation?

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Objectives: Human immunodeficiency virus (HIV) can provoke peripheral and central nervous system disease. With respect to central nervous system disease a consensus conference from 2005 differentiated asymptomatic neuro-cognitive impairment (ANI), mild neuro-cognitive deficit (MNCD) and HIV-associated dementia (HAD). To date, it is unclear whether CNS disease is due to infectious or immunological mechanisms. Methods: In a prospective cross-sectional analysis, 33 HIV-positive, homo-and bisexual men from early (CDC A 1+2, B1+2) and late (CDC A3, B3, and C 1-3) stages without and with highly active antiretroviral therapy (HAART) were examined neuropsychologically and with MRI scans. Additonally, they underwent venous and lumbar puncture. Routine laboratory parameters (CD4+-cell count and HI-viral load in blood, cells, protein content,

glucose, lactate and HI-viral load in cerebrospinal fluid (CSF)) as well as cytokine patterns in CSF have been analysed. Results: In early stages of HIV-infection inflammatory signs (CSF cell elevation and high IgG-index) pre-dominated; CSF viral load and pro-inflammatory cytokines were up-regulated in untreated and down-regulated in treated early stage patients. In late stages there were no acute inflammatory markers up-regulated in CSF, but protein content and lactate were high, especially in late stage patients without HAART. HI-viral load was negative or low in treated and very high in untreated late stage patients. Anti-inflammatory cytokines were upregulated in both, patients on and not on HAART. Conclusions: Obviously, in early stages of the infection, HIV provokes an acute inflammatory reaction which is only in part suppressed by HAART. In late stages, chronic inflammation plays the major role independent from the presence of the virus and HAART. These results must be confirmed in bigger patient cohorts.

P12
HIV gp120 accelerates amyloid-Abeta production by promoting the interaction of BACE with APP in ceramide-rich membrane domains

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Amyloid-beta(Abeta) deposition appears to be accelerated in some patients infected with HIV. To study a potential mechanism for this phenomenon we created a triple transgenic line of mice that express gp120 with knock-in mutations in human amyloid precursor protein (APP) and human presenilin 1 (PS1). In 2-month old mice, diffuse amyloid-beta 1-42 (Abeta) deposition was apparent in the hilus region of APP/PS1 and APP/PS1/gp120 mice. By 6 months of age, the number and size of Abeta plaques and activated microglia associated with the Abeta were increased in APP/PS1/gp120 mice compared with APP/PS1 mice. There was a trend that brain levels of the bioactive lipid ceramide were elevated in gp120- and APP/PS1/gp120 transgenic mice, but not in APP/PS1 mice. To determine the mechanisms by which gp120 promoted A formation we exposed human neuroblastoma cells expressing human APP and primary neurons to gp120IIIB. BACE expression and APP maturation were increased by gp120, and gp120 dose dependently (10-1000 pM) increased beta-(BACE) and gamma-secretase activities within 6 h, and the production of Abeta within 24 h. There was no effect of gp120 on beta-secretase activity except at the highest dose of gp120 tested. Based on evidence that pro-amyloidogenic processing of APP occurs in ceramide-rich lipid rafts, and findings that gp120 enlarges rafts by increasing ceramide, we next determined if gp120 promoted the physical association of BACE and APP in these microdomains. A 6 h treatment with gp120 increased the co-localization of APP and BACE in lipid rafts. When we prevented gp120 from increasing lipid raft size by inhibiting de novo ceramide synthesis, BACE activity and Abeta production were reduced. These data suggest that gp120 promotes beta-site cleavage of APP by increasing the amounts of BACE and APP and by promoting their association in lipid rafts. Moreover,our findings suggest a mechanism for accelerated amyloidogenesis and cognitive impairment in HIV dementia.

P13
HIV-gp120 Induces Up-regulation of the α7-nAChR: Implications for HIV Associated Neurocognitive Disorder

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Over 30 million people are infected with HIV. Approximately 30% of HIV+ patients develop HIV associated neurocognitive disorder (HAND), a neurodegenerative disease that leads to severe cognitive, motor and behavioral disturbances. The molecular mechanisms by which HIV infection leads to neurocognitive decline are not fully understood. Several hypotheses have emerged to explain the development of HAND, including neuroinflammation mediated by infected microglia and direct neuronal toxicity by HIV proteins. HIV-gp120, a neurotoxic glycoprotein on the envelope of HIV-1 interacts with several membrane receptors including CD4, CCR5, CXCR4 and nicotinic acetylcholine receptors (nAChRs). However, the role that nAChRs may play in the development of HAND has not been investigated. We utilized SH-SY5Y neuroblastoma cells as a model to study the effects of gp120 on the expression and function of the α7-nAChR. Confocal microscopy experiments with fluorescently labeled α-Bungarotoxin (α-Bgtx), a selective ligand for nAChRs, show that gp120-treated cells present a 50-60% increase in α-Bgtx binding. In addition,

electrophysiological recordings present a 77-167% increase in ACh-stimulated currents. A pharmacological approach using AMD3100, SDF1 and PD98059, suggests that CXCR4 mediated activation the MAP Kinase pathway is responsible for the gp120-induced up-regulation of α7-nAChRs. In addition, qRT-PCR experiments on gp120-treated cells show an increase in α7-nAChR and EGR-1 (a transcription factor for the α7-nAChR) mRNA levels. The α7-nAChRs are ligand-gated channels highly permeable to Ca2+ and excess intracellular Ca2+ can induce apoptosis. Interestingly, blocking α7-nAChRs with α-Bgtx reduces gp120-induced apoptosis in SH-SY5Y cells. Moreover, qRT-PCR experiments on HIV+ post-mortem brain tissue from donors with or without HAND suggest higher levels of α7-nAChRs mRNA in HAND+ samples. In summary, our data suggest that HIV-gp120 mediated activation of the CXCR4 receptor induces up-regulation of α7-nAChRs causing cell death. These suggest that α7-nAChRs may contribute to the development of HAND and could be an important therapeutic target.

P14
CSF sphingomyelin/ceramide C24:1 ratio can predict declines of neurocognitive status in HIV-infected patients

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Sphingolipids are bioactive lipids that play important roles in regulating cellular signaling. Perturbations in sphingolipid balance that result in the accumulation of one or more sphingolipid species can cause cellular dysfunction and trigger neurodegeneration. In this study we sought to determine if CSF sphingolipid content could be used as a surrogate marker for cognitive status in patients from diverse ethnic and geographical sites and to determine the relationship of these CSF biomarkers to neuropathology. The study group consisted of 292 HIV-infected men and women sampled from the Johns Hopkins, CHARTER, Hawaii and Puerto Rico cohorts. Longitudinal CSF samples from two consecutive clinic visits were available on 184 HIV-infected subjects. Control samples were collected from HIV-negative subjects (n = 29) and patients with normal pressure hydrocephalus (n = 27). In longitudinal analyses of HIV-infected subjects, a specific decrease in the sphingomyelin/ceramide ratio for C24:1 predicted a decline in neurocognitive status. Each log-unit decrease in this ratio was associated with a 39% ($p = 0.047$) increased odds of worsening cognitive status. However, after a worsening in cognitive status had occurred, sphingomyelin/ceramide ratios for several species including C24:1 were increased. These data suggest that sphingomyelin/ceramide balance becomes perturbed early in the neurodegenerative process, reflecting an increase in ceramide content. After neurodegeneration has stabilized, this balance is again shifted to reflect a decrease in ceramide content, perhaps due to the metabolism of ceramide to other products. In preliminary analysis of MRI data we found positive correlations between ceramide and sphingomyelin levels and choline in frontal and parietal regions. Since sphingomyelin synthesis involves the addition of a phosphocholine group to ceramide, these data suggest that imaging brain choline may be an indirect measure of sphingolipid content. These findings identify a lipid-based biomarker that can predict worsening cognitive status in a diverse group of HIV infected subjects.

P15
Hypertriglyceridemia in ART-treated HIV+ individuals: Potential impact on HIV sensory polyneuropathy

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HIV infection, aging and combination antiretroviral therapy (CART) are associated with hypertriglyceridemia (hyperTG) and oxidative stress, which may augment risk for sensory neuropathy (SN). We evaluated the prevalence and clinical correlates of SN among HIV+ and HIV- research volunteers at a single baseline (BL) visit. SN was defined as ≥1 clinical sign on a standardized exam including reduced distal vibratory or sharp sensation, and reduced ankle reflexes. Blood TG levels were measured. Concurrent

medications and concomitant SN risk factors were evaluated including age, diabetes mellitus, nadir and current CD4 count, history of alcohol abuse/dependence and prior or current use of HIV protease inhibitors, potentially neurotoxic dideoxynucleoside antiretrovirals, and statins, typically given to lower cholesterol and TG. Of 436 HIV+ subjects averaging 52 years old, most were men (86%), on CART (75%), with good virologic suppression (median plasma HIV RNA 1.7 log10 copies/ml) and immune recovery (median CD4 current/nadir, 458/105 cells/mm3). 27% of HIV+ subjects met criteria for SN and 48% of these reported pain, paresthesias or numbness. HIV+ subjects had higher mean TG levels than HIV- (245 ± 242 mg/dl vs 160 ± 97 mg/dL; $p < 0.003$). Among HIV+ individuals, those with TG levels in the highest tertile showed significantly greater risk of SN (odds ratio [OR] 2.9; 95% confidence interval [CI], 1.7-4.9) compared to those in the lowest tertile (reference). Risk remained elevated after adjusting for medication use and for other factors significantly associated with SN including age, nadir CD4 and diabetes mellitus (adjusted OR 2.9 CI, 1.4-6.1). Conclusions: HIV+ individuals frequently have elevated TG levels. After adjusting for concurrent SN risk factors and medications, SN frequency was highest among HIV+ individuals with TG levels in the top tertile. Clinical implications: Future studies should evaluate whether lowering TG by diet or medical therapy can protect against disabling SN.

P16
Immunosuppression Increases Latent Infection of Brain by JC Polyomavirus

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Background. Progressive multifocal leukoencephalopathy (PML) is caused by reactivation of JC polyomavirus (JCV). Increased JCV reactivation in kidney, as indicated by JCV viruria is reported during immunosuppression however the relevance of systemic to neural reactivation remains unknown. Patients and Methods. Brain and kidney from 138 immunologically well characterised non-PML patients were assessed for JCV LT and VP1 DNA using nested PCR. Autopsy findings were reviewed and all brains underwent full neuropathological examination. Three pathologically proven PML cases served as controls. Patients were classified as immunosuppressed if they were on immunosuppressive medication, were HIV positive with CD4 count below 500 cells/mm^3, or if they had widespread metastatic tumour, multi-systems failure or septicaemia in conjunction with a significantly decreased lymphocyte count ($< 0.5 \times 10^9$ cells/L). Results. JCV LT DNA was detected in 31% of kidney and 30% of brain from non-PML patients. Of the non-PML patients with brain JCV LT DNA, 66% did not have kidney JCV LT DNA. Brain JCV LT DNA was independent of presence of kidney JCV LT DNA ($p = 0.69$). JCV VP1 DNA was detected in 12% of non-PML kidney and 8% of non-PML brain. JCV LT DNA was more likely to be found in the kidney ($p < 0.001$) and brain ($p = 0.009$) of immunosuppressed than immunocompetent patients. A higher proportion of HIV/AIDS patients were JCV LT DNA positive in the kidney and brain as compared to other immunosuppressed patients. HIV/AIDS patients with brain JCV LT DNA had lower CD4 counts than those without brain JCV LT DNA ($p = 0.05$). Conclusion. Immunosuppression drives increased brain JCV latency independent of systemic latency. A small percentage of patients with latent JCV LT DNA show VP1 DNA, raising the possibility of late gene replication in a minority of severely immunosuppressed individuals.

P17
Infection of Peripheral Blood Mononuclear Cells by JC Polyomavirus is Independent of Immunosuppression

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Background: JC polyomavirus (JCV) is the aetiological agent of progressive multifocal leukoencephalopathy (PML), a deadly demyelinating disorder of the central nervous system occurring in immunosuppressed patients. Previous studies have highlighted the involvement of lymphocytes in JCV dissemination during PML however it is unclear whether lymphocytes are sites of viral latency during immunocompetence or simply transient vector for viral dissemination. Methods. Spleen from 37 autopsy patients and peripheral blood mononuclear cells (PBMCs) from 70 blood donors were assessed for JCV large T (LT), regulatory region (RR) and viral protein (VP)1 DNA using PCR. Autopsy and clinical findings for all patients were reviewed to exclude presence of PML in JCV DNA positive samples. Patients were classified as immunosuppressed if they were on immunosuppressive medication, were HIV positive with CD4 count below 500 cells/mm3, or if they had widespread metastatic tumour, multi-systems failure or septicaemia in conjunction with a significantly decreased lymphocyte

count (<0.5 × 10^9 cells/L). Results. JCV LT DNA was detected in 84% of non-PML spleen and 94% of non-PML PBMCs. The presence of JCV LT DNA in both spleen and PBMCs was independent of patient immune status. Analysis of RR DNA revealed the presence of archetypal JCV in all cases. The presence of JCV VP1 DNA was highly dependent on patient immune status ($p = 0.007$), detected in 35% of non-PML spleen and 33% of non-PML PBMCs from immunosuppressed patients. Patients with HIV/AIDS were significantly more likely to have JCV VP1 DNA than any other immunosuppressed patient group ($p = 0.02$). The CD4 counts of HIV/AIDS patients with JCV VP1 DNA were significantly lower than those of HIV/AIDS patients without JCV VP1 DNA ($p < 0.001$). Conclusion. Latent JCV persists in lymphocytes independent of patient immune status. The dependence of JCV VP1 DNA on patient immune status suggests that immunosuppression modulates viral latency.

P18
Risk of JC Virus Reactivation in Pediatric Patients with Crohn's Disease

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Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) of unknown etiology that results in significant morbidity and health care costs. The disease seems to occur when the intestinal immune cascade and inflammatory pathways are triggered by an antigen in genetically susceptible individuals, resulting in the clinical symptoms. Various medications, including 5-aminosalicylates, antibiotics, corticosteroids, and immunomodulators have traditionally been used to control inflammation. Recently, the introduction of biologic agents has produced an astounding transformation by halting or slowing the progression of CD, and the tumor necrosis factor-α inhibitors, such as infliximab and adalimumab, and adhesion molecule inhibitors, such as natalizumab, are used in the CD treatment. Although the remarkable efficacy of biological therapy has resulted in significant success in CD management, susceptibility to infections remains a concern. The human polyomavirus JC (JCV) reactivation in CD after biological therapy and its association with progressive multifocal leukoencephalopathy (PML), has been found in 3 patients with multiple sclerosis (MS) and CD, linked to treatment with natalizumab. Therefore, the use of natalizumab was suspended but was subsequently resumed for MS and for CD, only through a special restricted distribution programme. On this basis, the purpose of our study was to assess the risk of reactivation of JCV in 23 patients with CD, who did not respond to standard therapy and subjected to infliximab infusion, attending the Department of Pediatrics of "Sapienza" University of Rome. As controls, we enrolled 19 patients with CD responding to standard therapy and 14 patients with other intestinal diseases. During monitoring, the patients were subjected to blood and urine sampling and to endoscopy for biopsies, to assess the presence of JCV DNA. The samples positive for JCV were subjected to sequencing analysis to search the presence of mutations within transcriptional control region (TCR). The results showed the presence of JCV DNA in 3/23 patients treated with infliximab and in 1/19 patients responsive to standard therapy. The major finding of this study is that monitoring of JCV infection revealed a viral reactivation 8 months after starting infliximab therapy and none of the patients evidenced symptoms suggestive of PML. Analysis of the TCR sequences obtained from plasma and urine of examined subjects, showed a structural organization similar to that of the archetype. In contrast, analysis of TCR sequences obtained from biopsies of patients with CD has revealed the presence of rearranged structures. Although our result must be validate in larger groups of patients and with longer follow-up period, it enriches the data present in literature about the possible viral reactivation in response to infliximab treatment.

P19
The role of neurofibromatosis type 2 in regulation of JC virus gene expression and T-antigen mediated transformation

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The human polyomavirus, JC virus (JCV), is the causative agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) and also belongs to the growing list of potential human oncoviruses. JCV displays a strong tropism toward glial cells and typically replicates only under

immunosuppressive conditions. 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, which can also function as an oncogene by inactivating tumor suppressor proteins including p53 and pRb. 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 nuclear binding partner for T-antigen. NF2 is a cytoplasmic scaffolding protein shown to have tumor suppressor function, and has been localized to the nucleus where it's role is less clear. The association with T-antigen in the nucleus of neuronal origin tumors suggests a potential role for NF2 in regulating expression of the JCV promoter. To investigate this possibility, human glioblastoma U87-MG cells were cotransfected with JCV early or late promoters, along with T-antigen and NF2. Results show that NF2 in both the presence and absence of T-antigen, reduced viral promoter activity in a reporter assay suggesting that NF2 acts as a negative transcriptional regulator. However, preliminary studies could not detect NF2 in direct association with the JCV promoter as demonstrated by a chromatin immunoprecipitation (ChIP) assay, suggesting that NF2 interacts with T-antigen independent of the JCV promoter. These results suggest that NF2 may act as a negative regulator of JCV gene expression which may have implications in suppression of JCV reactivation as well as in JCV-induced tumorigenesis.

P20
Human beta-defensins and chemokine receptors in the central nervous system: potential factors in HIV infection

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Human beta-defensins (hBD) are antimicrobial peptides secreted by epithelial cells and astrocytes. hBD2 and -3 inhibit R5 and X4 isolates of HIV by dual mechanisms that include direct virion inactivation and intracellular inhibition. We have shown that intracellular inhibition is mediated through the chemokine receptor CCR6, a cell surface receptor for hBD2. CCR6 engagement triggers intracellular signaling that induces elevated expression of the antiviral protein APOBEC3G. CCR6 is expressed on cell types that are most susceptible to HIV infection, including memory CD4+ T cells, Th17 cells, α4β2 cells, and dendritic cells. Importantly, CCR6 has also been reported to be expressed by microglia. We have analyzed CCR6 expression on the peripheral blood counterpart of microglial cells, i.e., monocyte-derived macrophages (MDM), and find that its expression is readily induced by LPS stimulation. Recent reports have shown that CCR2, expressed on macrophages, is also a receptor for hBD2 as well as MCP-1. We and others have shown that MCP-1 levels are positively correlated with the severity of HIV-associated dementia. hBD2 competes with MCP-1 for CCR2 binding and activation, and it inhibits MCP-1-mediated chemotaxis. This suggests that defensins could mitigate CNS infection by HIV in two ways, one through intracellular inihibition of HIV replication in microglia and T cells by APOBEC3G and the other by minimizing migration to the CNS and activation of CCR2+ macrophages. Further study of these hBD2-mediated antiviral activities could lead to a better understanding of HIV infection of the CNS as well as identify potential targets with which to treat infection and its sequelae in the CNS and periphery. This study was supported by awards R01NS066842 from NINDS, NIH (A.G-D). Mark K. Lafferty was a trainee under Institutional Training Grant T32AI007540 from NIAID, NIH.

P21
VZV reactivates subclinically in 21% of HIV+ individuals

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Multiple neurologic and ocular disorders can result from reactivation of varicella zoster virus (VZV) in the absence of rash in both normal and HIV-infected individuals. Because the incidence of zoster is increased in HIV+ individuals, we determined the prevalence of subclinical VZV reactivation. Paired serum and CSF samples were obtained from 180 HIV + subjects who attended a single neurology clinic. The mean interval between HIV diagnosis and CSF collection was 4.6 years, ranging from 3 days before until 17 years after. There were 151 (84%) men and 29 (16%) women ranging in age from 18 to 71 years (mean age 40 years). Of the 180 HIV+ subjects, 136 (76%) had AIDS and 17 (9.4%) were non-AIDS; the AIDS status was unknown in 27 (14.6%). Antibodies directed against all VZV glycoproteins were detected

by ELISA in CSF of 45/180 individuals. The antibody specificity index (ASI) was then calculated to confirm intrathecal synthesis of anti-VZV IgG. Of the 45 subjects with anti-VZV glycoprotein antibodies in their CSF, 43 had a positive ASI (≥1.5), 38 of whom had no known history of zoster. In the remaining five HIV+ patients with a history of zoster, the VZV+ ASI was detected from 2 days to 6 years after zoster, revealing that in HIV+ patients, VZV infection of the CNS persists for years. Overall, VZV reactivates subclinically in 38/180 (21%) of HIV+ individuals, 7.6 times more often in HIV+ subjects with no history of zoster than in HIV+ subjects who develop zoster.

P22
Axonal Transport Limits Virus Induced Demyelination

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Several neurovirulent viruses including herpes simplex, rabies, influenza, and Borna disease viruses utilize axonal transport as a strategy for disseminated infection and evasion of the host immune system. Our laboratory has generated isogenic recombinant demyelinating (DM) and non-demyelinating (NDM) Mouse Hepatitis Virus (MHV) strains that differ only in the host attachment spike glycoprotein. Both DM and NDM strains are capable of infecting neurons, however, these strains differ in their capacity to translocate to white matter. DM MHV strain infection begins in the neuronal cell body and propagates centripetally to the axon, with subsequent axonal degeneration and demyelination. NDM strains are unable to propagate from gray to white matter and as a result, are unable to induce demyelination. The DM strain spreads intra-axonally in an anterograde manner within gray matter and upon reaching the white matter, viral particles infect oligodendrocytes and microglia/macrophages presumably via direct cell–cell contact. In contrast, the NDM strain is unable to propagate to the white matter secondary to an inability to infect oligodendrocytes and microglia/macrophages. Recruitment of microglia/macrophages following DM MHV strain infection provides at least one mechanism of demyelination. In fact, we have demonstrated ultrastructural evidence of macrophage-mediated myelin stripping in the absence of any significant B or T cell infiltration. Since the two strains differ only in their spike protein, spike protein must be essential for axonal transport and subsequent oligodendrocyte infection. An understanding of the mechanism(s) by which viral particles interact with and take advantage of the normal axonal transport system is critical to the development of effective therapy and the spike protein is a logical target to prevent and/or ameliorate viral infection and demyelination. Our virus induced demyelination model provides a unique system to elucidate the mechanisms of viral axonal transport and its role in diseases of the nervous system.

P23
Chronic innate immune activation in the immunopathogenesis of HIV infection

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Infection by the human immunodeficiency virus (HIV) is characterized by functional impairment of immune responses, particularly those mediated by T lymphocytes, the causes of which are largely unexplained. We proposed a model in which the chronic activation of innate immune response mediated by plasmacytoid dendritic cells (pDC) is a key contributor to several aspects of HIV immunopathogenesis: 1) apoptosis of uninfected CD4 T cells; 2) increased expression of immunosuppressive ligands such as PD-L1; 3) upregulation of T cell activation markers; 4) chemoattraction of CCR5+ CD4 T cells at the site of infection, thus favouring systemic diffusion of the virus; and 5) indoleamine 2,3 dioxygenase (IDO)-mediated suppression of T cell responses and alteration of the balance between Th17 and regulatory T cells. We manipulated HIV by depleting

envelope-associated cholesterol to different degrees, in order to generate virions with reduced ability to activate pDC. We used this modified HIV virions to show that upregulation of costimulatory molecules and partial activation of antigen-presenting cells (APC) can be dissociated from the induction of type I IFN and IDO-mediated immunosuppression *in vitro*. Extreme cholesterol withdrawal rendered HIV a more powerful recall antigen for stimulating memory CD8 T cell responses in individuals with pre-existing immunity against HIV. This effect was strictly dependent on the inability of cholesterol-depleted HIV to activate pDC and type I IFN production. Thus, although IFN-alpha/beta may act as potent inhibitors of HIV replication during the acute phase of infection, the prolonged activation of pDC during the chronic phase may eventually become harmful for the immune system. Because of the tight link between peripheral immune status and the CNS, the elucidation of the basic mechanisms of HIV pathogenesis has important implications on HIV cognitive disorders.

P24
Molecular Neuropathology of the Dopamine system in NeuroAIDS

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In vitro studies have documented that co-administration of cocaine and HIV-1-proteins damages neurons to a greater extent than either viral proteins or cocaine alone. Few studies have examined this interaction using animal models. To model the onset of HIV-1-infection in relation to a history of drug use, the current research compared behavior and extracellular dopamine and metabolite levels following Tat1-86 infusions in rats with and without a history of cocaine experience. Animals receiving a behaviorally sensitizing regimen of cocaine demonstrated a decrease in extracellular dopamine concentration in the nucleus accumbens, consistent with evidence describing upregulation of dopamine transporter uptake. Contrary to this effect, Tat1-86 microinfusion into the nucleus accumbens following the sensitizing regimen of cocaine caused a significant increase in extracellular dopamine levels (nM) within 48 hours with no difference in percent of baseline response to cocaine. After 72 hours, Tat+cocaine treated animals demonstrated a blunted effect on potassium-stimulated extracellular dopamine release with a corresponding decrease in expression of behavioral sensitization to cocaine challenge. A persistent decrease in extracellular dopamine metabolite levels was found across all time-points in Tat-treated animals, regardless of experience with cocaine. Dopamine alterations have also been found using HIV-1 transgenic rats for animal models, with direct HIV-protein induced effects on the dopamine transporter. These animal studies 1) provide evidence for divergent neurochemical and behavioral outcomes following Tat-treatment; 2) indicate that HIV-1 effects are contingent upon experience with cocaine, and 3) the rat animal models (acute and chronic exposure) provide further evidence for dopaminergic alterations with HIV-1 protein exposure.

P25
Unique protein signatures for HIV-1 and HCV mono-infection versus co-infection

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Hepatitis C virus (HCV) is one of the most common viruses to co-infect individuals with human immunodeficiency virus type 1 (HIV-1). Of the 1 million individuals infected with HIV-1 in the United States, approximately 30-40% are co-infected with HCV. The objective of this study was therefore to investigate the proteome profiling of HIV, HCV mono- and co-infected patients. PBMCs were prepared from the age-gender-matched clinical samples: HIV-1-/HCV-, HIV-1+/HCV-, HIV-1-/HCV+ and HIV-1+/HCV+. Lysates were subjected to a 2D-GE LC-MS/MS proteomic analysis to sequence peptides. Proteins were identified by searching the raw MS/MS data in IPI human database. 271 protein spots were detected by gel image analyses on the control, a total of 184, 224 and 145 protein spots were detected in HIV-1, HCV and HIV-1/HCV treated samples. Of these, 153, 177 and 91 proteins were respectively increased in expression after HIV-1, HCV and HIV-1/HCV treatments. Conversely, 31, 47 and 54 spots in the HIV-1, HCV and HIV-1/HCV treatments respectively had decreased expression relative to control. LC-MS/MS and Mascot database matching resulted in successful identification of 31 unique and differently expressed proteins. The proteome of HIV-1, HCV and HIV-1/HCV-treated PBMC primarily affected

collagen-binding protein 2, cytoskeletal proteins (tropomyosin, gelsolin and profilin), chaperones and co-chaperones (HSP90 and stress-induced-phosphoprotein), and metabolic proteins (GTP-binding nuclear protein Ran, gluthatione S-transferase). The identification of collagen-binding protein 2 in HIV-1 and HIV-1/HCV, as well as a protein highly similar to CBP2 in HCV points to a novel molecular mechanism in agreement with previous clinical observations. Furthermore, the identified changes highlight protein signatures possibly involved in viral survival and replication that could be used potentially as drug targets. The specific protein fingerprints revealed through this study, could therefore help to understand the mechanisms underlying HIV-1 and HCV coinfections. Supported by US Public Health Service/NIH R01 AI077414 & NIH-RCMI Biomedical Proteomics Facility 2G12RR03035.

P26
Mechanisms for cellular control of HIV replication in HIV reservoirs of the brain

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HIV can invade the brain and persist in this organ for decades. HIV reservoirs in the brain are a potential source of virus for dissemination. Furthermore, they can contribute to neurodegeneration, which can be a problem even in optimally treated HIV-infected individuals. Once established, HIV reservoirs cannot be purged from the brain. Therefore targeted therapies are required for maintaining HIV quiescence in this organ. This requires a profound knowledge of virus-host interactions for regulation of HIV replication in brain cells. The potential target cells for HIV persistence in the brain include neural progenitor cells and astrocytes. Detailed studies of HIV replication in astrocytes demonstrate that these cells are capable of very efficiently restricting HIV production during persistent infection. However, they can also transiently increase virus production in response to external stimuli. My laboratory has demonstrated that the HIV regulatory factor Rev plays a crucial role as target in the control of HIV replication in astrocytes. We showed that astrocytes can attenuate Rev function and interfere with its localization and trafficking properties. To understand the molecular mechanisms of Rev control in astrocytes, we have identified novel cellular interaction partners of Rev in astrocytes. These include specific hnRNPs and a new family of proteins we call Risp (Rev-interacting HIV suppressors). Knock-down experiments indicate that the hnRNPs are positive regulators of HIV replication, whereas Risp proteins are suppressors of HIV production in persistently infected astrocytes. Our results indicate that host cell interaction partners of Rev are critical determinants of HIV production in astrocyte reservoirs. Future studies will focus on developing strategies that support and enhance cellular control mechanisms for negative regulation of Rev activity.

P27
Simian varicella virus open reading frame 63/70 expression is required for efficient virus replication

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Both human varicella zoster virus (VZV) and simian varicella virus (SVV) cause chickenpox in their natural host, after which both viruses become latent in ganglionic neurons. Thus, SVV infection of non-human primates has served as a model to study varicella virus pathogenesis. The SVV open reading frame (ORF) 63 is duplicated in the terminal repeat region as ORF 70 and shares 52% amino acid identity with VZV ORF 63/70. Like VZV latency in human ganglia, SVV ORF 63/70 is transcribed and translated in latently infected monkey ganglia. There are conflicting reports regarding the requirement of VZV ORF 63 expression for virus replication in culture. Using a bacmid clone containing the complete SVV genome, we constructed SVV mutants with stop codons in SVV ORF 70 (single), stop codons in both SVV ORFs 63 and 70 (double) and a revertant in which the engineered stop codon in

ORF 63 was changed back to the wild type (revertant). Transfection of Vero cells with wild type bacmid, single and double mutants as well as the revertant bacmid all produced a cytopathic effect. However, the double mutant virus grew extremely slow in culture. Our results demonstrate that while SVV ORF 63 is not required for replication in Vero cells in culture, its expression helps to produce robust virus replication. The requirement of SVV ORF 63 for successful infection of monkeys is under investigation.

P28
Cocaine/Sigma receptor-mediated induction of ALCAM: Implication for increased monocyte adhesion and migration in HAND

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Monocyte adhesion and trafficking associated with advanced HIV-1 infection is often exacerbated in cocaine-abusing, HIV-infected individuals. The underlying mechanisms are, in part, attributable to cocaine-mediated increase in impairment of blood brain barrier integrity. Up-regulation of adhesion molecules is a major determinant of the compromised barrier. In recent years, identification of a novel activated leukocyte cell adhesion molecule (ALCAM) has been implicated in the transmigration of leukocytes across the endothelium of the systemic vasculature. Its role in the transmigration of HIV-infected leukocytes in the context of cocaine abuse however, remains poorly defined. In the present study, we demonstrate cocaine-mediated induction of ALCAM in human brain microvascular endothelial cells. Furthermore, this effect of cocaine was mediated through the activation and translocation of the sigma receptor to the lipid raft domains of the plasma membrane, with subsequent activation of the platelet-derived growth factor (PDGF)-β receptor. Signaling by PDGF-βR resulted in sequential activation of mitogen-activated protein kinases (MAPKs), Akt, and nuclear factor κB (NF- κB) pathways leading to induced expression of ALCAM. Functional implication of up-regulated ALCAM was confirmed in cell adhesion and transmigration assays. *In vivo* relevance of these findings was further corroborated in cocaine-administered mice that demonstrated both increased monocyte adhesion as well as transmigration into the CNS. Neutralizing antibody to ALCAM ameliorated this effect. Taken together, these findings implicate cocaine-mediated induction of ALCAM as a mediator of increased monocyte adhesion and transmigration into the CNS of cocaine-treated mice. Understanding the regulation of ALCAM expression may provide insights into the development of potential therapeutic targets for neuroinflammation associated with HIV infection and cocaine abuse.

P29
Characterization of stages of monocyte maturation/differentiation that facilitate their transmigration across the blood brain barrier and infection by HIV: Implications for NeuroAIDS

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The prevalence of human immunodeficiency virus 1 (HIV) associated neurocognitive disorders resulting from infection of the CNS by HIV continues to increase despite the success of antiretroviral therapy. Although monocytes are known to transport HIV into the CNS, there are few specific markers that identify monocyte subpopulations susceptible to HIV infection and/or capable of infiltrating the CNS. We cultured human peripheral blood monocytes and characterized the expression of the phenotypic markers CD14, CD16, CD11b, Mac387, CD163, CD44v6 and CD166 at different stages of monocyte/macrophage (Mo/Mac) maturation/differentiation. We determined that a CD14+/CD16+/CD11b+/Mac387+ Mo/Mac subpopulation preferentially transmigrates across the BBB in response to CCL2. Genes associated with Mo/Mac subpopulations that transmigrate across the BBB and/or are infected by HIV were identified by cDNA microarray. We showed that JAM-A, PECAM and PrPc are necessary for monocyte transmigration across the BBB. We also showed that HIV infected monocytes appear to transmigrate preferentially across the BBB transcellularly. Uninfected monocytes appear to use the paracellular route. Our findings contribute to the understanding of monocyte maturation, infection and the mechanisms used for their transmigration into the brain during the pathogenesis of NeuroAIDS.

P30
Increased interaction between the δ-opioid and CXCR4 chemokine receptor is implicated in the reduced CXCL12/CXCR4 signaling in MOR-deficient mice

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The chemokine CXCL12 and its specific receptor (CXCR4) have been increasingly studied in the central nervous system (CNS) due to their critical roles in neuronal and glial physiology. CXCR4 is also one of the major HIV-1 co-receptors and is involved in HIV neuropathogenesis. Recent evidence suggests that drugs of abuse, including opiates, can facilitate progression to neuroAID and that MOR is a key regulator of CXCR4 in the brain; however, the molecular basis of the opioid/chemokine interaction are not fully understood and may involve different mechanisms in neuronal and glial cells. Our previous studies demonstrated that MOR stimulation specifically up-regulates the protein ferritin heavy chain (FHC) - an inhibitor of CXCR4 - in neurons and suggested additional mechanisms could be operative in glial cells. In this study, we investigated CXCR4 function in brains and astroglia cultures deprived of MOR. Reduced coupling of CXCR4 to G proteins was found in brain slices and tissue homogenates of MOR-/- mice compared to wild type (WT) controls. CXCR4-induced signaling was also reduced in glial cultures from MOR-/- mice, as shown by analysis of CXCR4 downstream targets (Akt and ERK1/2). Pharmacological studies with δ-opioid receptor (DOR)-specific ligands suggested that DOR-CXCR4 interactions are implicated in the inhibition of CXCR4 in MOR-deficient cells both *in vitro* and *in vivo*. Moreover, increased CXCR4/DOR co-immunoprecipitation was found in brain tissue and cultured glia from MOR-/- mice. Importantly, CXCR4 function was restored by pretreatment with a DOR antagonist. Overall, these findings indicate that DOR play a crucial role in the regulation of CXCR4 in glia, likely via silent receptor heterodimers. The data also suggest that the opiate system interferes with normal CXCR4 function in different ways and depending on receptor subtypes.

P31
Soluble CD163 as a plasma marker of HIV disease activation: Implications of macrophage-mediated immune responses

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CD163, a hemoglobin scavenger receptor expressed exclusively on monocyte/macrophages, is shed upon activation during inflammation as soluble CD163 (sCD163). Previously, we have demonstrated that monocyte expansion from bone marrow during simian immunodeficiency virus (SIV) infection correlated with plasma sCD163, rate of AIDS progression and severity of SIV encephalitis. Here, we examined sCD163, a plasma marker of monocyte/macrophage activation, as a measure of innate immunity during HIV infection and its potential clinical use as an index of immune stimulation. sCD163 was elevated in plasma of chronic (>1 year) HIV-infected individuals prior to antiretroviral therapy (ART) compared to HIV-seronegatives. With effective ART sCD163 declined, but did not return to HIV-seronegative levels, suggesting the presence of residual macrophage activation even with undetectable virus. In early HIV-infected individuals (≤1 year), sCD163 surprisingly returned to non-HIV-infected levels with ART, in contrast to chronic-infected subjects. Changes in plasma viral loads were associated with changes in sCD163 during early HIV-infection. With ART interruption in early HIV-infected subjects, both sCD163 and plasma viral load spiked and rapidly returned to baseline with re-initiation of ART, linking virus replication and macrophage activation in these subjects. sCD163 is normalized by effective ART only during early HIV infection, not in chronic HIV disease, suggesting a benefit of early initiation of ART in maintaining monocyte/macrophage activation at normal levels. Overall, this study points to the utility of sCD163 as a marker of monocyte/macrophage activation that occurs with HIV infection and underscores the significance of innate immune activation during HIV pathogenesis. This work was supported by NIH-NS37654 (KW), NIH-NS40237 (KW), NIH-AI071915 (ER), NIH-NS051129 (ML), NIH-MH58076 (PI: Igor Grant, RJE, SL), NIH-MH62512 (RJE, SL).

P32
The alpha7-nicotinic acetylcholine receptor is up-regulated in a transgenic mouse model that expresses the HIV-1 coat protein gp120: potential implications in the pathogenesis of HIV-1 associated dementia

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Infection with Human Immunodeficiency Virus 1 (HIV-1) has expanded to become a great public health challenge, both nationally and globally. Also, a significant percentage of the affected population can develop severe neurological impairments of the central nervous system (CNS) that can culminate in dementia, myelopathy, and myopathy. One of these neurological diseases is know as HIV-1 associated dementia (HAD), which has a high prevalence among HIV-infected patients. Several proteins that form an integral part of the HIV-1 virus have been evaluated for their role in CNS injury; and studies demonstrate that the HIV-1 gp120 envelope protein can be a mediator for the neurological deficits seen in HAD. Various mechanisms have been proposed to account for the injury seen in HAD and can involve ion channels. One receptor evaluated is the alpha7 nicotinic acetylcholine receptor (alpha7-nAchR), which might be involved in the events preceding injury in the brain. Our laboratory has shown that alpha7-nAChRs are up-regulated in lung, spleen, and peritoneal macrophages in a gp120-transgenic mouse model. Furthermore, quantitative RT-PCR experiments on transgenic mice's brain have shown a significant increase in alpha7-nAChRs mRNA levels in the striatum, a region shown to be affected by HAD; suggesting a link between alpha7-nAChRs, gp120, and HIV/HAD pathogenesis. Since studies have shown that alpha7-nAChRs have the highest calcium permeability among all nicotinic receptors subtypes, we hypothesize that the alpha7-nAChRs up-regulation will lead to a higher calcium influx into the cell, which can trigger apoptosis, providing a mechanism for injury in the brain. By determining the functional role of the gp120-induced alpha7-nAChRs up-regulation, we will gain a more complete understanding of disease progression, which in turn could lead to novel therapeutic strategies that would increase life expectancy and overall quality of life in people living with HIV/AIDS. Supported by NIH grants 2U54NS43011 and MH080661.

P33
Proteomic Analysis of Serum from Patients with HIV-associated Leukoencephalopathies for the Identification of Potential Biomarkers of Disease

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Progressive Multifocal Leukoencephalopathy (PML) is a demyelinating disease of Central Nervous System (CNS) caused by reactivation of the human polyomavirus JC Virus (JCV) in immunocompromised and HIV-positive patients. After the introduction of Highly Active Antiretroviral Therapy (HAART) for HIV treatment, a PML-like leukoencephalopathy not associated with viral detection in cerebrospinal fluid (CSF), known as Not Determined Leukoencephalopathy (NDLE), has been observed. Since the etiopathogenesis of NDLE is unclear and the discrimination between PML and NDLE is currently based on the detection of JCV DNA within CSF, we performed a proteomic study on PML and NDLE sera for identification of diagnostic and prognostic markers of disease by means of Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF). Sera samples from five PML and five NDLE patients were collected. The sera were depleted of albumin and IgG and the resulting serum proteins were separated on two dimensional gel electrophoresis (2DE). Data were analysed using Ludesi Redfin Software and the differentially expressed spots were cut from gels and tryptic digested. Peptides were purified with C18 Zip-Tip and spectra were obtained with a Microflex-LRF MALDI-TOF. The mass lists obtained from chromatograms were used for databases search on Mascot (Matrix Science) and Aldente (Expasy). Seventeen proteins differentially expressed between the two case groups were selected for MALDI-TOF analysis and four of these were identified by means of databases as known proteins. We have detected an increase of hemopexin, haptoglobin-beta chain, wnt2B in PML sera and an increase of vitamin D-binding protein (DBP) in NDLE sera. These preliminary data, that will be validated by increasing the number of studied samples and by western blotting, are at this moment very promising.

P34
The Role of Buprenorphine and CCL2 on Monocytes and the Blood Brain Barrier

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Human immunodeficiency virus 1 (HIV-1) enters the CNS early in the course of infection and results in neurocognitive impairment in a significant number of infected individuals. Our laboratory previously

demonstrated that HIV infected monocytes cross the Blood Brain Barrier (BBB) and alter BBB permeability in response to the chemokine CCL2, a potent attractant for monocytes. This inflammatory process is enhanced by opiate abuse. Many HIV infected opiate abusers have altered CNS disease with increased inflammation and neuronal injury that contribute to neurologic impairment. Buprenorphine has recently been used in the management of opioid dependence. However, the effects of Buprenorphine on the transmigration of uninfected and HIV infected monocytes into the CNS parenchyma and on the BBB are unknown. We are examining the effects of Buprenorphine and CCL2 on BBB integrity and on adhesion molecules of human monocytes and brain microvascular endothelial cells (BMVEC) required for transmigration. Using western blotting, coimmunoprecipitation and proteomics we demonstrated that Buprenorphine and CCL2 change the expression and the level of phosphorylation of signaling proteins on BMVEC and monocytes including JAM-1, ZO-1 and PECAM that are involved in transmigration of monocytes across the BBB. Our novel data demonstrate some of the mechanisms by which CCL2 contributes to the pathogenesis of NeuroAIDS and how Buprenorphine may impact these processes. This work is supported by NIDA, Grant # 5P20DA026149-02.

P35
Valproic acid for the treatment of myelopathy associated with HTLV-1/ Tropical spastic paraparesis (HAM/TSP)

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Background: Corticosteroids have been reported as beneficial, especially for HAM/TSP patients in the early phase of onset of symptoms. In our experience, the more frequent repetition of pulse therapy could be beneficial, with 25-30% improvement in neurological symptoms over the years. More recently, a group of Martinique and France made use of valproic acid in these patients with good results. Objective: To prospectively evaluate the effectiveness of valproic acid (20mg/kg/day) in individuals with HAM/TSP, associated with conventional therapies. Methods: This open and prospective study on TSP/HAM patients in regular follow-up. Neurological evaluation scale of the Research Institute Evandro Chagas (IPEC Scale) was used at 12 weeks, blindly by one of the authors (JV). Results: A total of 43 patients were included, but two cases were excluded from analysis due to difficulties in attendance. 24 patients (mean age 45 years and seven were men) were eligible for this preliminary analysis for 48 weeks of follow up. The average score on the scale IPEC was 15.5 ± 5 and 14 ± 5 at baseline and after 48 weeks, respectively (p = NS), there was no change in the other scales used, regardless of whether or not corticosteroids or valproic acid alone. The only improvement reported by patients was the improvement of intestinal constipation. Conclusion: There was no action to improve the use of valproic acid, but the study will complete 96 weeks of follow up for the long-term evaluation of these strategies. Support: Fapesp, CNPq, FFM.

P36
Relevance of intrathecal oligoclonal anti-EBV antibodies in multiple sclerosis

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It has recently been suggested that Epstein-Barr virus (EBV) could be a potential causative infectious agent in Multiple Sclerosis (MS). We quantified by ELISA technique cerebrospinal fluid (CSF) and serum levels of anti-EBV IgG in 100 relapsing-remitting (RR) MS patients, classified according to clinical and Magnetic Resonance Imaging (MRI) evidence of disease activity, in 109 patients with other inflammatory neurological disorders (OIND) and in 87 patients with non-inflammatory neurological disorders (NIND). Anti-EBV nuclear antigen-1 (EBNA-1) and anti viral capsid antigen (VCA) IgG levels were expressed as arbitrary units and quantitative intrathecal synthesis of anti-EBNA-1 and anti-VCA IgG was determined by Antibody Specific Index (ASI). The presence of EBV-specific CSF oligoclonal IgG bands (OCB) was assessed in MS patients by antigen-specific immunoblotting. CSF concentrations were higher in OIND than in MS ($p < 0.0001$) and NIND ($p < 0.01$) for anti-VCA IgG, and in MS than in NIND ($p < 0.01$) and in OIND than in NIND ($p < 0.05$) for anti-EBNA-1 IgG. Serum levels of anti-EBNA-1 IgG were more elevated in MS than in OIND and NIND ($p < 0.0001$). Serum titers of anti-EBNA-1 IgG were inversely ($p < 0.001$) correlated with EDSS. An intrathecal IgG production of anti-VCA and anti-EBNA-1 IgG was present only in a small proportion of MS and controls (range from 1.1 to 6.4%). EBV-specific CSF-restricted OCB were detected in 25/100 (25%) MS patients. These findings indicate that an intrathecal release of EBV-specific oligoclonal IgG can occur in a subset of patients with MS in whom an EBV brain persistent infection may act

as a cofactor in the development of the disease. Serum anti-EBNA-1 IgG seem to mark MS patients compared to other inflammatory and non inflammatory conditions. Work supported by FISM (2008-R-12) and by Programma di Ricerca Regione-Universite 2007-2009.

P37
Cytokine/chemokine expression in the brain of the HIV-1 transgenic rat during endotoxin tolerance

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Animals previously exposed to a few sub-lethal dosages of endotoxin develop endotoxin tolerance (ET), a decreased responsiveness to a subsequent lethal endotoxin challenge. In this study, we hypothesized that persistent presence of HIV-1 viral proteins affects the neuroinflammation profile. HIV-1Tg and F344 rats (n = 12 ea) were randomly assigned to receive two injections (i.p.) of either 250 ug/kg LPS or saline at 8:00 AM and at 5:00 PM on Day 1, followed by one injection of either 5 mg/kg LPS or saline at 8:00 AM on Day 2 (n = 3). Two hours later, brains and blood were collected for RNA and serum preparation, respectively. The sera were tested for IL-1beta, TNF-alpha, Kc/Gro, IL-4, IL-5, IL-13, and IFN-gamma. We found that the response to the lethal LPS dose was obviously tempered in both HIV-1Tg and F344 rats, suggesting that both strains of animals were in an ET state at the end of experimental regimen. We then examined total RNA prepared from the brains for 84 key genes of chemokines, chemokine receptors, cytokines, cytokine receptors, and inflammatory molecules. We found that, during ET, there are 21 genes up-regulated in the brains of both HIV-1Tg and F344 rats. In the F344 rat, 18 chemokines and chemokine receptors and 3 cytokines and other inflammatory proteins are up-regulated. In contrast, in the HIV-1Tg rat, 13 chemokines and chemokine receptors and 8 cytokines, cytokine receptors, and other inflammatory proteins are up-regulated; however, the increase in the chemokine (c-x-c) ligand genes in HIV-1 Tg rats was substantially lower than that in the F344 rats. In the HIV-1Tg rats, 4 chemokines/chemokine receptor genes were down-regulated, whereas no down-regulation of chemokine/chemokine receptors was detected in the F344 rats. The presence of HIV-1 viral proteins may alter the expression profile of inflammatory molecules during ET (NIH DA007058 & DA016149 to SLC).

P38
Activated CD8 Lymphocytes inhibit Neural Stem Cell Proliferation: Role of Interferon Gamma

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Chronic inflammation is a characteristic of many viral infections of the CNS, including Herpes Simplex virus (HSV)-1. Using a murine model of HSV-1 encephalitis (HSE), we have shown that gamma-interferon producing T lymphocytes persist in the brain long after viral clearance. This chronic inflammation is associated with significant neuropathology and spatial memory defects, reminiscent of the disease seen in patients surviving HSE. Currently, therapeutic interventions to treat neurological sequelae associated with HSE are grossly inadequate. Although garnering the potential of endogenous or transplanted neural stem cells (NSCs) to replace lost adult brain tissue is being widely investigated, the impact of inflammation on NSC function, which may contribute to neurodegeneration, remains relatively unexplored. In the present study, we observed that activated CD8 lymphocytes profoundly suppressed NSC proliferation. Luciferase activity measured from luciferase (luc)+ murine NSCs cultured with activated, MHC-matched CD8 lymphocytes (luc-) was two to five-fold lower than similar co-cultures with un-stimulated lymphocytes. On the other hand, activated CD4 lymphocytes, did not suppress NSC proliferation. This differential lymphocyte effect was confirmed by demonstrating decreased BrdU uptake by NSC when co-cultured with activated CD8 cells. Interestingly, neutralizing antibodies to IFN-gamma, but not an isotype control antibody, reversed the impact of CD8 lymphocytes on NSC proliferation. Additional experiments using antibodies specific to the IFN-gamma receptor complex demonstrated that IFN binding to the R1 subunit mediated both CD8 T lymphocyte and IFN-gamma effects on NSCs. In addition, activated CD8 lymphocytes decreased nestin expression levels in NSC, suggesting induction of an altered cell differentiation state. Experiments are currently underway to determine IFN-gamma receptor expression patterns on NSCs and to elucidate its role in lymphocyte-mediated NSC response. The studies presented here begin to elucidate the mechanisms by which lymphocytes alter neuro-restoration, which may ultimately impact the long-term sequelae commonly seen following viral encephalitis.

P39
Neurologic Manifestations of Human Immunodeficiency Virus-2: Dementia, Myelopathy, and Neuropathy in West Africa

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Background: While well-documented in HIV-1, neurologic sequelae have not been systematically evaluated in HIV-2. An existing cohort in rural Guinea-Bissau was neurologically evaluated in this study. Methods: Local researchers were trained to obtain histories, and administer focused portions of the testing. After obtaining informed consent and excluding for confounding comorbidities, sixty-seven individuals (22 HIV-2 participants, 45 seronegative controls) were evaluated and analyzed. HIV+ individuals were divided into CD4 < 350 and CD4 ≥ 350 for analysis. HIV-associated neurocognitive disorders (HAND), assessed by the International HIV Dementia Scale (IHDS); distal sensory polyneuropathy (DSPN), and myelopathy were the main outcome variables. Results: In univariate analysis, there was no difference in IHDS scores among groups. When analyzed by primary education attainment, IHDS scores were nonsignificantly higher ($p = 0.06$) with more education. There was no significant difference in DSPN prevalence among groups; overall 45% of the sample had DSPN. There were no cases of myelopathy. In multivariate analysis, higher IHDS scores were significantly correlated with older age (coefficient = -0.11, $p < 0.001$). Logistic regression analysis showed that older age (odds ratio (OR) 95% CI = 1.04-1.20), lower CD4 count (OR 95% CI = 0.996-0.999), and higher BMI (OR 95% CI = 1.02-1.43) significantly predicted the presence of DSPN. Conclusion: This study suggests the IHDS may be an ineffective HAND screen in settings of lower educational attainment. Similar to HIV-1, DSPN seems to occur with more severe (lower CD4 count) HIV-2 disease. As neurological disease continues to increase in HIV-1, a better understanding of the viral-host interactions in HIV-2 and its consequent neurological diseases may provide an avenue for tackling the epidemic problems of HIV-1.

P40
Rituximab associated progressive multifocal leukoencephalopathy in the setting of rheumatoid arthritis

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Background: Progressive multifocal leukoencephalopathy(PML) has increasingly been associated with therapeutic monoclonal antibodies, most clearly in the case of natalizumab for multiple sclerosis and efalizumab for psoriasis. Many more cases are reported associated with rituximab, but the analysis of drug induced risk has been challenged by the frequent use of this drug for malignant conditions where PML has long been a known risk, and by polypharmacy. We report a case of rituximab associated PML in a healthy rheumatoid arthritis patient with minimal risk for PML. Case Description: A 73 yo WF with 3 year history of rheumatoid arthritis and no history of malignancy, HIV, or chemotherapy. She had minimal therapy for RA before receiving one course of rituximab. She developed progressive sensorimotor and speech symptoms referable to the left hemisphere 5 months after rituximab. MR and CSF were diagnostic of PML. She received mefloquine experimentally, but her disease progressed, including painful spasms or seizures. An MR scan in October 2009 shorty before death, demonstrated enlarging lesions with slight contrast enhancement. She died ~3 months after onset of symptoms. An autopsy was performed that demonstrated aggressive PML with marked inflammatory changes. Lesions had both CD8 and CD20 positive cells infiltrating, sometimes in perivascular pattern. Conclusion: PML has been an extremely rare complication in rheumatoid arthritis even when aggressive therapy has been used. This patient with minimal risk of PML developed PML symptoms 5 months following rituximab therapy, and progressed to an inflammatory PML with rapid progression to death in the subsequent 3 months. This case, and four other cases of rituximab associated PML in RA, strengthen evidence that rituximab may increase risk for PML. Prescribers needs to consider this risk.

P41
Assessing the role of cytosolic RNA sensors, RIG-I and MDA5, in SIV infection

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The role of cytosolic RNA sensors, RIG-I and MDA5, during retroviral infection remains unexplored. Studies on HIV innate immune responses have mainly involved analysis of endocytosis-dependent TLR function in pDC's and monocytes using

synthetic RNA molecules or HIV-1-encoded ligands. In this study, pigtail macrophages were used to assess the relative contributions of RIG-I/MDA5- and TLR-mediated induction of innate immune responses. Monocyte-derived pigtail macrophages were either transfected with siRNA to RIG-I and MDA5 or pretreated with chloroquine, to block cytosolic RIG-i/MDA5 signaling and the endocytosis-dependent TLR response, respectively. IFN-α/β and IFN-stimulated gene (ISG) levels were then measured by quantitative real-time PCR. *In vivo*, the temporal expression patterns of RIG-I and MDA5 were assessed using brain protein lysates from SIV-infected M. nemestrina monkeys from acute to terminal phases of disease progression. Protein levels were measured by western blot and immunohistochemistry. The IFN response was abrogated in cells pretreated with chloroquine and MDA5 knockdown experiments, suggesting a role for both TLR- and MDA5-mediated IFN response to SIV infection in macrophages. In the macrophage- and astrocyte-rich white matter region of the brain, a distinct temporal expression pattern for RIG-I and MDA5 was observed during the acute stages of SIV infection. Specifically, MDA5 induction was immediate and transient, while RIG-I expression increased gradually but the level was sustained. A generalized upregulation of both RIG-I and MDA5 expression was observed during the terminal phase of infection. The type I IFN response to SIV infection in macrophages signals through both the cytosolic RNA sensor MDA5, as well as the endocytosis-dependent TLR receptors. Furthermore, the difference in cellular and temporal expression patterns between RIG-I and MDA5 implies unique roles for each in eliciting the innate immune response during primary SIV infection.

P42
HIV Tat, methamphetamine, and MMP-dependent activation of microglia

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Microglial activation is thought to contribute to neuronal injury occurring with methamphetamine (MA) and select HIV proteins. While multiple mechanisms may contribute to MA and HIV associated microglial activation, one possibility is that matrix metalloproteinases (MMPs) play a role. This possibility is supported by the fact that minocycline, a potent inhibitor of MMP expression and activity, abrogates microglial activation. It is also supported by recent work in which select MMPs mediate microglial activation occuring in murine models of Parkinson's disease. In the present study, we have focused on the potential for HIV Tat and MA to stimulate increased expression and/or release of specific MMPs, and on the potential for released MMPs to stimulate ectodomain shedding of intercellular adhesion molecule-5 (ICAM-5). We have also examined the possibility that the shed ICAM-5 ectodomain can interact with microglial integrins to stimulate microglial activation. We find that Tat and MA can both stimulate increased MMP release from neural cells. We also find that MA stimulates ectodomain cleavage of ICAM-5 from cultured cells, and that this is reversed by their pretreatment with a broad spectrum MMP inhibitor. Moreover, an acute dose of MA, administered *in vivo*, is associated with cleavage of ICAM-5 in murine hippocampus and striatum. Additional studies with the recombinant ICAM-5 ectodomain suggest that the shed molecule can directly activate microglia, as determined by increased release of proinflammatory molecules, and that this activation is in some part dependent on the ability of the ectodomain to interact with the integrin LFA-1. While future studies will address the issue of whether ICAM-5 activated microglia can stimulate neuronal toxicity, these data support an emerging appreciation of MMPs as important mediators of microglial activation.

P43
Apelin-mediated neuroprotection through regulation of NMDA receptor function: implications for HIV-associated excitotoxic injury

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Excitotoxic neuronal damage via activation of the N-methyl-D-aspartate receptor (NMDAR) has been implicated in a variety of neurological disorders, including HIV-associated neurocognitive disorders (HAND). *In vitro* models of HIV-induced neurotoxicity suggest that activation of certain neuronal G-protein coupled receptors (GPCRs), such as chemokine receptors, can counteract injury through modulation of NMDAR signaling. The APJ receptor is a recently described GPCR that, like chemokine receptors, can serve as a co-receptor for HIV entry and can promote survival in peripheral cells. Furthermore, APJ and its ligand apelin are highly expressed in neurons *in vivo* and *in vitro*, and we

have previously shown that recombinant apelin protects against glutamate- and HIV-induced neurotoxicity. Extending these studies, we hypothesize that apelin promotes neuronal survival during HIV infection by activating cell survival signaling and/or inhibiting excitotoxic cell signaling. Using heterologous systems and primary rodent brain cultures in an *in vitro* model of HIV-induced excitotoxicity, we found that: (i) recombinant apelin activates cell survival signaling pathways to provide neuroprotection against excitotoxicity; (ii) recombinant apelin inhibits excitotoxic cell signaling by attenuating NMDAR activity and subsequent calpain activation, and by modulating NMDAR phosphorylation. These studies suggest that apelin/APJ signaling can counteract excitotoxic neuronal damage via modulation of NMDARs. Ongoing studies will identify potential functions for apelin in HIV-infected individuals, specifically determining associations between apelin expression and neurocognitive status. Further definition of apelin in HIV infection and the mechanism (s) of apelin-mediated neuroprotection will improve our ability to develop therapeutics for HAND and other neurodegenerative disorders.

P44
Cocaine and HIV-mediated disruptions of hypothalamic signaling in hypothyroidism

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Over the last two decades, consequences of HIV infection of the CNS on disease severity and clinical neuropsychiatric manifestations have changed. These changes are due, in part, to improved control of peripheral infection by new anti-retroviral medication regimens and more efficient CNS penetration of combination anti-retroviral therapies (cART). While the life spans of HIV infected patients have been prolonged with successful cART, the spectrum of cognitive alterations observed in these patients has broadened, as well. Recent studies report that there does not appear to be a single prototypical pattern of neuropsychological impairment associated with HIV, but rather it includes diverse manifestations. Some comorbidities, such as substance abuse or depression, likely play significant roles in the neuropsychiatric profiles of HIV infected patients. Newly recognized factors contributing to neurocognitive impairments include ageing and unanticipated side effects from cART. Likewise, disturbances in neuroendocrine functioning are emerging as a potentially important contributor to HIV-associated neurocognitive alterations. Our data show for the first time HIV infection of the hypothalamus and altered levels of thyroid hormone processing enzymes. Likewise, decreased vasopressin and oxytocin immunoreactivity in hypothalamic neurons was also observed in HIV patients. Thus, HIV infection of the CNS may contribute to changes in hormone signaling in the CNS, thereby resulting in abnormal hypothalamic-pituitary-thyroid axis signaling and neuropsychiatric dysfunction. Supported by NIDA029523 to TDL.

P45
The relationship between adaptive stress responses and HIV replication in macrophages and its impact on neurotoxicity

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HIV infection of the central nervous system (CNS) results in HIV-associated neurocognitive disorders (HAND) in 30% of patients despite use of antiretroviral therapy. HIV-infected monocyte-derived-macrophages (HIV/MDM), the primary CNS HIV reservoir, promote neurodegeneration through release of excitatory neurotoxins and induction of neuroinflammation. We hypothesized that cellular adaptive stress responses, which can be triggered by virus infection, are not only activated in HIV/MDM but also modify neurotoxin release and inflammatory cascades associated with neurodegeneration. We developed an *in vitro* model of HIV neurotoxicity utilizing human HIV/MDM and primary rat cortical neurons. With this system, we have demonstrated that HIV infection of MDM results in the modulation of the integrated stress response (ISR). HIV infected MDM demonstrate a biphasic induction of phosphorylated-eif2α, with peaks of activation early after infection (24 hrs) and during high replication periods. Phosphorylated-eif2α is a key indicator of cellular stress and directly attenuates protein translation and promotes molecular chaperones. In addition, HIV replication reduces expression of heme oxygenase 1 (HO-1), a component of the anti-oxidant response element (ARE), indicating pathological oxidative stress. We found that pharmacological induction of phosphorylated-eif2α or the ARE attenuates HIV replication, demonstrating that these stress responses are part of an anti-viral cellular defense in the macrophage. We also found that activation of

the antioxidant response, but not phosphorylated-eif2α, attenuates neurotoxin production in HIV/MDM. Our results suggest that pharmacological agents that activate the antioxidant response have therapeutic potential for HAND, through suppression of HIV replication and neurotoxin production in HIV/MDM. Our ongoing studies are defining the roles for the antioxidant response in suppressing inflammatory mediators and neurotoxin production by HIV/MDM. Understanding how cellular adaptive stress responses respond to HIV infection and the consequences of this activation on HIV replication and neurotoxin production will improve our understanding of HAND pathogenesis and provide insights for therapeutic strategies.

P46
Lower Than Expected Maraviroc Concentrations in Cerebrospinal Fluid Still Exceed the 50% Inhibitory Concentration for Wild-Type CCR5-tropic HIV-1

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HIV-associated neurocognitive disorders continue to be common. Antiretrovirals achieving higher concentrations in cerebrospinal fluid (CSF) are associated with HIV suppression in CSF and better cognitive functions. The objective of this study was to measure total concentrations of a newer antiretroviral, maraviroc (MVC), in CSF and to compare them with matched plasma concentrations and *in vitro* inhibitory concentrations. Eight subjects with HIV-1 infection enrolled based on use of 300 or 600 mg/d of MVC and availability of stored CSF and matched plasma. MVC was measured in 10 CSF and plasma pairs by reverse phase high performance liquid chromatography with tandem mass spectrometry and HIV RNA by quantitative reverse transcription polymerase chain reaction. MVC concentrations were compared to the 50% inhibitory concentration (IC50) for wild-type CCR5-tropic HIV-1 (0.51 ng/mL). Subjects were predominantly middle-aged white men with AIDS. Median concurrent CD4+ cell count was 220/ul with 50% below 200/ul. HIV RNA were detectable in 70% of plasma and 30% of CSF specimens. MVC was present in all CSF specimens with a median total concentration of 2.35 ng/mL (range 0.2-18.3). The median plasma concentration was 92.5 (total) and 12.6 (unbound) ng/mL with a median CSF-to-plasma ratio of 0.029. MVC concentrations in CSF exceeded the IC50 of wild-type CCR5-tropic HIV-1 in all specimens but one by a median of 4.6-fold. The single subject with CSF MVC below the IC50 was on 300 mg/d of MVC without ritonavir. The observed CSF concentrations are substantially lower than expected based on plasma concentrations and MVC physicochemical characteristics. Despite this, they exceeded the IC50 in 90% of specimens, suggesting that MVC may contribute to control of HIV in the central nervous system.

P47
Glutamate excitotoxicity, cortical and subcortical neuronal damage as potential markers of HIV-associated neurocognitive disorder (HAND): A 1H-MRS and neuropsychological study

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Background: Chronic brain HIV infection and aging may lead to new neuropathological processes. Methods: 54 HIV+ adults aged 54 ± 7 on HAART (Plasma and CSF (N = 26) HIV RNA < 50 cp/mL in 96%; median CD4 = 526) and 11 demographically-comparable controls underwent neuropsychological testing, mood examination, blood tests, and single voxel 1H-MRS at 3T: Right frontal white matter (FWM), posterior cingulate cortex (PCC) and right caudate area (Caud) metabolites were quantified using jMRUI with baseline and water corrections. Regression models investigated factors associated with brain metabolites: 1. Neurocognitive model; 2. HIV biomarker model, 3. Cardiovascular model, 4. Multivariate model combining factors at $p < .10$. Overall neurocognitive impairment was defined by the Global Deficit Score. Results: Relative to controls,

HIV+ individuals demonstrated significantly increased FWM glutamine/glutamate (Glx) (d = .64; p < .04); decreased PCC N-Acetylaspartate (NAA) (d = .50; p = .07), and PCC increased myo-Inositol/Creatine (mIo/Cr) (d= .57; p < .16); and decreased Caud NAA (d = .59; p < .02). Higher FWM Glx was associated with lower PCC NAA (r = -.33; p < .02). 20% of the HIV+ group were classified as neurocognitively impaired versus 0% in the HIV- group; p < .05. Caud NAA was decreased in the impaired HIV+ group (d = .50; p < .20). Memory performance; serum β2-microglobulin, HIV duration and Framingham cardio-vascular risk were retained in the multivariate model and showed: β2-microglobulin negatively correlated with FWM Glx (p < .02), but positively with PCC mIo/Cr (p < .05). Lower memory performance (p = .05) was associated with lower PCC NAA. Cardio-vascular risks were associated with lower PCC NAA and lower memory performance only in initial models (p < .05). In the HIV+ group, higher depressive, and apathy complaints were associated with lower Caud NAA (p < .05). Conclusion: Ongoing excitotoxicity despite viral suppression was found in areas of the brain that have been classically involved in HIV infection, linked to injury in new areas. This suggests a hybrid development: classical HAND plus new pathological process involving posterior cortical areas.

P48
Analysis of a conserved HIV-1 LTR downstream element in the context of HIV-1 transcriptional regulation in cells of the monocyte-macrophage lineage

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Transcription factor binding sites present both upstream and downstream of the transcriptional start site in the human immunodeficiency type 1 (HIV-1) long terminal repeat (LTR) are necessary for activity in the cells of the monocyte-macrophage lineage. Two downstream CCAAT enhancer binding protein (C/EBP) binding sites have previously been identified within the simian immunodeficiency virus (SIV) LTR. TRANSFAC analysis of the HIV-1 subtype B LAI full-length LTR sequence revealed three potential C/EBP binding sites. One hundred full-length subtype B, 38 subtype A, 115 subtype C, and 25 subtype D LTR sequences were complied, aligned, and analyzed for the presence of downstream C/EBP binding sites. HIV-1 subtypes B, C, and D also contain a potential downstream C/EBP binding site (DS3) with a similar physical position and nucleotide sequence. Interestingly, this potential C/EBP binding 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). Preliminary results showed that NFAT has a stronger affinity for this element as compared to C/EBP but it was also observed, in competition gel shift analysis, that this element is able to compete with another weak upstream C/EBP binding site (US1) with respect to C/EBP binding. These results suggest that there is a very intricate utilization of the transcription factors and their cognate binding elements in HIV-1 LTR. This utilization not only depends on the cellular phenotype but also is responsive to a range of extracellular stimuli that can potentially influence the levels and/or availability of important regulatory transcription factors in context of HIV-1 transcription.

P49
Suppression of HIV-1 Transcriptional Elongation by p27SJ DING Phosphatase

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HIV-1 gene transcription is controlled by the cooperation of viral and host factors which bind to specific DNA sequences within the viral promoter spanning the long terminal repeat, LTR. Previously we showed that a novel plant DING phosphatase, p27SJ, suppresses HIV-1 gene transcription by binding to the viral protein Tat and preventing its nuclear import. Here, we describe the inhibitory effect of p27SJ on the phosphorylation of RNA polymerase II C-terminal domain (RPII CTD) and NF-κB p65. Inhibition of RPII CTD phosphorylation leads to the suppression of the association of RPII CTD with the LTR. Inhibition of binding of RPII CTD to LTR by p27SJ resulted in the suppression of LTR transcription and elongation and a decrease of LTR transcriptional activity. Mapping of the region within the LTR that is affected by p27SJ revealed that both NF-κB and RPII CTD binding sites were important. Our data suggest a possible mechanism by which p27SJ can regulate HIV-1 LTR expression can be through the dysregulation of NF-kB p65 and preventing its phosphorylation and binding to the LTR. Here, we also show that the cooperativity between NF-kB and RPII CTD was affected by p27SJ. Our data suggest a possible mechanism by which p27SJ DING

phosphatase can control HIV-1 LTR expression by inhibiting phosphorylation of CTD RPII and suppressing LTR transcription and elongation, and by interfering with the function of the NF-κB family of cellular proteins. Supported by a grant awarded by NIH/NIMH to SA.

P50
Activation of HIV-1 LTR by Rad51 in microglial cells

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Infection with HIV-1 induces a variety of biological alterations to the host that are beneficial to the life cycle of the virus but may have adverse effects on the host cell. Here we demonstrate that expression of Rad51, a major component of the homologous recombination-directed DNA repair (HRR) pathway, is induced upon HIV-1 infection of microglial cells. Activation of Rad51 expression positively impacts on HIV-1 LTR transcription through a region of the viral promoter known for binding the inducible transcription factor NF-κB. Rad51 showed the ability to form a complex with the p65 subunit of NF-κB and regulate the level of p65 interaction with LTR DNA encompassing the κB motif. This study provides evidence for reciprocal interaction of HIV-1 and a host DNA repair protein that impacts on expression of the viral genome. These results also point to the ability of HIV-1 to recruit proteins involved in DNA repair that are necessary for retroviral DNA integration, efficient replication and prevention of viral-induced cell death.

P51
A novel deletion in JC Virus agnoprotein causes productive infection of cortical pyramidal neurons

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Objective: To study the molecular characteristics of JC Virus in an HIV-negative patient who presented with grey matter brain lesions and died from fulminant JCV encephalopathy (JCVE) caused by productive infection of cortical pyramidal neurons(1). Methods: We performed whole JCV genome PCR amplification, as well as PCR screening and sequencing of multiple brain areas of the JCVE patient and immunofluorescence (IF) staining with anti-JCV agnoprotein and anti JCV VP1 capsid protein antibodies. Results: We identified a novel JCV variant with archetype-like regulatory region containing a 143 bp deletion in the Agno gene, reducing the 71 aa agnoprotein to a putatitve 10 aa agno peptide. PCR screening encompassing the deletion site showed that this was the predominant strain, named JCVCPN1, in the brain autopsy sample of the JCVE patient. This strain was present in the cortex of each frontal, parietal and temporal lobes. However, PCR screening using primers located within the deletion site indicated that this strain co-existed with JCV with intact agno gene, which was also found in the CSF and plasma of this patient. Double IF staining with anti-agno antibody encompassing the entire agnoprotein (generous gift of Dr Safak) colocalized with VP1 staining in the cortical areas of the JCVE case, while anti-agno antibody directed against the C terminus of the protein (generous gift of Dr Sawa) showed limited staining and rare co-localization with VP1 capsid protein. Conversely, both anti-agno antibodies colocalized with VP1 staining in a control brain with Progresive Multifocal Leukoencephalopathy. PCR screening of 11 CSF samples from PML patients revealed the presence of another agno-deletion mutant Conclusions: The agno deletion variant JCVCPN1 displays a novel cellular tropism and causes a productive infection of hemispheric cortical pyramidal neurons. Concomitant presence of undeleted agno strain in the same patient suggests dual JCV infection. 1. Wuthrich, C., X. Dang, S. Westmoreland, J. McKay, A. Maheshwari, M. P. Anderson, A. H. Ropper, R. P. Viscidi, and I. J. Koralnik. 2009. Fulminant JC virus encephalopathy with productive infection of cortical pyramidal neurons. Ann Neurol 65:742.

P52
The HIV-1 Tat Protein Preferentially Interacts with Pur-alpha in Disrupting Pur-alpha and Pur-gamma Co-localization in KG-1 Oligodendroglial Cells

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Pur-alpha is a demonstrated activator of HIV-1 TAT-dependent transcription. Little is known about a

related family member Pur-gamma. It has been reported that Pur-gamma functions in very early mouse embryogenesis, between days e14 and birth, after which its levels decline. Pur-alpha levels concomitantly rise and peak at days 18-25 after birth. We report here, however, that in human KG-1 oligodendrocytes Pur-gamma is expressed as well as Pur-alpha. Oligodendrocytes are not infected by HIV-1, but JC virus, which is strongly influenced by nearby HIV-1 infection, can infect them. In studies based on immunofluorescence microscopy, Pur-gamma is very nuclear in localization in these cells, whereas Pur-alpha is minimally present in the nucleus while having a very perinuclear presence. This may reflect the presence of a strong nuclear localization signal in Pur-gamma, whereas Pur-alpha has both nuclear localization and nuclear export signals. Pur-alpha immunofluorescence is localized to a structure juxtaposed to the nucleus in the KG-1 cells. The components and dynamics of this structure, indicating a possible role in RNA transport, will be discussed. A fraction of Pur-alpha is also co-localized in the nucleus with Pur-gamma. In addition, there is localization of Pur-alpha around the perimeter of the nucleus, where there is a noticeable lack of Pur-gamma immunofluorescence. The HIV-1 Tat protein is not produced in KG-1 cells, but it is actively accumulated by these cells. When HIV-1 Tat is added to the KG-1 cells, there is a loss of co-localization of Pur-alpha and Pur-gamma prior to entry of Tat into the nucleus. Pur-gamma localization becomes almost exclusively nuclear. Our studies examine Tat effects on Pur-gamma localization over a time course encompassing entry of Tat into the nucleus. Our previous published studies reveal that at times later than 48h, Tat and Pur-alpha co-localize as they enter the nucleus. A dynamic shift in localization of Pur-alpha and Pur-gamma in the presence of HIV-1 Tat suggests a possible role for Pur-gamma in Pur-alpha-associated activities influenced by HIV-1.

P53
Regulation of complement component C3 and its cognate receptor C3aR by HIV-1 viral protein R (Vpr)

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HIV/SIV infection is known to induce the synthesis of C3 in macrophages and brain and contribute to the pathogenesis of NeuroAIDS. Since, Vpr is one of the HIV-1 encoded proteins that play an important role in viral pathogenesis, and inhibition of complement synthesis and activation may represent a putative therapeutic approach, we analyzed whether Vpr induces C3 and C3aR expression. Our studies utilizing C3 promoter-luciferase and C3aR promoter-luciferase constructs demonstrate that Vpr induces both the promoters in promonocytic cells and astrocytes. This Vpr-mediated transactivation involves transcription factors, C/EBP and NFkappaB since overexpression of the inhibitory isoform of C/EBP-beta, liver inhibitory protein (LIP) and the dominant negative IkappaBalpha mutant attenuates Vpr-mediated C3 promoter activation respectively. Furthermore, over-expression of IkappaB alpha mutant attenuates Vpr mediated transactivation of the minimal promoter construct (-199 bp) of C3 harboring the C/EBP binding site. These results suggest that there is an interaction between C/EBP and NFkappaB in C3 regulation by Vpr.

P54
Morphine induces inflammation and oxidative stress without influencing HIV-1 viral replication in human brain-derived cells

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Opioid-abusing cohorts have a high incidence several characteristic features that increase the incidence of contracting blood-borne pathogens and sexually transmitted diseases. They utilize shared devices to inject drugs. These individuals also exhibit a high-level of psychological distress and as a result practice unsafe sex. It is therefore not surprising that almost 30% of HIV-1-infected individuals in USA are linked with injection drug use. HIV-1 disease progression is accelerated due to a lack of adherence to antiretroviral treatment regimen and onset of comorbid conditions. As well, HIV-1-infected opioid-abusers exhibit severe neurological dysfunction and neuropathology at autopsy. The underlying molecular basis of these clinical observations needs elucidation. Previously, we have reported that morphine induces differential miRNA and protein expression that potentially impacts inflammation and oxidative stress in human monocyte derived macrophages. In this study, we utilized human fetal brain-derived cells to determine a) If morphine and HIV-1 infection have a concomitant effect on inflammation and oxidative stress b) If morphine affects HIV-1 viral replication. To mimic heroin abuse, we utilized a chronic morphine abuse model, wherein cells were subjected to 0.1 μM morphine treatment at 3-4 day intervals for duration of 21 days. IL-6 concentration was found to increase

with increasing duration of treatment in microglia, astrocytes and mixed brain cell cultures. Highest levels of IL-6 were secreted from mixed brain cell cultures and astrocytes secreted the least. At day 3 of the treatment, mixed brain cell cultures had 3-fold and 8-fold greater secretion of IL-6 as compared to microglia and astrocytes, respectively. At the end of treatment, all cell-types had equivalent levels of IL-6 secretion. Human monocyte-derived macrophages derived from three different donors were infected with HIV-1 JR-FL. In all three donors, HIV-1 replication was not affected by morphine in a statistically significant manner. Similarly, morphine did not increase viral replication in microglia, mixed brain cell cultures or astrocytes infected with HIV-1 YU-2 or HIV-1 YU-2/MuLV, respectively. Of note, IL-6 secretion was similar from morphine-treated or HIV-1 infected morphine treated cell-cultures. Reactive oxygen species were detected in microglia and mixed brain cell cultures but not in astrocytes. Microglia had the highest concentration at day 2 after treatment. These results, suggest that chronic opioid abuse may lead to sustained inflammation and oxidative stress in the brain without affecting HIV-1 replication. In this study, morphine and HIV-1 do not seem to have a concomitant effect. Yet these disparate triggers induced similar pathological response in the brain.

P55
Valproic acid inhibits the release of sCD40L induced by non-nucleoside reverse transcriptase inhibitors in Human Immunodeficiency Virus infected individuals

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Despite the use of highly active antiretroviral therapies (HAART), which are able to efficiently control viral load, a majority of Human Immunodeficiency Virus type 1 (HIV-1) infected individuals still develop HIV-1 associated neurocognitive disorders (HAND), indicating that host inflammatory mediators, in addition to viral proteins, may be contributing to these disorders. Consistent with this notion, we have previously shown that levels of the inflammatory mediator soluble CD40 ligand (sCD40L) are elevated in the plasma and cerebrospinal fluid (CSF) of HIV-1 infected, cognitively impaired individuals receiving conventional HAART treatment. Here we demonstrate that treatment with the non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz (EFV) results in an accumulation of circulating sCD40L in HIV-1 infected individuals. Consistently, increased levels of sCD40L were also observed in a suspension of EFV-treated washed human platelets, which are the main source of circulating sCD40L. Based on our recent findings that valproic acid (VPA) displays anti-platelet activity, we hypothesized that VPA would inhibit sCD40L release induced by EFV. Indeed, we now reveal that VPA, a known glycogen synthase kinase 3 beta (GSK3-beta) inhibitor, was able to inhibit the release of sCD40L in HIV-1 infected individuals receiving EFV, and in washed human platelets, in a GSK3-beta dependent manner. Furthermore, VPA did not alter platelet counts in mice receiving oral treatment of this drug and, although their response was dampened, these platelets were still functional in the presence of VPA. These results have important implications in the development of adjunctive therapies for HAND, which are currently lacking, and highlight the potential use of VPA in this manner.

P56
Anti-inflammatory actions of the opioid receptor antagonist, β-funaltrexamine: Role of TLR-4 and NF-κB?

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Astroglial mediated inflammation is instrumental in the neuronal damage observed in neuroinflammatory pathologies. For instance, astrocyte-derived chemokines, such as CCL2 and CXCL10 have been implicated in the pathology of neuroAIDS. CNS influx of microbial products, including lipopolysaccharide (LPS), is a consequence of blood-brain barrier disruption associated with neuroAIDS and other neuropathologies. We have characterized the effects of LPS on CCL2 and CXCL10 expression in normal human astrocytes (NHA). Using an inhibitor of NF-κB nuclear translocation (SN50), we have demonstrated that LPS-induced CCL2 and CXCL10 expression in NHA is NF-κB dependent. Subsequent investigation indicated that β-funaltrexamine (β-FNA), an opioid receptor antagonist, inhibited chemokine expression in NHA. Furthermore, we have utilized HEK-293 reporter cells (HEK-Blue4) to assess signaling through the toll-like receptor (TLR)4-NF-κB pathway. Initial findings demonstrate that TLR4 signaling is also inhibited by β-FNA. To date, our data suggest that dysregulation of NF-κB activation may be involved in the anti-inflammatory actions of β-FNA. Ongoing studies are expected to further elucidate the mechanism by which β-FNA

inhibits inflammatory signaling, particularly in human astrocytes. Insights gained are expected to be instrumental in the development of therapeutic strategies to prevent and/or treat neuroinflammation.

P57
Herpes simplex virus type 1 (HSV-1) induces multiple cleavages of Amyloid Precursor Protein (APP) and Abeta accumulation in human and rat neuronal cells

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Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that, following primary infection, establishes a lifelong latent infection in the trigeminal ganglia. During the episodes of reactivation, newly produced viral particles travel back to primary infection sites, but they may also move upward to the central nervous system, causing productive but generally asymptomatic infections. Epidemiological and experimental findings suggest the involvement of HSV-1 in the pathogenesis of Alzheimer's disease (AD). The present study was aimed at investigating: i) the effects of productive HSV-1 infection on APP processing; ii) the intra- and/or extracellular Abeta accumulation; iii) the intracellular localization of APP C-terminal fragments (CTFs). We found that in both human neuroblastoma cells and rat cortical neurons HSV-1 triggers multiple APP processing, which results in the intracellular production of various neurotoxic species. In particular, in infected cells, we found the formation of APP fragments (APP-Fs) of 35 and 45 kDa (APP-F35 and APP-F45) that comprise portions of Abeta. Western blot analysis of cell lysates treated with formic acid suggests that APP-F35 is an oligomeric form of Abeta. Consistently, immunoprecipitation studies and ELISA assay for extracellular Abeta showed that HSV-1 increases the levels of Abeta aggregates. Intracellular Abeta40 and Abeta42 accumulation was also observed following HSV-1 infection. The HSV-1-induced multiple APP cleavages were produced by cellular enzymes involved in the amyloidogenic APP processing pathway, i.e. β- and γ-secretases, and caspase-3 like enzymes. Finally, in either cell models we observed the release of CTFs from their physiological membrane localization and their translocation into the nucleus, thereby potentially triggering gene-dependent cell death. Collectively, our findings indicate that HSV-1 infection leads to the production of APP-Fs and to Abeta accumulation at both intra- and extracellular level, thus suggesting HSV-1 could play a co-factorial role in AD pathogenesis.

P58
HIV-1 gp120 up-regulates α7 nicotinic acetylcholine receptor in monocytes-derived macrophages

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The infection and depletion of the immune system represent the most fundamental consequences in HIV-1 infection. HIV-1 entry into target cells is mediated by viral envelope gp120 binding to CD4+ cells resulting in viral infection. It is known that acute exposure of HIV-1 envelope gp120 activates calcium mobilization mediated by CXCR4 and CCR5 in primary human macrophages. This gp120-induced calcium mobilization has been proposed to trigger signaling pathways that contributes to the overall immunological deficit observed during HIV infection. However, the mechanism for this unique calcium signaling is unknown. Here we report that the acute gp120-induced calcium mobilization was abrogated by pre-incubation of monocyte-derived macrophages (MDM) with α-bungarotoxin, an antagonist of the highly calcium-permeable α7-nicotinic acetylcholine receptor (α7-nAChR), a channel recently described in human macrophages. We also found that chronic gp120 exposure induce an up-regulation of α7-nAChR in MDMs. Chronic exposure of MDMs to gp120 displayed a 3-fold increase in intracellular calcium concentration [Ca2+]i as compared with

control cells. Flow cytometry and confocal imaging studies for α7-nAChR expression in samples from 30 women (20 HIV-seropositive and 10 HIV-seronegative) revealed a significant increase in the α7-nAChR expression level in MDMs from an HIV-seropositive women group ($p < 0.05$). We also demonstrated that physiological concentrations of choline activate α7-nAChR promoting extracellular calcium entry into MDMs. Moreover, patch clamp experiments in MDMs revealed choline-induced currents and by using a potent positive allosteric modulator, PNU-120596, characteristic α7-nAChR currents were identified. Finally, cytokines quantification suggests an anti-inflammatory phenotype in gp120-exposed MDMs. Altogether, these results demonstrate that the α7-nAChR may contribute to signaling pathways that contributes to the immunological deficit observed during HIV infection. Along these lines, the present study suggests that the α7-nAChR could be a potential target for pharmacological intervention to prevent the inflammatory and apoptotic responses of immune cells during HIV infection.

P59
Protective effect and mechanism of curcumin on TNF-alpha-induced neuronal damage in rat hippocampus

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Our previous research has proved curcumin could improve learning and memory ability in memory disorder rats induced by gp120 (Dong J, *et al*, 2009). Based on that, the present study observed protective effect of curcumin on neuronal damage in rat hippocampus induced by TNF- alpha which is one of the most important inflammatory cytokines in HIV-1-associated dementia (HAD). Curcumin could prevent the growth inhibition of neuron induced by TNF- alpha. After treatment of curcumin, cell body of neuron recovered back to normal shape, pyramid or ellipse. Axon, many dendrites and secondary branches grew from cell body and formed network structure. Curcumin also could prevent the rapid influx of Ca2+ induced by TNF-alpha and maintain the Ca2+ homeostasis of neuron cytoplasm. To explore the functional protective effect of curcumin on neurons, we did electrophysiological experiments to observe effect of curcumin on long term-potentiation (LTP) inhibition induced by TNF-alpha and N-methyl-D-aspartate (NMDA). TNF- alpha and NMDA both could obviously inhibit LTP of hippocampal slices of rats. Initial slope of excitatory postsynaptic potential (EPSP) couldn't keep stable and gradually went down. However, curcumin could prevent LTP inhibition induced by TNF- alpha and NMDA. These results indicate that the protective effects and mechanism of curcumin is that it can prevent the rapid Ca2+ influx induced by TNF- alpha and maintain the Ca2+ homeostasis in neuron cytoplasm through NMDA receptor.

P60
The Functional Protective Effect and Mechanism of Curcumin on IL-6 Induced Neuronal Damage in Rat Hippocampus

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Curcumin is an extract from the plant curcuma longa which has multiple pharmacal activities, such as anticarcinogenic, anti-inflammatory, and anti-HIV-1. Our previous study has proved curcumin could improve learning and memory ability in memory disorder rats induced by gp120. Based on that, the present study was performed *in vitro* in rat brain slices to determine the functional effect of curcumin on IL-6-induced neuronal damage in rat hippocampus, and to dissect the molecular mechanism of the impact of curcumin on IL-6 neurotoxicity. *In vitro* brain slices recording techniques were used to record the excitatory postsynaptic potential (EPSP) of CA1 pyramidal layer of rat hippocampal brain slices. High frequency stimulation (HFS) was given on Schaffer branches to induce long-term potentiation (LTP). The initial slope of EPSP was then measured in the treatments of IL-6 or NMDA with or without curcumin. The results showed that compared with the control group incubated with artificial cerebrospinal fluid (ACSF) only, IL-6 and NMDA markedly inhibited LTP of hippocampal brain slices of rats $p < 0.05$). The inhibition of LTP by IL-6 or NMDA was partially but significantly prevented by addition of curcumin (IL-6 versus IL-6 plus curcumin, $p < 0.05$; NMDA versus NMDA plus curcumin, $p < 0.05$). IL-6, curcumin and NMDA had no effects on basal synaptic transmission of hippocampal slices. These results indicate that curcumin exerts a preventive effect on IL-6-induced neuronal damage in rat hippocampus, probably through act on the NMDA receptor of neuronal cytomembrane to increase intracellular Ca2+ influx.

P61
Identification of Active Loci of a Human Endogenous Retrovirus in Neurons of Patients with Amyotrophic Lateral Sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) is characterized by the progressive loss of motor neurons, of unknown etiology. Previous studies showed reverse transcriptase in serum of ALS patients at levels comparable to HIV-infected patients; however, the source and significance of the retroviral elements is uncertain. Methods: Expression of a human endogenous retrovirus (HERV-K), was determined in autopsy brain tissue of patients with ALS and compared to control populations, by real time polymerase chain reaction followed by sequencing of the amplified genes and confirmed by immunostaining. Results: HERV-K pol transcripts were increased in patients with ALS compared to those with chronic systemic illness, but could not be detected in Parkinson's disease or in the accidental death controls. Sequencing revealed several actively transcribed loci in the HML-2 and 3 subfamilies of HERV-K, with a specific pattern of expression including intact open reading frames and the transcription of a unique locus in ALS. The frequency of intact pol transcripts was highest in the motor cortex and the reverse transcriptase protein was localized to cortical neurons of ALS patients. HERV-K expression strongly correlated with TDP-43, a multi-functional protein known to be dysregulated in ALS. Conclusions: We have identified a specific pattern of HERV-K expression in ALS, which may potentially define the pathophysiology of ALS. Targeting of activated genome-encoded retroviral elements may open new prospects for the treatment of ALS.

P62
Borna Disease Virus infects human primary neural stem cells and impairs neurogenesis

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Borna disease Virus (BDV) is a non-segmented, negative-strand RNA virus, capable of infecting a large number of vertebrates. In mammals, persistent infection of the central nervous system (CNS) leads to behavioural disorders. BDV is a recognized pathogen in veterinarian field but it also infects humans and might be involved in mental diseases, such as schizophrenia. Neurogenic niches such as the subventricular zone and the sugranular zone of the dentate gyrus as well as neural progenitors cells have been shown to be infected by BDV in experimentally infected rats and it was suggested that neural progenitors might be involved in BDV-induced pathogenesis. Interestingly, there has been argument lately in favour of a role for hippocampal neural stem cells in the neurophysiopathology of schizophrenia. The aim of our study was to investigate whether primary human neural stem cells (hNSC)are permissive to BDV and whether BDV might alter the physiology of these cells. Human primary NSC cultures were established from the CNS of human embryos. We demonstrated that they are highly permissive to the viral BDV strain He80, isolated from a diseased horse. BDV-He80 productively replicates and disseminates in hNSC. The morphology, survival and nestin expression were not altered in BDV-infected NSC although the virus persists for at least 12 passages. On the contrary, BDV strongly impaired neurogenesis when differentiation was induced by withdrawal of growth factors. Both, a decrease in the number of newly formed neurons and a decrease in their survival was observed. This was specific to neurogenesis since astrogliosis was not altered. In conclusion, we provide evidence that BDV is capable of damaging human neurogenesis and we demonstrate a new mechanism by which BDV might impair neural function in infected individuals. These results may help to understand the behavioural disorders associated with BDV infection.

P63
Analysis of Tryptophan and Its Metabolites in CSF of SIV-Infected Pigtail Macaques by GCMS/MS

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Despite the ability of HAART therapy to control viremia and prevent the development of AIDS, approximately 30% of HIV-infected patients develop mild cognitive and motor deficits – a component of HIV-associated neurological disease (HAND). Identification of a biomarker that is predictive of HAND would allow doctors to prescribe neuroprotective agents to those patients most at risk for developing neurological disease. The amino acid tryptophan and its metabolites are potential candidates for biomarkers of neuronal health, as tryptophan is the

precursor to neurotransmitter serotonin. During HIV infection tryptophan is diverted to the kynurenine pathway by the enzyme indoleamine 2,3-dioxygenase (IDO), resulting in production of several neuroactive metabolites, including the neurotoxic NMDA receptor agonist quinolinic acid (QA) and the neuroprotective NMDA receptor antagonist kynurenic acid (KA). The balance between serotonin, trp, and these kynurenine metabolites may be critical to neuronal function. Using a GCMS/MS assay (Eckstein *et al*, 2008), we have extended our analysis to all kynurenine metabolites, seven neurochemical pathways, and all 20 amino acids plus GABA to provide an unprecedented examination of longitudinal CSF samples from an accelerated model of HIV CNS disease in pigtailed macaques. Preliminary data from end-stage disease show CSF tryptophan levels declining with severity of CNS disease; however, the most severe animals had levels similar to uninfected controls, which may be a result of breakdown of the blood-brain barrier. Further studies will elucidate the earliest detectable point at which tryptophan metabolism changes in the CSF, whether tryptophan deficits result in changes in levels of neurotransmitters such as serotonin, and whether various treatments such as HAART ameliorate neurotoxic effects of tryptophan metabolism.

P64
Evaluation of biomarkers for the prognosis and diagnosis of different forms of leukoencephalopathies: a longitudinal study

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Leukoencephalopathies in HIV+ and HIV- patients need to be evaluated to define prognostic and diagnostic markers of disease. To achieve this aim, clinical, virological and neurological features of leukoencephalopathies affecting HIV+ and HIV- patients were analysed. Fiftyfive cases showing lesions at MRI were enrolled in a longitudinal survey and divided according to the diagnosis: 20 HIV+ PML, 6 HIV- PML, 29 HIV+ Not Determined Leukoencephalopathies (NDLE). Mean length of follow up was 39.2 months (0.7-43). At the enrolment, mean CD4+ cells count was higher ($p = 0.008$) in NDLE (309/µl; 233-401), than in PML patients (106.5/µl; 75.5-166), while mean values of CSF HIV viral load (VL) was higher ($p < 0.01$) in PML than in NDLE patients. The SNRS score was 100% (90-100) in NDLE and 75% (37-100) in HIV+ PML ($p = 0.057$). The P100 latency Multimodal Evoked Potential resulted increased in HIV+ PML patients, compared to NDLE patients. CSF JCV DNA VL showed an higher mean value in the HIV+ PML patients who died within one year (log 5,72 copies/ml) and in HIV- PML patients (log 5,62 copies/ml, $p < 0.005$), than in the patients still alive (log 3,74 copie /ml). The Transcriptional Control Regions of JCV were characterized by sequencing and the transcription factor binding sites for sp1, cre, p53 and ap1 were more represented in strains from HIV+ PML patients with fatal outcome and HIV- PML patients than from PML patients with benign clinical course. None of the searched viruses (JCV, LPV and Human Herpesviruses) was found in the NDLE patients, confirming that viruses are not involved in the pathogenesis of NDLE. The main prognostic markers of PML seem to be CSF JCV viral load, TCR rate of rearrangements, whereas CD4 cell count, CSF HIV RNA, SNRS score, and P100 latency potential seem to be markers of differential diagnosis between PML and NDLE.

P65
HIV infection of human astrocytes disrupts blood brain barrier integrity by a gap junction dependent mechanism

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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 enable 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 astrocytes end feet and dysregulated astrocyte signaling that result in BBB compromise, suggesting an important role for these few infected astrocytes in NeuroAIDS. All alterations in BBB structure and integrity induced by HIV infected astrocytes were gap junction and possibly maxi channel (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.

P66
HIV-tat alters Connexin43 trafficking and expression, enabling HIV-infected astrocytes to maintain gap junctional communication with uninfected cells

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As people with AIDS live longer, the prevalence of cognitive impairment is increasing, despite antiretroviral therapy. The mechanisms that mediate CNS dysfunction in general do not correlate with active viral replication, suggesting that mechanisms of amplification participate in the spread of these toxic signals within the central nervous system. We demonstrated that gap junction channels participate in the diffusion of toxic signals between HIV infected astrocytes and uninfected astrocytes. However, it was difficult to understand why HIV infection, an inflammatory condition, did not reduce gap junctional communication. We found that HIV-tat protein, but not gp120, vif or nef, increased expression of Cx43 in HIV-infected cultures by a mechanism that involves, increased expression of Cx43 mRNA, binding of tat to the Cx43 promoter, and higher expression of Cx43 protein in the cytoplasm and at the membrane, resulting in maintain/enhanced communication with neighboring cells even after HIV infection. Our findings describe a novel mechanism of toxicity within the brain, triggered by low numbers of HIV-infected astrocytes and amplified by gap junctions, contributing to the pathogenesis of NeuroAIDS.

P67
The novel role of astrocye ATP receptors, and hemichannels of pannexin and connexin, in the pathogenesis of NeuroAIDS

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HIV entry into the central nervous system (CNS) results in neurological dysfunction. The mechanisms that mediate CNS dysfunction are still not well understood, and include inflammation, viral presence and/or replication. In the current ART era where CNS viral replication and inflammation are reduced, the mechanisms that mediate cumulative damage need further study.. We demonstrated that gap junctions and maxichannels (ATP receptors, hemichannels of connexin and pannexin-1, play a critical role in viral entry, amplification of apoptosis of uninfected cells, and inflammation. Our findings describe a novel mechanism of toxicity within the brain, triggered by low numbers of HIV-infected astrocytes and amplified by gap junction and maxi channels, contributing to the pathogenesis of NeuroAIDS.

P68
Tunneling nanotubes (TNT), a novel communication system in the pathogenesis of HIV/AIDS

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We have novel data that demonstrated that HIV proteins and perhaps virus can spread by an intracellular pathway, named tunneling nanotubes (TNT), without an extracellular component. TNT are long cytoplasmatic bridges that accomplish long-range directed communication between cells. The proposed function for TNT is the cell-to-cell transfer of large cellular structures such as vesicles, organelles and small molecules (second messengers and toxic signals). We identified that TNT may serve as an additional way for HIV, HIV proteins, and signaling molecules to spread infection and toxicity between connected human macrophages, contributing to the disease process as well as to the pathogenesis of HIV/AIDS and NeuroAIDS. Our preliminary data and published studies indicate that HIV increases the formation of TNT, enabling HIV proteins to spread between connected cells that may result in a faster time course of HIV dissemination. Thus, our hypothesis is that TNT formation induced by HIV infection enables HIV to spread between connected cells by an intracellular route without an extracellular component. We are examining the contribution of this novel intracellular communication system to amplify HIV infection between macrophages and other CNS cells. TNT in macrophages are highly expressed during the active spread of the virus and decline after the cultures are

infected. We identify that TNT in macrophages are totally distinct from the ones described in T cells, in that they do not shared cytoplasmic connection rather communicate by a synapse-like mechanism. Thus, further investigation is necessary to identify the specific mechanisms by which HIV-1 induces the formation of TNT and utilizes these processes, as well as to design new therapies to reduce viral spread and toxicity by this intracellular pathway within the CNS.

P69
Extracellular HIV-1 Vpr induces a decrease in astrocytic glutathione and ATP levels that impacts secretion of neuroprotective factors and HIV-1 neuropathogenesis

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Human immunodeficiency virus type 1 (HIV-1)-infected CD4+ T cells and cells of the monocyte-macrophage lineage cross the blood-brain barrier and spread viral infection to cells of the central nervous system (CNS). Within the CNS, they secrete inflammatory cytokines, chemokines, and the viral proteins Tat, gp120, nef, and viral protein R (Vpr), impairing survival of uninfected bystander cells. Increasing levels of soluble Vpr have been found in the serum and cerebrospinal fluid of HIV-infected patients, correlating with disease progression. Additionally, patients in late-stage disease, or with acquired immunodeficiency syndrome (AIDS), suffer from different inflammatory conditions and manifest excessive intracellular oxidation. Consistent with these observations, we have demonstrated that Vpr-containing conditioned media decreases astrocytic glutathione (GSH) and ATP pool levels. Furthermore, extracellular (EC) Vpr was found to transactivate viral gene expression from integrated proviral DNA in astrocytes. Based on these results, and the aforementioned clinical manifestations, we hypothesize a correlation between enhanced EC HIV-1 Vpr in the CNS and a decline in astrocytic GSH levels with disease progression. In addition, EC Vpr both directly and indirectly causes astrocytic oxidative stress and reactivation of viral gene transcription, which may seed infection from persistently infected astrocytes to the CNS compartment. Moreover, by lowering intracellular energy storage, Vpr may also affect ATP-dependent glutamate clearance, which consequently impairs secretion of neuroprotective factors and neuronal survival. These results support the hypothesis that HIV-1 extracellular Vpr deregulates the neuronal-astrocytic network, thus providing new therapeutic approaches to ameliorate the clinical course of HIV-associated neurocognitive impairment.

P70
Comparative Analysis of VZV Protein 62 and 63 in the Three Branches of Human Trigeminal Ganglia and in Dorsal Root Ganglia

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Varicella zoster virus (VZV) is a human alphaherpesvirus that, after primary infection, hematogenously spreads to the sensory ganglia where it can establish lifelong latency. Reactivation of VZV commonly affects the V1 (ophthalmic) branch of the trigeminal ganglion (TG) and often the dorsal root ganglia (DRG). We investigated the distribution of latent VZV (protein 62 and protein 63) in the neurons projecting to the 3 branches of the human TG and in the DRG. In 12 TG, neurons were allocated to the different branches (ophthalmic, maxillary (V2), mandibular (V3)) of the TG by post-mortem tracing with DiI or neurofilament staining. These 12 ganglia and an additional 12 TG were compared to 22 dorsal root ganglia. Latent VZV protein 62 and protein 63 were detected via immunohistochemistry with monoclonal antibodies on frozen sections. The percentage of VZV-positive neurons in a x400 field of view was analyzed. It was found to be highest in the neurons projecting to the V1 branch (protein 62: 22 ± 6.4; mean ± SE; protein 63: 8.9 ± 4.8) compared to the V2 (protein 62: 18.0 ± 5.9; protein 63: 7.1 ± 3.9) and the V3 (protein 62: 17.5 ± 5.7; protein 63: 4.5 ± 3.6), although this difference did not reach statistical significance. Protein 62 was, however, significantly more abundant in all branches. When the 24 TG were compared to the 22 DRG, latent VZV protein expression was found at similar frequencies in the sensory ganglia and protein 62 was significantly more abundant than protein 63. In conclusion we directly

compare the expression VZV protein 62 and protein 63 in sections from the same TG or DRG. Each protein showed similar frequencies in the TG and the DRG, reflecting the route of primary VZV infection via the bloodstream. Protein 62 was detected at significantly higher frequencies than protein 63, the functional significance of which warrants further investigation.

P71
Glia utilize RIG-I and DAI to perceive disparate viral pathogens of the CNS

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The rapid onset of potentially lethal neuroinflammation is a defining feature of viral encephalitis. Glial cells are ideally positioned to respond to invading pathogens of the central nervous system (CNS) and produce key inflammatory mediators following exposure to neurotropic viruses. However, the mechanisms by which resident CNS cells perceive such challenges have not been defined. Recently, several cytosolic pattern recognition receptors, including retinoic acid-inducible gene I (RIG-I) and DNA-dependent activator of IFN regulatory factors (DAI), have been described that appear to function as intracellular sensors of RNA and DNA viruses, respectively. Interestingly, recent studies suggest that RIG-I may also be able to recognize DNA pathogens in a polymerase III-dependent manner. However, little is known regarding the expression of these molecules or their function in the CNS. Our studies indicate that microglia and astrocytes constitutively express mRNA encoding RIG-I and DAI, and both of these proteins can be detected in resting cells. Furthermore, expression of these intracellular viral sensors is upregulated following infection, with elevated DAI levels following DNA viral challenge and induced expression of RIG-I when microglia and astrocytes were exposed to disparate RNA viruses including vesicular stomatitis virus and Sendai virus. Interestingly, DNA viruses were also capable of inducing RIG-I expression consistent with the previously reported ability of this receptor to mediate cellular responses to such pathogens. Importantly, reportedly specific ligands for these viral sensors were found to elicit glial immune responses and targeted knockdown of these receptors limits such responses following viral challenge and attenuates the production of soluble neurotoxic mediators by these cells. These studies point to these novel intracellular pattern recognition receptors as key components in the recognition of viral pathogens by resident CNS cells and may represent an important mechanism underlying the damaging neuroinflammation and neuronal cell death associated with acute viral CNS infections.

P72
HIV-1 Tat Modulates Intercellular Communication in Human Brain Cells

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Due to the lack of sufficient animal models, cell culture system has paved the way in early investigations in neuropathogenesis underlying HIV Associated Neurocognitive disorder (HAND). Gap junction channels play a major role in intercellular communication between neuron and glial cells by allowing the passage of various ions (like K+, Na+) and second messengers (cAMP, Ca2+), thereby regulating different signaling pathways. Previous studies have reported an increase in the level of connexin (Cx)-43 in primary human astrocytes upon infection with live HIV virus. But it is largely unclear whether this effect is attributed due to any particular protein of the virus. Moreover, the underlying signaling pathways and the consequences of this increase in gap junction communication needs to be worked out. We have established human neuron-astrocyte co-culture system using the cells differentiated from human fetal brain derived neural precursor cells, in an attempt to closely mimic the *in vivo* conditions of human brain. In our study, we observed a significant increase in gap junction communication proteins (Cx40, Cx26 and Cx36) post treatment with HIV-1 Tat protein. Further studies are in progress to understand the consequence of this increase in connexins on cellular toxicity and signaling mechanisms that may lead to these alterations. Study was supported by NBRC core funds and PG is recipient of Junior Research Fellowship from Council of Scientific and Industrial Research, New Delhi, India.

P73
Mechanism of Dopamine Mediated Increase in HIV Infection of Human Macrophages

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As HIV infected individuals live longer with the success of combinatorial antiretroviral therapy, neurological complications are emerging as a major health issue, especially among HIV-infected drug abusers. HIV positive individuals who abuse drugs such as cocaine and methamphetamine show changes in the incidence and severity of neuropathology and in the development of HIV-associated neurological disorders (HAND). The mechanism(s) by which drug abuse alters the development of

neurological disease remains unclear, but cocaine, methamphetamine and other drugs of abuse mediate their effects by increasing extracellular dopamine in the CNS. We hypothesize that dopamine modulates HIV infection in macrophages through dopamine receptors, resulting in the exacerbation of HIV induced neuropathology in HIV infected drug abusers. Our findings demonstrate that dopamine increases HIV replication in primary human macrophages by increasing the number of macrophages infected with HIV, at least in part, through the activation of D2-like dopamine receptors. Preliminary data indicate that increased infection is due to enhanced HIV entry into macrophages in the presence of dopamine. Our data also demonstrate that dopamine activates macrophage dopamine receptors and alters macrophage signaling. These data indicate a significant role for dopamine in the response of the CNS to HIV infection, as well as in the modulation of other macrophage functions. Further, these data suggest that drug-induced increases in CNS dopamine may be a common mechanism by which drugs of abuse mediate the development of HAND in HIV infected drug-abusing individuals. Supported by NIDA.

P74
Soluble and Cell-associated Insulin Receptor Dysfunction Correlates with Severity of HAND in HIV-infected Women

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Objective: To determine if changes in soluble and cell-associated insulin receptor (IR) and IR substrate-1 (IRS-1) were associated with the presence of severity of HIV-associated neurocognitive disorders (HAND) in a cohort of HIV-seropositive women. Background: Blood sugar metabolism abnormalities have been identified in HIV-infected individuals and associated with HAND. This abnormality may occur as a result of chronic HIV infection, antiretroviral treatment (ART) side effects, aging, genetic predisposition, or a combination of these factors. This may increase the morbidity and mortality in this population. Methods: 34 HIV-seropositive women and 10 controls without history of diabetes were evaluated. Soluble IRs (sIR, alpha and full length) in plasma and CSF samples was determined by ELISA. Membrane IR quantification was performed in CSF white cell pellets (WCP). WCP were permeabilized and incubated with anti-IRS-1 or anti-phospho-tyrosine-IRS-1 antibodies and FITC secondary antibodies; levels were analyzed by flow cytometry. Cognitive performance progression evaluated in 23 women who completed at least 3 evaluations. Cognitive status did not change (n = 10), progressed to a worse (n = 8), or improved (n = 5). Results: HIV-seropositive women had a significantly increased level of sIR full length in plasma ($p < 0.001$) and CSF ($p < 0.005$) compared to controls. When stratified by HAND, increased plasma sIR full length was associated with the presence ($p < 0.001$) and severity ($p < 0.005$) of HAND. A significant correlation was observed between plasma sIR full length levels and HAND progression ($p = 0.02$) where higher levels correlated with improvement and lower with worsening ($p < 0.05$). A significant decrease in IRS-1 tyrosine-phosphorylation was associated with the presence ($p < 0.02$) and severity ($p < 0.02$) of HAND. Conclusions: This study provides evidence for increase IR secretion and decrease IRS-1 phosphorylation in HIV-seropositive women. This IR dysfunction may have a role in progression of HAND and could represent a biomarker for the presence and severity of HAND. Partially supported S11NS046278, U54NS43011, P20RR11126, G12RR03051.

P75
Role of CD4+ and CD8+ T cell responses against JC virus in patients with PML

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Objective: To assess the role of CD4+ and CD8+ T cells against JC virus in the clinical outcome of progressive multifocal leukoencephalopathy (PML). Methods: We tested 110 subjects, including 6 early PML patients (PML-E), within one year of symptoms onset; 13 PML progressors (PML-P), who died within one year of symptoms onset; 42 PML survivors (PML-S), still alive one year after symptoms onset; 6 healthy HIV+ controls (HIV+); 8 patients with leukoencephalopathy of unknown origin (LeukoX); and 35 healthy subjects (HC). PBMC were isolated over a Ficoll-Paque gradient and stimulated with a JCV peptide library covering the entire VP1 protein.

We assessed cellular immune response against JCV by IFN gamma ELISpot, measuring the response of all T cells, and Intracellular Cytokine Staining (ICS), measuring CD4+ and CD8+ T- cell responses. Results: There was a good correlation between ELISpot and ICS results in PML-S: 71.4% when tested within 6 months of symptoms onset (PML-S < 6 months) and 81.8% when tested later (PML-S > 6 months). Only 18.2% of PML-P had a positive ELISpot in comparison to 68.8% of PML-S < 6 months and 83.3% of PML-S > 6 months (p 0.018 and 0.0004). ICS showed a lower cytotoxic CD8+ response in PML-P (16.7%) compared to PML-S < 6 months (100%) and PML-S > 6 months (93.4%), (p = 0.0047 and 0.0029) and a trend in the CD4+ responses in PML-P (50%) compared to PML-S < 6 months (85.7%) and PML-S > 6 months (100%) (p = 0.2657 and 0.0206). PML-P had weaker ELISpot responses than PML-S, LeukoX patients and HC. Conclusion: ELISpot and ICS are high throughput assays that can be used regardless of the HLA composition of the subjects. Both showed that the cellular immune response against JCV is associated with better clinical outcome. ICS demonstrated that it is principally mediated by CD8+ T cells. These assays will be useful as prognostic markers and to guide the management of PML patients.

P76

Bone marrow-derived mesenchymal stem cell infection and transformation by JC virus in *in vitro* and *in vivo* models

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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, studies have demonstrated the transforming ability of JCV early protein, T-antigen, in culture and animal models. Also, JCV T-antigen has been detected in a variety of human tumors, including brain neoplasms. JCV infection most likely occurs in childhood and persistent virus has been detected in tonsilar stromal cells and cells of the bone marrow hematopetic lineage. In this study, we demonstrate that bone marrow-derived non-hematopoetic 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 and develop neural-like tumors in animals. A subset of cancer cells isolated from T-antigen induced tumors is positive for T-antigen and CD133, a marker of human cancer stem cells. These cells have the ability to induce secondary tumors upon implantation into Nude mice that develop more rapidly than those initiated by the parental cells. In light of earlier reports on the potential of MSCs to differentiate into a variety of lineages characteristic of different tissues and their ability to traffic to distant tissues, including brain, our data suggests a potential common origin of T-antigen associated tumors. The identification of JCV expression in these cells may be critical for understanding of JCV latency, distribution, and oncogenic potential in diverse tissues. Further, our data provides an experimental model system for studying of molecular and cellular events leading to transformation of bone marrow-derived MSCs and the ability of these cells to develop organotypic tumors from JCV T-antigen transformed mutipotent stem cells.

P77

Dissecting the role of Sp transcription factor binding sites on viral transcription in HIV-1 long terminal repeats derived from HIV-1 dementia patients

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Introduction: HIV-1 penetrates the central nervous system (CNS), causing neurocognitive impairment including HIV-associated dementia (HAD). Macrophages and microglia are sites of productive HIV-1 infection within the CNS. In contrast, although astrocytes are extensively infected, they undergo a restricted infection. Multiple blocks to virus production in astrocytes have been reported, including decreased transcriptional activity of the HIV-1 long terminal repeat (LTR). We recently demonstrated that LTRs isolated from a panel of demented HIV patients are compartmentalized and sequence analysis revealed mutations in regions associated with transcriptional activation which differentiated matched CNS and lymphoid-derived LTRs. Significantly, sequence differences were observed in the region spanning the three Sp transcription factor binding sites previously reported to be essential for both basal and activated transcriptional activity. These data suggest that Sp binding sites may play a

role in mediating the restricted transcriptional activity of CNS-derived LTRs in astrocytes. Methods: HIV-1 LTR sequences were PCR amplified and cloned from a cohort of five HAD autopsy subjects consisting of matched CNS- (brain, cerebral spinal fluid, or spinal cord) and lymphoid- (lymph node, spleen, or peripheral blood mononuclear cells) derived isolates. Using a luciferase reporter system, transcriptional activity of HIV-1 promoters isolated from the CNS and lymphoid compartments was analysed in T cells and in SVG astrocyte cells. Electrophoretic mobility shift assays (EMSA) were used to analyse recombinant Sp1 binding to radiolabeled DNA probes (encompassing the three Sp sites in combination or separately) derived from each unique LTR. Densitometry was used to determine the relative affinity of each DNA probe for Sp1 binding. Results: Transcriptional assays showed that CNS-derived LTRs had restricted basal transcriptional activity in SVG astrocyte cells. EMSA performed using DNA probes with all three Sp sites in combination revealed differences between CNS and lymphoid-derived LTRs in overall Sp1 binding affinity. When Sp sites were analysed in isolation we identified CNS-derived promoter distal Sp sites (Sp-III) with markedly reduced Sp1 binding affinity when compared to lymphoid-derived sites. Heterogeneity was observed in the Sp1 affinity of CNS and lymphoid-derived promoter medial (Sp-II) and promoter proximal (Sp-I) Sp binding sites. Changes in Sp1 binding at promoter medial (Sp-II), and promoter proximal (Sp-I) sites had minimal affect on overall LTR affinity. In contrast, changes at the promoter distal (Sp-III) site largely dictated overall LTR affinity. Furthermore, we identified sequence changes both within and flanking the Sp sites that were responsible for reduced Sp1 binding. Discussion: The reduced Sp1 binding potential of CNS-derived LTRs suggests these viruses have a reduced capacity to initiate viral transcription, which correlates with the restricted transcription observed in astrocytes. Taken together, these data suggest unique transcriptional mechanisms exist within astrocytes, ultimately affecting the fate of viral infection and the development of latency.

P78
Glia as architects of accelerated CNS inflammation and injury with opioid abuse in neuroAIDS

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We and/or others have previously reported that opioids can increase HIV-1 replication in infected cells, and exacerbate the neurotoxic effects of Tat and gp120 *in vitro* and/or *in vivo*. Using a GFAP-driven, doxycycline (DOX)-inducible Tat transgenic mice model, morphine exposure was shown to cause synergistic increases in Tat-induced MCP-1/CCL2 production, astrogliosis, and microgliosis in the striatum following 2, 7 or 10 days of exposure. Although there was no indication of neuron death at these time points, sustained Tat induction and/or morphine co-exposure significantly reduced spine density. Morphine also potentiated Tat-induced formation of bead-like varicosities and/or fragmentation in the dendrites of striatal neurons. Importantly, the neuronal injury coincided with deficits in rotarod performance and alterations in morphine-dependent anti-nociceptive behavior. To identify the cell and molecular targets of opioids in the interactive pathology, neurons, astrocytes, and microglia from wild-type or mu opioid receptor (MOPr) null mice were cultured individually or co-cultured using a variety of paradigms and experimental outcome measures. Results showed that (i) HIV Tat or gp120 were intrinsically neurotoxic in the absence of glia, while (ii) morphine-enhanced Tat/gp120 neurotoxicity was entirely dependent on MOPr+ astroglia and microglia, and (iii) the synergistic neurotoxicity was abolished when MOPr+ neurons were cultured with MOPr null glia. The presence or absence of the MOPr on neurons did not affect the synergistic neurotoxicity. Thus, morphine's ability to potentiate Tat or gp120-induced neurotoxicity seems completely dependent upon the presence of MOPr+ glia. We propose that opioids and HIV-1 Tat/gp120 act synergistically to directly destabilize glial function, and that aberrant glia-neuron signaling causes neuron injury and dysfunction. Our results provide insight into the cellular basis by which opioids exacerbate neuroAIDS. We propose that MOPr-expressing glia are likely to be important targets for therapeutic intervention for HIV-opioid abuse co-morbidity. Support: DA19398, DA18633, DA27374.

P79
ATP Signaling is Critical for HIV Entry into Primary Human Macrophages

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HIV infection results in HIV-associated neurocognitive disorders (HAND) in approximately 50% of infected individuals. This dysfunction results from HIV-infected monocytes crossing the blood brain barrier, release of HIV, and subsequent infection of CNS macrophages, microglia, and astrocytes. Infected macrophages are central to the disease process as they release inflammatory mediators and neurotoxic factors. A more complete understanding of the mechanisms that mediate HIV infection of macrophages is necessary to develop interventional strategies for HAND. We propose that large pore ion channels in maxi channels (pannexins and ATP receptors) play a significant role in HIV infection of macrophages. We demonstrate that inhibition of ATP receptors using oxidized ATP (oATP), a P2X7 receptor inhibitor, results in a significant dose-dependent decrease in HIV replication in primary human macrophages and a significant decrease in the percentage of macrophages infected. This effect is consistent using several R5 viral strains, ADA, BaL, and YU-2. Treatment with several other P2X receptor inhibitors, including suramin, NF 279, and A-740003, results in similar inhibition. This indicates that extracellular ATP plays a significant role in HIV infection of macrophages. We propose that HIV binding to macrophages results in ATP release and subsequent calcium influx that facilitates viral entry. In support of this, our data suggest that treatment of macrophages with gp120, the HIV surface protein, results in rapid induction of ATP release. Furthermore, oATP blocks HIV entry into macrophages, indicating that ATP signaling is critical early in the viral life cycle. We are examining the role of calcium flux on HIV entry and of ATP receptors in gp120-induced calcium influx. Our data provide compelling evidence that host cell receptors other than those directly bound by HIV are critical for viral entry into macrophages and may lead to the development of novel therapeutics for the treatment of HAND.

P80
Prevalence and HIV disease correlates of HAND in the pre-CART and CART eras

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Prevalence and HIV disease correlates of HAND in the CART versus pre-CART eras Robert Heaton, Ph. D., Donald Franklin, B.S., Ronald Ellis, M.D., Ph.D., Scott Letendre, M.D., J. Allen McCutchan, M.D., and Igor Grant, M.D., for the CHARTER and HNRC Groups University of California, San Diego Combination antiretroviral therapy (CART) has greatly reduced the incidence of opportunistic disease and mortality in HIV-infected individuals, but studies of its effect on neurocognitive impairment (NCI) have been mixed. Here we examined NCI in the pre-CART and CART eras in persons at various stages of HIV infection. 857 adults (HIV-, n = 179; non-AIDS, n = 516; AIDS, n = 162) from the pre-CART era (1988-1995) were compared to 937 (HIV-, n = 94; non-AIDS, n = 336; AIDS, n = 506) from the CART era (2000-2007). Treatment era cohorts received comparable, comprehensive neuromedical and neuropsychological evaluations, and similar, rigorous screening to exclude non-HIV CNS comobidities. Overall, 40% of HIV-infected individuals had NCI in the CART era vs. 33% in the pre-CART era. Analysis by CDC Stages showed a significantly higher rate of NCI for CART era in CDC A only (36% post-CART vs 25% pre-CART, $p = .001$). Infected CART era individuals were more likely to be on ARVs (70% vs. 47%; $p < .0001$), had higher current (but lower nadir) CD4 cell counts, were more likely to have an undetectable plasma viral load (65% vs. 5%; $p < .0001$), and had much longer estimated duration of infection (9.9 vs 2.8 yrs, $p < .0001$). History of severe immunosuppression (low nadir CD4) was the only robust predictor of NCI in both eras. NCI remains prevalent despite CART. Of interest, more CART era non-AIDS cases have NCI than pre-CART. This suggests negative CNS effects of longer survival in a pre-AIDS state during which the brain remains exposed to repeated fluxes in HIV and/or chronic immune stimulation.

P81
Entry and fusion of La Crosse virus

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La Crosse Virus (LACV) is a leading cause of pediatric encephalitis and asceptic meningitis in the Midwestern United States where its primary mosquito vector is native. The LACV glycoproteins (Gn and Gc) play a critical role in the neuropathogenesis of LACV infection as the primary determinant of neuroinvasion. A 22-amino-acid hydrophobic segment within Gc (1066-1087) was recently identified as the LACV fusion peptide. To define the role of the fusion peptide in virus entry, fusion, and neuropathogenesis, a panel of recombinant LACV (rLACV) with mutations in the fusion peptide was generated using a novel, reverse genetics system. Both conformational and non-conformational antibodies detected expression of Gn and Gc in cells infected with WT or rLACVs. However, replication of recombinant fusion peptide mutants was significantly

reduced compared to wild-type (WT) virus in muscle cells (G8), primary rat cortical cultures, and mosquito cells (C6/36). Using a fusion-from-within (FFWI) assay to measure virus induced cell-to-cell fusion, the fusion peptide mutants demonstrated decreased fusion relative to WT LACV in BHK-21, G8, and C6/36 cells which is consistent with previous data from our laboratory testing the fusion efficiency of these specific mutations in a plasmid-based, cell-to-cell fusion assay. To determine the effects of these mutations within the LACV fusion peptide on virus mediated neuronal loss, a MAP2 ELISA performed on primary rat neuronal cultures demonstrated dendritic loss similar to WT LACV for the fusion peptide mutants. In conclusion, viruses with changes in the fusion peptide domain have reduced fusion and growth phenotypes, supporting a critical role for the fusion peptide in virus fusion and entry. Interestingly, these viruses maintained their ability to cause neuronal loss *in vitro*, suggesting that the fusion peptide of LACV Gc is a determinant of properties associated with neuroinvasion, but not of neurovirulence.

P82
Evidence for dynamin- and clathrin-mediated endocytosis of La Crosse virus

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La Crosse virus (LACV) is a leading cause of pediatric encephalitis and aseptic meningitis in the Midwestern United States where its primary mosquito vector is endemic. The LACV glycoprotein Gc plays a critical role in the neuropathogenesis of LACV as the primary determinant of neuroinvasion and serves as the viral attachment protein. Many viruses, including LACV, require a low-pH dependent conformational change during endosomal maturation to mediate fusion. Although it has been established that LACV enters cells by receptor-mediated endocytosis, the mechanisms involved in its internalization are ill defined. Here, we used chemical and molecular inhibitors of entry pathways in concert with imaging studies to better define LACV's route of entry and the role of clathrin in this process. Imaging studies clearly demonstrated that LACV colocalizes with clathrin. In addition, the inhibition of dynamin- and clathrin-mediated endocytosis using dynasore, chlorpromazine and hypertonic media resulted in decreased fusion indices in a fusion from within assay and significant decreases in virus replication. The contribution of clathrin and dynamin to virus entry were further supported by studies demonstrating that, LACV infection could be rescued in the presence of these inhibitors by adsorbing virus at 4oC to abate endocytosis and subsequently exposing these cultures to low-pH media (pH 5.5) for 30-60 seconds, thereby allowing the virus to fuse with the plasma membrane to initiate infection. In conclusion, the inhibition of clathrin-mediated endocytosis by chemical and molecular means has provided strong evidence for clathrin-mediated endocytosis as the route of entry for LACV. Future studies will determine the roles of cholesterol and caveolae as well as macropinocytosis in LACV entry.

P83
A unique brain HIV sequence database reveals genetic and biological properties of HIV variants associated with CNS compartmentalization and dementia

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HIV- associated neurological disorders (HAND) are complex phenomena requiring analysis of large datasets for better understanding of underlying mechanisms. HIV sequences in brain are evolutionarily divergent compared to those in other tissues due to the infected target cell population (macrophages/microglia), immune selection pressures, and timing of viral transit into the CNS. Env is the main determinant of neurotropism. Numerous labs have generated matched brain and non-brain env sequences. However, methods to perform meta-analysis across these sequences are currently lacking. To address this need, we created a unique database for sequence datasets of HIV env in the brain that will soon be public. The database structure facilitates flexible sequence queries linked to uniform annotations of patient data such as HIV disease markers and neurological diagnoses. The database consists of datasets from 20 publications, representing approximately 3000 sequences from 77 patients (~1500 from brain, 1000 from blood/lymphoid tissues, and 500 from other tissues). We used a standardized anatomical ontology to capture neuroanatomical detail, while maintaining ontological relationships between brain regions. Using database queries to compare HIV sequences between brain and non-brain tissue sites, or within different regions of brain, we demonstrated: 1) genetic variants associated with brain compartmentalization or dementia based on analysis of 792 env sequences from 25 patients. These variants enhance macrophage tropism, with a subset exhibiting additional phenotypes relevant for neurovirulence, such as increased shedding of the viral

envelope glycoprotein associated with induction of neuronal apoptosis in primary brain cultures. 2) evidence of brain-specific adaptive selection at specific env residues associated with these phenotypes; 3) genetic compartmentalization (Slatkin-Maddison test, $p < 0.05$) within a subset of distinct brain regions based on 109 pairwise comparisons between 20 unique brain regions, or between left and right hemispheres, in 5 of 11 patients. Thus, the analysis of meta-data reveals genetic and biological properties of HIV variants in the brain that contribute to development of HAND.

P84
Transcriptional regulation of microglia activation phenotype in HIV associated neurocognitive disorders involves p53, c-Maf and Twist2

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Microglia have pro-inflammatory, neurotoxic actions as well as anti-inflammatory functions that promote recovery and repair. The transcriptional control of these specific microglia behaviors is not well understood. During neuroinflammation due to HIV infection, the transcription factor p53 accumulates in microglia and microglia p53 expression is required for the *in vitro* neurotoxicity induced by the HIV gp120 coat protein. We also observed that p53 knock out (p53-/-) microglia do not induce pro-inflammatory genes or secrete pro-inflammatory cytokines following stimulation by pro-inflammatory cytokines. Additionally, p53-/- microglia demonstrate increased phagocytic activity and expression of genes associated with alternative activation in macrophages. In cortical tissue sections from patients with HIV associated neurocognitive disorders (HAND), nuclear p53 accumulation (a marker of p53 transcriptional activity) was largely excluded from the population of microglia with immunoreactivity for the alternative activation marker CD163. Taken together, these data suggest that p53 influences microglia behavior, promoting a pro-inflammatory phenotype, while p53 deficient microglia demonstrate behaviors associated with alternative activation and anti-inflammatory macrophage functions. To investigate potential mechanisms by which p53 impacts microglia behavior we analyzed global gene expression profiles obtained from cultured wild type and p53 deficient microglia to identify common transcriptional regulatory elements suppressed by p53 or activated by p53 deficiency. This analysis identified the c-Maf transcription factor, a positive regulator of alternative activation in macrophages, as a downstream target of p53 in microglia. We further identified the transcription factor Twist2, which is known to promote c-Maf expression, as upregulated by p53 deficiency. Furthermore, we observed that microglia require p53 for cytokine dependent induction of miR-155, a microRNA known to repress c-Maf expression and promote classical activation in macrophages. Taken together, these findings suggest that p53 has a novel role in determining how microglia respond to inflammatory stimuli by regulating the expression of c-Maf. These findings also suggest that oxidative stress induced activation of p53 in microglia may contribute to the pathogenesis of HAND by influencing microglia behavioral phenotype.

P85
Herpes simplex virus-1-induced reactive oxygen species stimulate cytokine production in murine microglia

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Production of reactive oxygen species (ROS) in the central nervous system contribute to neuronal damage during numerous pathological states. We have previously reported that non-productively infected microglial cells were the major source of inducible nitric oxide synthase during experimental murine herpes encephalitis. In the present study, oxidation of 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was used to measure the production of intracellular ROS in cultures of primary murine microglia at 3, 8, 24, 48, and 72 h following infection with herpes simplex virus (HSV)-1. The levels of intracellular ROS were found to be highly elevated by 48 h post-infection (p.i.). Correspondingly, the majority of this virus-induced ROS production was blocked by diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase. Interestingly, inhibition of NADPH oxidase also decreased virus-induced cytokine and chemokine production and phosphorylation of p38 MAP kinase. In addition, HSV was found to induce oxidative damage as assessed by 8-isoprostane levels. Finally, inhibitors of NADPH oxidase were found to block virus-induced apoptosis and oxidative damage. Taken together, these data demonstrate that HSV-induced cytokine and chemokine responses by microglia, as well as oxidative damage and apoptosis, are mediated through oxidative stress responses.

P86
Different correlates suggest different pathogenic pathways for hiv associated minor neurocognitive impairment versus HIV associated dementia

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HIV-associated neurocognitive disorders (HAND) include asymptomatic neurocognitive impairment (ANI), minor neurocognitive disorders (MND) and HIV-associated dementia (HAD). Various studies showed that HIV enters into the central nervous system (CNS) via CD16+ infected monocytes. A persistent replication of HIV from the CNS reservoir (mainly perivascular macrophages and microglia) could be the main pathogenic mechanism for HAND. The chronic inflammation induced by HIV replication in the CNS might also contribute as the potential neurotoxicity induced by the long-term use of antiretroviral agents specially those with high CNS penetration. We measured monocyte receptor expression by 9-color flow cytometry (CD45, CD14, CD16, CD69, CD163, CCR2, CX3CR1, CXCR4 and CCR5) and soluble factors by ELISA assay (sCD163, sCD14, BAFF, CCL-2, CCL-5, CX3CL1 and CXCL-12) in blood and CSF samples from 20 HIV+ patients treated with combination anti-retroviral therapy (cART) who developed HAND. We found an increase in the percentage of blood monocytes, surprisingly higher in patients with minor cognitive disorders compared with demented one ($p = .0017$). Moreover, this increase was mainly due to the quiescent CD14+/CD16-monocyte subset. This suggests that MND/ANI could rather be the result of events occurring in the periphery. ELISA assays of inflammatory markers suggested dissociation between intra- and extracerebral patterns of inflammation. Indeed, sCD163 level in the serum was inversely correlated with BAFF and MCP-1 levels in the CSF. CSF lymphocytes, including mostly CD8 but also CD4 lymphocytes, were CCR5-bright and CD69-bright. Of note, SDF1(CXCL-12) concentration in the CSF correlated with CSF lymphocytes count, suggesting a role for this chemokine in lymphocyte attraction. Our results suggest that common mechanisms govern both monocyte and lymphocyte migration from the periphery to the brain. CNS invasion by activated monocytes may alter the blood-CSF barrier and allow subsequent entry of lymphocytes. Finally, MND/ANI pathogenesis could be associated with blood specific feature whereas dementia rather relates with an autonomic cerebral state. Thus, MND/ANI might be considered as a clinical entity rather than a pre-HAD state.

P87
Rabies virus infection injures neurons by inducing oxidative stress

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Recent studies have provided evidence that rabies virus infection induces neuronal process degeneration, involving both dendrites and axons, in mice infected with the challenge virus standard (CVS) strain of rabies virus by hindlimb footpad inoculation (J Virol 82:513, 2008). Because of similarities of morphologic changes in experimental rabies with diabetic neuropathy and other diseases, we have hypothesized that neuronal process degeneration occurs as a result of oxidative stress. CVS infection of dorsal root ganglia (DRG) neurons was studied in cultured adult mouse DRG neurons. Two days after plating they were infected with CVS. Immunostaining was evaluated in CVS- and mock-infected cultures for neuron specific β-tubulin, rabies virus antigen, and for amino acid adducts of 4-hydroxy-2-nonenal (4-HNE) (marker of lipid peroxidation and hence oxidative stress). Neuronal viability (by trypan blue exclusion), TUNEL staining, and axonal growth were also assessed in the cultures. CVS infected 33-54% of cultured DRG neurons. Neuronal viability and TUNEL staining were similar in CVS- and mock-infected DRG neurons. There were significantly more 4-HNE-labelled puncta at 2 and 3 days post-infection in CVS-infected cultures than in mock-infection and axonal outgrowth was reduced at these time points in CVS infection. Treatment with the antioxidant N-acetyl cysteine (1 mM) prevented the reduction in axonal outgrowth that occurred in CVS infection. Axonal swellings with 4-HNE-labelled puncta were associated with aggregations of actively respiring mitochondria. NF-κB is activated in CVS infection and the peptide inhibitor SN50 (3μM) reduced axonal swellings in CVS-infected DRG neurons. These data suggest that NF-κB may be a critical bridge between CVS infection and oxidative stress. Oxidative stress is likely important *in vivo* in rabies and

explains previous observations on degeneration of neuronal processes.

P88
JC virus and not BK or EB virus can migrate across an *in vitro* model of the human blood-brain barrier

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To enter the brain, viruses must migrate across the blood-brain barrier (BBB), a monolayer of endothelial cells joined together by intracellular tight junction proteins. Tight junction proteins are significant because they restrict paracellular transport, thereby providing the "barrier" function to the BBB. Mechanisms suggested to explain how viruses cross the BBB include: indirect infection of brain endothelial cells (BECs), trafficking across BECs inside of infected immune cells, and movement of cell-free virus directly across the BECs. Although BECs are exposed to a variety of cell-free viruses circulating in the blood, only a select few of these viruses actually cause infection within the CNS. Here, we examined brain uptake of three different viruses using an *in vitro* model of the human BBB. Real-time PCR was used to track migration of Epstein-Barr Virus (EBV), BK virus (BKV) and JC virus (JCV) across a monolayer of the human brain endothelial cell line, tHBEC XIII. Although JCV was transported across the BBB in a time-dependent manner, both EBV and BKV were not taken up by the BECs. JCV can be transported within hours and pharmacologic studies suggest that this occurs via a clathrin-mediated mechanism. Viral entry was also confirmed by electron microscopy. Our results indicate that the movement of cell-free JCV across the BBB is a specific and regulated process that could significantly contribute to the spread of progressive multifocal leukoencephalopathy (PML).

P89
α4β1 Integrin mediates the recruitment of immature dendritic cells across the blood-brain barrier during experimental autoimmune encephalomyelitis

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Dendritic cells (DCs) within the CNS are recognized to play an important role in the effector phase and propagation of the immune response in experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis. However, the mechanisms regulating DC trafficking into the CNS still need to be characterized. In this study, we show by performing intravital fluorescence videomicroscopy of the inflamed spinal cord white-matter microvasculature in SJL mice with EAE that immature, and to a lesser extent, LPS-matured, bone marrow-derived DCs efficiently interact with the CNS endothelium by rolling, capturing, and firm adhesion. Immature but not LPS-matured DCs efficiently migrated across the wall of inflamed parenchymal microvessels into the CNS. Blocking α4 integrins interfered with the adhesion but not the rolling or capturing of immature and LPS-matured DCs to the CNS microvascular endothelium, inhibiting their migration across the vascular wall. Functional absence of β1 integrins but not of β7 integrins or α4β7 integrin similarly reduced the adhesion of immature DCs to the CNS microvascular endothelium, demonstrating that α4β1 but not α4β7 integrin mediates this step of immature DCs interaction with the inflamed blood-brain barrier during EAE. Our study shows that during EAE, especially immature DCs migrate into the CNS, where they may be crucial for the perpetuation of the CNS-targeted autoimmune response. Thus therapeutic targeting of a4 integrins affects DC trafficking into the CNS and may therefore lead to the resolution of the CNS autoimmune inflammation by reducing the number of CNS professional APCs.

P90
Role of PINCH protein in neuronal response in HIV infection of the CNS

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During HIV infection of the CNS, neurons are damaged by viral proteins, such as Tat and gp120, or by inflammatory factors, such as TNF-α that are released

from infected and/or activated glial cells. Host responses to this damage may include the induction of survival or repair mechanisms. In this context, previous studies report robust expression of a particularly interesting cysteine histidine rich protein called PINCH, in the neurons of HIV patients' brains, compared to nearly undetectable levels in HIV-negative individuals (Rearden, 2008), suggesting PINCH's involvement in neuronal signaling during HIV infection of the brain. To address potential triggers for PINCH induction in HIV patients' brains, an *in vitro* system mimicking some aspects of HIV infection of the CNS was utilized. We investigated neuronal PINCH expression, subcellular distribution and biological consequences of PINCH sequestration upon challenge with Tat, gp120 and TNF-α. Our results indicate that in neurons, TNF-α stimulation increases PINCH expression and changes its subcellular localization. Furthermore, PINCH mobility is required to maintain neurite extension upon challenge with TNF-α. PINCH may function as a neuron-specific host-mediated response to challenge by HIV related factors in the CNS. Supported by NIMH085602 to TDL.

P91
T Cell Receptor-independent activation of Th-17 cells by HIV Tat protein: Role in HAND and IRIS

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Background: Immune Reconstitution Inflammatory Syndrome (IRIS) is characterized by deterioration in clinical status after the initiation of anti-retroviral therapy despite improvement of virological and immune function. It may involve the CNS, even in the absence of opportunistic infections and rapidly progresses to cause severe encephalitis resulting in death. CNS-IRIS is largely mediated via activated-T cells. This paradoxical infiltration of the brain with T cells and accompanying low peripheral CD4 lymphocytes due to HIV infection represents a diagnostic challenge and a treatment dilemma. Methods and Results: T cells isolated from peripheral blood mononuclear cells (PBMCs) from normal human donors (n = 5) were treated with HIV Tat protein (15.6-250 nM) for 72 hours. Granzyme B and IL-17 were measured by ELISA in supernatants as an indicator of cytotoxic T cell activation. Tat induced T cell activation in a dose-responsive manner. To characterize the epitope of Tat that activates T cells, they were exposed to 15-mer Tat peptides overlapping by 10 amino acids derived from HIV clade B. Tat 29-43 showed maximal granzyme B production ($p < 0.001$). To determine if viral clade influences Tat-mediated T cell activation, cells were exposed to Tat-derived from HIV clades A, B, C and D (200 nM). Clades B, C and D but not clade A caused T cell activation ($p < 0.05$). To determine the mechanism of T cell activation by Tat, cells were treated with 200 nM Tat protein in the presence or absence of pharmacological agents and compared by non-parametric analysis. Cytokine production was attenuated by 10 μM TPCK (NF-κB blocker; $p < 0.01$), 100 nM RO-32-0432 (pan-protein kinase C inhibitor; $p < 0.05$), 30 μM chlorpromazine and 500 μM amiloride (clathrin mediated endocytosis inhibitors; $p < 0.05$), but was not affected by 3 ug/ml filipin (inhibits caveolae endocytosis). To determine if T cell activation by Tat was dependent on the T cell receptor (TCR), cells were exposed to Tat with or without nocodazole (5mg/ml; inhibits LCK phosphorylation). Nocodazole had no effect on T cell activation. To determine the effect of Tat-mediated T cell activation on HIV susceptibility, PBMCs were treated with 200 nM Tat for 72 hours, and infected with HIV NL4-3. One week later, HIV replication was quantified by measuring p24 antigen in culture supernatants. Tat increased HIV susceptibility in PBMCs ($p < 0.05$), which was specifically blocked using an anti-Tat antibody (1:1000, $p < 0.01$). Conclusions: 1) HIV Tat protein activates T cells leading to release of cytokines and cytotoxic granules. The mechanism likely involves endocytosis of Tat, protein kinase C and NF-kB signaling but may be independent of the TCR. Prevention of Tat-mediated T cell activation by inhibition of Tat endocytosis may represent novel therapeutic approach for preventing the development IRIS. 2) Viral clade may impact immune activation in the context of HIV infection and may contribute to the development of IRIS. 3) Tat-activated T cells are also more susceptible to HIV infection setting up a positive feedback loop in IRIS. Acknowledgements: Supported by NIH grants: R01NS056884 and F31NS063855. Muznabanu Bachani for technical assistance.

P92
Role of JCV induced disbalance of chemokines in neuron-glia interaction

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Axonal damage has recently been recognized to be a key predictor of outcome in a number of CNS white matter diseases including multiple sclerosis and leucodystrophies. There have been limited studies of axonal loss in Progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the CNS

resulting from destruction of oligodendrocytes upon productive replication of JC virus (JCV). Recent imaging and pathological studies of the CNS have shown the presence of neuronal damage and axonal loss in PML lesions. Neurons relay on healthy glial network, hence neuronal-glial interactions are crucial for the maintenance of brain homeostasis and neuronal survival. Although effect of JCV in regulation of cellular functions has been investigated in astroglial cells, the impact of JCV on neuronal cells via glial cell infection remains entirely unclear. We analysed expression of chemokines released by JCV Mad1 infected primary human neurospheres and found increased levels of Gro-α, ENA-78, GCP-2, CXCL16 and IL-8/CXCL8 upon infection. Quantitative RT-PCR demonstrated that levels of mRNAs for Gro-α, ENA-78, GCP-2 and IL-8/CXCL8 also were higher in JCV infected samples compared to uninfected controls. Increased expression of GCP-2 was found in AIDS-PML autopsy brain samples but not in controls. Higher levels of CXC chemokines Gro-α, CXCL16, CXCL11/I-TAC and GCP-2 along with CCL27 and CCL11 were detected in supernatants from primary brain mixed culture incubated with brain slices from AIDS-PML samples compared with HIV+/no PML or controls (no brain pathology). The aberrant production of chemokines may contribute to the developing of neuronal injury during JCV infection.

P93
Neurotropic HIV-1 viruses induce the Kynurenine Pathway (KP) more effectively compared to non-neurotropic viruses in monocyte derived dendritic cells (MDDCs)

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Background: HIV-1 activates the KP and its catabolite quinolinic acid (QUIN), which is elevated in HAND. DCs are one of the primary target cell types and play an important role during the initial activation of CD4 + T cells and dissemination of virus. There is increasing evidence for the presence of DCs in brain during disease. Recently, both the products of the KP, particularly QUIN, and KP related tryptophan depletion have been found to cause T cell apoptosis and hyporesponsiveness. We hypothesized that strains of HIV differ in their ability to induce the KP leading to neurotoxicity and immune paresis, which further contributes to viral persistence through impaired T cell related clearance. Methods: Monocytes are isolated from PBMCs using CD14+ magnetic beads and cultured for 7 days followed by overnight infection by various HIV isolates. The neurotropic strains JRFL, C158 and C124 and the non-neurotropic strain BaL were all added at the same viral TCID50 concentration. QUIN levels were analysed by gas chromatography-mass spectrometry by collecting culture supernatants at days 3,4,5 and 6 following infection. The effect of MDDCs on T cell apoptosis was assessed by adding culture supernatants from infections at day 3 to PHA activated CD4+ T cells. CD4+ T cells were stained with CFSE and incubated for 5 days and the T cell repertoire was assessed by flow cytometry using CD3, CD4, CD45, CD25, CD127 and caspase 3 antibodies. Results: Our results consistently indicated significantly high levels of QUIN production by JRFL ($p < 0.0027$), C158 ($p < 0.007$) and C124 ($p < 0.009$) compared to BaL. However, analysis of viral replication showed ten-fold lower levels of viral copy numbers in JRFL ($p < 0.026$), C158 ($p < 0.023$) and C124 ($p < 0.023$) compared to BaL. We also observed higher T cell apoptosis with MDDC infection by neurotropic viruses (JRFL; $p < 0.05$) and C124 $p < 0.03$). Furthermore, MDDC infection with neurotropic viruses leads to T cell activation and increase in T regulatory cells. A significant correlation was seen between elevated QUIN levels and T regulatory cells with the neurotropic viruses. Conclusions: There are significant differences in the ability of strains of HIV to induce the KP, with HAND related strains having the greatest ability. This has implications for understanding the basis of neurovirulence. Furthermore, such KP induction probably plays a significant role in viral persistence in the brain through induction of tolerance and facilitation of neurotropism. Finally, the poor induction of the KP by BaL has significant implications for studies of neuropathogenesis that have used BaL.

P94
CCR5 gene polymorphisms are not associated with HIV infection and progression in North Indians

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Background: Chemokine receptors are thought to be involved in HIV infection and disease progression. Objectives: The aim of our study was to investigate the association of CCR5 polymorphisms with HIV-1 C-clade infection and progression of AIDS in North Indian population. Results: We recruited 132 HIV-1

seropositive individuals and followed them for disease progression. CCR5 9029 and CCR5 59653 polymorphisms were genotyped using direct sequencing and genotype frequencies were compared using Fischer exact test. 46 patients (34.1%) were typical progressors (TPs) and 86 patients (65.9%) were non progressors (NPs) over 2 years duration. CD4+ T cell counts were found to be significantly higher among NPs as compared to TPs; the viral loads were significantly lower in NPs ($P < 0.05$). No significant difference was observed in levels of CXCR4 and CCR5 between the both the groups. We did not observe any significant difference in distribution of variants genotypes and alleles of CCR5 9029 and CCR5 59653 polymorphisms between HIV1 + NPs and TPs. These genetic variants did not show any association with clinical characteristics. Conclusions: Our preliminary results suggest a lack of an association of CCR5 polymorphisms with HIV infection in our population; however, further studies in larger cohorts are required to confirm this observation. (supported by funding from NIH Grant #RO1 NS 055653).

P95
Ibudilast, A Pharmacologic Phosphodiesterase Inhibitor, Prevents Human Immunodeficiency Virus-1 Tat-Mediated Activation of Microglial Cells

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Human Immunodeficiency Virus-1 (HIV-1)-associated neurocognitive disorders (HAND) occur, in part, due to the inflammatory response to viral proteins, such as the HIV transactivator of transcription (Tat), in the central nervous system (CNS). Given the need for novel adjunctive therapies for HAND, we hypothesized that Ibudilast would inhibit excess production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha) in microglial cells. Ibudilast is a non-selective cyclic AMP phospohdiesterase inhibitor that has recently shown promise as a treatment for neuropathic pain via its ability to attenuate glial cell activation. Accordingly, here we demonstrate that pre-treatment of microglial cells with increasing doses of Ibudilast inhibited Tat-induced synthesis of TNF-alpha by microglial cells in a manner dependent on serine/threonine protein phosphatase activity. Ibudilast had no effect on Tat-induced p38 MAP Kinase activation, and blockade of adenosine A2A receptor activation did not reverse Ibudilast's inhibition of Tat-induced TNF-alpha production. Interestingly, Ibudilast reduced Tat-mediated transcription of TNF-alpha, via modulation of nuclear factor-kappa B signaling, as shown by quantitative Real-Time PCR (qRT-PCR) and luciferase reporter assays. Together, our findings shed light on the mechanism of Ibudilast's inhibition of Tat-induced TNF-alpha production in microglial cells and may implicate Ibudilast as a potential novel adjunctive therapy for the management of HAND.

P96
Chondroitin sulfate-E induced enhancement of Japanese encephalitis virus infection

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Proteoglycans are major components of the cell surface and the extracellular matrix, which are composed of glycosaminoglycans (GAGs) and core proteins. GAGs are long unbranched polysaccharides consisting of a repeating disaccharide unit, each of which can be sulfated in variable position and quantities, and have diverse functions in the body. Several studies have suggested the relationship between GAGs and viral infection, and have found that soluble GAGs inhibit certain viral infection. In this study, we found soluble chondroitin sulfate-E (sCS-E), which is one of GAGs involved in neuritogenesis and neuronal migration, enhanced JEV infectivity in a mouse neuroblastoma cell line (neuro2A) and primary rat neuron. Although sCS-E inhibited the binding of JEV to cell surface, it eventually resulted in enhancement of viral infection through interruption of JEV-induced interferon (IFN) synthesis. In neuro2A, shRNA knockdown of N-acetylgalactosamine 4-sulfate 6-O-sulfotransferase, which synthesizes CS-E from CS-A by catalyzing transfer of sulfate, led to increased constitutive IFN expression, and it resulted in the reduction in the susceptibility to JEV. In 17-day-old rats (JEV resistant), intracerebral administration of CS-E with JEV led to significantly increased viral load as compared with mock-treatment. Because it has been reported that CS-E is widely expressed in developing brain and gradually decreases after birth, this study imply that brain CS-E may be a host factor involved in the susceptibility of neurons to JEV that is more lethal in newborn than in adult animals.

P97

MHV-68, a model virus for Epstein-Barr virus infections, enters the brain independent of functional B-lymphocytes via vessel endothelial cells and causes immune-mediated damage as well as chronic tissue infection

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Molecular details on cerebral gamma herpesvirus infection are lacking. Infection of mice with the murine gamma herpesvirus MHV-68 mirrors human cerebral Epstein-Barr virus (EBV) infection. Here we show that MHV-68 can be detected intracerebrally from day 3 after nasal inoculation of newborn BALB/c mice; maximum viral loads were gained on day 9. After that viral loads decrease without completely disappearing during 6 months of follow-up. Viral spreading occurred haematogeneously. MHV-68 invaded the CNS via cerebral endothelial cells. CNS infection was independent of preceding respiratory replication or the presence of functional MHV-68-positive B-lymphocytes. Beginning on day 9 cerebral inflammation affected all brain regions, with a maximum between days 16 and 35. Brain inflammation persisted during follow up. It was dominated by T-lymphocytes with marked cytotoxic activity leading to necrosis present between days 9 and 14 only. Inflammation increased with viral loads and decreased with age at infection. Rate and extent of inflammation were lower in animals with Th1-type immune response compared to animals with Th2-type immune response. Immunodeficient mice showed highest viral loads but no increased rate of necrosis. Our data suggest that immune-mediated cell damage and chronic tissue infection are key players in gamma herpesvirus-associated cerebral disease.

P98

Lineage-dependent effects of opiates and HIV-1 proteins on CNS progenitors lead to white matter pathology

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Injection drug abuse and HIV are interlinked epidemics. Epidemiological and experimental evidence suggest that co-exposure to opiates can worsen HIV-related CNS neuropathology. Since HIV does not infect neurons, CNS pathology is due to direct toxic effects of viral proteins released from infected immune cells and/or secondary inflammatory processes promulgated by glial cells. The potential for HIV and opiate interactions is extensive since opioid receptors are widely expressed by neural/glial precursors, their progeny, and also by microglia. We find that critical CNS progenitor functions are adversely affected by exposure to HIV proteins and/or morphine; co-exposure synergistic affects certain outcome measures. *In vitro* parameters affected by HIV-1 Tat and/or morphine include migratory capacity, chemokine/cytokine secretion, and proliferation/lineage. There are robust interactive effects of Tat and morphine on differentiation among progenitors expressing Sox2 or Olig2 transcription factors. For endpoints thus far examined, progenitors appear less sensitive to effects of gp120. *In vivo* studies using inducible Tat-transgenic mice demonstrate increased susceptibility of Sox2+ and Olig2+ progenitor populations, which may contribute to subsequent deficits in oligodendroglia (OLs) within the adult brain, and to alterations in white matter. Numbers of CC-1+ OLs are reduced in the corpus callosum of adult Tat-transgenic mice co-exposed to morphine, and the morphology of the cells is quite abnormal. At the light microscopic level, Tat and morphine co-exposure resulted in many OLs with truncated, aberrant and/or fragmented processes, and also increased the presence of TUNEL+ OLs in striatum/corpus callosum. Electron microscopy revealed marked OL degeneration, including cells with dense, marginalized nuclear heterochromatin or frank pyknosis, as well as myelin deficits. Overall, our findings show that CNS progenitors are selectively vulnerable to individual or combined effects of HIV proteins and opiates, in a manner that shows regional variation and lineage-specificity. Support: DA24461, DA19398.

P99

Cys80 of JC Virus Capsid Protein, VP1 is Essential for Intrapentamer Disulfide Bond and Pentamer Formation

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Objective: Human pathogenic JC virus (JCV) belongs to the polyomavirus family which includes simian virus 40 (SV40). JCV capsid consists of 72 capsomeres of a major structural protein VP1. Five VP1 monomers interdigitate and form a capsomere. Disulfide bonding is known to facilitate formation of SV40-VP1 pentamer, yet its mechanism remains unknown. Here, we have tested the hypothesis that JCV-VP1 assembles into a pentamer via disulfide bonds and identified the VP1 cysteine-pairs that contribute to the pentamer formation and to the formation of infectious virions. Methods: The VP1 cysteine mutants were created by inverse PCR method, replacing each of six cysteine residues with alanine. Six mutants harboring multiple VP1 cysteine mutations were also made. The mutants were analyzed by transfecting mutant genomes into SVG-A cells, and JCV growth was immunocytochemically monitored. The pentamer formation was assessed by synthesizing VP1s *in vitro* (in which disulfide bonding is facilitated to occur), and by sucrose gradient sedimentation. Efficiency of VP1 oligomerization in HeLa cells was also tested by separating VP1-oligomers on nonreducing-SDS-PAGE condition followed by anti-VP1 immunoblotting. Results: We found that a mutation at Cys80 or Cys247 in VP1 lead to loss of viral propagation. The rate of pentamer formation of Cys80 VP1, but not of Cys247 VP1, was significantly reduced compared to those of wild type. Further, the fact oligomerization of Cys80 mutant VP1 was impaired *in vitro* implies the involvement of other cysteine(s) with Cys80 in disulfide bonding in promoting the pentamer formation. Analysis of five quadruple cysteine-pair mutants showed that mutant VP1s with intact cysteine-pair of Cys80-Cys200, Cys80-Cys247 or Cys80-Cys260 formed pentamer. Conclusions: Formation of intrapentamer disulfide bonds between Cys80 and Cys200, Cys247, or Cys260 are necessary for guiding the formation of dimer through pentamer. Cys247, in addition to interaction with Cys80, contributes to virion formation that assembled from pentamer.

P100
Macrophage colony stimulating factor regulation by NF-Kappa B in HIV-1 infected macrophages: induction of survival pathways responsible for myeloid viral reservoirs and NeuroAIDS

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Monocytes/macrophages play important roles in the pathogenesis of HIV infection and in HIV associated dementia. Previous studies have identified a monocyte/macrophage subset (CD16+/CD163+) that is increased in peripheral blood during HIV infection. Expansion of these cells correlates directly with viral load and inversely with CD4+ T cell count, belowa critical threshold. Macrophages and microglia accumulating in CNS tissue in HIV dementia also express this phenotype. Furthermore, CD16+ monocytes have been demonstrated to be preferentially infected by HIV-1. Taken together, these observations suggest that factors controlling monocyte/macrophage homeostasis may be important in the disease process and for the design of therapeutics. With this concept in mind, we have begun to investigate the regulatory mechanisms controlling M-CSF production in monocytoid cell lines and in primary macrophages. M-CSF promotes differentiation of macrophages from monocytes, increases viral production, and upregulates both CCR5 and CD4 receptors leading to increased susceptibility to infection. HIV-1 infection is also known to induce M-CSF production thus forming a positive feedback loop. Tumor necrosis factor alpha (TNF-a) is one of several inflammatory cytokines secreted in response to HIV-1 infection of T-cells and macrophages and is known to upregulate M-CSF, although the mechanism of these effects are not sufficiently understood. We found that TNF-a promotes M-CSF secretion in macrophages and activates the -1310/+48 bp M-CSF promoter in Mono Mac1 cells. Inhibitors of the NF-kB pathway, including an IKK inhibitor (sc-514) and an IkB activator (dexamethasone) diminish this response. We identified four putative NF-kB and four C/EBP binding sites within the M-CSF promoter. Our findings using M-CSF promoter constructs mutated at individual NF-kB locations suggest these sites are redundant in M-CSF regulation. Quadruple mutations for NF-kB sites were not activated by TNF-a or inhibited by dexamentasone while the C/EBP mutants were responded the same as the wild type promoter. Consistant with these findings TNF-a increased and dexamethasone diminished NF-kB

translocation to the nucleus in Mono Mac-1 cells. TNF-a treatment promoted p65 binding to the M-CSF promoter in U1 cells. In summary, our findings demonstrate that NF-kB induces M-CSF expression on a promoter level via multiple functional NF-kB binding sites. Considering that NF-kB signaling is activated in HIV-1 infected cells, we are investigating which factors induce M-CSF and NF-kB in myeloid cells. Vpr is a known modulator of the NF-kB pathway and may promote survival of HIV-1 infected cells, consistent with induction of M-CSF.

P101
Toll-like receptor 2 (TLR2) is important for limiting HSV-1 replication and development of lethal herpes simplex encephalitis in mice

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Herpes simplex virus type 1 (HSV-1) is a ubiquitous human pathogen that resides in the nervous system for the life of the host. In rare instances, HSV-1 crosses into the central nervous system (CNS) and results in a focal, necrotizing encephalitis. Antiviral therapy only partially reduces disease severity, suggesting a significant proportion of the neurological damage is due to an over-active immune response. Limiting early HSV-1 replication at the primary infection site would theoretically reduce the viral load capable of infecting the CNS. Toll-like receptors (TLRs) on immune and stromal cells are among the first sensors of microbial infection. The importance of TLR9 in HSV-1 animal models has been documented. TLR9 senses viral dsDNA in the endosome and leads to type I interferon production and inhibition of viral replication. In contrast, the role of surface-expressed TLR2 in anti-HSV immunity remains unclear. In a murine model of cutaneous lip HSV-1 infection, we show that mice lacking TLR2 (TLR2-/-) are increasingly susceptible compared to intact mice. Eighty percent of TLR2-/- mice succumb to CNS infection between five and eight days post infection, whereas only forty percent of WT mice die by day ten. Viral loads in TLR2-/- mice are slightly elevated in both peripheral and CNS tissues, however, we believe there are other factors contributing to the increase in mortality. Studies are underway to examine the onset and magnitude of CNS inflammation. Future studies using an intracerebral infection route will delineate a protective vs. pathological consequence of TLR2 ligation within the brain parenchyma. These studies are geared toward understanding the optimal conditions necessary to limit early HSV-1 replication and generating more efficient therapies for patients with HSV-1 encephalitis.

P102
Human Immunodeficiency Virus & Decreased Dopamine Availability in the CNS: Relationship with Neuropsychological Performance

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Background: HIV-1 in the CNS is localized in varying concentrations in different brain regions particularly in the subcortical regions, causing neurodegeneration in the basal ganglia, and other regions of high dopaminergic activity and can lead to decreased availability of dopamine (DA) and possibly resulting in neurological and neuropsychological (NP) problems. There is, however, a paucity of studies on the direct relationship between the decreased availability of DA in different regions of post mortem brains and performance in different NP functions during life. Objective: The present study investigated the relationship between the DA availability in fronto-cortical and the basal ganglia regions of individuals with HIV/AIDS, and the antemortem performance of different NP functions. The relationship between HIV-1 RNA levels in different brain regions and different NP functions was also investigated. Results: A 45% decreased in DA concentration in the substantia nigra (SN) of HIV-1 infected individuals was observed and was found to be significantly correlated with the low level of performance (T-Scores) in learning and memory. In case of homovanillic acid (HVA), changes in the levels in the basal ganglia, caudate and putamen was significantly correlated with the level of performance (T-Scores) in motor functions, speed of information processing and attention/working memory. A significant inverse correlation was found between the levels of HIVRNA in the frontal cortex and abstract/executive function and attention/working memory. There were no significant correlations found between HIVRNA in other brain regions and NP performance. Conclusions: These findings suggest that the decrease in the availability of DA in the SN, and changes in the levels of HVA in different brain regions are, in part, related with the lower level of performance in some of the NP functions in individuals with HIV/AIDS. The study was supported by the NIH Grant #s RO1NS43982; R21 NS062669, RO1 NS055653.

P103
Rabies virus as an Ariane's thread to unravel pathways committing neurons towards death or survival

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The acute myelo-encephalitis caused by rabies virus (RABV) is responsible for 55 000 deaths a year, ranking it among the ten most infectious global diseases. RABV has developed a unique strategy to ensure its propagation into the nervous system: its virulence correlates with the ability of the infected neurons to survive. By contrast, attenuation of laboratory strains is linked to their ability to trigger cell death. The capacity of RABV to promote neuronal survival or death depends upon the cellular partners recruited by the PDZ-binding site (PDZ-BS) of its envelope protein G (1). Neuronal survival requires selective association of the PDZ-BS with the PDZ domain of two closely related serine-threonine kinases (MAST1 and MAST2). A single amino acid change in the PDZ-BS triggering neuronal death, allows G to recruit additional PDZ partners, notably a tyrosine-phosphatase, PTPN4. Silencing this phosphatase abrogates RABV-mediated apoptosis. It is now established that MAST2 functions as an inhibitor of neurosurvival and PTPN4 as an inhibitor of cell death, so that RABV Gs probably antagonize PTPN4 and MAST2 functions by disrupting the interactions of their PDZ domains with their respective cellular ligands (1). Structural data confirm this hypothesis. G provides now a unique tool to understand how these different primary PDZ-PDZ-BS interactions commit neurons towards death or survival. (1) Prehaud *et al*, Sci Signaling 2010.

P104
Pathogenesis of multiple sclerosis: activation of herv-f(c)1; human endogenous retrovirus

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We have provided evidence that HERV-F(c)1 on chromosome X plays a role in the causation of MS. We tested 3 close-lying markers for association in the cohort consisting of 350 patients and 500 controls. One marker rs391745 was lowest with a p-value of 4*10-6 (2-sided) for association with disease, when calculating on the basic of C-allele carriers. This value reminded significant after Bonferroni correction (p = 0.0009). Here we report increased expression of HERV-F(c)1 gag protein in primary human lymphocytes from 40 MS patients. The p-value for CD19- positive B lymphocytes was 0.0158* and for monocytes 0.0026*. Subsequently, when we analyzed only MS patients in the acute stage of the disease we observed more than 4-fold increased in the expression of HERV-F(c)1 gag protein as compared to a control group. The p-values were: CD4 lymphocytes $p < 0.0001$, CD8a lymphocytes $p = 0.0001$, monocytes $p = 0.0045$. External calibration curves based on T7 *in vitro* transcribed gag-pol F(c)1 fragment and quantitative RT-PCR with primers specific for HERV-(c)1 gag and pol were used to determine absolute RNA virus copy numbers present in plasma from healthy controls and MS patients. Accumulation of HERV-F(c)1-specific RNAs with 10-fold increased in absolute copy numbers was detected in acute MS plasmas over that in controls ($p < 0.0001$). To further extend our understanding of HERV-F(c)1 acting as a pathogen in MS, we studied the infectious properties of the virus. To our knowledge we are the first to report that HERV genomes can be infectiously transmitted to non-human cells in culture, without reconstitution of the full sequence of the virus and cloning into expression vectors. Cell-free retrovirus produced by the MS1859 and MS1851 cell strains (established from MS patients) infected EB185JC-Orangutan B-lymphocytes and CV-1- African Green Monkey Kidney Fibroblasts. RT-PCR analysis confirmed that infected cells contain copies of integrated HERV-F(c)1 genome and expressed viral RNA.

P105
The influence of exercise on hippocampal NPC proliferation and survival is mediated by regulation of the CREB/BDNF pathway in gp120 transgenic mice

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Backgrounds Human immunodeficiency virus (HIV) infection associated in the central nervous system (CNS) causes neurocognitive disorders (HAND) are accompanied with brain atrophy. In these patients, Iimpairment of adult neurogenesis in the hippocampus likely contributes to thememory and cognitive dysfunction. Although running exercises canhave been reported to enhance neurogenesis, the underlyingdetails of molecule mechanisms of the enhancement are not well understood. Objective The aim of this study is tTo determine the impact of exercise on proliferation and survival of the adult hippocampal neural progenitor cells (NPCs) in a HIV-gp120 transgenic model in which the expression of gp120 is regulated by a GFAP promoterline and to understand the molecular mechanisms involved in exercise-induced alteration in NPC proliferation and its survival. in gp120 transgenic mice Methods 8 week-old wild type and gp120 transgenic mice were used. Sedentary control mice were housed in standard cages for 20 days. Mice of the running group wereprovided allowed access to running wheels for 3 days, 10 days or 20 days. Another Mice of the

detrained group wasere provided with a running wheels for the first 10 days, and the wheels were then removed from their cages and the mice were housed in standard cages for anotherthe next 10 days. To assess neural progenitor cell (NPC) proliferation, a single dose of 200 mg/kg of BrdU was given and euthanized 2 hours later. For study of survival of NPCs, the mice were given 50 mg/kg injections of BrdU were given at 2 hour intervals for 6 hours and euthanized on day 1 or day 21. To determine the levels of brain-derived neurotrophic factor (BDNF) and phosphorylation of the transcription factor, cAMP-response-element binding protein (CREB) in the hippocampus, immunohistochemistry and western blotting were performed. Results At baseline, the NPC cells were lower in the gp120 mice. In both, wild type and gp120 mice Aa significantn increase in BrdU-positive cells took place at 3 and 10 days of exercise followed by for both groups. But a significant decrease in cells at 20 days was noted. The NPCs in the wild type returned to levels comparable to the sedentary group while the gp120 group maintained a higher number of NPCs. Similarly the drop in NPCs following detraining was greater in the wild type compared to the gp120 mice. shows that proliferation is not necessarily exercise dose dependent in either wild type or gp120 mice. In the wild type group, cell level return to that of the control group after 20 days of running. While in the gp120 group, cell level remained much high than the control group after 20 days of exercise, but cell level decreased substantially between 10 and 20 days of running. And in wild type group, detraining actually lead to a much greater drop in the number of BrdU positive cells compared to the control group. While in the gp120 group, BrdU positive cells returned to that of the control group after detraining.Interestingly, in both 30% of newborn cells survived 20 days after proliferation both in wild type and gp120 mice. But 80% of newborn NPCscells survived after 20 day running compared to 30% in the sedentary group. Partial protection was seen following 10 days of exercise, however, it was associated with. Early 10-day period of running protected newborn cells partially both in wt and gp120 transgenic mice. On the other hand, sedentary for 10 days and running for 10 days has no protective quality in either the wt or the gp120 mice. Thus continuous exercise has the greatest benefit on the survival of newborn cells and early exercise was a greater benefit than exercise after sedentary period. 10 days of exercise led to a marked increase in hippocampal BDNF protein and CREB phosphorylation in both wild-type and gp120 transgenic mice. Detraining decreased hippocampal BDNF protein levels and CREB phosphorylation. Conclusions: 1) Expression of gp120 in astrocytes leads to a decrease in NPCs which can be overcome by exercise. 2) Exercise leads to an early increase in NPC proliferation, the survival of which is maintained by only sustained exercise. 3) Cessation of exercise decreases NPC function below those of sedentary animals. 4) Exercise regulates NPC function via effects on BDNF and phosphorylation of CREBIt thus appears that running exercise lead to increased phosphorylation of the transcription factor CREB and increased BDNF expression. BDNF can then exert its morphogenetic and neurogenic effects on the brain.

P106
Alterations in Brain Metabolism during the First Year of HIV-Infection

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Objectives: Monocyte infection by human immunodeficiency virus (HIV) and subsequent migration into the brain early during infection likely induces metabolic changes that can be observed with magnetic resonance spectroscopy (MRS). The purpose of this study was to examine changes in brain metabolism during the first year of HIV infection and to determine if these changes were related to plasma viral levels or monocyte populations. Methods: Single-voxel MRS were performed on nine HIV+ subjects identified during acute HIV infection and nine seronegative control subjects at study entry. HIV + subjects were examined within 90 days of an indeterminate Western Blot, then again two and six months later, over the course of early infection. Blood samples were collected at imaging time points to allow for viral RNA and monocyte subset quantification. Results: Compared to controls, HIV+ subjects showed decreased choline and "glutamate + glutamine" (Glx) in the frontal cortex (FC) as well as increased myo-inositol (MI) in the basal ganglia (BG). Choline levels (ratios and concentrations) were elevated in both the FC and white matter (WM) of HIV+ subjects after two and six months. Higher choline concentrations in the WM and BG and lower N-acetylaspartate (NAA) levels in the BG and FC were associated with greater numbers of activated monocyte populations (CD14highCD16low and CD14lowCD16high). Conclusions: Lipid metabolism, as observed by MRS, is changing during the initial year of HIV infection. Associations between NAA, choline and activated monocyte counts suggesting early neuronal dysfunction, concurrent with glial activation, are related to immunologic events in the periphery.

P107
Interface between beta-catenin and IFNgamma signaling to regulate productive HIV replication in astrocytes

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Canonical Wnt/beta-catenin is a neuroprotective pathway regulating many functions of neurons and glia, including growth, differentiation, and formation of synapses. Its dysregulation is linked to a number of neurodegenrative diseases. Previously, we demonstrated that astrocytes exhibit robust endogenous level of beta-catenin signaling. Inhibiting beta-catenin by pre-treatment with IFNgamma and/or loss of function studies induced HIV productive replication in astrocytes by 4-6-fold. Inversely, activating beta-catenin in HIV permissive targets (T cells and macrophages) potently inhibited HIV replication. These findings define beta-catenin as a host restrictive pathway for HIV replication across a number of compartments. Using primary fetal astrocytes and astrocytoma cell lines, we evaluated here the interplay between IFNgamma and beta-catenin signaling that impacts HIV replication in astrocytes. We demonstrate that IFNgamma mediates HIV productive replication in astrocytes by inhibiting beta-catenin signaling in a Stat 3 dependent manner. IFNgamma induced an antagonist of the beta-catenin pathway (Dickkopf related protein-1, DKK1) by 2.5-fold in primary fetal astrocytes. Inhibiting DKK1 and Stat3 abrogated the ability of IFNgamma to induce HIV replication in astrocytes, while inhibiting Stat1 or GSK3beta had no effect. These findings point to cross talk between IFNgamma and beta-catenin signaling that may exert both biologic and virologic effects in central nervous system. Greater understanding of the relationship between beta-catenin signaling and biologic factors that modulate it in the context of HIV could lead to new strategies to target direct and indirect pathologies of HIV in CNS. This work was funded by NIH grants RO1 NS060632 and R21 A1077329 to LA.

P108
Selective loss of synaptic protein MARCKS in HIV-1 encephalitis is mediated by Tat-induced NMDA receptor excitation

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Background: One of the neuropathological characteristics of HIV-1 associated neurocognitive disorder (HAND) is the loss of pre- and post-synaptic structures. Because both synapses and dendrites have the ability to regenerate, it is critical to understand the mechanism of synaptodendritic damage in HAND and to identify molecular targets for potential therapeutic intervention. Methods and Results: We first examined select synaptic proteins: myristoylated alanine-rich C-kinase substrate (MARCKS), growth associated protein 43 (GAP-43), synaptophysin, and postsynaptic density protein 95 (PSD-95), in postmortem brain tissues from HIV-1 infected patients. We found that HIV-1 infected patients had significantly lower MARCKS levels compared to HIV-1 negative group. Within the HIV-1 infected group, patients with encephalitis had even less MARCKS compared to the non-encephalitis group. While HIV-1 infected patients also had significantly reduced GAP43 levels compared to HIV-1 negative group, encephalitis only showed a trend towards reduction in the level of GAP43. No significant changes related to HIV-1 infection were observed in synaptophysin and PSD-95 levels. We then determined if MARCKS is involved in Tat-induced synaptodendritic damage in rat primary cortical neurons. Subtoxic concentration of clade B HIV-1 Tat protein significantly reduced the densities of dendritic spine in rat primary cortical neurons of 21 days-in-vitro after 18 hrs treatment. MARCKS level was also significantly decreased by Tat treatment. However, NMDA receptor blockers and downstream signal inhibitors can inhibit Tat-induced decrease of MARCKS level, and can also rescue the dendritic damage caused by Tat. These data suggest that MARCKS may play an important role in Tat-induced synaptodendritic damage. Conclusion: There is selective loss of MARCKS in brain tissues of patients with HIV encephalitis. This may be mediated via Tat-induced NMDA receptor activation.

P109
Infiltrating Cells and Resident CNS Cells Producing IL-6 Contribute to Acute Seizures Following Viral Infection

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Epilepsy has an incidence of about 1-3% and affects about 2.5×10^6 Americans and greater than 5×10^7 individuals worldwide. Seizures can occur singly or temporally. Infections such as viral infections

are often associated with seizures in children and adults. Many of the seizure disorders induced by infection are often refractory to anti-epileptic drugs and about 30% of individuals with epilepsy are refractory to current anti-seizure medications. Our study is attempting to define innate immune responses that are induced following viral encephalitis that contribute to seizures. We have developed a novel viral infection-induced seizures model in C57BL/6 mice. C57BL/6 mice were infected with 2×10^5 pfu of the DA strain of Theiler's murine encephalomyelitis virus or mock infected. Approximately 50% of mice developed acute seizures between days 3 and 10 post infection. DA virus was cleared after 3 weeks following infection. After a latent period of about 2 months, about half of the mice developed spontaneous seizures, epilepsy. Motor function and coordination were impaired in mice that had seizures. Pyramidal neurons and inflammatory changes in the hippocampus correlated with seizures. IL-6 gene expression in the central nervous system (CNS) was found to be upregulated early following infection. Interestingly, fewer IL-6 deficient mice developed seizures than wild-type infected mice. Bone marrow chimera studies revealed that both infiltrating cells and resident CNS cells were important for the development of seizures. In conclusion, the innate immune responses are important in the development of acute seizures following DA virus CNS infection. Neuronal death correlated with the development of seizures. IL-6 is an important cytokine that contributes to the development of seizures. This novel model of epilepsy represents a "hit and run" phenomenon where a virus infection initiates damage in the CNS. The virus is cleared by the immune response but the initial damages results in disease after the virus is gone. Further characterization of this model will aid in understanding virus-host interactions that lead to seizure disorders in humans.

P110
Lentiviral Vector Mediated Expression of sTNFR-Fc as A Novel Therapeutic Intervention for NeuroAIDS

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Human immunodeficiency virus type 1 (HIV-1) infection mediated production of tumor necrosis factor-α (TNF-α) from immune-activated mononuclear phagocytes is implicated with the neuropathogenesis progression toward HIV dementia. TNF-α, a proinflammatory cytokine and neurotoxin, is known to directly induce neuronal injury and death. To develop a novel therapeutics for the treatment of neuroAIDS, we constructed and characterized a lentiviral vector that expresses a soluble TNF receptor (sTNFR)-Fc fusion protein, intended to inactivate TNF-α. To facilitate detection of transduced cells, a report gene encoding the green fluorescent protein (GFP) was also included in the expression cassette using an IRES element. Several cell lines including murine microglia, human microglia and neuroblastoma were effectively transduced using high-titer vector stocks and transduction efficiencies were determined to be as high as 100%. Transduced cells showed no morphological alteration or viability loss as compared to control cells. Expression of sTNFR-Fc from these transduced cells was detected by RT-PCR and the sTNFR-Fc secretion was demonstrated using Western blotting. ELISA confirmed high level of sTNFR-Fc production in the media of all the transduced cell cultures (up to 520.0 ng/mL). The secreted sTNFR-Fc was stable in culture medium, and its biological activity was determined by *in vitro* protein binding assay using recombinant TNF-α. In addition, the secreted protein was shown to be protective for primary neurons from HIV-1 Tat-mediated neurotoxicity. Mouse bone marrow derived macrophages expressing sTNFR-Fc were shown to transmigrate directly into areas of diseased brain with active HIV-1 encephalitis in a murine model of human disease. These results establish the feasibility of using lentiviral vectors to express sTNFR-Fc as a potential novel therapeutic intervention for HIV dementia.

P111
Effect of time delay after necropsy on analysis of ganglia from rhesus macaques latently infected with simian varicella virus

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Clinical, pathological and virological features of latent simian varicella virus (SVV) infection in rhesus macaques parallel latent varicella zoster virus (VZV) infection in humans. Results generated from human ganglia delayed in sampling due to coroner timeframes have raised concern about virus reactivation after death. To directly address this issue, we inoculated two rhesus macaques intrabronchially with SVV and euthanized both monkeys 12 weeks

later. Tissues from one monkey were processed immediately after euthanasia. Tissue from the second monkey was maintained at 40C for 30 hours before processing, as is done in humans. Based on the abundance of SVV DNA in peripheral blood mononuclear cells, viremia in the first monkey was greater during primary infection. In both monkeys, SVV DNA was detected only in ganglia, but not in lung or liver. Transcripts specific for SVV ORFs 61, 62, 63 and 66 were detected in ganglia from both monkeys, and small amounts of RNA specific for SVV ORFs 29 and 40 were detected in the first monkey. In multiple ganglia of both monkeys, SVV ORF 61 specific RNA was detected at high levels, SVV ORF 63 protein was detected in the neuronal cytoplasm, and limited numbers of CD3-positive T cells were seen. Our analysis revealed that a 30-hour delay in time after death did not affect detection of viral DNA, expression of viral RNA or protein or detection of CD3-positive T cells in ganglia of latently infected monkeys.

P112
A Growth Factor Attenuates HIV-1 Tat and Morphine Induced Damage to Human Neurons: Implication in HIV/AIDS-Drug Abuse Cases

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The neuropathological abnormalities of human immunodeficiency virus (HIV)-1 patients abusing illicit drugs suggest extensive interactions between the two agents, which increase the rate of progression of neurodegeneration. The role of HIV-1 transactivating protein, Tat has been elucidated in mediating neuronal damage via apoptosis, a hallmark of HIV Associated Neurocognitive Disorders (HAND), but the underlying mechanisms involved in enhanced neurodegeneration by illicit drugs still remain elusive. In an attempt towards understanding the possible interactions, we exposed human neurons differentiated from primary human neural precursor cells (hNPCs) and human neuroblastoma cell line, SHSY5Y with HIV-1B Tat and Morphine and observed that morphine exacerbates HIV-Tat induced apoptosis in human neurons as well as in SHSY5Y as assessed by TUNEL. We also observed that HIV-Tat in presence of morphine increased reactive oxygen species (ROS) production via NADPH oxidase, disrupted mitochondrial homeostasis and increased caspase-3 activity in SHSY5Y cells. In addition to this, we found that pretreatment of cells with Platelet derived growth factor (PDGF-BB) protects neurons from HIV-Tat and morphine induced cell damage. PDGF-BB was able to alleviate ROS production, maintained mitochondrial membrane potential and decreased caspase-3 activation. Preliminary studies reveal involvement of PI3K and MAPK pathways in PDGF mediated abrogation of HIV-Tat and morphine induced toxicity. Detailed investigations are currently in progress to elucidate cellular and molecular pathways and to explore novel factors that might be involved in PDGF mediated protection against HIV-Tat and morphine. Study was supported by NBRC core funds and SM is recipient of Junior Research Fellowship from University Grant Commissions, New Delhi, India.

P113
Predictors of incident distal neuropathic pain in HIV-infected individuals in the era of combination antiretroviral therapy

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Background and Objectives: Distal neuropathic pain (DNP) is a common symptom of HIV-associated sensory neuropathy (HIV-SN). The rate at which DNP accumulates and factors predicting its onset in the era of combination antiretroviral therapy (CART) are not known. Methods: We evaluated HIV+ volunteers without DNP at entry into the CHARTER study. Enrollees underwent biannual neuromedical and psychiatric assessments. Incident DNP was defined as self-report of pain, burning, or aching in the distal legs and feet at any follow-up visit. Potential baseline (BL) risk factors for incident DNP examined using mixed effects logistic regression were: Age (>50 years); Sex; Ethnicity; history of DSM-IV substance abuse or dependence (substance A/D; eg, alcohol, cannabis, opioids, amphetamines); current depressed mood (Beck Depression Inventory, BDI>17); D-drug exposure; current CD4; nadir CD4; Hepatitis C co-infection; diabetes mellitus. CART history and plasma viral load were combined (CART X PVL) as follows: A) current CART, PVL undetectable (N = 194); B) current CART, PVL detectable (N = 134); C) past CART (N = 46); D) CART naive (N = 87). Results: Among 471 subjects with 1,987 study visits (median follow-up 18 months [IQR 6,

30]), 115 (24%) developed DNP. In multivariate analysis, significant predictors of greater probability of incident DNP were: past (not current) CART (CART X PVL group C), BDI>17 and any substance A/D. Age > 50 years was marginally significant (p = 0.06). Conclusions: Despite availability of CART, new complaints of DNP in HIV still occur. Risk factors associated with incident DNP were discontinuation of CART, depressed mood, and substance A/D. The association of depressed mood and pain has been observed in other conditions. Discontinuation of CART and exposure to substances of abuse may reflect toxicities that contribute to incident HIV-SN and subsequently DPN. Further analysis of risk factors for HIV-SN with and without DPN is needed to clarify this.

P114
SIV Gag Escape from CD8+ T Cell Control in the CNS During Anti-retroviral Treatment

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HIV frequently causes neurologic impairment even with HAART. Although associations between MHC class I alleles and AIDS have been reported, the role specific MHC class I alleles play in restricting development of HIV-induced neurologic disease has not been examined. Using a well-characterized SIV/pig-tailed macaque model of HIV CNS disease, we demonstrated that the MHC class I allele Mane-A*10 plays a major neuroprotective role by limiting SIV replication in the brain. The odds of developing CNS disease (SIV encephalitis) were over six times higher for animals that did not express the Mane-A*10. Correspondingly, animals that expressed Mane-A*10 had significantly lower amounts of SIV RNA, activated macrophages, and neuronal dysfunction in the CNS than Mane-A*10 negative animals. Mane-A*10 positive animals with the highest CNS virus burdens contained SIV Gag escape mutants at the Mane-A*10-restricted SIV Gag KP9 epitope whereas wild type KP9 sequences dominated in the brain of Mane-A*10 negative animals with comparable CNS viral burdens. To extend these studies, we examined emergence of SIV Gag K165R escape mutants (within the Mane-A*10-restricted KP9 Gag epitope) in CSF and plasma of SIV-infected macaques treated with combination anti-retroviral therapy to contain virus replication. Three key, novel findings of these studies were 1) K165R SIV Gag escape mutants emerged in CSF and plasma during the decaying phase of viremia after initiating HAART treatment before complete suppression of viral replication, 2) SIV K165R Gag escape mutants were archived in latent proviral DNA reservoirs in the brain in animals receiving HAART that suppressed viral replication, and 3) replication competent SIV K165R Gag escape mutants were present in the resting CD4 T cell reservoir isolated from HAART-treated SIV-infected macaques. Together, our findings illustrate that it is crucial to define the relationship between MHC class I alleles, effector CD8+ T cell responses, and viral escape in HIV/SIV CNS disease.

P115
Presentation of human T-cell leukemia virus type 1 (HTLV-1) Tax protein by dendritic cells: the underlying mechanism of HTLV-1-associated neuroinflammatory disease

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of a debilitating neurologic disorder, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). This disease features a robust immune response including the oligoclonal expansion of CD8+ cytotoxic T lymphocytes (CTLs) specific for the viral oncoprotein Tax. The key pathogenic process resulting in the proliferation of CTLs and the presentation of Tax peptide remains uncharacterized. We have investigated the role of APCs, particularly dendritic cells (DCs), in priming of the anti-Tax CTL response under both *in vitro* and *in vivo* conditions. We investigated 2 routes (direct versus indirect) of Tax presentation using live virus, infected primary CD4+/CD25+ T cells, and the CD4+ T-cell line (C8166, an HTLV-1-mutated line that only expresses Tax). Our results indicated that DCs are capable of priming a pronounced Tax-specific CTL response in cell cultures consisting of naive PBLs as well as in HLA-A*0201 transgenic mice (line HHD II). DCs were able to successfully direct the presentation of Tax through infected T cells, live virus, and cell-free Tax. These observations were comparable to those made with a known stimulant of DC maturation - a combination of CD40L and IFN-γ. Our studies clearly establish a role for this important immune cell component in HTLV-1 immuno/neuropathogenesis and suggest that modulation of DC functions could be an important tool for therapeutic interventions.

P116
The Transcription Factor Spi-B Binds Unique Sequences Present in the Tandem Repeat Promoter/Enhancer of JC Virus and Supports Viral Activity

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Progressive Multifocal Leukoencephalopathy (PML) is an often fatal demyelinating disease caused by lytic infection of oligodendrocytes with JC virus (JCV). Development of PML in non-immune suppressed individuals is a growing concern with reports of mortality in patients treated with monoclonal antibody therapies including natalizumab. JCV can persist in the kidneys, lymphoid tissue, and bone marrow. JCV gene expression is restricted by non-coding viral regulatory region sequence variation and cellular transcription factors. This study demonstrates that the transcription factor Spi-B binds to sequences present in the JCV promoter/enhancer and may affect early virus gene expression. Four potential Spi-B binding sites are present in the promoter/enhancer elements of PML-associated variants Mad-1, Mad-4, and the non-pathogenic archetype. Spi-B sites present in the promoter/enhancers of PML-associated variants alone bound protein expressed in JCV susceptible brain and lymphoid derived cell lines. Expression of exogenous Spi-B in non-permissive cells increased early viral gene expression. Strikingly, mutation of the Spi-B core in a binding site unique to Mad-4 was sufficient to abrogate viral activity in progenitor-derived-astrocytes. These results suggest that Spi-B could regulate JCV gene expression in susceptible cells, and may play an important role in JCV activity in the immune and nervous systems. Interestingly, Spi-B is upregulated in lymphoid cells capable of harboring latent JCV infection in response to Natalizumab treatment, and the Mad-4 binding site important to viral infection has been reported in a patient that developed PML during Natalizumab treatment suggesting that Spi-B may be a contributing factor in the development of PML.

P117
JCV T-antigen interaction with Cul7- A critical role of ubiquitination in cancer transformation induced by the JC virus

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A convincing body of evidence suggests that ubiquitination and the ubiquitin proteasome degradation pathway play a key role in neoplastic transformation. Ubiquitination, as post-translation modification, is involved both in functional regulation and degradation of specific cellular targets known as proto-oncogenes and tumor suppressors. Oncogenic viral proteins interact both with proto-oncoproteins and tumor suppressors leading to the modulation of their cellular function by several mechanisms including ubiquitination. Interestingly, viral oncoproteins themselves can also be regulated by this post-translation modification. Additionally, viruses can assemble their own E3 ligases or regulate the activity of cellular E3 ligases. E3 ligases, involved in the final step of the ubiquitination process, are the enzymes that provide the specificity for the interaction with target substrates by the means of a large number of proteins. Recent studies on the potential correlation between viral infection and oncogenesis, have addressed the emerging role of the ubiquitination system as a possible mediator for cancer transformation. In this scenario we hypothesized that JCV T-antigen may interferes with the ubiquitination system and we investigated a possible interaction between JCV T-antigen and the E3 ligase Cul7. To prove our hypothesis we performed co-immunoprecipitation and co-immunofluorescence experiments using the glioblastoma cell line U87MG. Our results indicate that JCV T-antigen and Cul7 interact in the cytoplasmic compartment. In addition, JCV T-antigen stabilizes Cul7. These observations suggest that JCV T-antigen can modulate Cul7 E3 ligase activity leading to oncogenesis. Further study addressing the biological significance of this interaction will decipher the cellular processes modulated by JCV T-antigen and Cul7 and will indicate new avenues for therapeutic intervention.

P118
Advanced Oncolity vectors for treatment of brain tumors

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Oncolytic vectors (OV) are attenuated lytic viruses that depend on characteristic defects in the anti-viral response of cancer cells to preferentially replicate and spread in tumors. OV derived from herpes simplex virus type 1 (oHSV) have shown promise in the clinic, but their efficacy is limited due to poor replication in tumor cells. We sought to develop a new class of oHSV vectors which can replicate essentially as unattenuated virus in glioma cells, but are blocked for replication in normal neurons. To Achieve this goal, (i) we developed suitable methods for targeting infection of specific cell types by directing vector attachment to unique cell surface receptors (e.g. EGFR); (ii) we engineered the essential viral proteins ICP4 and ICP27 which will be express only in the absence of specific miRNAs that are down-regulated in gliomas (e.g. miR-124). Expression is strictly controlled by naturally occurring micro-RNAs (miR) that are differentially expressed in normal brain neurons, neural precursor cells (NPCs) and tumor cells. Unlike current oncolytic viruses, our vectors do not have defective genes and our preliminary data show dramatically improved virus replication in tumor cells without raising toxicity for normal tissue.

P119
Progressive multifocal leukoencephalopathy in an HIV patient receiving successful long term HAART

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Progressive multifocal leukoencephalopathy (PML) has been traditionally associated to severe immunosuppression and described mainly in HAART naive patients with low lymphocytes CD4+ count. In last years some cases of PML have been described in HIV patients with higher lymphocytes CD4+ count shortly after initiation of HAART and in association to the immune reconstitution inflammatory syndrome (IRIS). We report on a rare case of PML, not IRIS associated, who occurred in a HIV positive patient with a lymphocytes CD4+ count > than 700/μL and with an undetectable HIV viral load resulting from a long term HAART. The neurological sign at onset consisted in focal myoclonus, an unusual clinical manifestation of PML that is probably due to motor cortical involvement from adjacent demyelinating lesions. Several aspects in this case: the high lymphocytes CD4+ count, the undetectable HIV viremia, the long term HAART and the initially negative JCV PCR on CSF, were highly misleading for establishing the diagnosis of PML. Despite the HAART and the intravenous, followed by oral, steroid treatment, the patient's condition dramatically deteriorated. She died one year after the onset of neurological symptoms. The post mortem histological and electron microscopy examination of the brain confirmed the diagnosis of PML. The absence of recent restoration of immune response, the absence of contrast enhancement of demyelinating lesions in brain RMN and the scanty inflammatory infiltration in pathological study, ruled out the possibility of PML-IRIS. Moreover not other immunosuppressive conditions, described as being associated with PML in HIV negatives, were identified. This case confirm that a severe immunosuppression or an IRIS is not required to the development of PML in HIV positives. The diagnosis of PML should always be considered in patients with consistent neurological symptoms, even with high lymphocytes CD4+ level and a full viral suppression resulting from a long term HAART.

P120
Detection of Borna Disease Virus among Iranian schizophrenia patients: A molecular and cellular approach

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Introduction: Borna disease virus (BDV), a neurotropic, negative-stranded RNA virus, might be associated with certain human mental disorders such as severe depression and schizophrenia. Several research groups have reported that psychiatric patients had a significantly higher prevalence of BDV serum antibodies than normal controls. In addition, a significantly higher presence of BDV RNA from peripheral blood mononuclear cells was identified in mental patients than in controls. This study was designed to measure the prevalence of BDV infection in Iranian schizophrenia and schizoaffective patients and further characterization of this strain. Method: 33 schizophrenia and schizoaffective patients beside 38 control subjects were examined. All BDV markers including Antigen, Antibody and

Circulating Immune Complexes (CIC) were checked for all sera samples. Also, the presence of BDV p24 and p40 RNA in peripheral blood mononuclear cells for BDV positive patients was conducted by RT-PCR and nested PCR. Results: BDV CIC positivity was detected in 22.2% of psychiatric patients (6/27). The control group was 5.26% (2/36) positive. The incidence of BDV CIC was significantly higher in psychiatric patients than healthy individuals. In addition, BDV Ag positivity was 12% for mental patients (4/33) and 2.6% for control subjects (1/37). One of the BDV positive patients with both high titer of all markers and positive nested RT-PCR was selected as the source for possible isolation of Iranian infectious BDV strain, by co-cultivation with a proper human cell lines. Conclusion: Our data support the finding that BDV infection might be a contributory factor to the pathogenesis of schizophrenia in the Iranian population. The worldwide prevalence of human BDV infection and also identification of its genetic and biological properties can help to find morbidity risks and investigation of transmission models of this neurotropic virus. The study for isolation and characterization is still going on.

P121
Requirement of p38 MAPK activity in monocytic and neuronal cells for HIV gp120-induced neurotoxicity

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HIV-1 envelope protein gp120 interacts besides CD4 with G protein-coupled chemokine receptors CXCR4 and CCR5 on host cells. This interaction, independent of infection, triggers downstream signaling that has been implicated in neurotoxin production and the pathogenesis of HIV-1 associated neurocognitive disorders (HAND). We previously showed in cerebrocortical cell cultures from rodents containing microglia, astrocytes and neurons, that overall inhibition of p38 mitogen-activated protein kinase (MAPK) signaling abrogated the neurotoxic effect of HIV-1 gp120. However, the time course of p38 MAPK activation and the contribution of this kinase in the various cell types remained unknown. In this study, we found that for HIV gp120-induced neurotoxicity to occur, active p38 MAPK is required in monocytic lineage cells, namely macrophages and microglia, and neuronal cells. In cerebrocortical cell cultures HIV-1 gp120 stimulated a time-dependent overall increase of active p38 MAPK and the activated kinase was primarily detected in microglia and neurons. Interestingly, both increased activation of p38 MAPK and neuronal death in response to gp120 were prevented by prior depletion of microglia, or in the presence of CCR5 ligand CCL4 or of p38 MAPK inhibitors. In human monocytic THP-1 cells and primary monocyte-derived macrophages (MDM), HIV gp120 stimulated production of neurotoxins was abrogated by prior introduction into the cells of a dominant-negative p38 MAPK mutant or p38 MAPK siRNA. On the other hand, toxic supernatants from gp120-stimulated mononuclear cells caused in microglia-depleted cerebrocortical cell cultures consisting of neurons and astrocytes an increase of activated p38 MAPK and neuronal apoptosis. In contrast, the neurotoxic effects of cell-free supernatants from gp120-stimulated monocytic THP-1 cells were prevented in the same cerebrocortical cell cultures pretreated with a pharmacological inhibitor of p38 MAPK. Thus, p38 MAPK signaling was critical upon exposure to HIV gp120 for both the neurotoxic phenotype of monocytic cells and subsequent toxin-initiated neuronal apoptosis. Supported by NIH grants R01 NS050621 and R01 MH087332 (to M.K.).

P122
Monoamine oxidase activity in an SIV/macaque model of HIV CNS disease

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The essential monoaminergic neurotransmitters dopamine and serotonin are diminished in the brain and CSF of HIV-infected patients, although the exact mechanism of loss remains elusive. One proposed theory for the loss of dopamine centers on increased oxidative stress in HIV CNS disease and the exquisite susceptibility of dopaminergic neurons to oxidative damage. Hydrogen peroxide is a toxic byproduct of monoamine oxidase (MAO), one of the primary enzymes responsible for dopamine and serotonin metabolism. *In vivo* experimental activation of this enzyme increases oxidation of glutathione, thereby creating oxidative stress. Since deficits in monoaminergic neurotransmitters and oxidative stress are both important contributors to HIV-associated CNS disease, MAO makes an attractive target for treatment. Inhibitors of MAO have been investigated both clinically and in animal models of HIV CNS disease with mixed results, ranging from minor neurocognitive improvement and increased brain dopamine

levels to no change in neurocognition and worsening neuropathological lesions. However, direct contribution of MAO activity to HIV neuropathogenesis has never been established. Using an accelerated, consistent SIV/macaque model of HIV CNS disease, we found MAO activity in the basal ganglia was elevated in SIV-infected macaques compared to uninfected controls in late stage disease. Furthermore, MAO activity correlated with CNS lesion severity and viral load in the basal ganglia. These data directly implicate increased MAO activity in the neuropathogenesis of SIV infection for the first time and suggest reevaluation of the potential therapeutic value of MAO inhibition in HIV/SIV CNS disease.

P123
Prion protein appears to function in neuron differentiation, nanotube contacts, and cell-to-cell transmission of TSE agents

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Host prion protein (PrP) is most abundant in neurons where its functions are unclear. We used rat neuronal precursor cells, transduced with the temperature sensitive SV-40 T antigen just before terminal differentiation, to find if proliferative arrest was sufficient to cause an increase in PrP. Proliferative arrest at 37.5oC induced a 7-fold increase in PrP by 2 days. Very few cells incorporated BrdU, and T antigen was markedly reduced, indicating effective arrest (J Cell Biochem, epub 6/2010). Moreover, additional neuritic processes with abundant plasma membrane PrP connected many cells. PrP also concentrated between apposed stationary cells, as well as on extending growth cones and their filopodia. Stationary cells with high PrP could be maintained for 30 days in their original plate, and they reverted to a proliferating low PrP state at 33oC. Ultrastructural studies confirmed an increase in nanotubes, adherent junctions and apparently open synctial regions between high PrP cells. Notably, nanotubes are conduits for the exchange of viruses between cells. The association of PrP with dynamic recognition and contact structures that have known viral functions indicates its specific role in CJD and other TSEs. It suggests that host PrP is the essential host receptor that binds TSE infectious particles to facilitate their transfer between cells. High PrP cells were successfully infected with a rat adapted FU-CJD agent, and we are currently titrating infectivity in low and high PrP cells using a rapid tissue culture assay with no background signal (J Neurovirol 14:352-61, 2008).

P124
Role of neurotrophins in neuroAIDS

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HIV-associated dementia (HAD), a severe form of neurological symptoms occurring in people living with HIV-1, is comprised of motor alterations, cognitive impairments and often depression. Neuronal degeneration is seen in these individuals. The current highly active antiretroviral therapy does not necessarily reduce HAD. Thus, adjunct therapies aimed at reducing HIV-induced neurodegeneration must be developed. We have examined the neuroprotective activity of the neurotrophin brain-derived neurotrophic factor (BDNF) against the HIV envelope protein gp120. This protein reproduces some feature of HAD when injected into rodents. BDNF blocked the neurotoxic effect of gp120 both *in vitro* and *in vivo*. Intriguingly, gp120 reduces the expression of BDNF in the rat brain. This down–regulation was also observed in postmortem brains of HAD subjects, suggesting that lack of neurotrophic support is a risk factor for developing HAD; nevertheless, little is known about the molecular mechanisms of BDNF neuroprotection. The strain of gp120 used engages the chemokine receptor CXCR4. This receptor is also implicated in HIV entry into immunocompetent cells, but it is also highly expressed in the brain. Therefore, we have examined whether BDNF modulates the expression of CXCR4 and other chemokine receptors. Pharmacological concentrations of BDNF significantly decreased CXCR4 levels, whereas reduced BDNF expression, as seen in BDNF heterozygous mice, up-regulated CXCR4 mRNA. Thus, the neuroprotective property of BDNF against gp120 appears to be linked to its ability of down-regulating CXCR4. We suggest that BDNF could be an ideal adjunct therapy for HAD, because, not only may it inhibit viral entry but it may also prevent neuronal degeneration and loss of synaptic connections. Supported by HHS NS040670 and DA026174.

P125
Polyomavirus reactivation and relationship to cellular immunity in patients with Relapsing Remitting Multiple Sclerosis on Natalizumab therapy

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Introduction: Natalizumab reduces clinical relapses and the risk of sustained progression of disability in patients with relapsing remitting multiple sclerosis (RR MS). It has been associated with the development of progressive multifocal leucoencephalopathy (PML), a rare demyelinating disease caused by JC virus. Reactivation of BK virus has been shown to occur in association with various types of immunosuppression and is known to cause morbidity in the immunocompromised host. Aim: To evaluate the relationship between the prevalence of JC and BK in MS patients receiving Natalizumab and the degree of immunosuppression as evidenced by change in CD4+ count and CD4+/CD8+ ratio over time. Methods: This is an ongoing prospective, longitudinal, observational study that started in January 2007. We sampled serum and urine specimens at baseline and at 3-monthly intervals over the course of the study period. Results: A total of 86 subjects with active RRMS received Natalizumab in our Department. We report one case of PML after 40 doses in a patient who was persistently JC viruric but not viraemic. Reactivation of BK virus occurred in urine in 17 patients at a mean of 13.5 doses. No significant cellular immunosuppression related to treatment was noted in the group. Conclusion: We report BK reactivation in urine related to Natalizumab but no significant relationship to viraemia was seen and no BK related morbidity was evident. Further studies on the significance of BK virus in MS remain to be done but it may have consequences for the group in terms of infection- related adverse effects. Our findings further support evidence of an association between Natalizumab therapy and the development of PML.

P126
Prevalence of HIV-Related Neurocognitive Complications in Catalonia: Results from a Cross-Sectional Multicenter Study

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Background: Prevalence of neurocognitive complications in HIV-infected patients has been described in some countries, although this has not been established in Catalonia (Spain) yet. Methods: Seven centers in Barcelona participated in this multi-site cross-sectional study aimed to validate a brief, reliable and useful instrument for the assessment of HIV-associated neurocognitive impairment (NCI). 268 HIV-infected patients were recruited and assessed by a comprehensive neuropsychological tests battery covering 7 neurocognitive areas, and demographic, medical and emotional data were also collected. NCI was defined as performing 1 or 2 standard deviations below the mean in at least 2 areas, and presence of confounding comorbidities was particularly controlled for data comparisons. HIV-associated neurocognitive disorders (HAND) classification was applied in a subgroup of 166 patients. Results: Subjects were mostly middle-aged (42 years) men (80.9%), treatment-experienced (82.8%), HCV seronegative (75.3%), with undetectable plasma viral load (64.8%), a median CD4 cell count of 476 cells/μL, and nadir CD4 cell count of 235 cells/μL. 129 patients (48.1%) showed NCI and 124 (49.4%) cognitive complaints, 60 (49.1%) from those with NCI. Considering confounding comorbidities, 88 subjects (32.8%) had presence of a CNS-related opportunistic infection, referred drug abuse, were on psychiatric treatment, or presented other circumstance considered as major influence for impairment coexistence. Excluding those individuals, 77 subjects (42.7%) had NCI, 51 (40.8%) from those on ART treatment. According to a multivariate model, factors more strongly related to NCI were AIDS diagnosis, nadir CD4 cell count, time with HIV, time on ART treatment, and education level. With regard to HAND classification, 166 subjects were eligible to be evaluated, and their distribution was as follows: asymptomatic NCI: 37 (51.4%), mild neurocognitive disorder: 28 (38.9%), and HIV-associated dementia: 517 (9.7%). Conclusions: About half of our HIV-infected patients from Catalonia present HIV-associated NCI (48.1%). This result indicates a comparable rate of neurocognitive complications as found in other countries. Effective interventions must be found in the goal of neurocognitive functioning protection for HIV-infected patients.

P127
T-cell-induced chronic microglial cell activation following MCMV encephalitis

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Murine cytomegalovirus (MCMV) brain infection stimulates microglial cell-driven proinflammatory chemokine production which precedes the presence of brain-infiltrating systemic immune cells. Activation of resident murine microglia (CD45intCD11b+) was detected until 30 d following MCMV brain infection, as assessed by MHC class II expression. The

induction of MHC class II on these cells was found to be time-dependent with maximal expression at 30 dpost-infection (p.i.). Activated microglia were also found to be a major source of TNF-α. We went on to investigate the phenotypes and infiltration kinetics of peripheral leukocyte trafficking into MCMV-infected murine brains. Flow cytometric analysis of brain-infiltrating leukocytes at 5, 15, and 30 d.p.i., was performed to assess their phenotype. A predominantly macrophage (CD45highCD11b+Ly6Chigh) and neutrophil (CD45highCD11b+Ly6G+) infiltration was seen early during infection (i.e., 5 d p.i.), with low levels of IFN-γ production by CD45highCD3 +CD8+ cells. By 15 d p.i., the phenotypic profile shifted to a predominantly lymphocytic (CD45highCD3+) infiltrate and there was marked increase in the levels of IFN-γ production at 15 and 30 d p.i., by CD8+ T-cells. This lymphocyte infiltrate was detected until 30 d.p.i., with CD8+ and CD4+ T-cells present at a 3:1 ratio, respectively. At 30 d p.i., antigen specific CD8+ cells were identified as the major source of IFN-γ. Interestingly; immediate early viral antigen (IE1) expression was also detected at this time point. We then investigated the role of IFN-γ in chronic microglial activation by using IFN-gamma -knockout (GKO) mice. At 30 d p.i., GKO mice demonstrated similar phenotypic brain infiltrate when compared to wild-type mice (Wt), however MHC class II expression on microglia isolated from these GKO mice was significantly lower compared to Wt mice. Taken together, these results suggest that MCMV brain infection results in chronic microglial cell activation and long-term persistence of antigen specific CD8+ T-cells producing IFN-γ Microglial cell activation was found to be dependent on IFN-γ production by viral Ag-specific T-cells.

P128
Varicella Zoster Virus Vasculopathy: Histological and Immunohistochemical Analysis of Virus-infected Arteries

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VZV vasculopathy, a cause of ischemic and hemorrhagic stroke, results from productive virus infection of cerebral arteries. Our histological and immunohistochemical analysis of VZV-infected cerebral arteries from three patients with VZV vasculopathy revealed: (1) neointimal hyperplasia, (2) a duplicated/disrupted internal elastic lamina, and (3) a thickened intima with minimal fibrosis, composed predominantly of smooth muscle actin (SMA)+ cells, but not CD31+ endothelial cells. Since no animal model exists to study the pathogenesis of VZV vasculopathy, we explanted normal human cerebral arteries in culture, infected them with VZV and conducted the same analyses as in VZV-infected arteries from patients with VZV vasculopathy. Histological and immunohistochemical changes in VZV-infected cerebral artery explants paralleled changes seen in cerebral arteries of patients with VZV vasculopathy. Infected cerebral artery explants contained increasing amounts of viral DNA and viral RNA over time as well as VZV antigen. Parallel histological and immunohistochemical changes in VZV-infected human cerebral arteries and in human cadaveric cerebral artery explants indicate its usefulness to study the pathogenesis of VZV vasculopathy. Overall, VZV-induced accumulation of SMA+ cells in the hyperplastic intima may lead to reduced elasticity and arterial occlusion - a possible mechanism for stroke.

P129
VZV transcription in latently-infected human ganglia

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Identification and characterization of the entire varicella zoster virus (VZV) transcriptome in latently infected human ganglia will further our understanding of latency and changes that initiate reactivation. Earlier studies to determine the full extent of latent VZV transcription were hindered by the inability to detect multiple low abundance viral transcripts by mirco- and macro-array and PCR analysis. To date, only five transcripts mapping to open reading frames (ORFs) 21, 29, 62, 63, and 66 have been detected and sequence-verified. We recently developed a novel multiplex RT-PCR assay that allows rapid and sensitive detection of transcripts corresponding to all 68 unique VZV open reading frames (ORFs). We analyzed 27 trigeminal ganglia from 14 subjects obtained within 24 hours after death from men and women, age 16 to 83 years. Ninety-six percent of ganglia contained VZV DNA by PCR. Multiplex RT-PCR analysis identified 10 VZV transcripts mapping

to: (1) ORFs 63, 29 and 62, previously detected and sequence verified; (2) ORFs 4 and 40, previously detected by in situ hybridization; and (3) ORFs 11, 68, 43, 41 and 57, not previously detected. Overall, latently-infected human trigeminal ganglia contain transcripts corresponding to multiple VZV ORFs, of which ORF 63 is the most prevalent.

P130
Genome wide expression profile of microRNA in HIV-1 positive patients with neuropathogenesis: A novel potential biomarker for early diagnosis

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HIV-associated dementia (HAD) is commonly observed in AIDS patients worldwide and more prevalent in patients with drug abuse history. Following infection with HIV-1, infected individuals exhibit a remarkable variation in virus replication and disease progression including neuropathogenesis and dementia. Several factors are causally linked to the disproportionate disease pattern including differences in host genetic background, immunological control, viral and host cellular gene expression. It has been suggested that a genome wide analysis may provide a true picture about the related genes underlying disease induction. In this regard, we hypothesize that non-coding microRNAs (both host and viral) in HIV-1 infected individuals are regulated differentially, thus altering viral and host gene expression, immune responses and disease outcome. Using both *in vitro* infected PBMC culture and PBMCs derived from HIV-1 seropositive individuals with and without neuropathogenesis, we evaluated genome wide microRNA (miRNA) profile as well as their corresponding mRNA profile. Preliminary results indicate that, among the 704 miRNA tested, 55 miRNA were differentially regulated in the infected PBMC compared to its uninfected/mock infected control cells. Further statistical analyses indicate that 15 miRNA are upregulated significantly ($p < 0.05$) in multiple donors ($N = 9$). Functional and target gene analysis revealed that many of the virus induced miRNA dyregulated genes are involved in immune gene expression, differentiation and proapoptosis. Together these results suggest that virus infection/exposure upregulated several miRNAs that target genes, which are required for defense and virus replication. Future studies using samples from infected individuals with and without dementia will identify disease specific miRNA regulation. Results from this study will have significant impact on biomarker discovery and potential antiviral targets as identified in cancer patients and other diseases.

P131
Comparative study of neurocognitive impairments between seropositive HIV-1 Clade C and seronegative individuals

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Background: HIV transmission in India is occurring more widely. Evidence suggests that the Clade C is the most common in India and literature suggest that it impacts neurocognitive functioning. But there is paucity of research data concerning neuropsychological impairment in the subjects with HIV-1 Clade C. Aim: To study the neurocognitive functioning in individuals with seropositive HIV-1 Clade C and compare the same with healthy control group. Method: 68 seropositive HIV-1 Clade C in the age range of 18-45 years of either gender, with atleast 5 years of education, with CD 4 count ≤400 cells/μl and the control group consisted of age and sex matched 67 healthy subjects, were assessed on HNRC (HIV Neubehavioural Research Center) neuropsychological battery for cognitive impairments. Subjects in both groups were also assessed on BDI (Beck's Depression Inventory), WHOQOL (WHO Quality of Life Scale) and STAI (State and Trait Anxiety Inventory). Both the groups were compared using t-test and Chi-Square test. Results: Comparison of both groups showed that subjects with HIV differed significantly from the control group on the domains of memory -IHDS [psychomotor score $t = 2.64$, $p = .009$; total score $t = 2.67$, $p = .009$], attention and working memory - spatial span test [forward score, $t = 2.06$, $p = .041$; backward score $t = 2.91$, $p = .004$; total score $t = 2.83$, $p = .005$] and executive functioning "WCST [non Perseverative errors $t = 2.04$, $p = .043$; categories completed $t = 2.34$, $p = .021$; trial to complete first category $t = 2.69$, $p = .008$ and CAT (total error score $t = 2.78$, $p = .006$)]. Additionally both the groups differed significantly on the ratings of WHOQOL [physical domain $t = 5.23$, $p = .000$; independence level $t = 2.34$, $p = .020$; social relationship $t = 2.86$, $p = .005$; spirituality/religion $t = 5.23$, $p = .000$; total score $t = 2.86$, $p = .005$] and STAI - trait anxiety score $t = 2.14$, $p = .034$. Conclusion: HIV seropositives were significant impairment in the domains of memory, attention, working memory and executive functioning in comparison to HIV

seronegatives. Acknowledgements: This study was made possible by funding from NIH Grant No. RO1 NS 055653.

P132
Involvement of the endogenous HERV-F(c)1 retroviral locus on the human X chromosome in the etiology of multiple sclerosis

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Two views have dominated the discussions of the etiology of multiple sclerosis in recent decades: It could be a genetic disease, or it could be a disease caused by an infectious agent. The genetic view is most clearly expounded by studies of twins showing that concordance between monozygotic twins is approximately 25 percent, while concordance between dizygotic twins is only 2-3 percent. The alternative view, that multiple sclerosis is caused by an infectious agent, is most clearly backed by animal studies. The obvious intersections of a genetic and a viral etiology are the human endogenous retroviruses. 48 intact or near-intact human endogenous retroviruses were treated as inherited loci in an analysis based on genetic epidemiology. We investigated DNA from 350 patients with verified multiple sclerosis living in Western Denmark, as well as 500 controls living in the same part of the country. Females constituted 62 percent of cases and 67 percent of controls. A total of 220 markers were tested. A striking cluster of significant markers occurred in chromosome X at approximate position 97100000 around the HERV-F(c)1 proviral locus (NCBI genome build 37.1). The marker rs391745 was lowest with a p-value of 4*10-6 (2-sided) for association with disease, when calculated on the bases of C-allele carriers. This value remained significant after Bonferroni correction ($p = 0.0009$). We retested the association of rs391745 and multiple sclerosis in two other Danish cohorts. Rs391745 was again associated with MS ($p = 0.01$) in one of them. The p-value for all three cohorts combined was 0.00001 (2-sided). This was significant after Bonferroni correction ($p = 0.003$). Finally, we performed a scan of the region surrounding HERV-F(c)1. The association occurred in a 20 kb region around the provirus. In contrast, the nearest known genes lie 141 kb upstream and 57 kb downstream, respectively.

P133
Chronic morphine modulates TLR-4 expression and signaling in microglial cell line BV2

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Opiate use and abuse have been known to suppress a number of immune responses, and therefore, have been postulated to serve as cofactors in the progression of opportunistic infections. The high frequency of bacterial sepsis observed in drug abusers has been shown to be in part due to impairment of innate immunity. Microglial cells are the first and main form of active immune defense in the central nervous system (CNS). They mediate pathogen clearance as well as potentiate inflammatory pathways, which although largely beneficial may be harmful to the CNS. Morphine's effect on microglia has not been very well documented. Our study examines effects of morphine on TLR-4 mediated inflammatory pathways and bacterial clearance in microglial cell line BV2. We note that chronic morphine treatment of the microglial cell line BV-2 leads to an increase in TLR-4 expression by inducing TLR-4 promoter activity, mRNA levels and receptor's surface expression. Consequently, chronic morphine treatment enhances LPS mediated TLR-4 activation, leading to increased secretion of inflammatory cytokines such as TNF-α, IL-6 by modulating NF-κB activation. In addition to inflammatory mechanisms, TLR-4 activation has been implicated in enhancing phagocytic and bactericidal activity. Although, chronic morphine treatment alone inhibits phagocytosis and bacterial clearance in microglia, however, paradoxically, in the presence of LPS, morphine enhances phagocytic and bacterial killing ability of microglia by TLR-4 receptor upregulation. These studies further expand understanding of morphine mediated immune modulation and consequences drug abuse can have on neuro-immune interactions.

P134
Downregulation of JCV T-antigen by hypoxia and glucose deprivation in medulloblastoma

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Recent studies have reported the detection of the human neurotropic virus, JCV, in a significant population of brain tumors, including medulloblastomas. Accordingly, 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, play a role in the oncogenic function of JCV T-antigen. Here we demonstrate that T-antigen expression is suppressed by both hypoxia and glucose deprivation in medulloblastoma cells that endogenously express T-antigen. This regulation appears to occur at the post-translational level because of little change in primary transcripts and decreased T-antigen protein stability upon hypoxia or glucose deprivation. Mechanistic studies indicate that glucose deprivation-mediated suppression of T-antigen is partly mediated by AMP kinase (AMPK), an important sensor of the AMP/ATP ratio in cells. We have found that AMPK activation inhibits T-antigen expression, whereas AMPK inhibition prevents hypoxia and glucose deprivation-mediated suppression. In addition, T-antigen downregulation is partially dependent on reactive oxygen species (ROS) production during glucose deprivation. Functional studies indicate that T-antigen prevents cytotoxicity induced by glucose deprivation and prevents ROS induction as well. These observations point to the possible involvement of JCV T-antigen in cell survival from key processes regulating medulloblastoma proliferation and invasion. Studies are in progress to identify the molecular pathways involved in the regulation of T-antigen by hypoxia and glucose deprivation and the biological importance of this downregulation in tumor pathogenesis.

P135
Association of astrocyte-elevated gene-1 (AEG-1) with necrosis and induction by hypoxia and glucose deprivation in glioblastoma

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Glioblastomas continue to rank among the most lethal primary human tumor. Despite treatment with the most rigorous surgical interventions along with the most optimal chemotherapeutic and radiation regimens, the median survival is just 12-15 months after diagnosis for patients with glioblastoma. One feature of glioblastoma associated with poor prognosis is the degree of hypoxia and expression levels of hypoxia-inducible factor-1 α (HIF-1α). HIF-1α expression allows metabolic adaptation to low oxygen availability, partly through upregulation of VEGF and increased tumor angiogenesis. Here, we demonstrate an induced level of astrocyte-elevated gene-1 (AEG-1) in high-grade as compared to low-grade astrocytomas and association of AEG-1 with necrotic areas in glioblastoma. AEG-1 has the capacity to promote anchorage-independent growth and cooperates with Ha-ras in malignant transformation. In addition, AEG-1 was recently demonstrated to be an oncogene that can induce angiogenesis in glioblastoma. Results from *in vitro* studies show that AEG-1 is induced by hypoxia in a HIF-1α-dependent manner and that PI3K inhibition abrogates AEG-1 induction during hypoxia. Furthermore, we show that AEG-1 is induced by glucose deprivation and that prevention of intracellular reactive oxygen species (ROS) accumulation prevents this induction. Additionally, AEG-1 knockdown results in increased glucose deprivation-induced cytotoxicity, indicating that AEG-1 induction is necessary for cells to survive this type of cell stress. These observations link AEG-1 overexpression in glioblastoma with hypoxia, glucose deprivation, and angiogenesis; thus, targeting these pathways may lead to improved therapy for glioblastoma in the future. Studies are in progress to identify the molecular pathways involved in the regulation of AEG-1 by hypoxia and the biological importance of AEG-1 activation in tumor pathogenesis.

P136
Development of co-selected single nucleotide polymorphisms in the viral promoter precedes the onset of human immunodeficiency virus type 1-associated 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 at position 5 of Sp site III (5T)) increased in frequency as disease severity increased. Additionally, the 3T variant correlated with HIV-1-associated dementia. LTR sequences derived by 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, demonstrating 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 evolve in their native patient-derived LTR backbone structure demonstrated a spectrum of basal and Tat-mediated transcriptional activities, using the IIIB-derived Tat and co-linear Tat derived from the same molecular clone containing the 3T/5T LTR SNP. In silico predictions utilizing co-linear envelope sequence suggested that the patient's 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 HIV-1 genomic swarm may evolve during the course of disease in response to selective pressures that lead to changes in prevalence of specific polymorphisms in the LTR, env, and/or tat that could predict the onset of neurological disease and result in alterations in viral function.

P137
Novel Pathways of combination antiretroviral therapy (cART)-mediated Toxicity in the Central Nervous System

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The incidence of HIV-associated dementia has been decreasing with the advent of combination antiretroviral therapy (cART) due to efficient systemic viral control; however, the prevalence remains high, partly due to increased life expectancy. Neurotoxicity in the peripheral nervous system has been observed as a side effect of antiretroviral drugs, but the contribution of cART to HIV-associated neurocognitive disorders is unknown. Alterations in lipid and protein metabolism in addition to mitochondrial damage-induced production of reactive oxygen species (ROS) are known to play a role in peripheral antiretroviral drug toxicity. Thus, we hypothesize that cART compounds induce mitochondrial and endoplasmic reticulum (ER) stress, leading to neuronal damage, contributing to the changing clinical and pathological picture seen in HIV positive patients. To test this hypothesis, we treated primary rat cortical neurons with commonly prescribed antiretroviral compounds from two classes, nucleoside reverse transcriptase inhibitors (NRTI) and HIV protease inhibitors (PI) in therapeutically relevant doses and assessed mitochondrial stress, ER stress and neuronal damage. Our data suggest PIs (indinavir, ritonavir and saquinavir) are neurotoxic alone or in combination with NRTIs (zidovudine, stavudine and zalcitabine), as detected by loss of MAP2 and caspase and calpain activation. Zidovudine, ritonavir, and saquinavir in combination induced early increases in mitochondrial and ER stress as detected by increased BiP, p-eIF2alpha and ATF6-beta expression. Moreover, similarly treated primary human macrophages displayed a comparable ER stress response, and supernatants from these macrophages induced mitochondrial stress in neurons. Overall, our results suggest neuronal responses to antiretroviral drugs are class specific, and therapeutically relevant drug combinations induce mitochondrial and ER stress in relevant cell types, culminating in neuronal damage. Understanding the underlying mechanisms of the toxicity will provide new strategies to develop adjunctive therapies to complement cART, as well as potential diagnostic means for testing the neurotoxicity of new cART treatments.

P138
NFkB activation promotes immune activation in HTLV-I-associated myelopathy/tropical spastic paraparesis

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Background: Human T lymphotropic virus type I (HTLV-I) is the etiologic agent of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic inflammatory disorder of the central nervous system. Evidence suggests that viral-induced immune activation plays a key role in the pathogenesis of HAM/TSP. The HTLV-I-encoded transactivating protein Tax is known to activate nuclear factor

kappa B (NFkB), a key host signaling pathway regulating immune response, but the contribution of the NFkB pathway to the immune activation associated with HAM/TSP has yet to be fully defined. To further delineate the role of NFkB activation in HAM/TSP, we examined NFkB activation in peripheral-blood mononuclear cells (PBMC) from subjects with HAM/TSP, and tested the effect of NFkB inhibition on key ex vivo correlates of immune activation in HAM/TSP. Materials and Methods: We examined the role of NFkB activation during immune activation associated with HAM/TSP by using small molecule NFkB inhibitors, including a newly developed selective inhibitor of NFkB, PBS-1086. NFkB activation was measured by a DNA-binding ELISA on nuclear extracts from PBMC of subjects with HAM/TSP and healthy donors to detect nuclear translocation of NFkB subunits. Immune activation in HAM/TSP PBMC was measured by tritiated thymidine incorporation, a marker for spontaneous proliferation, and by FACS analysis of the lymphocyte activation markers CD25 and CD69 and the pro-inflammatory cytokine STAT5. Proviral loads in untreated and inhibitor-treated HAM/TSP samples were measured by real time PCR. Results NFkB activation was assessed in peripheral-blood mononuclear cells (PBMC) from subjects with HAM/TSP and in healthy donors (HD). Nuclear translocation of the NFkB RelA was significantly higher in PBMC from subjects with HAM/TSP compared to HD ($p = 0.032$) following short-term (20 h) culture, indicating increased activation of the NFkB pathway in HAM/TSP. Treatment with the small molecule inhibitor PBS-1086 reduced NFkB activation ($p < 0.01$). PBS-1086 reduced expression of CD25 and CD69 in HAM/TSP PBMC as well as phosphorylation of STAT5 in a dose-dependent manner ($p < 0.01$ for all). PBS-1086 also inhibited spontaneous lymphoproliferation of HAM/TSP PBMC in a dose-dependent manner. PBS-1086 treatment resulted in a mean proviral load reduction of 20% compared to untreated PBMC in a 72 h culture. Discussion These results indicate that NFkB activation plays a critical upstream role in the immune activation associated with HAM/TSP, and identify the NFkB pathway as a potential therapeutic target for immune modulation in HAM/TSP.

P139
Risk factors for dementia among HIV+ individuals in Uganda

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Background: There is increasing recognition for the importance of HIV-associated dementia (HAD) in sub-Saharan Africa. However, only few studies have attempted to characterize its prevalence and associated risk factors. This knowledge gap is significant given that HIV+ persons in this region are living longer due to the increased availability of HAART; and improved survival might result in a "second epidemic" of cognitively-impaired HIV+ persons in this region. Therefore, we sought to examine the prevalence of HAD in sub-Saharan Africa as well as the factors that predispose HIV+ individuals to HAD. Methods: Participants were 125 HIV+ persons (68% women, mean age = 37 years, mean education = 8 years, mean CD4 count = 242 cells/μL) recruited from the Infectious Diseases Clinic at Makerere University, Uganda. They underwent detailed sociodemographic, medical history, immune status, functional, neurologic, and neuropsychological evaluations. Diagnosis of HAD was made based on consensus conference involving study neurologists and neuropsychologists. Univariate logistic regression was used to identify candidate correlates of HAD. All univariately significant variables were then entered into a multivariable stepwise logistic regression to identify, more parsimoniously, risk factors for HAD in this cohort. Results: Forty-two percent of the cohort (52 of 125) had HAD. Univariate correlates of HAD were advanced age (OR = 1.09, p = .003), female sex (OR = 2.46, p = .030), low education (OR = 1.15, p = .007), history of syphilis (OR = .318, p = .016), and subjective memory complaints (OR = 3.34, p = .007). Neuropathy was marginally significant (OR = 1.96, p = .076). In the multivariable analyses, advanced age (Step 1, OR = 1.09, p = .003), female sex (Step 2, OR = 4.33, p = .002), low education (Step 3, OR = 1.16, p = .010), and history of syphilis (Step 4, OR = .346, p = .047) were associated with an increased odds of HAD. Conclusions: HAD is common in this sub-Saharan Africa cohort, and is associated with advanced age, female sex, and low education. The association with history of syphilis is ambiguous, especially as patients in Uganda often receive this diagnosis when they develop a rash.

P140
Large T antigen promotes JC virus replication in G2 arrest by inducing G2 checkpoint signaling

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Large T antigen (TAg) of the human polyomavirus JC virus (JCV) possesses DNA binding and helicase activities, which are required for replication of the viral genome. We have investigated the relation between JCV replication and the host cell cycle in order to identify the cellular machinery required for JCV replication. We show that JCV-infected cells expressing TAg accumulate in G2 phase of the cell cycle as a result of the activation of ATM- and ATR-mediated G2 checkpoint pathways. The induction of G2 arrest is caused by not only viral DNA replication, but also TAg itself. Analysis of TAg mutants with different subnuclear localizations suggested that the association of TAg with cellular DNA contributes to the induction of G2 arrest. Abrogation of G2 arrest by inhibition of ATM and ATR, Chk1, and Wee1 suppressed JCV genome replication. In addition, abrogation of G2-M transition by Cdc2 depletion disabled Wee1 depletion-induced suppression of JCV genome replication, suggesting that JCV replication is facilitated by G2 arrest resulting from G2 checkpoint signaling. Moreover, inhibition of ATM and ATR by caffeine suppressed JCV replication and propagation after establishment of infection in cells. The observation that oligodendrocytes productively infected with JCV *in vivo* also undergo G2 arrest suggests that G2 checkpoint inhibitors such as caffeine are potential therapeutic agents for JCV infection.

P141
European study on the comparative expression of Human Endogenous Retrovirus and Epstein-Barr Virus, in Multiple Sclerosis

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Human endogenous retroviral family W (HERV-W) RNA in circulating virion particles (Multiple Sclerosis associated RetroViral element, MSRV) has been associated with the evolution and prognosis of Multiple Sclerosis. HERV-W encodes a powerful immunopathogenic envelope protein (ENV) that activates a pro-inflammatory and autoimmune cascade through interaction with Toll-Like Receptor 4 (TLR4) on antigen-presenting cells, and triggers superantigen-like dysregulation of T-lymphocytes. As a potent TLR4 agonist, HERV-W/ENV antigen can be an upstream inducer of immunopathogenicity in disease. In parallel, Epstein-Barr Virus (EBV) has been shown to express latency genes in B-lymphocyte follicles of MS brains and such Herpesviridae are known to transactivate HERV-W elements such as the one expression reverse transcriptase activity detected in MS (MSRV). In the present study, we have addressed the question of a potential interplay between HERV-W elements and EBV by analyzing expression markers of both exogenous and endogenous viruses in patients with MS and normal controls from multiple European countries. These were measured by quantitative PCR, ELISA for the detection of specific antigen or antibody. Overall, 100 MS patients, 150 Healthy controls and groups of 30 to 50 controls with other neurological diseases, chronic infections or autoimmunity were studied. Positive antigenaemia for HERV-W Envelope protein (Env) was detected in serum from more than 75% of MS cases, but not in infectious diseases (HBV, HCV, HIV infections) nor in Lupus, nor in other neurological diseases. A cluster of about 45% of positive patients were nonetheless found in Chronic Inflammatory Demyelinating Polyneuropathies (CIDP). Moreover, when about 150 Healthy controls were tested, 4% displayed low but positive Env antigenaemia, indicating the existence of "asymptomatic" carriers. The difference between MS and controls (except CIDP) was very significant but no difference was found between the different MS clinical forms (remitting-relapsing-RRMS-, Secondary progressive-SPMS- and Primary Porgressive -PPMS-. Nonetheless, Immunohistology with different monoclonal antibodies specific for various epitopes of HERV-W Env protein detected this antigen in MS plaques at different stages, on post-Mortem brain sections. Quantitative RT-PCR for specific HERV-W subtype associated with MS (MSRV), also showed significant difference for MSRV RNA expression in peripheral blood mononuclear cells (PBMC) between MS and controls, but not between MS clinical forms. Interestingly, quantitative PCR for MSRV subtype showed significant increase of MSRV DNA copy number in PBMC between MS and controls, but also increased between RRMS and SPMS along with clinical score. In parallel, the same patients showed significant difference for EBV EBNA-1 antigen serology, but not for EBV DNA copy number in PBMC. This difference was limited to few MS patients. Our results confirm the strong association between MS and MSRV-type HERV-W element, with evidence of DNA replication and copy number increase with disease evolution, as well as the presence of its neuroinflammatory protein (Env antigen) in the great majority of MS patients. Nonetheless, parallel results on EBV antigens and genomic detection indicate that

this Herpesviridae member may play a role in activating or boosting MSRV expression in B-lymphocyte reservoir when, e.g., forming follicles in MS brains with EBV-positive sub-population, as elsewhere reported. This study thus presents a rationale for presenting the interplay between environmental viruses (EBV) and Genes consisting in Endogenous retroviral elements leading to the chronic expression of a neuroinflammatory protein (MSRV/HERV-W Env) underlying the evolution of MS disease. Given that transactivation of HERV-W/MSRV element was also shown to occur with other Herpesviridae, the latter can be cosnidered as co-factors in the triggering of a common "endogenous" pathogenic pathway activated and fueled by the expression of HERV-W MSRV element and its Env protein. This HERV-W protein at the crossroads of viral triggers and MS neuroinflammatory cascade, is therefore our lead therapeutic target for specific immunotherapy with neutralizing humanized antibody. It is now produced under GMP condition for preparing clinical trials.

P142
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 peripheral blood-derived LTRs (a C-to-T change at position 3 of C/EBP site I (3T) and at position 5 of Sp site III (5T)) increased in frequency as disease severity increased. Additionally, the 3T variant 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 418 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. Results obtained to date have demonstrated that the 3T and 5T variants have been identified in the DREXELMED Cohort in the HAART era. SNP density within the entire HIV-1 LTR was determined by comparison to the conB reference sequence. 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, SNPs have been identified that associated with CD4 T-cell count and viral load. In addition, SNPs were identified that associated with change in CD4 T-cell count and change in viral load. 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 that may be predictive of more advanced stage HIV disease and that may result in alterations in viral function.

P143
Ferritin heavy chain, CXCR4 and opiates in neuroAIDS: a multispectral image analysis within the human cortex

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HIV progression and development of neurological complications are directly and indirectly affected by substances of abuse, including opiates. However, the mechanisms involved in the deleterious action of opiates as it concerns neuroAIDS remain undefined. One unresolved issue relates to the interaction between opioid receptors and the HIV co-receptor, CXCR4, which is abundantly expressed in both the immune and nervous system. Our previous studies have shown that prolonged morphine treatment down-regulates CXCR4 function *in vitro* (i.e. rat cortical neurons) and *in vivo* (rat brain) with no apparent changes in receptor levels. However, we found that stimulation of mu-opioid receptors (MOR) 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 (or DAMGO) on CXCR4 both *in vitro* and

in vivo. CXCR4 and its natural ligand (CXCL12/SDF-1) are primarily involved in fundamental neuronal and glia functions, including neurotransmission, differentiation, and survival. Thus we hypothesized that alterations of CXCR4 function induced by opioids may contribute to HIV neuropathology, via regulation of FHC. The present study is a semi-quantitative assessment of FHC protein expression within brain tissue samples from control subjects and HIV-infected individuals (including opiate users) using multispectral immunohistological image analysis. The goal is to gather novel insight into the molecular mechanisms of opiate-induced neurological impairment involving disruption of CXCR4 signaling. The data suggest that a deficit in CXCR4 function is associated with over-expression of FHC within neurons in opiate abusing and neuroAIDS patients. This is the first systematic assessment of FHC expression within the human cortex in both disease and drug abuse states and disputes earlier reports purporting the absence of FHC within human neurons. The clinical implication for this line of research extends beyond the drug abusing HIV positive population to suggest the potential involvement in neurological complications secondary to therapeutic opiate administration in patients with neuroinflammatory disease.

P144

Activation of multiple sclerosis-(MS)-associated retrovirus (MSRV) and syncytin-1 in astrocytes and in PBMC of MS patients by the Epstein Barr Virus and its gp350 protein

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system, with demyelination and gliosis. A potential virus involvement in MS exist for the Epstein Barr virus (EBV), and for two elements of the W family of human endogenous retroviruses (HERV-W): MSRV, that forms free virions, and syncytin-1, the ERVWE1env protein; both retroelements have neuropathogenic properties. In culture, MSRV/HERV-W genes/proteins are activated by HSV-1 and its immediate-early protein, and binding to CD21 of EBVgp350 envelope glycoprotein transactivates HERV-K18 in B cells. In the past we studied MSRV in MS patients in various temporal and clinical stages; in all cases, striking parallelisms between MS behaviour and MSRV/HERV-W presence/load were found. By simultaneous detection of MSRV and HHV-6, we found a direct correlation between MS and MSRV presence/load, but not for HHV-6. MS brains over-express MSRVenv and syncytin-1 transcripts, with respect to controls, while EBV presence was not detected. Since late EBV seroconversion is a strong risk factor for MS development, we performed *in vitro* experiments on PBMC from MS patients and MSRV(+) controls, as well as on U87 MG astroglioma cells, that were exposed to EBV or to purified EBVgp350. Co-cultures with the EBV-producer B95-8 cell line in Transwell plates were also performed. When exogenously given to cell cultures, EBV stimulates the expression of MSRVenv and syncytin-1. This effect is obtained also by addition of purified EBVgp350, both in glial cells and PBMC, and is abolished by anti-EBVgp350 antibody. The EBVgp350 effect on MSRVenv and syncytin expression is abolished also by siRNA directed against the NF-kB p65 subunit, suggesting that EBVgp350 action on both retroelements involves the NF-kB pathway. The EBV-HERV-W/MSRV interactions suggest the possibility of a direct role of neuro-toxicity effector for MSRV, and an indirect one for EBV in MS pathogenesis.

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Short and long term effect of methamphetamine withdrawal: Proteomic profiling of plasma from HIV-infected patients

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Introduction: Use of methamphetamine (METH) with concomitant HIV-1 infection can increase the risk of central nervous system (CNS) injury. We still lack reliable biomarkers that reflect the molecular mechanisms underlying the neurocognitive impairment associated of either of these diseases. We postulated that application of unbiased proteomic profiling of plasma could lead to new markers of disease and insights into neuropathogenic disease processes. Patient cohort: Samples were provided by HIV Neurobehavioral Research Programs (HNRP) at the University of California San Diego which have been investigating the impact of METH on the brain in HIV-infected and uninfected individuals. We used samples from the same individual at 2 time points. Four cohorts of 8 samples each were used. Three cohorts consisted of

HIV-infected patients with i). Persistent METH use; ii). Long-term METH abstinence; iii). Short-term METH abstinence. Fourth cohort, control, consists of HIV- and METH- individuals. Experimental approach: We selected MudPIT with 8-plex iTRAQ approach as primary method of profiling. Plasma samples were immundepleted from 14 most abundant proteins, digested with trypsin, labeled with iTRAQ labels fractionated using isoelectric focusing mode (first dimension) and further fractionated using RP-HPLC. For mass spectrometry data acquisition we used an ABI 4800 MALDI-TOF/TOF instrument equipped with Protein Pilot® software for database search and peptide/protein quantitation. Results: Summarizing, we have identified and quantitated 450 proteins belonging to various functional classes such as regulatory, structural, enzymes etc. Preliminary analysis showed that among others levels of proteins such as plasminogen isoform 1, guanine nucleotide regulatory protein, vitamin D binding protein and ceruloplasmin circulating in plasma are changed by METH. Differential expression of these proteins will provide new insights into host's response to the viral infection. To make these proteins relevant as diagnostic biomarkers larger cohorts are needed. Support: This work was supported, in part, by NIH Grant P20DA026146-01; P01 DA12065; P30 MH62512.

P146
Targeting and Evaluating Therapy of HIV Brain Disease: Strategies to Identify Novel Objective CSF Biomarkers

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As attention has turned from the more severe disease caused by HIV—defined initial is the AIDS dementia complex (ADC) and now referred to as HIV-associated dementia (HAD)—to milder disease identified by impaired neuropsychological test performance in neurologically asymptomatic and symptomatic individuals, it is more important than ever that we develop laboratory-based biomarkers of active disease. Because milder "neurocognitive impairment" in those with HIV is likely etiological heterogeneous and may reflect either static residual or slowly progressive injury, it is important that to have objective biomarkers of ongoing HIV-related injury to both target therapy and evaluate its effects. We have explored the combined use of cerebrospinal fluid (CSF) viral (sensitive assays to detect low-level infection), immunological (including neopterin as a marker of macrophage activation across the spectrum of disease) and neural (including neurofilament light chain, NFL, and amyloid precursor protein) biomarkers. We have also used these CSF biomarkers as guiding pathogenetic vectors to analyze the results of high-throughput proteomic methods to indentify novel biomarkers and pathogenic networks in ADC and milder disease.

P147
Infection of differentiated human neural stem cells with varicella zoster virus

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Primary infection of humans with varicella zoster virus (VZV) 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 provide an alternative means to study the VZV-host relationship in neurons, we infected differentiated human neural stem cells (NSCs) *in vitro*. NSCs persist in the subgranular layer of the dentate gyrus of the hippocampus and subventricular zone of the lateral ventricle and support neurogenesis in the adult brain. Cultured NSCs proliferate and give rise to spherical clusters, "neurospheres". These self-renewing multipotent cells can be induced to differentiate into neurons, astrocytes and oligodendrocytes after adhesion to specific substrata depending on culture conditions. Herein, NSCs were differentiated in culture dishes coated with poly-L-lysine and mouse laminin in the presence of FGF-2, NGF, BDNF and retinoic acid. Immunostaining with neuronal (MAP2a and Neu N), astrocyte (GFAP) and oligodendrocyte (CNPase) markers revealed that differentiated neurons constituted approximately 90% of the cell population. These neurons were maintained in culture for up to four weeks. NSCs, two weeks after differentiation and human lung fibroblast (HLF) cells, were infected with cell-free VZV (zostavax vaccine) at an MOI of 2500 pfu/dish. A cytopathic effect (CPE) developed in infected HLF cells after seven days. Importantly, no CPE developed in neurons even two weeks after VZV infection. VZV DNA and virus-specific transcripts were detected in infected neurons, and dual immunofluorescent staining revealed the presence of VZV IE63 in healthy appearing neurons. Neither the tissue culture medium nor a homogenate prepared from the VZV-infected neurons was capable of producing a CPE in HLF cells. The potential of this model to further study the VZV-neuronal relationship will be discussed.

P148
Role of c-jun N-terminal kinase in varicella zoster virus-induced apoptosis in human fibroblast cells

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We previously demonstrated that varicella zoster virus (VZV) infection of MeWo cells and simian varicella virus infection of Vero cells cause apoptosis by the intrinsic pathway and involve downregulation of Bcl-2. VZV activates c-jun N-Terminal kinase (JNK), a stress signaling kinase known to play a role in cell death, in human fibroblasts. The objective of this study was to determine if JNK is involved in VZV-induced apoptosis in cultured human lung fibroblasts. VZV infection of fibroblasts produced activation of caspase-3 and accumulation of cleaved PARP. Incubation of VZV-infected cells with SP600125, a JNK inhibitor significantly decreased levels of these apoptotic markers. A Taqman low density array (TLDA) was performed to determine the modulation of 84 genes in the apoptosis pathway in VZV-infected fibroblasts in the absence and presence of SP600125. After VZV infection, there were significant changes in gene expression patterns involving upregulation of proapoptotic genes and downregulation of anti-apoptotic genes. For example, (i) the Fas ligand that triggers the extrinsic pathway of apoptosis and (ii) Bim which causes release of cytochrome c leading to the intrinsic pathway of apoptosis were induced and (iii) Bcl-XL expression which prevents release of cytochrome c was decreased in VZV-infected fibroblasts. JNK inhibition resulted in significant normalization as shown by decreased expression of Fas ligand and Bim and upregulation of Bcl-xL. Our findings suggest that JNK is involved in coordinated expression of cellular apoptosis pathway genes that facilitate productive VZV infection in non-neuronal cells.

P149
Activated type 1 IFN monocyte phenotype correlates with MRS changes in HIV infection

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Neurological impairment continues to occur with HIV-1 infection in spite of highly active retroviral therapy. Reducing viral replication with HAART has significantly diminished the severity of cognitive impairment with few HIV-infected individuals progressing rapidly to dementia. To identify subtle neural damage caused by HIV infection, we measured brain metabolites in individuals chronically infected with HIV using proton magnetic resonance spectroscopy (1H MRS). To determine the possible impact of peripheral activation on brain function, we compared viral load, monocyte gene expression, plasma variables and neuropsychological testing with brain metabolites in the frontal white matter (FWM), anterior cingulated cortex (ACC) and basal ganglia (BG). Viral load correlated inversely with the neuronal marker N-acetylaspartate (NAA) in the ACC ($R = 0.49$). A type 1 IFN-driven monocyte phenotype significantly correlated with changes in the ACC and FWM including an inverse correlation with ACC and FWM NAA, FWM myoinositol (ml) and FWM creatine (Cr) levels. Monocyte IFN response genes, IP-10 and LGALS3BP, strongly correlated with their respective plasma concentrations. Increasing plasma IP-10 concentration also had a strong inverse correlation with ACC NAA concentrations ($R = 0.69$) as well as ACC and FWM choline (Cho, $R = 0.5$) and Cr ($R = 0.4$) concentrations. LGALS3BP plasma levels correlated inversely with ACC NAA concentrations ($R = 0.4$) and correlated with BG ml ($R = 0.5$). Furthermore, ACC NAA was significantly decreased in individuals with neurocognitive impairment. We conclude that chronic activation driven by type 1 IFN responses alters brain function in HIV infection. Immune activation, with a characteristic type 1 IFN response was a better predictor of HIV-induced changes in brain metabolites than viral load.

P150
Depletion of dendritic cells enhances susceptibility to cell-free infection of HTLV-1 in CD11c-DTR transgenic mice

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Human T-cell leukemia virus type 1 (HTLV-1) is associated with two immunologically distinct

diseases: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATL). The genesis of these diseases is believed to be associated with the route (mucosa versus blood) and mode (cell-free versus cell-associated) of primary infection as well as the modulation of dendritic cell (DC) functions. To explore the role of DCs during early HTLV-1 infection *in vivo*, we used a chimeric HTLV-1 with a replaced envelope gene from Moloney murine leukemia virus to allow HTLV-1 to fuse with murine cells, which are generally not susceptible to infection with human retroviruses. We also used a CD11c-DTR transgenic mouse model system that permits conditional transient depletion of CD11c + DCs. We infected these transgenic mice with HTLV-1 using both cell-free and cell-associated infection routes in the absence and presence of DCs. The ablation of DCs led to an enhanced susceptibility to infection with cell-free, but not cell-associated HTLV-1 in both the CD4 and non-CD4 fractions, as measured by the proviral load. Infection with cell-free virus in the absence of DCs was also found to have increased levels of Tax mRNA in the non-CD4 fraction. Moreover, depletion of DCs significantly dampened the cellular immune response (IFN-γ+CD8+ T cells) against both cell-free and cell-associated virus. These results uniquely differentiate the involvement of DCs in early cell-free versus late cell-associated infection of HTLV-1 and highlight a significant aspect of viral immunopathogenesis related to the progression of ATL and HAM/TSP after the initial infection.

P151
Measles virus regulation of suppressors of cytokine signalling (SOCS) proteins in the CNS

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Subacute sclerosing panencephalitis (SSPE) is a rare but fatal CNS complication of measles virus (MV) infection associated with long term persistentence. There are no effective treatments available. While studies of cytokine expression have been carried out in SSPE brain tissue and MV mouse models little is know about cytokine regulation in the CNS which is under the control of suppressors of cytokine signalling (SOCS) proteins. These are negative regulators of the JAK/STAT pathway and SOCS genes are rapidly up-regulated in response to a range of stimuli including infectious agents. SOCS proteins have been detected in SSPE brain and virus modulation of their expression would have direct bearing on the inflammatory response, virus clearance and hence persistence in the CNS. We have previously shown that wild type (WT) MV gives rise to a persistent infection in the CNS of C57/B6 mice (modeling the situation in SSPE) in contrast to the acute infection produced by rodent adapted virus. In WT infection the percentage of antigen positive cells (both neurons and oligodendrocytes) increase with time after mouse infection. However viral replication occurs at a low level as shown by quantitative RT-PCR and is associated with limited apoptosis (Abdullah *et al*, 2009, Neuropath. Appl. Neuropathol. 35,473-486). These results suggests that WT virus is able to regulate the immune response to avoid immune mediated clearance. Using RT-PCR we have determined that SOCS3 mRNA is found at similar levels in both non-infected and infected animals. However, SOCS 2 mRNA is minimal in non-infected mice and WT infection causes significant up-regulation. It is likely that SOCS2 suppresses the interferon gamma response leading to persistent infection and we are currently examining infection in SOCS2 "knock out" mice. Understanding of SOCS protein regulation may allow novel therapeutic strategies to be developed for SSPE and other persistent CNS infections.

P152
Targeted Nanoparticles for Gene Silencing: HIV-1 and Drugs of Abuse

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Gold nanoparticles (GNPs) and nanorods (GNRs) are beneficial for a range of biomedical applications due to their biocompatibility and surface plasmon resonance. Their surfaces can be modified to incorporate cationic charges which facilitate stable electrostatic complexation with anionic genetic materials such as small interfering RNA (siRNA), for the purpose of targeted gene silencing. The dual epidemics of drug abuse and human immunodeficiency virus (HIV-1) infection coincide with one another. Drugs of abuse are risk factors for acquiring infection with HIV-1 and can contribute to viral pathogenesis. The 32-kDa dopamine- and adenosine 3',5'-monophosphate-regulated phosphoprotein (DARPP-32) is recognized to be critical to the pathogenesis of drug addiction. Previous studies demonstrate that drugs of abuse enhance HIV-1 infection. Therefore, the effects of methamphetamine and heroin on DARPP-32 expression and the effects of DARPP-32-siRNA gene silencing on HIV-1 infection were investigated. Nanoplexes (GNRs complexed with DARPP-32 siRNA) were used for RNA interference in monocyte-derived macrophage (MDM). The shift in the localized longitudinal plasmon resonance peak of GNRs was used to show an interaction with siRNA. The uptake of nanoplexes into cells was determined

using plasmonic enhanced dark-field imaging of GNRs. Gene and protein expression were analyzed using quantitative PCR and Western blot analysis. MDM were infected *in vitro* using HIV-1; p24 antigen expression was determined using ELISA. Methamphetamine, morphine, and cocaine significantly upregulated DARPP-32 expression in MDM. GNRs complexed with DARPP-32 siRNA (nanoplexes) were used for RNA interference. A red shift occurred in the localized longitudinal surface plasmon resonance peak of the GNRs demonstrating an interaction with DARPP-32 siRNA that was confirmed by gel electrophoresis. Uptake of nanoplexes within the cytoplasm was observed through dark-field imaging. Effective gene silencing by the nanoplexes was evidenced by a reduction in the expression of DARPP-32 in MDM, with no observed cell cytotoxicity. DARPP-32 silencing decreased p24 antigen production in MDM infected with HIV-1 *in vitro*. Moreover gene expression for DARPP-32 was increased in MDM isolated from HIV-1 subjects. Silencing of DARPP-32 using siRNA against DARPP-32 may provide a novel gene therapy strategy to overcome drug addiction. Bioconjugated nanoparticles have great potential for studying intracellular processes at the single-cell level using high-resolution imaging. The inertness and non-toxicity of GNRs render them promising materials for biomedical and clinical applications.

P153
High prevalence of antibodies against the neurotropic polyomavirus BK in sera from patients affected by multiple sclerosis

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Viral agents seem to be linked to multiple sclerosis (MS). This association is based on evidence of (i) early exposure to viruses and MS onset; (ii) increased prevalence of MS disease in specific regions; (iii) likelihood of developing MS being more prevalent in high-risk areas; (iv) altered immune-responses to different viruses; and (v) common features among animal models and other human diseases, where viral agents cause diseases with long incubation periods, relapsing-remitting, and demyelination. Increased antibody titles to a particular virus and viral isolation from MS specimens have also been reported. Antibodies against neurotropic polyomaviruses have been found in human sera, while their DNA sequences have been detected in normal brain tissues or brain tumours. In this study, sera from patients affected by MS and controls, represented by sera from patients with other neurologic diseases, both inflammatory (OIND) and non-inflammatory (NIND), and from healthy donors, were investigated for the presence of antibodies against BKV, JCV and SV40. Sera were analyzed by enzyme immunoassay (EIA) for antibodies against viral-like particles (VLPs) of the viral protein 1 (VP1) belonging to BKV, JCV, SV40. A positive result was considered for values higher than 0.200. As it was shown that SV40 reactivity could be due to the binding of high titles antibodies to the BK virus, serum samples were pre-incubated for one hour at 37°C with BKV VLPs before being tested for anti-SV40 seroreactivity. Our study has indicated that the prevalence of BKV antibodies in sera from MS patients is higher than that detected in normal individuals, while levels of antibodies against BKV and JCV are lower in MS patients compared to those of normal subjects. Our preliminary data, obtained with a limited sample size, suggest an association between BKV and MS, while no association was found for JCV and SV40.
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P154
The Y-chromosome translocation, Yaa, dampens the murine inflammatory response to HSV and protects mice from lethal HSV encephalitis

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Herpes simplex virus (HSV) is the most common cause of sporadic fatal viral encephalitis in the developed world. Toll-like receptors (TLRs) 2, 3 and 9 are crucial for resistance to fatal encephalitis in mice and/or humans. TLR7 signaling is required for murine resistance to another cause of viral encephalitis – West Nile Virus, but has not been implicated in HSV neuropathogenesis. We hypothesized that B6.Yaa mice, which bear an extra copy of TLR7 along with several other genes, would be more resistant to HSV encephalitis compared to their consomic partner, B6, and that TLR7-/- mice would be more susceptible. Four-week old male mice were inoculated with 3×10^6 PFUs of neurovirulent HSV-1 Strain 17+ in the footpad. Mice were monitored daily for clinical disease and weight loss. The B6.Yaa mice had significantly decreased mortality (20%) as compared to B6 (60%), and significantly decreased

clinical scores following infection. Tissues were harvested post-infection for analysis of viral load, expression of host immune response transcripts, HSV-specific transcripts, and FACS analysis. The brains of B6.Yaa mice had decreased copies of viral genomes as measured by quantitative real-time PCR, as well as decreased expression of HSV-specific transcripts. Interestingly, the B6.Yaa mice, bearing an extra copy of TLR7, also had lower levels of host immune response transcripts in both the cortex and draining lymph node of the footpad at 4 and 6 days post-infection as compared to B6 mice. FACS analysis of draining lymph nodes confirmed these results showing decreases in frequency, number, and activation of CD4+ and CD8+ T cells in B6.Yaa mice as compared to B6. Preliminary data suggests that TLR7-/- mice have increased mortality and clinical score as compared to both B6 and B6.Yaa using this model. Thus, TLR7, which primarily recognizes RNA-containing ligands, may also control infection by this neurotropic dsDNA virus.

P155
Inhibition of interferon response by Cystatin B: A proposed mechanism for HIV persistence in macrophage reservoirs

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Cystatin B expression is positively correlated with HIV replication and decreased levels STAT-1 phosphorylation in monocyte-derived macrophages (MDM). However, the players and the mechanism by which this occurs is unknown. We hypothesize that Cystatin B inhibits the interferon (IFN) response and regulates STAT-1 phosphorylation by interacting with additional proteins. To test if Cystatin B inhibits the type I IFN (α/β) response, we performed luciferase reporter gene assays in Vero cells, which are IFN deficient. Cells were transiently transfected with an empty vector or a vector expressing Cystatin B, together with a plasmid construct containing the ISG54 promoter fused to firefly luciferase and the SV40 promoter fused to Renilla luciferase, and stimulated with IFN or left untreated. Interferon stimulated response element (ISRE)-driven expression of firefly luciferase in cells expressing Cystatin B was significantly inhibited when compared with cells transfected with the empty vector. To define the cognate protein-protein interactions in MDM, immunoprecipitates of Cystatin B protein complex was analyzed by LC-MS/MS. Cystatin B interacts with many different proteins including regulatory, glycolytic, redox, structural, transport, and signaling proteins. Among them, Major Vault Protein (MVP) was selected for verification by western blot because this protein inhibits JAK/STAT signals. Specific interactions by immunofluorescence and confocal imaging of HIV infected and uninfected MDM confirmed overexpression of MVP in HIV-infected cells. Our findings confirmed that Cystatin B interacts with proteins related with the regulation of STAT-1 phosphorylation and HIV replication and elucidated MVP as an important signaling candidate. This is the first study that correlates the interaction of cystatin B and MVP and IFN responses with HIV persistence in MDM and suggests novel targets for HIV restriction in macrophages, the principal reservoirs for HIV in the CNS. R01-MH08316-01, RCMI-NCRR-G12RR03051, SNRP-NINDS-1-U54NS431.

P156
CCL2 Disrupts the Adherens Junction and Increases Blood-Brain Barrier Permeability

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Alterations to blood-brain barrier (BBB) adhesion molecules and junctional integrity during neuroinflammation can promote central nervous system (CNS) pathology. The chemokine CCL2 is elevated during CNS inflammation and is associated with endothelial dysfunction. The effects of CCL2 on endothelial adherens junctions (AJ) have not been well defined. We demonstrate that CCL2 induces Src-dependent disruption of human brain microvascular endothelial AJ. β-catenin is phosphorylated and traffics from the AJ to PECAM-1, where it is sequestered at the membrane. PECAM-1 is also tyrosine phosphorylated, an event associated with recruitment of the phosphatase SHP-2 to PECAM-1, β-catenin release from PECAM-1, and reassociation of β-catenin with the AJ. Surface localization of PECAM-1 is increased in response to CCL2. This may enable the endothelium to sustain CCL2-induced alterations in AJ and facilitate recruitment of leukocytes into the CNS. Our novel findings provide a mechanism for CCL2-mediated disruption of endothelial junctions that may contribute to BBB

dysfunction and increased leukocyte recruitment in neuroinflammatory diseases including NeuroAIDS.

P157
Modulation of HIV-1 Replication, Inflammation, and Neurotoxicity by a Tobacco Cembranoid 4R: Therapeutic Implications for HIV-Associated Neurocognitive Disorders

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HIV-associated neurocognitive disorders (HAND) has become the most common neurologic complication of AIDS, affecting approximately 40-60% of HIV-infected patients. HAND is an encephalopathy induced by HIV-1 infection and fueled by immune activation of lymphocytes and macrophages. These activated cells have the capability to enter the brain and secrete neurotoxins of both host and viral origin affecting brain cells such as glial cells and neurons. We understand that there is a major need to identify a compound that can prevent or alleviate the damaging effects following HIV-1 infection in the brain. Our laboratory has identified a non-toxic compound from the tobacco leaf called cembranoid 4R that readily penetrates into the brain and has demonstrated anti-apoptotic, anti-inflammatory, and neuroprotective properties. 4R suppressed HIV-1 replication in PBMC by 4-fold, but that was not the case in human glial cells which 4R enhanced it by 2-fold. However, PBMC secretions attenuated HIV-1 infection in glial cells in the presence of 4R using a transwell system. We also demonstrate that 4R downregulated inflammatory chemokines such as RANTES, MIG, and IP-10 in PBMC whereas it upregulated inflammatory chemokines such as IL-8 and MCP-1 in human glial cells. These results suggest that 4R was able to modulate HIV-1 replication in PBMC and glial cells and consequently, this modulation was correlated with the expression of inflammatory chemokines. In addition, our results show that 4R was able to block significantly the neurotoxicity induced by HIV-1 Tat protein. In conclusion, this study revealed new properties of the novel compound 4R and provide new understanding of the mechanisms involved in the pathogenesis of HAND by which this compound may elicits its properties. Further knowledge of the mechanism by which 4R exerts these opposite effects on PBMC and glial cells could lead to the development of therapy for treating HAND.

P158
Cathepsin B secreted by HIV-infected macrophages contributes to neuronal apoptosis

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HIV-1 penetrates the central nervous system (CNS) early during infection as free particles. In late stage of the disease HIV crosses the blood-brain barrier mainly through the trafficking of infected-activated peripheral blood monocytes. Once in the brain HIV-1 infects mainly resident microglia and peripheral macrophages, which can lead to the development of HIV associated neurological diseases (HAND). Accumulating data suggests that alteration to the normal macrophage intracellular mechanisms caused by HIV-1 contributes to neuronal injury and apoptosis. Among the cellular proteins that could promote neuronal apoptosis, if not properly regulated, is cathepsin B (CATB), a cysteine protease of lysosomal origin involved in various important cellular processes including apoptosis. Recent work done in our laboratory demonstrated that monocyte-derived macrophages (MDM) secrete high levels of bioactive CATB that increases after HIV infection over time in culture, thus confirming a role of HIV in promoting dysregulation in the CATB system. We posit that secreted CATB contributes to increased neuronal death induced by HIV infection. To test this hypothesis we infected human MDM with HIV-ADA for up to 12 days. Neurotoxic potential of secreted CATB was determined after incubating the neuronal cell line SK-N-SH with MDM conditioned media (MCM) from control and HIV-infected cultures with or without CATB inhibitor (CA-074). Incubation of neuronal cell lines with HIV supernatants from HIV-infected MDM cultures resulted in a significant increase in the percentage of apoptotic neurons ($p < 0.05$), which was reverted to the levels of uninfected supernatants after the addition of CATB inhibitor. Our results demonstrate that HIV infection dysregulates CATB in macrophages, with increased bioactive CATB which contributes to neuronal apoptosis. These findings provide new evidence of the role of CATB in the neuropathogenesis of HIV. Supported in part by: R01-MH08316-01, R25-GM061838, G12RR03051 and SNRP-NINDS-1-U54NS431.

P159
Functional analysis of pRb2/p130 and Cdk9: implications in cancer, viral pathogenesis, drug development and gene therapy

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The putative pRb2/p130 tumor suppressor gene and cyclin-dependent kinase 9 (Cdk9) are two key battlegrounds in our research center. These molecules were identified and characterized in Dr. Giordano's laboratory in the early 1990s and they both became trend-setting topics in the areas of cancer research and cell biology. The retinoblastoma (Rb) gene family comprises three members: the Rb tumor suppressor gene pRb/p105, pRb/p107 and pRb2/p130, which are also known as pocket proteins, as their structure resemble the shape of a pocket. The three components of the Rb family share similar structural identities, arrest cell cycle progression in G1 phase, their phosphorylation patterns are cell cycle dependent and the inhibition of cell proliferation takes place in a cell-type-specific pattern, which indicates that the biological functions of the pocket proteins are not completely redundant. The inactivation of tumor suppressor genes is quasi-obligatory for the establishment of a malignant cell phenotype. Loss of tumor suppressor gene expression may occur via deletion mechanisms of particular chromosomal areas, epigenetic and/or genetic mutations, a combination of the two aforementioned events and because of certain viral factors. For instance, the human papilloma viral (HPV) E7 protein binds and neutralizes Rb proteins. The same occurs with polyoma viruses large T antigens. The large T antigen of the JCV binds pRb2/p130 and causes the release of E2F, which, in turn, exerts its effects on cell cycle regulation. Human pRb2/p130 gene is encoded in chromosome 16q12.2. A number of human tumor-derived cell lines exhibit deletions and /or mutations in this chromosomal area, such as hepatic, breast, prostate and ovarian cancers. Interestingly, retroviral-mediated gene transfer of human pRb2/p130 could inhibit human ling cancer cell proliferation *in vitro* and in xeno-transplanted nude mice. In addition to gene-based approaches for the expression of human pRb2/p130 in cancer cells, we developed a pRb2/p130-derived peptide termed Spa310, which is a 39 amino acids proteins spanning part of the spacer region. Interestingly Spa310 caused *in vitro* inhibition of cdk2 activity, substantial arrest of proliferation in mouse NIH/3T3 fibroblasts and inhibition of human lung tumor growth *in vitro* and in xeno-transplanted mice. In conclusion, gene-based interventions and use of small peptides derived from pRb2/p130 have in common the elimination of malignant cells by imparting cell cycle arrest through inhibition of cdk2 activity. Studies are currently in progress to understand the mechanism of Spa 310 entry into cells and tumor growth suppression in xeno-transplanted mice. Cdk9 Unlike other Cdks, Cdk9 is not directly involved in cell cycle regulation, but promotes RNA pol II-mediated transcription to sustain genetic programs related to cell proliferation, growth, survival and differentiation. Cdk9 cyclin partners are cyclin T1, cyclin T2a, cyclin T2b and cyclin K. The heterodimer "Cdk9/cyclin partner" is stable and constitutes a major component of the positive transcription elongation factor b (P-TEFb), which stabilizes the elongation of RNA pol II-driven RNA transcripts. The complex biology of Cdk9 can be summarized as follows: 1) The Cdk9-related pathway is a key component in the regulation of mammalian gene expression, which controls several cellular processes, such as cell growth, proliferation, survival and differentiation, depending on the cell context. 2) Cdk9/cyclin T1 are involved in the replication cycle of HIV-1. HIV-2, HTLV-1, EBV and HSV-1. 3) Deregulations in the Cdk9-related pathway are associated with various pathological conditions, which include cancer and cardiac hypertrophy. 4) The study of the mechanisms that lead to an aberrant Cdk9-related pathway is essential for the development of novel kinase inhibitors for the treatment of cancer, AIDS and cardiac hypertrophy and might shed useful insights into the study of the pathogenesis and progression of these maladies.

P160
Antiviral activity of NK cells in MS patients

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Multiple Sclerosis (MS) is a chronic autoimmune infiammatory disease believed to develop as a conseguence of the interaction of genetic susceptibility and environmental factors. Some evidences suggest an association between MS and herpesviruses which establish lifelong latent infections with occasional reactivation episodes, thus providing a persistent challenge for the immune system. Adaptive immune responses in MS patients have been widely studied with the aim to elucidate the T-cell mediated autoimmune mechanisms causing the disease. However,

the trigger of autoimmunity is still unknown. Recent observations suggest that Innate Immunity (II) might be a major factor in the ethiology of MS. II, mediated by Toll-Like Receptors (TLRs) has two important roles. It constitutes the first line of defense against pathogens, and it triggers adaptive immunity. Therefore, to verify whether II might play a role in MS, we studied the antiviral state induced in MS patients by activation of TLR9. We report that activation of II in MS patients does not result in antiviral protection, but confers increased susceptibility to herpesvirus infection. The study of TLR9 pathway did not reveal significant alterations compared to controls. However, we showed that activity of Natural Killer (NK) cells is altered. NK cells are an important effector of II. Therefore, alterations of NK cell functions could have an impact on autoimmune responses. The activity of NK cells depends on the balance of signals from activating and inhibitory receptors (killer immunoglobulin-like receptors, KIRs). Our results show that in the II of MS patients the inhibitory KIR2DL2 is upregulated with a consequent decrease of NK cell antiviral activity. These results suggest that MS patients might be more susceptible to herpesvirus infections than the healthy population. Furthermore, the defect of NK cell activity might be relevant for the ethiology of MS.

P161
Enhanced expression of OX40 by HTLV-1 Tax and its roles in the pathogenesis of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP)

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OX40, also known as CD134, is a member of the TNF family of co-stimulatory receptors expressed on activated T cells, and recent research has shown the importance of OX40-OX40L interactions in the development of immune-mediated diseases. HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic progressive neurologic disease associated with immunologically mediated damage to the central nervous system. In this study, we showed that OX40 was overexpressed in spinal cord infiltrating mononuclear cells of HAM/TSP patients by immunohistochemistry, whereas OX40 was not expressed on fresh peripheral blood mononuclear cells (PBMCs) but specifically expressed on the surface of HTLV-1-infected PBMCs along with the expression of the viral transactivator Tax only after short-term (16hrs) *in vitro* culture. We developed a panel of monoclonal antibodies against OX40, and tested their effect on Tax expression. Interestingly, when some of these monoclonal antibodies were added to the culture medium, both the percentage of CD4+OX40+Tax+ T cells and the HTLV-1 proviral load in PBMCs were significantly reduced after cultivation, suggesting specific elimination of OX40-positive HTLV-1 infected cells. This effect was significantly reduced when NK cells were removed from the culture, and was independent of each antibody's capacity to block the OX40-OX40L interaction. Notably, the frequency of CD4+OX40+Tax+ T cells per HTLV-1 infected PBMCs estimated by proviral load was found to be correlated with disease activity and severity in HAM/TSP patients. Collectively, these data provide new insight into the HAM/TSP pathogenesis and also suggest the potential of OX40 as a target molecule for immunotherapy of HAM/TSP.

P162
HIV-1 Tat upregulates expression of histone deacetylase-2 in human neurons: Implication for HAND

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Acetylation and deacetylation of histone proteins catalyzed by histone acetyltransferases (HATs) and histone deacetylases (HDACs) play an important role in chromatin remodelling and transcriptional regulation. Previous studies show an association of imbalances in protein acetylation and transcriptional dysfunction with a range of neurodegenerative conditions. Treatment with various HDAC inhibitors has emerged as promising new strategy for therapeutic intervention in neurodegenerative disorders. More recently, HDAC2 overexpression in neurons has been shown to modulate synpatic plasticity and memory formation in mice. However, the role of HDAC2 in the development of HIV-1-associated neurocognitive disorders (HAND) is not elucidated. We hypothesize that HIV-1 Tat protein may modulate HDAC2 expression leading to transcriptional repression of neuroprotective genes in neuronal cells, thereby contributing to the progression of HAND. Primary human neurons and SKNMC neuroblastoma cell line were incubated separately with HIV-1 Tat

protein, followed by gene expression using qRT-PCR and protein expression by western blotting and flow cytometry. Results indicate that HIV-1 Tat significantly upregulated HDAC2 in a time-and dose-dependent manner by primary neurons and SKNMC cells. Additionally, HDAC2 overexpression was associated with downregulation in Camk2a, CREB, and BDNF genes that regulate neuronal activity. This Tat induced modulation in expression was reversed by treatment with HDAC inhibitor trichostatin A or siRNA directed against HDAC2. These results suggest for the first time the possible role of HDAC2 in the development of HAND. These studies to unravel the mechanisms of HIV-1 neuropathogenesis may lead to development of new therapeutic strategies for HAND.

P163
Human Immunodeficiency Virus type -1 Clade B and C gp120 Inhibit NMDA-receptor mediated induction of Arachidonic Acid: Implications on Neuro-AIDS

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Aim: Previous studies have demonstrated that infections with HIV-1 clades differentially contribute to the neuropathogenesis of HIV-1-associated dementia (HAD) and HIV-associated neurocognitive disorder (HAND). The N-methyl-D-aspartate (NMDA) receptor and glutamine leads to up regulation of neurotoxins arachidonic acid (AA) and its byproducts of prostaglandin E2 (PGE2) and thromboxine A-2 (TBXA-2), are known to play a significant role in neuropathogenesis of HAD and HAND. We hypothesize that clade B and C gp120 proteins exert differential effects on human primary astrocytes by down regulation of NMDA-R, glutamate synthatase (GS) and upregulation of PGE2 and TBXA-2 gene expression and protein modification. Methods: RNA extracted from astrocytes treated with HIV-1 clade B- and C-gp120 proteins was reverse transcribed and analyzed by quantitative real-time PCR to determine NMDA-R, PGE2 and TBXA-2 gene expression. Cell lysates were analyzed by western blot to determine protein modification. Results: Our results indicate that HIV-1 clade B gp120 protein significantly down regulated NMDA-R, GS and upregulated PGE2 and TBXA-2 gene expression and their protein modification compared to HIV-1 clade C- gp120 protein. Conclusions: Thus, our studies for the first time demonstrate that HIV-1 clade B- gp120 protein appears to significantly increased neurotoxin arachidonic acid as compared to HIV-1 clade C- gp120 protein. This suggests a differential effect of HIV-1 clade B leading to increased neuropathogenesis and associated HAD and HAND.

P164
JCV Agnoprotein Stimulates Large T Antigen Binding to Origin of DNA Replication and Phe35 Plays a Critical Role in this Process

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Department of Neuroscience and Center for Neurovirology Laboratory of Molecular Neurovirology, Temple University School of Medicine, MERB-757, 3500 N. Broad Street, Philadelphia, PA 19140 JC virus (JCV) agnoprotein is a 71 aa small regulatory protein and has been shown to play critical roles in viral life cycle; however, its precise role in different stages of this process remains elusive. We have previously demonstrated that this protein is a target of protein kinase C (PKC) for phosphorylation at Ser7, Ser11 and Thr21 and the virus carrying agnoprotein phosphorylation mutants was unable to continue its life cycle due to defects found in virion formation and viral DNA replication (Sariyer *et al*, 2006, J. Virol. 80:3893-3903). We also showed that agnoprotein coding sequences contribute to viral replication cycle (Akan *et al*, 2006, Virology 349:66-78). Here we further investigated the involvement of agnoprotein in viral DNA replication cycle. DNA binding studies revealed that agnoprotein did not bind to viral DNA by itself, but it strongly stimulated large T antigen (LT-Ag) binding to its target sequences located within the origin of DNA replication. Mapping of agnoprotein indicated that N-terminus half of the protein (aa 18-42) was responsible for this stimulation. Based on the three-dimensional computer modeling of agnoprotein, this region adapts an alpha-helical structure and all three phenylalanine residues (Phe31, Phe35, Phe39) present within this helical region are aligned and stacked at the one side of this predicted helix. The possible structural and functional role of the Phe residues was examined by site-directed mutagenesis, DNA binding and viral DNA replication assays. It was surprising to observe that Phe35 was more critical among three residues with respect to agnoprotein-mediated functions. When mutated to Ala, Phe35 abrogated the agnoprotein-mediated stimulation of LT-Ag binding. Conversely it enhanced the viral DNA replication in several fold (~4 fold) more than WT. In addition, agnoprotein was found to be an activator of JCV gene expression when delivered into the nucleus of the transfected cells, which itself is a novel and previously unrecognized property of agnoprotein. Taken

together, these findings suggest that agnoprotein plays significant regulatory roles both in viral DNA replication and gene expression of JCV. Further investigation of its roles in these processes will help us dissect the important steps in JCV-induced disease, PML, which may eventually provide us opportunities to develop effective therapeutic intervention strategies against this deadly disease. This work was supported by the grants awarded to MS by NIH.

P165
Involvement of the JC virus Large T Antigen in Virion Assembly by Coupling of the Viral DNA Replication to the Encapsidation Process

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Department of Neuroscience and Center for Neurovirology Laboratory of Molecular Neurovirology, Temple University School of Medicine, MERB-757, 3500 N. Broad Street, Philadelphia, PA 19140 JC virus (JCV) is a member of polyomavirus family and the etiological agent of a deadly form of brain disease, known as progressive multifocal leukoencephalopathy (PML). It is a small nonenveloped DNA virus, whose assembly takes place in the nucleus of infected cells. The JCV virion is composed of one major (VP1) and two minor (VP2 and VP3) capsid proteins. These capsid proteins are synthesized in the cytosol and transported into the nucleus for virion assembly. The viral genome is situated in the innermost core of the virion, complexed with histones. It is assumed that VP2 and VP3 form the second layer in the capsid shell in contact with viral DNA. The mechanism of JCV virion assembly is completely unknown. Here we have investigated the possible involvement of JCV large T antigen (LT-Ag) in this process. Experimental evidence suggests that LT-Ag may mediate the coupling of the viral DNA replication to the JCV virion assembly. Coimmunoprecipitation and GST-pull down assays demonstrated that LT-Ag interacts with VP2 and VP3. These LT-Ag interactions were found localized in the C-terminus end of the capsid proteins. This region also overlaps with the DNA binding domains of each capsid protein. Further characterization of these interactions by DNA binding studies showed that VP2 and VP3 strongly induced the LT-Ag binding to its target sequences present within the origin of viral DNA replication. It is surprising that far C-terminus DNA binding region of each capsid protein was alone sufficient for this induction, confirming the findings from the mapping studies. In addition, heat shock protein family chaperons appear to play an important role in the observed interaction between capsid proteins and LT-Ag by bringing and tethering the capsid proteins to the N-terminus J domain of LT-Ag. 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 the JCV replication cycle and this may provide opportunities to develop effective strategies against JCV infections in the central nervous system. This work was supported by the grants awarded to MS by NIH.

P166
Regulation of Human Neurotropic JC Virus Replication By Alternative Splicing Factor SF2/ASF in Glial Cells

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The human neurotropic virus, JC virus (JCV) is the etiologic agent of the fatal demyelinating disease of the central nervous system, Progressive Multifocal Leukoencephlopathy (PML) that is seen primarily in immunodeficient individuals. Productive infection of JCV occurs only in glial cells and this restriction is, to a great extent, due to the activation of the viral promoter that has cell type-specific characteristics. Earlier studies led to the hypothesis that glial-specific activation of the JCV promoter is mediated through positive and negative transcription factors that control reactivation of the JCV genome under normal physiological conditions and suppress its activation in non-glial cells. Here we demonstrate that the alternative splicing factor SF2/ASF has the capacity to exert a negative effect on transcription of the JCV promoter in glial cells through direct association with a specific DNA sequence within the viral enhancer/promoter region. Our results show that down-regulation of SF2/ASF in fetal and adult glial cells increases the level of JCV gene expression and its replication indicating that negative regulation of the JCV promoter by SF2/ASF may control reactivation of JCV replication in brain. Our results establish a new regulatory role for SF2/ASF in controlling gene expression at the transcriptional level. This work was supported by grants awarded by NIH to K.K.

P167
JCV and SV40 Virions are Efficiently Released in the Absence of Agnoprotein but They are Deficient in Viral DNA Content: Implications for Involvement of Agnoprotein in Viral DNA Replication and Virion Biogenesis

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Department of Neuroscience and Center for Neurovirology Laboratory of Molecular Neurovirology, Temple University School of Medicine, MERB-757, 3500 N. Broad Street, Philadelphia, PA 19140 The regulatory role(s) of agnoprotein of polyomaviruses (JCV and SV40) during the viral life cycle is not completely understood. There are conflicting reports about their regulatory functions in the literature. Agnoproteins are highly basic small proteins and encoded by the late coding region of the viral genome. Here we investigated the regulatory role(s) of agnoprotein of JCV and SV40 in virion release using a point mutant (Pt) of each virus where the translation initiation codon (ATG) of agnoprotein was altered ablating protein expression. Analysis of both viral gene expression and replication using Pt mutant of each virus revealed that both processes were substantially down-regulated in the absence of agnoprotein compared to WT. This effect was particularly more drastic after 3 or 4 viral replication cycles. In order to further demonstrate the importance of agnoprotein for viral life cycle, complementation studies were performed. Stable expression of JCV agnoprotein in cells transfected with JCV Pt mutant genome elevated the level of the replication of the mutant virus closer to that observed for WT. Moreover, the behavior of both mutant viruses during viral life cycle was analyzed using viral release assays. Results showed that viral particles were efficiently released in the absence of agnoprotein but they were deficient in viral DNA content. These findings collectively suggest that agnoprotein plays an important role in viral DNA replication and virion biogenesis but a minor role, if any, in release of the viral particles from the infected cells. This work was supported by the grants awarded to MS by NIH.

P168
Extinction of Tumor Antigen Expression by SF2/ASF in JCV-transformed Cell Lines

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ABSTRACT Human neurotropic virus, JCV, induces a broad range of neural-origin tumors in experimental animals has been repeatedly detected in several human cancer more notably neural-crest origin tumors including medullablastomas and glioblastomas. The oncogenic activity of JCV is attributed to the viral early gene products, large T and small t antigen, as evident by the results from *in vitro* cell culture and *in vivo* transgenic animal studies. Recently, we have shown that alternative splicing factor, SF2/ASF, has the capacity to exert a negative effect on transcription of JCV in glial cells through direct association with a specific DNA motif within the viral promoter region. Here we demonstrate that SF2/ASF suppresses the expression of large T antigen and small t antigen in JCV-transformed tumor cell lines. Expression of SF2/ASF in such tumor cells ultimately hinder the transforming capacity of the viral tumor antigens. Moreover, downregulation of SF2/ASF in the viral-transformed tumor cell lines induces the growth and proliferation of the tumor cells. These observations demonstrate a new role of SF2/ASF in the JCV-mediated cellular transformation, and provide new avenue of research to understand pathogenesis of JCV-induced tumors through interplay between JCV oncogenic proteins and host factors, i.e., SF2/ASF. This work was supported by grants awarded by NIH to K.K.

P169
A Drosophila melanogaster model for human inherited prion diseases

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Prion diseases, or trasmissible spongiform encephalopathies (TSEs), are a group of fatal neurodegenerative diseases that affect humans and other mammalians. The “Protein Only Hypothesis” suggests that TSE are caused by a disease-associated and improperly folded form of PrPC, termed PrPSc. Human TSEs are biologically unique in that the disease process can be triggered by different phenomena: i) infection with prion infected tissues; ii) a sporadic event that generates PrPSc, and iii) inherited mutations in the human prion protein encoding gene PRNP. The aim of our work is to investigate human inherited prion diseases using a Drosophila melanogaster animal model, thus helping to advance our knowledge of the molecular mechanisms responsible of these pathologies that are presently not understood. We generated fruit flies transgenic for constructs carryng either the wild type or the mutated forms of human PrP. In particular, the mutations were: P102L, associated to GSS syndrome; D178N/129M associated to FFI; D178N/129V and E200K both associated to fCJD. With standard genetic

crosses, the different human PrP forms were actively expressed in the entire nervous system or in motor neurons. Flies expressing H-PrP exhibited a progressive loss of the ability to fly, followed by a progressive loss of the capability to walk. Moreover, they lost the control of legs movements: legs quivered and the flies often fell on a side. Locomotor activities were analyzed by specific behavioural tests. In order to evaluate the presence of tissue vacuolization/spongiosis, adult flies brains were examined by standard hematoxiyin/eosin stained sections at different time points when flies display severe age-dependent behavioural alterations. Our findings indicate that Drosophila could be a powerful tool to study human familial TSEs. In particular, H-PrP expression in transgenic flies gives rise to gross behavioural phenotypes that closely remind the neurodegeneration associated with altered function of prion diseases in humans.

P170
HIV-1 Specific T cell Immune Responses in Thai Individuals with neurological dysfunctions

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The role of HIV specific T cell immune responses in a neurologically well-characterized cohort in Bangkok, Thailand was investigated. HIV-infected volunteers (n = 25) that present with neurological impairment (NI) as well as CD4-, education-, gender-, and age-matched HIV-infected volunteers with no neurological impairment (N) (n = 25) were enrolled. All volunteers received antiretroviral (ARV) treatment at enrolment. PBMC at 6 months post ARV from 16 volunteers (11 NI and 5 N) were tested in an interferon-gamma ELISPOT assay. Cells were incubated with overlapping HIV peptides spanning Gag, Env and Pol from a CRF_01AE isolate (CM235/CM240). The most robust response was found against the Gag protein followed by Pol and then Env. However there seem to be clusters of peptides recognized by NI patients but not by N patients. In addition multi-parameter flow analysis to identify CD4 and CD8 T cell memory subsets, activation status as well as the involvement of regulatory T cells and Natural Killer cells showed that the cell profiles may differ between NI and N. In conclusion, HIV-specific T cell response may differ between HIV infected subjects with and without neurologic impairment.

P171
Regulation of MSRV (Multiple Sclerosis-associated retrovirus) and syncytin-1 by HIV-1 Tat in PBMC and U-87 MG

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Human immunodeficiency virus type 1 (HIV-1) affects the central nervous system of a majority of AIDS patients, by the mechanisms are not identified completely. MSRVenv and syncytin-1 are the env proteins of two retro-elements of the HERV-W family, that have potentially neutopathogenic properties, and thus have been proposed as co-factors triggering the immunopathogenesis of multiple sclerosis. Since ~10% of healthy humans are MSRV-viremic, we studied in PBMC and U-87 MG astrocytes the possible regulation of the above HERV-W retroelements by HIV and by HIV-induced proteins, by using real time RT-PCR assays that selectively identify either MSRV-env or ERVWE1-syncytin transcripts. The results highlighted that the expression of both elements is modified by HIV infection and by recombinant Tat treatment. Surprisingly we detected an opposite regulation of MSRVenv and syncytin-1, since MSRVenv transcription was up-regulated, whilst that of syncytin-1 was down-regulated. Syncytin-1 down-regulation is due at least in part to the HIV tat regulatory protein, this result was also confirmed by Intracellular flow cytometry of U87-MG astrocytes treated with HIV-Tat for 24h and stained by anti-HER-Wenv mAb. It is observed also in astrocytes transiently transfected with a syncytin-1 promoter-Luc reporter plasmid (URE-LTR, −436/+310)

and exposed to tat. The effect of tat on syncytin expression is abolished by the presence of siRNA directed against NF-kB p65, suggesting the involvement of the NF-kB binding site present in the cellular moiety of the promoter. Furthermore, HIV effects on MSRVenv and syncytin-1 suggest that the two retroelements are integrated in different regions of the human genome. These findings open the possibility that MSRVenv expression can induce the activation of pro-inflammatory cytokines and redox-reactant release (iNOS), at least in astrocytes and that couldt contribute to HIV-related neuropathology.

P172
Functional analysis of clonal cell lines containing the HIV-1 LTR 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 many binding sites shown to interact with multiple host and viral factors. Previous studies identified specific nucleotide sequence variations within CCAAT/enhancer binding protein (C/EBP) site I and Sp site III (3T, C-to-T change at position 3, and 5T, C-to-T change at position 5 of the binding site, respectively) that correlate with increased severity of HIV-1 disease and HIV-1-associated dementia. Stably transfected cell lines were developed utilizing bone marrow progenitor, T, and monocytic cell lines (TF-1, Jurkat, and U-937, respectively) to explore LTR phenotype associated with these genotypic changes from an integrated chromatin-based microenvironment. Macrophage-, T cell-, and dual-tropic LTRs were coupled to a plasmid encoding green fluorescent protein (GFP), and polyclonal HIV-1 LTR-GFP stable cell lines were developed. To examine the site of LTR integration within the genome as well as epigenetic modfications that may control LTR driven gene expression, clones were derived from each population of cells. These clones have been examined basally as well as with chemical and cytokine treatment and Tat transactivation. Results suggest that non-expressing clones cannot be induced to express under any circumstances examined, where as both intermediate and high expressing clones can be induced by chemical, cytokine, and histone deacetylase inhibitor treatment and Tat-mediated transactivation. Results demonstrate that the site of LTR integration and possible epigenetic modifications to viral and host DNA may determine whether the LTR will be transcriptionally active and if transcription can be induced or up-regulated. Further studies will begin to define the differences between transcriptionally silent and active LTR-containing cell clones.

P173
Venezuelan equine encephalitis virus infection induces a complex host innate immune response in brain

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Venezuelan equine encephalitis virus (VEEV) is an Alphavirus in the family Togaviridae. VEEV is a human pathogen and may cause encephalitis and death in young, old and immunocompromised individuals. VEEV is highly infectious in aerosolized form and has been developed as a bio-warfare agent. Inflammation plays a key role in the pathogenesis of VEEV infection in brain. However, the underlying molecular mechanisms are poorly understood. In this study mice were infected with V3000, a neurovirulent strain of VEEV in the left hind limb foot pad. Brains were isolated at predetermined time points post infection. Gene expression profiles in VEEV infected mice brain were evaluated using a global gene microarray and pathway specific toll-like receptors (TLR) and extracellular matrix and adhesion molecule (ECMAM) microarrays. Differentially expressed genes upon VEEV infection clustered in important immune pathways such as antigen presentation, inflammation, apoptosis and response to virus. TLR 1,2,3,7 and 9, chemokines, inflammatory cytokines, interferon, interferon regulatory factors and genes involved in signal transduction were upregulated in VEEV infected mice brain. In ECMAM study, several cell to cell adhesion molecules and extracellular matrix protein genes such as ICAM-1, VCAM-1, CD44, integrins and MMPs were differentially regulated. ICAM-1 knock out (IKO) mice infected with VEEV exhibited reduced inflammation in the brain and demonstrated a delay in initiation and severity of the clinical symptoms of disease as compared to VEEV infected wild type mice. These results show a complex innate immune response to VEEV infection in brain and improve our present understanding of VEEV induced pathogenesis. Present study will also help in

identification of genetic markers for diagnosis and targeted therapy of VEEV induced neurodegeneration. Further studies are in progress to understand the specific role of the differentially expressed genes. These studies were supported by grant from Defense Threat Reduction Agency project number 4.10019_07_US_B.

P174
Longitudinal Neuropsychological Test Scores Are Inversely Correlated with HIV DNA Copy Numbers in Activated Monocytes

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Background: HIV-associated neurocognitive disorders (HAND) incidence has decreased with antiretroviral therapy (ART) with little change in prevalence suggesting that ART does not fully eradicate HAND. Our lab has been studying the role of HIV DNA in neuropathogenesis hypothesizing that HIV DNA in surrogate cells alters the cellular phenotype. We conducted a systematic analysis of HIV DNA copy numbers from peripheral blood mononuclear cells (PBMC) subsets on subjects enrolled in Hawaii Aging with HIV Cohort. Methods: Sixty-eight subjects were selected based on having enough PBMC in the repository for at least 3 of the 5 annual visits. Baseline/annual evaluations included medical examination, neuropsychological testing, viral load, CD4/nadir counts. Summary neuropsychological test scores, NPZ-8, were averaged from 8 NP test scores. Frozen PBMC were separated into monocyte/non-monocyte fractions followed by separation into activated/non-activated fractions. DNA from each subset was assessed for HIV DNA copy numbers by multiplex PCR with HIV gag/beta-globin primers/probes. The NPZ8 score was regressed on log HIV DNA level and covariates using a mixed effects model to adjust for having repeated measurements on participants. Results: Activated monocyte HIV DNA was strongly associated with NPZ8 scores ($p < 0.0001$) independent of baseline/nadir CD4 cell counts, gender, or education. One unit increase in log HIV DNA copy in activated monocytes resulted in 0.3526 decrease in NPZ8 score; similarly noted with PBMC but not with CD14-negative cells nor non-activated monocytes. Conclusions: High HIV DNA is associated with lower cognitive function and remained high longitudinally with cognitive function remaining relatively stable over time. (Support by R01NS053345, P20RR011091).

P175
Genetic and functional characterization of the envelope glycoproteins (Env) from two highly neurotoxic, cerebrospinal fluid-derived HIV-1 isolates

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HIV-1 infects the CNS and replicates mainly in perivascular macrophages and microglia, leading to the development of HIV-associated neurocognitive disorders. Both direct effects of the virus and/or viral proteins, as well as bystander effects of the infection such as cellular activation and release of pro-inflammatory cytokines and excitotoxins may be involved in pathogenesis. Both the Env of viruses adapted *in vitro* to the growth in microglial cells, and those from pro-viruses in brain tissues from patients with HIV-1-associated dementia (HAD), display a characteristic phenotype featuring high macrophage tropism, low dependence on the primary receptor CD4, increased fusogenicity and altered sensitivity to various entry inhibitors. The primary HIV-1 isolates Jago and Doge were obtained from cerebrospinal fluid (CSF) from two HAD patients, and supernatants from HIV-1Jago- and HIV-1Doge-infected macrophages have been shown to promote neuronal death *in vitro* through excitotoxicity. Our goal was to characterize the Env of these highly neurotoxic isolates. Therefore, viral RNA was isolated and used for cDNA synthesis followed by: i) single-genome amplification and the generation of linear expression cassettes; and ii) conventional PCR and cloning into an expression vector. Genes obtained by both methods were used for genetic analyses and phenotypic studies (based on infection of target cells with luciferase-reporter, Env-pseudotyped viruses). Within each of the two isolates, we have obtained closely-related env genes but remarkable differences in the phenotype of the encoded Envs. Thus, some Envs appear to have low CD4 dependence and reduced sensitivity to the fusion inhibitor T-1249, suggesting increased fusogenicity and high macrophage tropism, while some others display much higher CD4 dependence and do not seem to be macrophage tropic. These differences could be related with the nature of the virus that may be present in CSF and that could derive from viruses present both in blood and brain.

P176
La Crosse Virus Replication and LACV Induced Neuronal Damage is Decreased in Minocycline Treated Primary Rat Neuronal Cultures

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La Crosse virus is a member of the California serogroup of bunyaviruses and an important cause of pediatric encephalitis and aseptic meningitis in the United States. Neuronal apoptosis has been demonstrated to be an important consequence of LACV infection both in culture and in a murine model of LACV encephalitis. Minocycline is a second-generation tetracycline derivative that has proven effective in the conventional treatment of bacterial infections and a variety of inflammatory conditions. It has also been shown that minocycline interferes with caspase activation and has possible neuroprotective and anti-inflammatory effects. Here, we demonstrate that minocycline treatment of primary rat neuronal cultures infected with LACV results in decreased virus replication, decreased caspase activation, and increased neuronal survival. These results suggest that minocycline warrants further consideration as a possible therapeutic intervention for LCV encephalitis. Additional studies examining the effects of minocycline treatment in a murine model of LACV encephalitis are in progress.

P177
Theiler's virus leader protein (L) induces apoptosis in murine macrophages by activating the p38 MAPK pathway but does not influence CNS persistence

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Theiler's murine encephalomyelitis virus (TMEV) is a highly cytolytic picornavirus that persists in the mouse central nervous system (CNS) in macrophages with apparent perpetuation of the infection by macrophage-to-macrophage spread. Infected CNS macrophages undergo apoptosis. Murine macrophages infected *in vitro* with the BeAn strain (moi = 10) undergo apoptosis (6-12 h pi) through the mitochondrial pathway that is Bax-mediated. After infection, p38 MAPK and p53 activation are required for induction of apoptosis in infected M1-D macrophages (2-6 h pi). Activated p53 transcriptionally up-regulates the Bcl-2 family proapoptotic BH3-only Puma and Noxa genes. Recently, we showed that expression (transient transfection) of only the leader (L) protein of the BeAn virus nonstructural proteins (VPg or 3B was not studied) resulted in apoptotic cell death. Mutation to remove the L CHCC Zn-finger motif, that is required to mediate inhibition of nucleocytoplasmic trafficking, abrogated L protein-induced apoptosis. We have now found that infection of M1-D macrophages with BeAn virus containing a mutated Zn-finger motif resulted in delayed cleavage of PARP and caspase-3 and reduction of apoptosis ($P < 0.02$) compared to wild-type BeAn virus (8-10 h pi). BeAn mutant L virus-infected M1-D cells also exhibited delayed activation of MKK3/6, p38 MAPK and p53 (at 2-6 h) compared to wild-type virus. Temporal analysis revealed that host protein synthesis was inhibited (> 6 h pi) by infection of wild-type BeAn infection, and that this inhibition was delayed in the L Zn-finger mutant virus infection. UV-inactivated wild-type virus did not activate the p38 MAPK signaling pathway. These results suggest that BeAn virus L protein leads to apoptotic cell death by activation of the p38 MAPK pathway early in infection and that the L Zn-finger motif is required for apoptosis. Finally, intracerebral inoculation of 6 week-old female CD1 outbred mice with the mutant compared to wild-type virus resulted in lower acute virus growth in brain. However, in contrast to prior literature, the set-point for persistence at the end of the acute growth phase (day 12 pi) and levels of persistent virus (virus RNA copies numbers) in the spinal cord were no different ($P > 0.05$).

P178
Non productively infected MAC387+ macrophages are a third population of macrophages involved in formation of SIV encephalitic lesions

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Active and continuous monocyte/macrophage traffic occurs throughout HIV and SIV infection, increases with the development of AIDS and is likely required for HIV and SIV-encephalitis. However, histopathological studies, by their nature, tend to focus on chronic, end stage disease. Using monocyte/macrophage markers and BrdU labeling, we sought to characterize macrophage differentiation/activation

in SIV-encephalitis in acute and chronic infection and HIV-encephalitis in chronic AIDS patients. HIV and SIV-encephalitic lesions were heterogeneously composed of recently infiltrated CD163-CD68-MAC387+ monocyte/macrophages, CD163+CD68+MAC387- perivascular macrophages and CD68+ or HAM56+ resident macrophages. Approximately 30% of the MAC387+ monocyte/macrophages detected in lesions 24 hours after BrdU injection were BrdU+, consistent with recent infiltration of these cells from bone marrow. Numerous MAC387+ cells were found in small perivascular cuffs with little active viral replication and few CD68+ macrophages. Severe active lesions with high viral loads had numerous CD68+ macrophages and multinucleated giant cells but fewer MAC387+ cells, and in some cases none. We did not find MAC387+ monocyte/macrophages that were productively infected or that express CD163 or CCR2 in any brain lesions, suggesting that they are more likely immune macrophages that amplify immune response consistent with M1-like macrophages. This is in contrast to infected perivascular macrophages that express CD163 and are potentially immunosuppressive M2-like macrophages. These results suggest that the formation of HIV- and SIV-encephalitic lesions is an active process through infection involving the recruitment and accumulation of macrophages. This process involves at least three subsets of macrophages: non-productively infected inflammatory MAC387+ monocyte/macrophages that are not found in the normal, non inflamed CNS, CD163+ perivascular macrophages and CD68+HAM56+ resident macrophages-microglia, the latter two of which are present in the CNS in non-pathogenic conditions, accumulate with disease and are reservoirs for productive viral replication.

P179
Herpes simplex virus type-1 and rabies virus neurotropism and spread in the CNS

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Neurotropic viruses, such as herpes simplex virus type 1 (HSV-1) and rabies virus (RV), may infect the CNS causing severe diseases, while they also have the potential to serve as vectors in gene therapy to the nervous system. In order to study neurotropic virus tropism and spread in the brain, an *ex vivo* system of organ culture of brain slices was established. This system was used to study HSV-1 infection, replication and spread in mice brain slices and to investigate the role of animal age, viral receptors, cellular proliferation and division ability, cell type, extracellular compounds such as those that belong to the extracellular matrix as well as intracellular factors that mediate viral replication and establishment of latent infection. HSV-1 spread was observed to occur per continuum and via axonal transport. The infection pattern of lentivirus pseudotyped by VSV-G and RV-G was compared to that of HSV-1. While the tropism of the VSV-G pseudotyped vector resembled HSV-1, the RV-G pseudotype infection was weak and more diffuse in the entire parenchyma. VSV-G pseudotype infected neurons but spared astrocytes, while the RV-G pseudotype infected astrocytes, but not neurons. Taken together these results indicate unique patterns of lentiviral and HSV-1 infections in the brain and furthermore, HSV-1 spread in the brain is determined both by anatomical neuronal networks as well as by intracellular factors.

P180
Adjunctive neuroprotective therapy for hand

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There are severe neurological complications that arise from HIV infection, ranging from peripheral sensory neuropathy to cognitive decline and dementia. The HIV proteins secreted from infected macrophages and astrocytes, gp120 and Tat, are cytotoxic to the surrounding neuronal cells. Mechanistically, this neurotoxicity may be mediated via HIV protein-induced oxidative stress. The goal of this study is to screen, identify and develop neuroprotective compounds relevant to HIV-associated neurocognitive disorders (HAND). We have screened more than 2000 compounds that included FDA approved drugs for protective efficacy against oxidative stress-mediated neurodegeneration and identified selective serotonin reuptake inhibitors (SSRIs) as potential neuroprotectants. Numerous SSRIs were then extensively evaluated as protectants against neurotoxicity as measured by changes in mitochondrial potential, neuronal cell death and axodendritic degeneration elicited by HIV Tat and gp120. While many SSRIs demonstrate neuroprotective actions, we found that paroxetine is potently neuroprotective against these toxins *in vitro* and *in vitro*. Interestingly, the inhibition of serotonin reuptake by paroxetine is not required for neuroprotection. We determined that paroxetine interacts selectively and preferentially

with brain mitochondrial proteins compared to proteins of liver mitochondria. Furthermore, paroxetine blocked calcium-dependent mitochondrial swelling from brain mitochondrial with 100 nM potency, similar to concentrations required for neuroprotection. Therefore, SSRIs such as paroxetine, may provide a novel adjunctive neuroprotective therapy to treat HIV patients with HIV neuropathy and dementia. Supported by P30MH075673 and R01DA024593.

P181
Surface expression of CXCR4 is altered in response to the mu-opioid agonist DAMGO leading to decreased HIV-1 replication in TF-1 human bone marrow progenitor cells

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About one-third of the cases of human immunodeficiency virus type-1 (HIV-1) infection leading to acquired immunodeficiency syndrome (AIDS) in the United States have been attributed to injection drug use, frequently involving the abuse of opioids. Previous *in vitro* and *in vivo* studies addressing the role of mu-opioid agonists in altering levels of the co-receptor CXCR4, and consequent HIV-1 replication, have yielded contrasting results. The bone marrow (BM) is believed to be a potential anatomical sanctuary for HIV-1. In this study, the CD34+CD38+ human BM progenitor cell line TF-1 was used as a model to investigate the effects of the mu-opioid receptor-specific peptide DAMGO on CXCR4 expression as well as infection of undifferentiated hematopoietic progenitor cells. The results revealed the presence of the mu-opioid receptor-1 isoform (MOR-1) in TF-1 cells. Flow cytometry experiments indicated that treatment with DAMGO resulted in a shift in the relative proportion of CXCR4+ cells to the low-expressing phenotype. This result is correlated with a >3-fold reduction in replication of the CXCR4-utilizing HIV-1 strain IIIB, indicating a potential role for high level CXCR4 expression in sustaining infection within these cells. These experiments provide insight into the role of MOR-1-mediated signaling with respect to inhibition of viral replication in BM progenitor cells.

P182
Disruption of Intracellular Vesicular Trafficking by Agnoprotein is Essential for Viroporin Activity and JC Virus Replication

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The human polyomavirus JC virus (JCV) is the causative agent of a fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). The JCV encoded agnoprotein, which is recognized as a viroporin recently, is implicated in plasma membrane permeabilization to ions and low-molecular-weight compounds during infection and is essential for the viral replication. Viroporins are a group of proteins interact with cellular membranes modifying permeability and which participate in the promotion of release of viral particles from infected cells. The 6K protein from Sindbis virus, M2 from influenza A virus, 2B and 3A from poliovirus, coxsackievirus 2B, 2BC and 3A, and hepatitis C virus p7 and NS4A represent examples of well-known viroporins. Despite growing research efforts to be directed towards discovery of new viroporins and analysis of their function during infection, little is known about the relationship between the viroporin function and host proteins. Here, we demonstrate that JCV viroporin, agnoprotein, interacts with the delta subunit of AP-3. This interaction prevents AP-3 mediated intracellular vesicular trafficking and promotes the transport of agnoprotein to the cell surface through suppression of targeting of agnoprotein to the lysosomal degradation pathway, resulting in plasma membrane permeabilization and facilitating virion release. These observations demonstrate a unique specific viral-host protein interaction implicated in the membrane permeabilization and virions release mediated by viroporin in JCV infection, and suggest that the viroporins of other viruses, which participates in the promotion of release of viral particles from infected cells, may be highly regulated by the specific interaction between viral and host proteins.

P183
Targeted delivery of siRNA to macrophages and microglia cells

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Inflammation mediated by TNF-α and the associated neuronal apoptosis characterizes a number of neurologic disorders. Macrophages and microglial cells are believed to be the major source of TNF-α in the central nervous system. Here, we show that suppression of TNF-α by targeted delivery of siRNA to macrophage/microglial cells dramatically reduces LPS-induced neuroinflammation and neuronal apoptosis *in vivo*. Because macrophage/microglia express the nicotinic acetylcholine receptor (AchR) on their surface, we used a short AchR-binding peptide derived from the Rabies virus glycoprotein as a targeting ligand. This peptide was fused to nona-D-arginine residues (RVG-9dR) to enable siRNA binding. RVG-9dR was able to deliver siRNA to induce gene silencing in macrophages and microglia cells from wild type, but not AchR-deficient mice, confirming targeting specificity. Treatment with anti-TNF-α siRNA complexed to RVG-9dR achieved efficient silencing of LPS-induced TNF-α production by primary macrophages and microglia cells *in vitro*. Moreover, intravenous injection with RVG-9dR-complexed siRNA in mice reduced the LPS-induced TNF-α levels in blood as well as in the brain, leading to a significant reduction in neuronal apoptosis. These results demonstrate that RVG-9dR provides a tool for siRNA delivery to macrophages and microglia and that suppression of TNF-α can potentially be used to suppress neuroinflammation *in vivo*.

P184
Delayed hospital presentation and delayed acyclovir administration- the major causes of morbidity and mortality in Herpes simplex encephalitis

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Background: Herpes simplex encephalitis (HSE) is a serious CNS infection with grave morbidity and mortality. The outcomes of patients have been significantly improved with antiviral therapy, acyclovir. Poor outcomes are associated with delayed initiation and inadequate treatment with acyclovir. Methods: We performed a retrospective review of patients diagnosed with HSE at Johns Hopkins Hospital (JHH) between January 1997 and April 2010. Suspected cases of HSE with non-detectable CSF herpes simplex virus DNA were excluded in this analysis. We investigated the prescribing pattern of acyclovir in our patients. Results: 37 patients were diagnosed with HSE. 20 patients had been transferred from an outside hospital. The average age was 46.3 ± 4.0 years old(range: 6 days to 79 years). Seventeen patients were immunosuppressed. The most common presentations were fever (30/34), encephalopathy (25/34), seizure (20/34) and headache (10/34). Only 4 patients had the triad of headache, fever and encephalopathy. The average time from symptoms onset to hospital admission was 3.1 ± 0.6 days (ranged 0-14 days). The average delay between presentation to hospital to administration of acyclovir was 1.5 ± 0.3 days (ranged 0-5 days): 2.2 ± 0.4 days (ranged 0-5 days) when presented to an outside hospital compared to 0.5 ± 0.2 days (ranged 0-2 days) when presented JHH. 25 patients completed 21-day course of acyclovir at 30mg/kg/day. 7 patients completed 14-day course. There were 5 cases of mortality. 11 patients had good outcome with Karnoffsky's score of 70 or better. 3 patients received corticosteroid in addition to acyclovir had a moderate outcome despite delayed acyclovir administration of 7 days respectively. Conclusion: The major delay in the administration of acyclovir for HSE appeared to be due to delayed presentation to hospital. A high index of suspicion for HSE and appropriate initiation, dosing and duration of acyclovir are critical in the management of HSE. Corticosteroid may play role in the management of HSE.

P185
Adalimumab-associated Progressive multifocal leukoencephalopathy in a patient with HIV infection

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Background Progressive multifocal leukoencephalopathy(PML) is a rare CNS infection caused by reactivation of JC virus. It occurs in individuals with severe immunosuppression especially in advanced HIV infection. PML has recently been reported in patients treated with monoclonal antibodies including natalizumab, efalizumab and rituximab. Anti-tumor necrosis factor (ant- TNFα) monoclonal antibody, adalimumab has been used in individuals with HIV infection. We describe a patient with HIV infection who developed PML 2 months after starting adalimumab for the treatment of psoriasis. Case A 40-year old male was diagnosed with HIV infection 6 years prior in 2004. He had not initiated anti-retroviral therapy due to high CD4+ T cell count(>500 cell/mm^3) and good viral suppression. He was

treated with adalimumab (first dose 80mg subcutaneously then 40mg subcutaneously fortnightly) for psoriasis in February 2010. He presented with 2-week history of mild confusion, abulia and left central facial weakness and left hemiparesis in April 2010. MRI brain revealed right frontal lobe T2/FLAIR signal hyperintensity, with some mass effect and mild contrast enhancement. Brain biopsy confirmed the diagnosis of PML. His CD4+ T cell count was 198cell/mm3 with a viral load of 2.4 million copies/mm3 on admission. He was treated with anti-retroviral therapy and enrolled into a PML-mefloquine clinical trial (ClinicalTrial.gov identifier: NCT00746941). He developed immune reconstitution inflammatory syndrome(IRIS) with obtundation and dense left hemiplegia. He responded to pulse IV corticosteroids with good effect. His CD4+ T cell count recovered to 567/mm3 2 months after the initiation of anti-retroviral therapy. At the last follow-up; his mental status had improved markedly but he continued to have dense left hemiparesis. Conclusion This is the first case report of adalimumab-associated PML in an individual with HIV infection. Monoclonal antibodies used therapeutically in individuals with HIV infection can be associated with severe opportunistic infections including PML.

P186
Immune reconstitution inflammatory syndrome occurring in progressive multifocal leukoencephalopathy with natalizumab for multiple sclerosis

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Objective: To study the outcome of patients with multiple sclerosis (MS) treated with natalizumab complicated by progressive multifocal leukoencephalopathy (PML) and the development of immune reconstitution inflammatory syndrome (IRIS). Methods: We reviewed the MedWatch database from Biogen-Idec (manufacturer of natalizumab, Tysabri) which comprised all natalizumab-related PML cases since its reintroduction. Between June 2006 and March 2010, 42 post-marketing cases of PML had been reported. Results: All (except two) natalizumab-related PML were managed by discontinuation of natalizumab and plasmapheresis/immunoadsorption (PLEX/IA). Patients were dichotomized into 2 groups: 18 patients had contrast-enhancement of PML lesions on neuroimaging before PLEX/IA (termed early-PML-IRIS'') and 24 patients developed IRIS after PLEX (termed late-PML-IRIS). Immunological rebound or IRIS is defined as worsening of neurological deficits during the immune reconstitution following discontinuation of natalizumab, corroborated by inflammatory changes on neuroimaging. Following PLEX/IA, CSF JC viral load increased by >10 fold in those with early-PML-IRIS but <2 fold in late-PML-IRIS. All patients had worse EDSS scores following PLEX: EDSS increased by 3.6 $\pm$ 0.3 in early-PML-IRIS and by 2.7 $\pm$ 0.5 in late-PML-IRIS. The neurological deficits trended higher in early-PML-IRIS (EDSS 8.7 $\pm$ 0.3 vs 7.1 $\pm$ 0.6 in late-PML-IRIS, $p < 0.05$). Mortality was comparable between the two groups, 27.8 $\pm$ 11% (5/18) vs 20.1 $\pm$ 8% (5/24). Corticosteroid therapy during IRIS was associated with better outcome, 7.4 $\pm$ 0.5 vs 9.1 $\pm$ 0.6, $p < 0.05$. Interpretation: Early immunological rebound after natalizumab appears to be associated with worse survival and neurological outcome. IRIS poses a challenge in the treatment natalizumab-assoicated PML. Attempts to "remove" natalizumab with PLEX/IA may result in fulminant IRIS with worse outcomes. Corticosteroid therapy needs to be systemically studied in the management of natalizumab-related PML.

P187
Evidence of microglial activation in HIV+ patients with neurocognitive impairment without encephalitis or productive HIV infection of the CNS

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HIV-associated neurocognitive disorders (HAND) describes different levels of neurocognitive impairment and is a common complication of HIV infection. The most severe of these, HIV-associated dementia (HIV-D), has decreased in incidence since the introduction of anti-retroviral therapy, however, more cases of less severe neurocognitive dysfunction, namely minor cognitive motor disorder (MCMD) and asymptomatic neurocognitive impairment (ANI), are now seen. The neuropathogenesis of HIV-associated neurodegenerative disorders is not completely understood but macrophages/microglia play a significant role in the development of the more severe HIV-D. Here, we report evidence of microglial activation in patients with HIV infection and varying degrees of neurocognitive impairment but without encephalitis that is similar to that seen in patients with HIVE, the neuropathology of HIV-D. These cells appear to be

activated by expression of CD163+ and CD16+, but many have a ramified morphology that is associated more with "resting" microglia. In contrast to HIVE, productive HIV infection of the CNS was not detectable by amplified immunohistochemistry or in situ hybridization. Microglial activation may suggest a common mechanism between the lesser and more severe HIV-associated neurodegenerative disease processes.

P188
Latent infection of brain cells in asymptomatic HIV – the central role of perivascular macrophage and modulation by progressive immunosuppression

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Introduction Establishing the site of brain latency of HIV in asymptomatic individuals is critical to understanding the nature of the central nervous system reservoir of HIV. Studies are limited as they rely on access to brain tissue from individuals who die prior to the onset of AIDS. The brain cell/s within which the virus is latent in asymptomatic HIV infection has not been established. Methods Triple nested PCR for HIV gag DNA was performed on laser dissected cell populations (400 cells per population); astrocytes, perivascular macrophage and microglia in five HIV asymptomatic individuals, two positive controls (HIV encephalitis) and two negative controls (non-HIV, non-neurological). Cells were targeted following immunoreaction for GFAP and CD68 in addition to morphologic criteria. Neuropathological analysis to detect associated evidence of brain reactivity in the absence of HIV p24 protein was also performed. Data were correlated with CD4 levels using a Spearman correlation. Results The level of sensitivity of the methodology was determined to be one or more copies of HIV DNA per triple-nested PCR. HIV DNA was detected in all cell populations in asymptomatic individuals but was most consistently found in perivascular macrophage. Microglial and astrocytic reactivity was observed in brain tissue from asymptomatic individuals. The percentage of PCR reactions detecting HIV DNA in perivascular macrophage correlated inversely with the peripheral CD4 count (Spearman correlation, rho = -0.93, p = 0.006). No such relationship was evident in other cell populations. Conclusions This study establishes that brain cell reservoirs of HIV exist during asymptomatic infection and are associated with brain reactivity. It provides evidence that perivascular macrophage are the pivotal cell driving HIV brain infection at times of increasing immunosuppression in asymptomatic individuals.

P189
The Neuroprotective Effects of Fluconazole against a Mitochondrial Toxin and HIV Tat are linked to the Akt pathway

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Background: Neuronal injury and cell death are directly related to the cognitive dysfunction observed in individuals with HIV infection. Therefore, a neuroprotective agent would be a powerful aid in the fight against this illness. Using moderate throughput screening assay on nearly 2000 compounds we identified we identified the FDA-approved antifungal agent fluconazole (Diflucan, Pfizer) as a neuroprotective agent against HIV Tat protein and 3-nitroproprionic acid, a toxin used to mimic the oxidative damage, reactive oxygen species production, and ensuing neurodegeneration resulting form HIV infection in the brain. We have now investigated the mechanisms underlying the neuroprotective effects of fluconazole. Methods and Results: Fluconazole (500 nM - 1 μM) protected against the mitochondrial toxin, 3-nitroproprionic acid (3NP) ($p < 0.05$) and HIV Tat protein ($p < 0.01$) as determined by an MTT-based cell survival assay. At 1μM fluconazole concentration, nearly complete protection was noted against both toxins. One hour preincubation with 10 μM fluconazole was sufficient to significantly decrease cleaved caspase 3 levels in cells exposed to 3NP for 18 hours. To further understand the neuroprotective effects of fluconazole, we than tested it's ability to modulate the Akt (PKB) pathway. One hour pre-incubation of neuronal cultures with fluconazole before the application of 3NP resulted in a dose-dependent increase of phosphorylated Akt (Ser473). Specifically, 1 μM fluconazole increased pAkt levels by 13%, while 10 μM fluconazole resulted in a 40% increase in pAkt levels. Conclusion: These results suggest that the PI3K/Akt pathway may be involved in the neuroprotective effects of fluconazole. Further studies to determine if PI3K/Akt inhibition can abrogate the protective effects of fluconazole against 3NP and the HIV Tat protein are underway.

P190
JCV and BKV urinary excretion increases during treatment with Natalizumab

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Natalizumab is a humanized monoclonal antibody targeting the alpha-4 beta-1 integrin, approved for treatment of patients with Multiple Sclerosis (MS) who have failed other therapies or have aggressive disease. The efficacy of the drug is very high, but 58 post-marketing cases of confirmed Progressive Multifocal Leukoencephalopathy (PML) have been reported among Natalizumab-treated patients. A total of 38 MS patients undergoing treatment with Natalizumab have been enrolled in this study and serum, urine, Peripheral Blood Mononuclear Cells (PBMCs) have been collected monthly in order to monitor JCV and BKV reactivation and to identify high-risk population. Viral loads were assayed by Quantitative Real-Time PCR and viral strains were molecularly characterized. The median number of follow-up was 8,5 (range: 1-16). At the enrolment, JCV and BKV were found in the urine of 11 (28,9%) and 5 patients (13,1%), respectively. During the follow up, after 3, 6 and 9 months, JCV was detected in the urine of 37%, 36,4% and 36,4% of the patients, while BKV was detected in the urine of 37%, 25% and 20% of the patients, respectively. 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 were genotype 1 (12/18 patients). As for BKV, there was a high prevalence of genotypes I (8/17 patients) and II (7/17 patients), followed by genotype IV (2/17 patients). No virus was found in sera and PBMCs, besides JCV in the serum from one patient and BKV in the PBMCs from two patients. The results obtained showed that JCV and BKV reactivate during the follow up, proving the relevance of viral load monitoring in clinical specimens in order to define risk factors of PML development.

P191
Treatment of Mice with HIV Encephalitis with a Novel Interferon-alpha Blocker

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Title: Treatment of Mice with HIV Encephalitis with a Novel Interferon-alpha Blocker Objective: To determine the efficacy of a novel interferon-alpha (IFNa) blocker on HIV-encephalitis (HIVE) SCID mice. Background: IFNa is increased in the CSF of HIV dementia patients compared to HIV patients without dementia. Blocking IFNa in a HIVE mouse model improves cognition and certain pathology of HIVE. *In vitro* data suggests that IFNa toxicity results in dendritic simplification. Currently there are no FDA approved therapeutics to use as IFNa inhibitors. NormferonTM-alpha (Vaccinia B18R gene product) has the greatest binding affinity of any known compound to IFNa and provides an important additional margin-of-safety against autoimmune reactions because of its minimal homology to endogenous human proteins. Design/Methods: SCID mice are injected intracerebrally (IC) with either uninfected (controls) or HIV-infected human macrophages. Preliminary studies examined the amount of IFNa by ELISA in brain of control and HIV mice. Control and HIV mice will be treated with NormferonTM-alpha or saline by intraperitoneal (IP) injection twice daily and sacrificed 10 days after IC inoculation of human cells. The brains will be analyzed for microgliosis, astrogliosis, neuronal integrity, amount of IFNa, presence of NormferonTM-alpha and PKR, a protein expressed after IFNa receptor engagement. Results: ELISA's indicate that HIV mouse brains have approximately 45 pg/ml IFNa. Our two doses of NormferonTM-alpha will be based on the assumption that 1% of NormferonTM-alpha, when injected IP, will cross the blood brain barrier. Since 250 pg/ml of NormferonTM-alpha neutralizes 5 pg/ml IFNa, we will administer 250 ng/ml HIV-infected and control mice. Conclusions/Relevance: Preliminary data have allowed us to determine the amounts of NormferonTM-alpha to administer to HIV and shown that NormferonTM-alpha crosses the BBB and ameliorates HIVE in mice. These studies would ultimately serve as proof of principle for testing NormferonTM-alpha in patients with HIV dementia.

P192
Cyclooxygenase-2 (COX-2) inhibitor blocks the production of West Nile virus (WNV)-induced neuroinflammatory markers in astrocytes

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Inflammatory immune responses in brain initially triggered to clear the WNV promote additional damage resulting in blood-brain barrier (BBB) disruption and neuronal death thus hampering clinical management of WNV-associated encephalitis (WNVE). However, the mechanisms by which WNV modulates these inflammatory responses are unclear. We previously demonstrated that matrix metalloproteinases (MMPs) play an important role in WNV-induced BBB disruption. COX-2 and its product prostaglandin E2 (PGE2) are known to induce inflammatory molecules such as cytokines and MMPs. The objective of this study was to characterize the pathophysiological consequences of COX-2 expression in WNV-infected human brain cortical astrocytes (HBCA) and mice brain tissue. C57BL/6 mice and HBCA cells were infected with WNV (NY99) and levels of COX-2 and PGE2 were determined at different time points after infection. Further, the expression and activity profile of key cytokines and MMPs was analyzed in the presence or absence of COX-2 specific inhibitor (NS398). WNV infection significantly induced mRNA and protein COX-2 expression in the HBCA cells and mice brain tissues. The increase in the expression of PGE2 in infected HBCA cells also coincided with the increase in the expression of COX-2 and virus titers. Treatment of infected HBCA cells with COX-2 specific inhibitor, NS-398, attenuated the expression of MMP-1, -3 and -9 in a dose-dependent manner. Similarly, the expression of IL-1beta, -6 and -8, which were markedly elevated in infected HBCA cells, exhibited a significant reduction in their levels in the presence of NS-398. In conclusion, our data suggest that astrocytes are one of the potential sources of COX-2 derived PGE2 in WNV infection. COX-2 derived PGE2 initiate downstream pathological events such as cytokine production and MMP signaling pathway, thus contributing to two major hallmarks of WNVE, neuroinflammation and BBB disruption. The ability of COX-2 inhibitors to modulate WNV-induced COX-2 and PGE2 signaling should be further investigated in an animal model as a potential approach for the clinical management of WNVE.

P193
JC virus antibody and viremia as predictors of PML in HIV-1 infected individuals

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Objective: We examined whether JC virus (JCV)-specific antibodies and viremia are predictors of PML in people with human immunodeficiency virus type 1 (HIV) infection. Methods: We identified 30 PML cases and 81 HIV disease-matched controls in the Multicenter AIDS Cohort Study and 53 PML cases and 159 matched controls in the Swiss HIV Cohort Study (SHCS). Blood samples were available from 2 years before diagnosis of PML to up to 1 year after diagnosis. JCV viremia was assayed by quantitative PCR and IgG, IgA and IgM antibodies to JCV capsids by ELISA. Results: Comparing antibody responses of the 83 PML cases versus the 230 controls, we found no difference in median JCV IgG, IgM or IgA levels at any time points. However, controlling for CD4+ lymphoctye counts and age, a 1 log10 increase in JCV capsid IgG was seen in PML cases at 6 months prior to diagnosis (hazard ratio (HR) 1.75, p?=?0.005). This association was strongest for SHCS patients developing PML (HR of 1.94; p?=?0.007). For 25% of PML cases, JCV specific antibodies were undetectable throughout the 2 year period prior to diagnosis. Low level JCV DNA was detected in plasma from 14 (17%) of 83 PML cases and 28 (12%) of 230 controls and was not significantly associated with an increased risk of PML. Interpretation: JCV viremia was not predictive of development of PML in HIV positive individuals, but some patients may show higher JCV IgG responses 6 months prior to diagnosis.

P194
Murine coronavirus pathogenesis: virus-host interaction

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Murine coronavirus, MHV, infection of the mouse provides a model for the study of encephalitis and chronic demyelinating diseases such as Multiple Sclerosis. The highly neurovirulent JHM strain spreads rapidly thoughout the central nervous system (CNS) inducing a minimal T cell response, resulting in lack of viral clearance and mortality of all infected mice. The more neuroattenuated A59 strain spreads less extensively in the CNS and induces a robust T cell response, resulting in viral clearance; surviving mice develop chronic demyelinating disease. In addition, JHM, unlike A59, can induce lethal CNS disease in the absence of the only

known MHV receptor, CEACAM1a; this mechanism likely contributes to the extensive viral spread observed during JHM infection *in vivo*. Thus, we suggest that the enhanced neurovirulence of JHM results from more efficient CEACAM1a-independent spread in the CNS and the induction of a weak antiviral T cell response. The type I interferon (IFN-α,β) response is essential for protection from MHV very early in infection, as demonstrated by the uniformly rapid death of interferon receptor knockout mice. Paradoxically, infection of several cell lines does not induce IFN-β and replication in these cells is not inhibited by pretreatment with IFN-β. However, in primary cell types such as pDCs and macrophages, IFN is induced by MHV infection and an antiviral state is established. Other primary cell types such as neurons and astrocytes fail to produce IFN following infection and, *in vivo*, likely depend on IFN produced by pDCs and macrophages for protection. Thus MHV induction of IFN-α/β and the ability to induce an antiviral state is cell type dependent and protection from MHV in the CNS requires the orchestrated activities of several cell types. The type I IFN response may involve different cell types in the immune privileged CNS as compared to other organs.

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Innate immune responses by neurons are regulated by miRNAs during lentivirus-associated CNS disease

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Interferon beta (IFNβ) production is an early innate immune response to lentiviral infection of the CNS. In our rapid and consistent SIV/pigtailed macaque model of HIV encephalitis, SIV enters the CNS within four days of infection, accompanied by a marked IFNβ response. We recently reported evidence that microRNAs participate in posttranscriptional tuning of IFNβ expression in cultured primary macrophages, which *in vivo* are both vehicles for viral import into the CNS and important contributors to the IFNβ response. *In vivo* relevance of these results is supported by differential expression of these miRNAs during the course of SIV infection and by locked nucleic acid in situ hybridization (LNA-ISH), revealing co-localization of IFNβ mRNA and miRNAs that target IFNβ in several CNS cell types. Unexpectedly, neurons in both uninfected and SIV-infected brain express IFNβ mRNA, while IFNβ protein expression varies greatly from uninfected to infected animals and during SIV CNS disease progression. This disconnect between mRNA and protein expression provides further evidence for miRNA-mediated posttranscriptional regulation of IFNβ. Our results also suggest that neurons, at least in primates, may play a heretofore unexplored role in immune response to CNS infection.

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Effects of HHV-6A Infection in the Common Marmoset

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HHV-6, a ubiquitous β-herpesvirus, is frequently associated with neurologic diseases including MS, mesial temporal lobe epilepsy (MTLE), encephalitis, and febrile illness. The two variants of HHV-6 include HHV-6B, the etiological agent of exanthema subitum (roseola) and HHV-6A. The pathogenesis and epidemiology of HHV-6A remain unclear, although it has been reported that this variant is more neurotropic than HHV-6B. Given the association with neurologic disease, the effects of a primary infection with HHV-6A are of interest. To examine this, we exposed C. jacchus marmosets to HHV-6A intravenously once a month for 4 months and monitored the resulting infection. In 3 of the 4 HHV-6A infected marmosets, we detected IgM responses one week post-inoculation. IgG responses were seen in 2 of the 4 infected animals one week post-inoculation, and by week 15 all the infected animals had IgG responses to HHV-6. In HHV-6A infected animals, the virus has not been consistently detected in the saliva, plasma, or PBMCs using nested PCR, suggesting that HHV-6A does not reside in the periphery at high frequencies. Infected animals began showing signs of neurologic disease after the second exposure to the virus. To date the disease has been monophasic in nature and mainly characterized by impairment of the sensory system. One marmoset developed a skin condition with blister like erythematous lesions, another marmoset developed a facial palsy, and another developed signs consistent with sensory ataxia. Currently, pathological studies are ongoing to determine the localization of the virus in tissue. Overall we find that primary HHV-6A infection is associated with rapid seroconversion and neurological symptoms in the marmoset. This

infection will provide a model system to study the immune response during primary herpesvirus infection and the resulting neurological disease.

P197
HIV-1 LTR genetic variation is impacted by use of illicit drugs in the DrexelMed Genetic Analysis Cohort

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HIV infection is prevalent among substance abusers. The effects of specific illicit drugs on HIV-1 disease progression have not been well established. 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. A prospective, longitudinal study was conducted on 418 HIV-1 seropositive drug users 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 users had both a lower current CD4+ T cell count as well as lower nadir CD4+ T cell count as compared to non-users. Drug users also had higher current viral loads and higher peak viral loads as compared to non-users. In addition, single nucleotide polymorphisms (SNPs) were identified that are unique to cocaine, marijuana, or non-users. In conclusion, illicit drug use seems to facilitate HIV-1 disease progression. In addition, use of drugs of abuse selects for genetic variations unique to mono- and multi-using HIV/AIDS patient cohorts.

P198
Role for Tumor Necrosis Factor-alpha in JC Virus Reactivation and Progressive Multifocal Leukoencephalopathy

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Human polyomavirus JC (JCV) infects about 80% of the population worldwide. Primary infection is asymptomatic in immunocompetent individuals and results in lifelong latency and persistent infection. JCV reactivation among the severely immunocomprised individuals may cause a fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). The NF-kappa-B and C/EBP-beta transcription factors play essential roles in regulating an array of cellular processes including inflammation. NF-kappa-B and C/EBP-beta also regulate the JCV promoter via a control element, KB, suggesting proinfammatory cytokines may reactivate JCV to cause PML, e.g., in HIV-1/AIDS. Since HIV-1 induces cytokines in brain, including TNF-alpha, we examined a role for TNF-alpha in JCV regulation. TNF-alpha stimulates both early and late JCV transcription. Further, the KB element conferred TNR-alpha responsiveness to a heterologous promoter. Immunohistochemistry of PML revealed robust labeling for TNF- alpha and TNFR-1. These data suggest TNF-alpha stimulation of the JCV KB element may contribute to JCV reactivation and PML.

P199
A mechanism of viral regulation of cellular gene expression in the CNS based on interferon control of multiple promoters of the PURA gene generating unique non-coding RNAs

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Purα is an evolutionarily conserved cellular protein that plays an important role in HIV-1 and JCV transcription and replication in the CNS. Purα functions involve binding to nucleic acids and altering their conformation and physical positioning. The distinct but related roles of Purα suggest a need for expression regulated differently depending on interactions with viral proteins. Here we report that human PURA (hPURA) transcription is regulated from three distinct and widely-separated transcription start sites (TSS). Each of these TSS is strongly homologous to a similar site in mouse chromosomal DNA. Transcripts from TSSs I and II are characterized by the presence

of large and overlapping 5′-UTR introns terminated at the same splice receptor site. TSS III, located within 80 bp of the translational start codon, generates a transcript with no introns. Here we show that PURA transcription at TSS II is regulated by viruses infecting the CNS. Transcription at TSS II is down-regulated through the presence of adjacent consensus binding elements for interferon regulatory factors (IRFs). Chromatin immunoprecipitation reveals that IRF-3 protein binds hPURA promoter sequences at TSS II *in vivo*. By co-transfecting hPURA reporter plasmids with expression plasmids for IRF proteins we demonstrate that several IRFs, including IRF-3, down-regulate PURA transcription. Infection of NIH 3T3 cells with mouse cytomegalovirus results in a rapid decrease in levels of mPURA mRNA and Purα protein. The viral infection alters the degree of splicing of 5′-UTR introns of TSS II transcripts. A characterization of PURA differential promoter regulation by HIV-1 and polyoma virus will be discussed. Results provide evidence for a novel mechanism of transcriptional control by multiple promoters used differently in various tissues and responding differently to viral signals. Viral infection alters not only the use of PURA promoters but also the generation of different non-coding RNAs from 5′-UTRs of the resulting transcripts, suggesting that a new aspect of viral infection in the CNS may be through generation of cellular, regulatory non-coding RNAs.

P200
Comparison of glial effects of Tat proteins from HIV-1 clades B and C on JC virus late gene transcription and on interaction with the immunomodulatory cytokine TGF-beta1

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In Africa HIV-1 infection involves primarily viral clades C and D, while in Western nations HIV-1 clade B predominates. We have shown that HIV-1 can exert a direct effect on JCV infection in the brain mediated by the HIV-1 protein, Tat, which is secreted by HIV-1-infected cells and is accumulated by JCV-infected oligodendrocytes. We sought here to determine whether there is any inherent difference in Tat of clades B and C to influence transcription of the JCV late gene promoter. For comparisons Tat genes of different HIV-1 clades were cloned into pCDNA3.1 and expressed under control of the CMV immediate early promoter. For this activation study the Tat first exon, generating a protein of 72 aa was used. Tat B-72 and Tat C-72 are 79% identical. Tat B, 86 aa, was also cloned under expression of its own LTR promoter. We find that Tat proteins of both clades B and C can stimulate transcription from the JCV late promoter and that there is no significant difference in their abilities to do so. A mutation of Tat C27 to Y abolishes the ability to activate both HIV-1 LTR and JCV late gene transcription. There is a major difference between Tat of clades B and C with regard to interaction with the immunomodulatory TGF-beta1 system. SMAD4 is a mediator of transcriptional effects of this cytokine, which alters transcription of many cellular genes and of JCV early and late genes. In the absence of Tat SMAD4 is localized throughout the cell with a preference for nuclei. In the presence of Tat B for 48 hrs SMAD4 is driven to become almost exclusively nuclear. Tat C does not have this effect. The effect of Tat B is seen whether Tat-72 is expressed from the CMV promoter or whether Tat-86 is expressed from the LTR promoter. Our conclusions at this point are that although Tat proteins of HIV-1 clades B and C do not differ in their direct effects on JCV late gene transcription, they may differ significantly in their effects on gene transcription mediated through the TGF-beta component of the innate immune system.

P201
Alcohol-mediated induction of PDGF: Role in Disruption of Blood Brain Barrier

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Alcohol abuse is prevalent among individuals infected with HIV-1. Increasing evidence suggests that alcohol and HIV-1 infection synergistically contribute to neuropathogenesis. The blood brain barrier (BBB) is critical for the maintenance of CNS homeostasis and for the regulation of the neural microenvironment. However, during both HIV-1 infection and alcohol abuse, disruption of this barrier facilitates the entry of HIV-infected monocytes into the CNS. The mechanisms by which the monocytes infiltrate into the CNS parenchyma under these conditions are elusive. In our earlier findings we had demonstrated up-regulation of the vascular permeant platelet-derived growth factor (PDGF)-BB in the newly migrating perivascular macrophages in the brains of macaques with Simian-HIV encephalitis. There is also evidence for the involvement of altered chemokine expression (i.e., CXCL10) in HIV-1 and alcohol associated neuropathogenesis. Together,

these findings led us to hypothesize that PDGF-BB may play a critical role in the disruption of the BBB and enhanced monocyte migration. Using Western blotting, we demonstrated increased expression of PDGF-BB in monocytes exposed to alcohol. Reciprocally, we also found that exposure of human brain microvascular endothelial cells (HBMEC) to PDGF-BB decreased tight junction protein ZO-1 expression and increased endothelial permeability, when assessed by permeability assays. Additionally, our initial data suggest that alcohol enhances PDGF-BB-induced CXCL10 expression in human astroglial cells. Overall, these findings suggest that PDGF-BB released from alcohol exposed monocytes results in disruption of BBB integrity in a tissue culture model with alterations in expression of tight junction and adhesion proteins. Also, PDGF-BB-modulation of astroglial function may contribute, in part, to BBB dysregulation. Taken together these findings underpin the role of PDGF-BB as a potential target in the therapeutical intervention of HIV-associated neurocognitive disorders, particularly in alcohol abuse.