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## P1
### Investigation of measles and veterinary morbillivirus cell entry receptors in the CNS

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Background: Neurons and oligodendrocytes are primarily infected in the measles virus (MV) complications subacute sclerosing panencephalitis and measles inclusion body encephalitis. The known cell entry receptor for both vaccine and wild type (WT) MV strains, signaling lymphocyte activation molecule (SLAM or CDw150) is specific to T and B cells, monocytes as well as activated dendritic cells. Although we have previously shown that human cofactor CD46 is expressed at a low level on a subset of neurons and oligodendrocytes in the human CNS, it is not a receptor for WT MV strains. Furthermore, neurons and oligodendrocytes are infected in their natural hosts by neurotrophic veterinary morbilliviruses which we and others have found do not use CD46. Therefore, the molecules which allow morbillivirus entry into these cell types in humans and other species are unknown.

Methods: Primary mixed, neural cell cultures (CD46 and SLAM negative) were infected with the Dublin WT strain of MV and selected veterinary morbilliviruses. We also used 2 human cell types (i) mature, CD46 negative neurons (hNT cells) derived from the human teratocarcinoma cell line, NT-2, by differentiation with retinoic acid and (ii) the human epithelial cell derived neuroblastoma cell line, SHSY5Y. Both human cell types express well characterised neuronal markers.

Results: We demonstrated for the first time that the WT Dublin strain of MV and particular veterinary morbilliviruses infect murine oligodendrocytes in addition to neurons. Both hNT and SHSY5Y cells are also infected by these viruses. Using a combined approach we have prepared a phage antibody library against SHSY5Y cells and have selected an antibody which also stains hNT cells and blocks infection by WT MV. Using the phage antibody we have detected a putative receptor protein for mass spectroscopy identification. We are currently investigating expression of this protein on murine neural cells and whether antibody blocking inhibits veterinary morbillivirus infections in the human cell types.

Conclusion: These studies should allow us to identify a novel neuronal receptor for WT MV and determine if species of veterinary morbilliviruses use a common or distinct receptor(s) to MV.

## P2
### In vitro and in vivo Infection of mouse neural cells lacking known human entry receptors with wild type, and vaccine strains of measles virus

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Background: Infection of the mouse CNS with wild type (WT) and vaccine strains of measles virus (MV) results in lack of clinical signs and limited antigen detection. It is considered that cell entry receptors for these viruses are not present on murine neural cells and infection is restricted at cell entry. However, it has not been resolved whether there is an inability of WT and vaccine MV strains to infect neural cells or if subsequent replication and/or spread of virus in the CNS are restricted.

Methods: We compared virus antigen and caspase 3 expression (for apoptosis) in primary mixed, neural cell cultures infected in vitro or prepared from mice infected intracerebrally with WT, vaccine or rodent neuroadapted viruses. In vitro Infection and apoptosis in murine brain endothelial cells (BEC) were also investigated. Viral RNA levels were examined in mouse brain by nested and real time RT-PCR.

Results: WT and vaccine strains were demonstrated for the first time to infect murine oligodendrocytes in addition to neurons and BEC despite a lack of the known MV cell receptors CD46 and signaling lymphocyte activation molecule (SLAM or CDw150). Unexpectedly, the percentage of cells positive for viral antigen was higher for WT MV than neuroadapted virus in both in vitro and ex vivo cultures. In the latter the percentage of positive cells increased with time after mouse infection and viral RNA (total and mRNA) was detected in brain for up to 20 days. Primary mixed neural cultures were negative for caspase 3 in WT and vaccine virus infections while significant apoptosis was observed in BEC cultures.

Conclusion: WT and vaccine MV strains can use an endogenous cell entry receptor(s) or alternative virus uptake mechanism in murine neural cells.

However viral replication occurs at a low level and is associated with limited apoptosis in neurons and oligodendrocytes. WT MV mouse infection may provide a model for the initial stages of persistent MV human CNS infections.

## P3
## Brain immunophilin response in HIV infected METH users

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Background: Chronic stress is associated with disruption of the glucocorticoid receptor (GR) homeostasis in the brain. Signaling through the GR requires its translocation to the nucleus. A key molecule in this process is the immunophilin FKBP52. Our previous studies have established that increased expression of brain immunophilins (IP) is a good indicator of neuronal stress in HIV infection. We hypothesize that methamphetamine (METH) use, a common comorbid factor in HIV infected patients, will further amplify the IP response in the brain.

Materials and Methods: To test our hypothesis we have initiated a retrospective autopsy study of IP and GR expression in the brain of HIV/METH users compared to HIV non-METH subjects. For in vivo studies we are using transgenic animals expressing HIV gp120 in the brain (gp120 TG) exposed to a METH binge regimen. The distribution of brain IP and GR are measured by quantitative immunohistochemistry.

Results: Our preliminary autopsy studies show that in the HIV/METH human brains the expression in the subcortical white matter (WM) of both FKBP52 and GR is significantly increased when compared to the HIV non-METH subjects. In the in vivo mouse model, the gp120 TG animals exposed to METH show impaired learning in the water maze testing, when compared to littermate controls. The necropsy studies show again increased GR and FKBP52 immunoreactivity in the frontal cortex.

Discussion: Increased FKBP52 expression in the WM of METH users is consistent with our previous finding of IP reactivity in degenerating axons. The preliminary analysis of increased GR reactivity in the WM indicates glial activation. Our results suggest a WM-associated substrate for the neuronal stress in HIV infected METH users, which in turn may be related to chronic neuroinflammation.

## P4
## HIV DNA in Cerebrospinal Fluid Cells as Viral Reservoirs

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Background: Advances in anti-HIV therapy are reflected through dramatic reductions in HIV RNA levels. Despite these outcomes, HAART has little impact on HIV-DNA levels. The presence of cells harboring replication-competent HIV-DNA allows for low levels of continuous replication, resulting in viral persistence. Since replication levels are often undetectable in HAART-treated patients, it is difficult to identify cellular reservoirs. Here we report the use of a fluorescently conjugated peptide nucleic acid (PNA) probe in fluorescence in situ hybridization-flow cytometry (FISH-FLOW) to detect HIV-DNA within infected cells. Due to the ability of PNA to readily cross the cell membrane, less manipulation is required. In addition, simultaneous labeling of surface antigens allows for cell type identification, which we further demonstrated in CSF cells.

Methods: Two HIV-infected cell lines (ACH-2 and OM10.1) and HIV-infected peripheral blood mononuclear cells (PBMCs) were incubated with PNA-Cy5, which has been shown to specifically target the pol region of HIV. PNA-Cy5 fluorescence was detected by flow cytometry. To identify monocytes, PBMCs were stained with CD14-FITC. Traditional assays to determine HIV DNA levels using DNA extraction and quantitative PCR were also performed to permit comparisons with the FISH-FLOW technique. We then assessed the feasibility of the technique on CSF cells from an HAD patient.

Results: Based on FISH-FLOW, approximately 52% of ACH-2 cells and 46% of OM10.1 cells were positive for HIV-DNA. Results from traditional assays showed that they contained $5.00 \times 10^5$ and $3.6 \times 10^5$ copies per $1 \times 10^6$ cells respectively. In HIV-positive PBMC samples from two patients, PNA FISH-FLOW indicated that 53% (patient #1) and 6% (patient #2) of the cells contained HIV-DNA. In these samples, monocytes represented 4% and 14% of total PBMCs, of which 0.4% and 4% contained HIV-DNA respectively. Results from CSF cells from a subject with HAD demonstrate the most HIV-DNA in CSF CD14+ cells, with the majority being CD14+/CD16+ cells.

Conclusions: The ability of PNA in FISH-FLOW to detect HIV-DNA was comparable to traditional assays. However, FISH-FLOW is more advantageous through its reduction in both time and labor. FISH-FLOW can be used for the identification of specific cellular populations containing HIV-DNA, throughout the course of infection. This assay may facilitate the use of HIV-DNA as a potential marker for the progression of HIV-related diseases such as HIV-Associated Neurocognitive Disorders (HAND) with its potential use in analyzing CSF cells. [Support by U54NS43011 (LMM, VW); R01NS053345 (MA, BS); R25MH080661 (MA, AB)].

## P5
### Characterization of HIV-1 sequence variations in the envelope and long terminal repeat sequences within the DrexelMed cohort

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Microglial cells and perivascular macrophages support most productive human immunodeficiency virus type 1 (HIV-1) replication within the brain, although both have low CD4 levels making macrophage tropism and preferential CCR5 utilization features of CNS-derived viruses and their envelopes. Sequence analysis has demonstrated both viral evolution within the CNS and compartmentalization of sequences between brain and peripheral tissues, suggesting the potential for adaptation within the brain environment. Previous studies of the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) have shown that specific variations within the C/EBP (3T) and Sp (5T) binding sites isolated from the peripheral blood correlate with disease progression and HIV-associated dementia in patient samples obtained prior to the introduction of HAART. The 3T/5T-containing LTRs may be representative of LTR genotypes that are preferentially retained because of specific functional properties involved in disease pathogenesis in the peripheral blood, CNS, and perhaps other compartments. We propose that certain envelope sequences are co-selected with the LTR and play an important role in determining the cell types infected within the peripheral blood and brain. Herein, we describe the cloning of a 4.3 Kb fragment of the HIV-1 provirus, including sequences extending from Vpr through the 3′ LTR, from the peripheral blood of patients enrolled in the DrexelMed HIV/AIDS patient cohort. Genomic sequence analysis of specific configurations of HIV-1 LTR transcription factor binding sites in association with specific envelope genotypes is presented. Future studies will address functional properties of these viral genetic signatures.

## P6
### Supernatants from HHV-6A and HHV-6B infected human macrophages induce death and loss of Map-2 in primary rat neuronal cultures

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Peripheral monocytes were isolated from human donors and differentiated into macrophages over 7 days in culture. These macrophages were subsequently infected with HHV-6A or HHHV-6B. Infected and mock infected macrophage supernatants were collected for six days and then applied to primary rat (Sprague Dawley embryonic day 17) neuronal cultures differentiated for 2 weeks. HHV-6A and HHV-6B infected supernatants were toxic to rat neurons as compared to primary cultures treated with mock supernatants as measured by loss of MAP-2 and PI staining. In addition, we examined caspase activation and the presence of cleaved PARP in these samples. This system mirrors previous studies from our group demonstrating HIV-induced neuronal loss in primary neuronal cultures treated with HIV infected macrophages. Collectively, these data suggest a common role for macrophages in neurodegeneration induced by viruses from disparate families. Co-infection experiments with HIV and HHV-6A or HHV-6B are underway.

## P7
### MDMx as a potential neuroprotective molecule in HIV-induced neuronal injury

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Human immunodeficiency virus (HIV)-associated neurocognitive disorder (HAND) is a serious complication of HIV infection, and can be associated with microgliosis, astrogliosis, neuronal loss and dendritic damage. The neuroinflammatory infiltrate, both HIV-infected and non-infected, activated macrophages/microglia (HIV M/M), has been linked to neuronal damage. We have previously hypothesized and shown that inflammatory changes in the CNS coincide with increased expression of cell cycle proteins E2F1 and phosphorylated pRb (ppRb) in the CNS of patients with HIV encephalitis, in a macaque model of simian immunodeficiency virus

encephalitis (SIVE), and in neurons in an in vitro model of HIV-induced neurodegeneration. Of these cell cycle proteins, E2F1 has been shown to bind MDMx, a member of the MDM2 family, and negatively regulate E2F1 induced apoptosis. We have previously shown decreased MDMx levels correlate with increased E2F1 levels in neurons in the brains of macaques with SIVE leading us to hypothesize that MDMx provides neuroprotection from HIV M/M in our in vitro model. To this end, we have observed decreased levels of MDMx in primary rat neuroglial cultures treated with HIV M/M supernatants. The observed decrease in MDMx protein levels was dependent on NMDA receptor activation and calpain activity consistent with our model of HIV-induced neurotoxicity. In addition, we have shown that MDMx is proteolyzed directly by calpain in vitro. To determine if increasing MDMx levels could protect against HIV-induced neurotoxicity, we overexpressed MDMx in neurons using an adeno-associated virus (AAV)-mediated expression system and found that MDMx provided partial protection from HIV M/M supernatant-induced neurotoxicity. Interestingly, the chaperone protein, 14-3-3, appears to stabilize MDMx in neurons pretreated with the calpain inhibitor, MDL21870. Finally, MDMx levels were decreased significantly in the midfrontal cortex of HIV-infected patients by immunofluorescence. These data suggest that following NMDA receptor mediated calcium influx, calpain activated degradation of MDMx occurs partially due to loss of 14-3-3 stabilization of MDMx in our in vitro model of HIV-induced neurodegeneration. Strategies to stabilize MDMx levels can provide protection not only in HAND, but in other neurodegenerative diseases where calpain activation contributes to the disease pathology.

## P8

### Pilot Studies of Neurocognitive Impairment Related to HIV-1 Infection in Abuja, Nigeria

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Background: There are approximately 30 million people in Sub-Saharan Africa with HIV/AIDS infection and in some areas the prevalence of HIV dementia in the absence of highly active antiretroviral therapy (HAART) has been documented to be as high as 50%. In Nigeria, where in recent years there has been rapid scale-up of treatment with HAART, the incidence and prevalence of HIV related neurocognitive impairment (NCI) and the rates of response to treatment are unknown. Therefore a pilot study was initiated in Abuja, Nigeria to examine the potential utility of applying an established neuropsychological (NP) screening battery and detailed NP testing to detect NCI and to identify viral signatures that may be associated with NCI among infected subjects.

Methods: 66 HIV-1 seropositive individuals and 56 seronegative control subjects were administered the International HIV Dementia Scale (IHDS) and a subset individuals (15 HIV infected patients and 11 controls) were also administered a detailed NP battery that is currently being utilized in diverse cultural settings. Blood samples from 7 infected subjects, 3 with evidence of NCI, were obtained for molecular analysis of HIV-1 strain, of which strains A and G are the most common in Nigeria.

Results: Analysis of unadjusted scores on the IHDS showed that 28.1% of the HIV-1 seropositive and 16.1% of seropositive individuals scored abnormally on the screen. The HIV seronegative and seropositive group administered the full battery was comparable with regard to level of education (the most influential variable on test performance) and all were young to middle aged adults. Results from the detailed NP test battery showed a mean effect size across the battery of 0.45, which is lower than that observed in some other international settings. Sequencing of partial pol and partial env amplicons from viral isolates revealed that 2 of the 3 patients with NCI were infected with subtype G virus and 1 with the circulating recombinant form (CRF) 02_AG; all 4 individuals without NCI were infected with CRF_02AG.

Conclusion: These studies demonstrate the feasibility for conducting these studies, which will be important for both establishing both the burden of NCI in the population of patients with HIV-1 infection in Nigeria and for assessing the specific HAART regimens that are currently being utilized for their effectiveness in the treatment of NCI.

## P9

### The Risk for HIV Associated Sensory Polyneuropathy is Increased by Metabolic Syndrome Components

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Background: Distal sensory polyneuropathy (DSPN) is the most common peripheral complication of HIV and combination antiretroviral therapy (cART). Both HIV and cART can increase the risk metabolic syndrome (MetS)-a cluster of components that cause atherosclerosis. We studied if MetS components predispose HIV+ participants (pts) to HIV associated DSPN.

Methods: From a cohort of HIV+ pts (n = 1,556), a smaller group (n = 130) had fasting laboratory tests and DPSN assessment- defined by symmetrical decreased reflexes or sensation in the lower legs. MetS was defined by the presence of $\geq 3$ of the 5 following: mean arterial pressure (MAP) $\geq 100$ mm Hg; triglycerides (TRG) $\geq 150$ mg/dl and high-density lipoprotein cholesterol (HDL-C) $< 40$ mg/dL; body mass index (BMI) $> 30$ kg/m2, plasma glucose (GLU) $\geq 110$ mg/dl; and type 2 diabetes (DM II). A multivariate logistic regression controlling for DSPN risks factors examined the association between DSPN and MetS components.

Results: After controlling for age, CD4 current, length of HIV infection, D-drug use ever, and past protease inhibitor use; MetS was not associated with DSPN ($p = 0.72$). However when each MetS component was assessed, elevated TRG was a risk factor for DSPN within the smaller group. For the larger cohort of HIV+ pts, self-reported DM II (OR = 2.3, $p < 0.01$) was a risk factor for DSPN.

Conclusions: The risk of HIV-associated DSPN was increased by DM II and elevated TRG, but not by other MetS components. Better control of these MetS components could reduce the risk of HIV associated DSPN.

## P10

### HIV and Aging: A Strain on Brain Function

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Background: Both HIV and aging can cause physical, immune, and cognitive frailty. Their interaction on brain function remains poorly characterized.

Methods: This cross sectional study obtained functional magnetic resonance imaging (fMRI) measures from 26 HIV infected (HIV+) and 25 seronegative (HIV−) subjects ranging from 20–62 years old. A Wilcoxon rank test assessed for differences in measured fMRI outcomes between HIV+ and HIV− groups. A multiple regression model studied the association and interaction between fMRI measures, HIV status, and age. An analysis of variance assessed the effect of highly active antiretroviral therapy (HAART) on fMRI values.

Results: HIV serostatus and age each independently affected fMRI measures but no significant interaction was present. fMRI measures in HIV+ subjects were equivalent to those obtained in HIV− subjects who were ~15–20 years older. The type of HAART regimen (protease inhibitor or non-nucleoside reverse transcriptase based) did not affect fMRI values.

Conclusion: fMRI could potentially be a non-invasive biomarker to assess the effects of HIV in the brain. Observed fMRI changes with HIV and aging suggest associated parallels in immune dysfunction. Frailty associated with ''normal'' aging could result from continued immunological maintenance in response to a variety of challenges that gradually deplete resources triggering increased brain functional metabolic demands. Persistent activation and rapid depletion of immune resources by HIV infection may augment brain functional metabolic requirements.

## P11

### The reproducibility of calibrated blood oxygen level dependent functional magnetic resonance imaging (BOLD-fMRI) in HIV infected (HIV+) subjects

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Background and Aims: Reproducibility studies of blood oxygen level dependent () functional magnetic resonance imaging (BOLD-fMRI) are needed to understand basic physiology of neurovascular coupling as a single scan may be affected by a subject's performance, variations in scanner hardware, or biological factors. We assessed the reproducibility of calibrated BOLD fMRI in both HIV infected subjects (HIV+) and healthy controls (HIV−).

Methods: BOLD and cerebral blood flow (CBF) responses were obtained from 8 HIV+ subjects and 10 HIV− controls, from 20–45 years old, on a 3 Tesla General Electric scanner at two separate scanning sessions separated by at least 3 months from each other. All subjects underwent calibrated BOLD studies consisting of both mild hypercapnia

and functional activation experiments. Mild hypercapnia provided a calibration method for calculating functional CMRO2 changes. Functional activation consisted of a black and white radial checkerboard flickering at 8 Hz. Clusters of CBF activated voxels within the visual cortex (VC) were assessed. The coefficient of variation (CV), a normalized measure of dispersion of a probability distribution, was determined for each calibrated BOLD measures outcome(functional changes in CBF, BOLD, and cerebral metabolic rate of oxygen consumption (CMRO2)) within both HIV+ and HIV− subjects. A variance components random effects model was used for each fMRI variable.

Results: The median age for HIV+ subjects and HIV− controls was similar with no significant differences observed for either sex or education. For HIV− controls the intrasubject CV values for functional BOLD, CBF, and CMRO2 were 8.8%, 10%, and 10.5% respectively. For these individuals total variability was greatest for functional CMRO2 (47.3%) compared to either CBF (30.1%) or BOLD (16%). For HIV+ subjects, intrasubject variability was greater for each of the functional measures −38.8% for CBF, 69.8% for BOLD, and 11.2% for CMRO2. Total variability was also increased for HIV+ subjects for each of these fMRI measures (61% for CBF, 74.6% for BOLD, and 17% for CMRO2).

Conclusions: HIV+ subjects have greater total variability than HIV− controls for measured functional changes in CBF and BOLD. In contrast, calculated functional CMRO2 changes were less variable for HIV+ subjects compared to HIV− controls. A possible breakdown in neurovascular coupling may occur in HIV+ subjects. Calibrated BOLD provides quantitative measurements compared to previous qualitative measure using functional overlap maps

## P12
## HIV and Methamphetamine Have Additive Effects on Functional Magnetic Resonance Imaging Measures

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Background: Human immunodeficiency virus (HIV) and chronic methamphetamine (METH) use each are associated with cerebral dysfunction as measured by functional magnetic resonance imaging (fMRI). We examined if an interaction occurred between HIV and METH within the lenticular nuclei (LN), a subcortical structure often affected by both.

Methods: Blood oxygen level dependent (BOLD) and arterial spin labeling (ASL) measurements of cerebral blood flow (CBF) were obtained from 24 HIV−/METH−, 8 HIV−/METH+, 23 HIV+/METH−, and 15 HIV+/METH+ subjects at 3 Tesla (General Electric) scanner. Baseline CBF and functional BOLD and CBF changes within the LN were recorded for a simple finger tapping paradigm (2 Hz). An analysis of variance (ANOVA) was performed to assess the association of HIV serostatus, history of chronic METH use (yes/no), and fMRI measures. The presence of a two-way interaction between HIV and chronic METH use was investigated with p-values from the Wald test presented.

Results: HIV serostatus and METH abuse each independently affected fMRI measures in the LN but no significant interaction was present. HIV-positive status was associated with lower baseline CBF (−10%, $p = 0.07$), larger functional BOLD (+0.06%, $p = 0.33$) and CBF (+32%, $p = 0.01$) changes. A history of chronic METH use was associated with lower baseline CBF (−16%, $p = 0.007$), larger functional BOLD (+0.22%, $p = 0.002$) and CBF (+33%, $p = 0.02$) changes.

Conclusions: Our results suggest a possible additive relationship between HIV and METH with the greatest changes in fMRI measures occurring in HIV+/METH+ subjects. Our findings are consistent with significant disruption of neuronal integrity in the LN primarily due to chronic METH use with an additional smaller contribution due to HIV− positive status. We conclude that fMRI could potentially be a non-invasive biomarker to assess the effects of HIV and METH in the brain.

## P13
## Novel Proteoglycan-related Molecular Targets in HIV-1 infection of Primary Human Macrophages: Implications for Neuropathogenesis

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HIV-1 neuropathogenesis and the likely role of the CNS as a sanctuary for viral re-emergence are two important consequences of HIV-1 infection. HIV-1-Associated Dementia (HAD) is a major problem among HIV-1-infected individuals. Viral reemergence has become a major obstacle in retroviral therapy, which is currently capable of lowering viral levels in the periphery and yet cessation of therapy is quickly followed by resurgent infection. For over 20 years CD4 has been considered to be the exclusive attachment receptor for HIV-1 gp120

and the two major coreceptors, CXCR4 and CCR-5, facilitate the process of viral fusion and efficient entry into specific target cells. However, recent data strongly suggest that HIV-1 can infect several target cells that have undetectable or very low levels of CD4 expression. We and other investigators have previously demonstrated that unspecified heparan-sulfate and chondroitin-sulfate cell-surface proteoglycans (HSPG and CSPG respectively) act as necessary HIV-1 attachment receptors on specific target cells, such as macrophages, brain microvascular endothelial (BMVECs), astrocytes, dendritic cells (DCs) and other cellular targets. Our current findings show that the transmembrane proteoglycan syndecan-1 specifically acts as a monocyte-derived macrophage (MDM) HIV-1 receptor, suggesting that syndecans (and possibly other cell-associated proteoglycans) may also act as HIV-1 receptors on brain microglia, which express low-levels of CD4. In addition, siRNA knockdown of D-glucuronyl C5-epimerase (GLCE), an enzyme responsible for the epimerization of D-glucuronic acid into L-iduronic acid of HS on cell-associated proteoglycans (increases the flexibility of HS chain thereby enhancing its ability to interact with proteins and various ligands), resulted in significant inhibition of HIV-1 replication (3- and 7-days post-infection) in primary human MDM, suggesting, for the first time, that GLCE is a novel target and plays a crucial role in viral interactions with proteoglycan HS-side chains and possibly in HIV-1 infection of brain microglia. Moreover, enzymatic digestion of cell-surface heparan-sulfate side-chains significantly inhibited HIV-1 YU-2 attachment and entry into human brain microglia, as indicated by the amount of p24 in cell lysates via ELISA. CNS and its cellular components remain an area of active investigation. Understanding the molecular role of cell-associated proteoglycans and their key biosynthetic enzymes, such as GLCE, in the infection of the human brain by HIV-1 is crucial in targeting this potential viral reservoir site.

## P14

### Transcriptomal analysis of Varicella-Zoster-Virus gene expression in patients with and without Post-Herpetic Neuralgia

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Varicella zoster virus (VZV) causes chicken pox (Varicella) as a primary infection following which it becomes latent in neurons in the dorsal root ganglia (DRG) and trigeminal ganglia (TG). It may then reactivate to cause shingles (herpes zoster), the most serious complication of which is post-herpetic neuralgia (PHN) which occurs in about 50% of individuals over 60 yrs. Despite its considerable clinical importance in terms of pain and long-term morbidity, current treatment of PHN is largely ineffective. The development of PHN following herpes zoster cannot be predicted. However, if PHN could be predicted very soon after zoster develops, then more intensive and longer anti-viral therapy with acyclovir might potentially influence its occurrence and/or severity. To address this possibility, we determined whether there are 'signature' VZV genes that are transcribed in the PHN-associated isolates at different levels compared with non-PHN isolates that could potentially predict the development of PHN at a very early stage. We used gene microarray technology to analyse viral gene expression in several VZV isolates obtained from patients with acute zoster who did or did not subsequently develop PHN. The results of the array analysis for transcription of VZV showed no significant differences in the expression of any ORFs between PHN and non-PHN samples. However, by applying a permutation test that estimates statistical significance of an ORF compared to randomly shuffled data of the same ORF, we identified 8 ORFs as having different average expression between PHN and non-PHN samples: ORFs 11b, 46b, 21, 41, 56, 16, 42, and 15. To further investigate these possible differences in mRNA levels of these 8 ORFs in the PHN-associated isolates and in non-PHN isolates we are currently completing these studies using TagMan RT-PCR assays which will yield more definitive and quantitative data. To compare the cytopathic effects of the PHN and non-PHN isolates, we performed growth curve experiments, using standard plaque forming unit (PFU) assays, on ten viral isolates (5 PHN and 5 non-PHN) grew in MeWo cells at days 0, 1, 2, 3, 4, and 5 post-infection. The growth curve patterns were very similar for all ten isolates indicating that the viruses associated with PHN were not simply replicating more efficiently in culture than those not associated with PHN.

## P15

### Changes in plasma proteomic biosignatures associated with therapeutic antiretroviral responses in HIV-1 infected patients

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Bioimaging, viral, and immune biomarkers support, but do not define, the tempo and progression of HIV-1 disease. Towards the goal of developing disease biomarkers we performed proteomic tests to identify plasma protein signatures linked to newly initiated antiretroviral therapies (ART). Two dimensional fluorescence difference gel electrophoresis (2-D DIGE) was performed on immunodepleted plasma after saturation labeling with Cy dyes. We found increased levels of proteins belonging to the complement cascade such as C3a, C3a precursor, C3c, C4a and C8 after ART. Pigment epithelium derived factor (PEDF) was also increased by ART. ART resulted in the downregulation of proinflammatory factors including ceruloplasmin precursor and kininogen paralleled viral load reductions and increased CD4+ T cell counts. For biosignature discovery select proteins, C3a and PEDF, were validated by ELISA. We conclude that ART modulates T cell reconstitution that parallels the complement system. As such, complement proteins uncovered in plasma provide potential surrogate markers for immune reconstitution and in monitoring therapeutic responses.

## P16

### cAMP signaling enhances HIV-1 LTR-directed transcription and viral replication in human bone marrow progenitor cells

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Several lines of evidence suggest that the bone marrow may serve as a reservoir for HIV-1 with infected cells of the myeloid lineage reseeding the virus into peripheral blood. In vitro and in vivo studies have demonstrated limited infection of hematopoietic progenitor cells. CD34+ bone marrow progenitor cells are partially refractile to HIV-1 infection, probably due to their low level expression of HIV-1 co-receptors CXCR4 and CCR5. However, it has been observed that under certain circumstances, this unresponsiveness to infection can be overcome. We have identified both the HIV-1 receptor CD4 as well as the co-receptors CXCR4 and CCR5 on the surface of human CD34+/CD38+ TF-1 bone marrow progenitor cell line. Therefore, this cell system was utilized as an in vitro model to determine effects of intracellular cAMP elevation on the alteration of HIV-1 infection. To this end, experiments revealed that cAMP elevation by the adenylyl cyclase-specific activator forskolin upregulated the viral co-receptor CXCR4 in TF-1 cells. This was accompanied by a concomitant increase in replication of a X4 HIV-1 strain in these cells, suggesting an increase in HIV-1 susceptibility. In parallel, an increase in X4-utilizing HIV-1 promoter activity was also detected upon augmentation of intracellular cAMP concentrations. Experiments focused on elucidating the underlying molecular mechanism for these observations have revealed the direct role of the protein kinase A pathway and the downstream transcription factor CREB. This assumes importance as HIV-1-infected patients have been found to have very high circulating levels of prostaglandin E2 (PGE2), a natural cAMP signaling pathway activator in vivo.

## P17

### Reovirus activates BMP signaling pathways in vitro and in vivo which contribute to neuronal survival following infection

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Introduction: Reovirus infection in vitro and in the CNS of neonatal mice is a classical model of viral pathogenesis. Understanding how viruses perturb host cell signaling pathways is critical to identifying novel antiviral targets. We now show that reovirus infection activates bone morphogenetic protein (BMP) signaling both in vitro and in a murine model of encephalitis and that activated BMP signal transduction can reduce virus-induced neuronal cell death.

Results: Two-day old NIH Swiss Webster neonatal mice were injected intracerebrally with reovirus serotype 3 (T3) and brains were harvested at specific time points. Both BMP receptor I expression and its downstream transcription factor, phosphorylated (serine 463/465) SMAD1, were up-regulated in immunoblots of infected whole brain lysates. Phosphorylated SMAD1 was exclusively seen in regions of brain known to be infected by reovirus including the cingulate cortex, hippocampus and the lateral nucleus of the thalamus. In these regions, phosphorylated SMAD1 was predominantly localized in uninfected neurons in close proximity to infected neurons, rather than in infected neurons themselves. In order to determine the role of BMP activation following reovirus infection, we pretreated HEK293 cells with BMP inhibitor or vehicle control followed 30 minutes later by mock or reovirus infection (T3 multiplicity of infection (MOI): 100). BMP inhibition enhanced virus-induced apoptosis at 8 and 24 hrs post-infection. These results suggested that BMP inhibition potentiated virus-induced cell death. We

next examined whether BMP activation would reduce virus-induced neuronal death. We pretreated primary mouse cortical neuronal cultures with BMP ligand (50ng/ml) 30min before T3 infection (MOI 100) and found a significant decrease in reovirus-induced apoptosis in treated neurons. Protection occurred in the absence of a significant effect on viral titer.

Conclusions: These data provide the first evidence for the activation of the BMP signaling pathways in the CNS following neurotropic viral infection and suggest that activation of BMP reduces, and inhibition of BMP potentiates, virus-induced neuronal cell death. These studies suggest that BMP signaling pathways may normally function as part of the host's protective innate immune response against CNS viral infection.

## P18
### Clinical Risk Factors Identify Seronegative HCV Co-infection in Substantial Minority of CHARTER Participants

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Background: Co-infection with Hepatitis C Virus (HCV) occurs in a substantial number of people living with HIV (PLWH) and might accelerate the progression of HIV related neurocognitive decline. The diagnosis may be missed due to falsely negative screening HCV antibody test. We hypothesized that clinical risk factors (RFs) would identify co-infected persons with higher risk of seronegativity (SN) for HCV.

Methods: 200 HIV-infected, SN-HCV participants (pts) of the CHARTER (CNS HIV AntiRetroviral Therapy Effects Research) study were selected. HCV RNA was measured using Roche Amplicor. Comprehensive neuropsychological testing was performed and overall performance was summarized by the Global Deficit Score (GDS). Variables were transformed as indicated by their distributions and routine statistical methods were used, including Fisher's exact test, correlation coefficients, and multivariate regression.

Results: Twenty-six (13%) pts had detectable HCV RNA. This was associated with IDU (57% vs. 30%, $p = 0.023$), high ($>50$) ALT (77% vs. 41%, $p = 0.001$), high AST ($>50$) (62% vs. 33%, $p = 0.008$), low ($<200$k) platelets (69% vs. 38%, $p = 0.005$), ethnicity (black/other; 62% vs 42%, $p = 0.09$), and undetectable HIV plasma RNA (58% vs. 36%, $p = 0.05$). Multivariate logistic regression analysis identified 5 independent RFs: IDU, high ALT, low platelets, black race, and undetectable HIV plasma RNA. A composite sum of the six RFs measure identified that 22/68 (32%) participants who had 2 or 3 RFs had SN-HCV. Among those without confounding comorbidities, SN-HCV was associated with worse GDS values (median 0.67 vs. 0.40, $p = 0.04$). Among those with SN-HCV, higher HCV RNA levels were associated with worse GDS values ($rho = 0.40$, $p = 0.07$).

Conclusions: A small number of clinical risk factors identified HCV viremia in a substantial minority of HCV seronegative PLWH. The clinical significance of this finding is heightened by the observation that those with SN-HCV had worse neuropsychological performance. This approach could help focus screening for HCV viremia in those at highest risk.

## P19
### Artificial microRNA targeted against viral polymerase inhibits Venezuelan equine encephalitis virus replication

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Venezuelan equine encephalitis virus (VEEV) is a member of the alphavirus in family togaviridae. VEEV is a mosquito-borne virus that may cause fatal human diseases. VEEV has been weaponized making it a potential biothreat agent. MicroRNAs (miRNA) are a class of small (19–28nt) endogenous RNA molecules that are key regulators of gene expression. Artificial microRNAs expressed through RNA polymerase II promoters using suitable vectors have been used in many gene silencing studies. VEEV RNA dependent RNA polymerase (RdRp) is central to VEEV replication. Therefore, we hypothesize that targeted inhibition of VEEV RdRp may efficiently inhibit VEEV replication. In this study, artificial microRNAs hairpins were designed against the nsp-4 region of the VEEV genome using Invitrogen Block-iT RNAi Designer. Five artificial microRNAs were cloned into pcDNA6.2-GW/EmGFP-miR (Invitrogen Inc.) which constitutively expresses green fluorescent protein as a marker. BHK cells were transiently transfected with microRNA constructs using lipofectamineTM 2000 (Invitrogen Inc.). Transfected cells were then infected with TC-83 (vaccine strain of VEEV) and were assayed for the inhibition of viral replication. Our results

show that microRNAs effectively inhibited VEEV replication in vitro suggesting that artificial microRNA may be developed as a potential therapeutic agent against alphavirus infection. These studies were supported by JSTO-CBD/DTRA Contract/ Grant/ Intergovernmental Project Order/ Project # 4.10019_07_US_B.

## P20
### Peripheral Macrophage Infection Is Associated with the Development of Simian Immunodeficiency Virus Encephalitis in Pigtailed Macaques

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The brains of individuals with lentiviral-associated encephalitis contain an abundance of infected and activated macrophages. It has been hypothesized that encephalitis develops when increased numbers of infected monocytes traffic into the central nervous system (CNS) during the end stages of immunosuppression. The relationships between the infection of brain and systemic macrophages during the development of lentiviral encephalitis are unknown. We examined the extent of macrophage infection in pigtailed macaques that did or did not develop simian immunodeficiency virus encephalitis (SIVE). At necropsy, macaques with SIVE had more infected macrophages in peripheral organs, with the exception of lymph nodes. T cells and NK cells with cytotoxic potential were more abundant in brains with encephalitis; however, T-cell and NK-cell infiltration in SIVE and human immunodeficiency virus encephalitis was more modest than that observed in classical acute herpes simplex virus encephalitis. These findings support the hypothesis that the development of lentiviral encephalitis occurs in hosts that are unable to control macrophage infection.

## P21
### Molecular mechanisms for decreased antibody response in aged humans

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Elderly humans have compromised humoral and cellular immune responses, which lead to reduced protection to infectious agents and to vaccines. Currently, available vaccines suboptimally protect the elderly population. The capacity to class switch the Immunoglobulin (Ig) H chain is critical to the effectiveness of humoral immune responses in mice and humans. We have previously shown that elderly humans have fewer percentages of CD19+ total B cells, switch memory B cells, and increased percentages of naïve B cells. Activated CD19+ B cells also have less E47, AID and IgG1 circle transcripts (CTs) with age. AID is critical for class switch recombination (CSR) and somatic hypermutation (SHM), both necessary for optimal function of Ig. More recently we have found the antibody hemagglutination inhibition (HI) assay response to the influenza vaccine is decreased in elderly (as others have previously shown) and this associates with the decrease in AID in the activated B cells from the same subjects. The mechanism that we have previously shown in mice and humans to regulate CSR (E47, AID, IgG CTs) in aging can now be used to evaluate the response to the influenza vaccine and other viral responses. This work is supported by NIH AG-23717 and AG28586 (BBB) and AG025256 and AI064591 (RLR).

## P22
### Morphine Enhances Tat-induced Activation in Murine Microglia

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Cumulating evidence shows that individuals who abuse opiates are more likely to develop HIV encephalitis (HIVE), a condition characterized by inflammation, leukocyte infiltration and microglial activation. Microglia, the resident macrophages of the brain, are essential to the progression of encephalitis first, as monocytes, sheltering the HIV when it invades the brain, and later as microglia acting as long term reservoir of the pathogen. The mechanisms, by which the HIV-1 transactivating protein Tat and opioids exacerbate microglial activation, however, are not fully understood. In the current study we explored the effects of morphine and HIV-1 Tat on the activation of mouse BV-2 microglial cells and primary mouse microglia. Both morphine and Tat exposure caused upregulation of the chemokine receptor CCR5, an effect blocked by the opioid receptor antagonist naltrexone. Morphine in combination with Tat also induced morphological changes in the BV-2 microglia from a quiescent to an activated morphology with a dramatic increase in the expression of the microglial activation marker CD11b as compared with cells exposed to either agent alone. In addition, the mRNA expression of inducible nitric oxide synthase (iNOS), CD40 ligand, IP-10 and the proinflammatory cytokines TNFa, IL-1β and IL-6, which were elevated with Tat alone, were dramatically enhanced with Tat in the presence of morphine. In summary, these

findings shed light on the co-operative effects of morphine and HIV-1 Tat on both microglial activation and HIV co-receptor upregulation, effects that could result in exacerbated neuropathogenesis.

## P23
### Proteomic Fingerprints of Primary Human Neurons treated with HIV-1 Clade B and C Proteins: Implications for Neuro-AIDS

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Background: It is suggested that the degree of neuro-AIDS vary according to the HIV-1 clade, but the molecular mechanisms behind the differences still remain unclear. Exploiting proteomics, we hypothesize that clade B and C-Tat and gp120 proteins induce differential protein profiles on primary human neurons.

Methods: In the current study, we used a differential proteomic analysis of primary human neurons treated with clade B and C-Tat and gp120 by two-dimensional gel electrophoresis (2DE), followed by liquid chromatography-tandem mass spectrometry and protein identification to establish homologies and dissimilarities in protein expression.

Results: Proteomic analyses indicated that 432 protein spots were detected in neurons in the size range 15–150 kDa with isoelectric point (pI) values between 4 and 9. Of the clade B and clade C 2D maps detected by image analysis, a total of 205 and 187 proteins were modulated with HIV-1 clade B-Tat and clade B-gp120 treatments respectively compared to 103 and 137 proteins expressed with HIV-1 clade C-Tat treatment and clade C-gp120 respectively. Our results suggest that HIV-1 clade B-gp120 protein upregulates significantly an amino-3-carboxymuconate-6-semialdehyde decarboxylase (ACMSD) signature characteristic of neuropathogenic-response as compared to HIV clade C-gp120. In addition, HIV-1 clade B-Tat protein significantly upregulates the expression of serotonin N-acetyltransferase (SNAT), which occurs to be exclusively associated with ACMSD-induction as compared to HIV clade C-Tat.

Conclusions: Taken together, these data suggest that HIV-1 clade B and C proteins have a differential protein profile. Further, our findings demonstrate for the first time that HIV-1 clade B-Tat and gp120 proteins appear to induce two specific enzymes closely associated with the generation of kynurenine and quinolinic acid which are two major molecules involved in the pathogenesis of HAD.

## P24
### Identification of a novel family of human proteins that restrict HIV replication in astrocytes by targeting Rev

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HIV can persist in the brain for decades by invading cell types like astroyctes that severely restrict HIV replication. Astrocytes suppress the activity of the HIV Rev protein, the key post-transcriptional activator of HIV gene expression, and cause abnormal subcellular localization of Rev. However, the cellular mediators of Rev-inhibition have been elusive. Here we report identification of a novel family of cellular Rev-interacting suppressor (RISP) proteins that interact with Rev in astrocytes. We demonstrate a direct relationship between RISP expression levels and the activity and localization of Rev in astrocytes. Knockdown of RISP expression in persistently HIV-infected astrocytes activated HIV production. Furthermore we demonstrate expression of RISP proteins in human brain tissues. Together our results identify the first family of cellular HIV restriction factors that target Rev and suggest the involvment of these proteins in controlling HIV production in the brain.

## P25
### Treatment of PBMC with the immunosuppressive drug Rituximab reactivates JC virus gene expression and replication in glial cells

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The human polyomavirus, JC virus (JCV), is the etiological agent of the fatal central nervous system demyelinating disease, progressive multifocal leukoencephalopathy (PML). While PML typically

occurs in patients with HIV infection and AIDS, recently, the development of PML is increasingly associated with immunosuppressive treatment for chronic autoimmune diseases, such as Crohn's disease, rheumatoid arthritis, and multiple sclerosis. Immune system control of JCV in the healthy population, where up to 80% of adults contain antibody against the virus, is not well understood. The first step in JCV reactivation requires the initiation of viral early gene transcription and the production of the viral early protein, T-Antigen to initiate the viral life cycle. Analysis of the non coding control region of the viral genome and the early promoter, in particular, has revealed that both tropism and transcription of the JCV are tightly regulated by T-Antigen in concert with multiple transcription factors as well as cytokines and soluble immunomodulators. We decide to investigate how one drug recently associated with the development of PML, Rituxumab, could potentially modify the pattern and expression level of cytokines produced by peripheral blood cells that may affect viral replication in JCV infected brain cells. A significant decrease in the level of the expression of cytokines including MCP-1 and IL-6 was observed in B cells upon treatment with Rituximab. Treatment of JCV infected primary astrocytes with soluble mediators collected from Rituximab-treated B cells showed an increase in viral load. Furthermore, the activity of the JCV early and late promoters in astrocytic cells was clearly increased in the presence of soluble factors released by PBMCs after treatment with the drug and this effect was amplified in the presence of T-Antigen. These results suggest that immunomodulators produced by PBMCs after treatment with Rituximab may create an environment for the reactivation of the JCV life cycle from a latent state leading to the development of PML. Thus, further investigation on specific immune system cells and soluble factors involved in this process will clarify the mechanisms involved in the early steps of JC virus replication.

## P26

### JC virus T-Antigen interaction with Neurofibrmatosis 2 (NF2) protein: effects on NF2 tumor suppressor function and JCV transcriptional activation

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The human polyomavirus, JCV, is the etiological agent of progressive multifocal leukoencephalopathy (PML). The detection of JCV DNA and the expression of JCV proteins in some human tumors including those of the brain and colon, have led to the hypothesis that JCV is able to contribute to the malignant phenotype of these tumors. JCV large T-Antigen is essential for viral replication and is able to interact with a number of cellular target proteins that are responsible for cell cycle regulation including the tumor suppressor proteins, p53 and pRb. In mice transgenic for the JCV early region, we have shown that T-Antigen also associates with the neurofibromatosis protein, NF2, in malignant peripheral nerve sheath tumors. NF2 functions as a tumor suppressor, and the NF2 gene is mutated or inactivated in most schwannomas and meningiomas. The development of these tumors depends on the loss of NF2 but the presence of tumors with wild type NF2 alleles suggests additional mechanisms for inactivating this protein. We therefore investigated the potential effect of T-Antigen on the tumor suppressor function of NF2. While NF2 is able to efficiently suppress the growth of brain tumor cells in vitro in the absence of T-Antigen, we observed that this function is abrogated in cell lines constitutively expressing T-Antigen. By immunoprecipitation, we found that T-Antigen interacts with the N-terminal domain of the NF2 protein encoded by the first 100 aa. This mutant represents the FERM domain of the protein, which is characteristic of band 4.1 cytoskeleton associated protein family members. In the context of JCV transcriptional regulation, T-Antigen mediated activation of the late promoter is strongly limited in the presence of NF2 while, in contrast, NF2 promotes the activity of the early promoter. However, in the absence of T-Antigen, NF2 promotes the activity of both the early and the late promoters suggesting that NF2 may have an independent role at the level of transcription regulation and may act as a transcription factor.

## P27

### Cocaine Abuse and HIV-1 Infection: Role of PDGF/PDGF-Receptor Axis in Disruption of Blood Brain Barrier

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Cocaine, often abused by HIV-infected patients, has been suggested to worsen HIV-associated dementia (HAD) via unknown mechanisms. Blood brain barrier (BBB) is critical for the maintenance of CNS homeostasis and for the regulation of the neural microenvironment. In HIV disease however, disruption of this barrier is what leads to the entry of HIV-infected monocytes into the CNS. The mechanisms by which the monocytes infiltrate into the CNS parenchyma still remain an enigma. In our earlier findings we had demonstrated up-regulation

of the vascular permeant platelet-derived growth factor (PDGF)-B in the newly migrating perivascular macrophages in the brains of macaques with Simian-HIV encephalitis. These findings let us to speculate that modulation of PDGF/PDGF-R axis could be a critical event in the disruption of BBB and enhanced monocyte migration. Mechanistically we demonstrated up-regulation of PDGF-B in monocytes infected with HIV or exposed to cocaine. Reciprocally, we also found that exposure of human brain microvascular endothelial cells (HBMEC) to cocaine resulted in phosphorylation of the PDGF receptor. Furthermore, PDGF-βR activation was associated with increased endothelial permeability. Additionally, we have also shown that both exogenous PDGF as well as cocaine can disrupt the integrity of the BBB in a tissue culture model, with alterations in expression of tight junction and adhesion proteins. Dissection of signaling pathways in PDGF & cocaine-mediated activation of PDGF-βR in HBMECs has implicated the roles of MAPK and FAK/Rho/PKC kinases. These cell culture studies were further validated in vivo in c57Bl/6 mice exposed to either cocaine or PDGF. Exposure of mice to either of these agents resulted in increased BBB permeability and transmigration, as evidenced by Evans blue extravasation & monocyte migration assays, respectively. Furthermore, PDGF-βR inhibitor, iminitab was able to abrogate cocaine/PDGF-mediated enhancement of BBB permeability and transmigration in vivo. Taken together, these findings underpin the role of PDGF-βR as a potential target in the therapeutical intervention of HIVE.

## P28
### Association of Genes Related to HSV-1 Infection with Alzheimer's Disease Risk

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Sporadic Alzheimer's disease (AD) appears to be the consequence of the interaction between combinations of genes and environmental factors (for example virus infections). Increasing evidences point to the possible involvement of herpes simplex virus type 1 (HSV 1) virus in Alzheimer's pathogenesis. We decided to test this hypothesis, by examining if human genes relevant to HSV 1 infection were associated with AD. Up to date, we have found that variants in genes participating in different phases of HSV 1 infection are associated with AD risk: these include i) TAP2, a major target used by HSV 1 to evade immune surveillance, ii) EIF2AK2 (eukaryotic translation initiation factor 2-alpha kinase 2), which codes for PKR, a protein that mediates the virus-induced shut-off of translation; and iii) a combined genotype of ADRB1 and GNB3 associated with increased production of cAMP–an inductor of HSV 1 reactivation in a human neuronal cell model subjected to adrenergic stress. All these results support the hypothesis that variants of human genes participating in HSV 1 infection modulate the susceptibility and/or clinical manifestations of AD. Currently, we are searching for novel target genes related with HSV 1 infection in human neuronal cells. To do this, we perform gene expression analyses in microarrays, followed by a kinetic study in quantitative PCR low density arrays, to select the responder genes/functions in a human neuronal cell model infected with HSV 1.

## P29
### Increased rate of monocyte turnover is predictive of AIDS and SIVE

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Cells of the monocyte/macrophage lineage are major targets for human immunodeficiency virus (HIV) in humans and simian immunodeficiency virus (SIV) in monkeys. Monocytes play critical roles in innate and adaptive immunity during inflammation. We hypothesize that specific subsets of monocytes expand with and drive central nervous system (CNS) and peripheral nervous system (PNS) disease. During SIV infection, monocyte traffic is accelerated and macrophages accumulate in tissues, including the brain and dorsal root ganglion (DRG), over the course of disease. In order to identify monocytes that recently emigrated from bone marrow, we utilized in vivo 5-bromo-2′-deoxyuridine (BrdU) labeling. BrdU, an analogue of thymidine, is incorporated into cellular DNA during replication, at the S-phase of the cell cycle. Monocytes are released from the bone marrow into the circulation shortly after the completion of this phase. By flow cytometry, we can detect BrdU incorporation in monocytes that have recently left the bone marrow and therefore monitor monocyte kinetics over the course of infection. We performed a longitudinal study with five SIV-infected, CD8-depleted rhesus macaques and four non-infected BrdU labeled controls. BrdU injection was performed periodically over the course of infection. Three of the SIV infected animals succumbed to AIDS at days 56, 77, and 89 post-infection and the first two were diagnosed with SIVE. These same animals had significant loss of epidermal fibers consistent with PNS disease. The percentages of BrdU monocytes in these animals

increased dramatically over the course of disease, peaking with the development of AIDS (pre-infection: 2.7%, 0.9% and 1.6%, necropsy: 11.0% (SIVE), 23.4% (SIVE) and 6.3% (no SIVE), respectively). Histopathologic studies showed evidence of BrdU labelled macrophages in brain lesions and in peripheral nerves near DRGs. The percentages of monocyte turnover correlated with disease progression and was a better predictor of AIDS than viral load. Our results suggest that levels of BrdU incorporation in monocytes correlate with disease progression and development of SIVE. BrdU labeled monocytes remained low (less than 2%) in the remaining SIV infected animals that did not develop AIDS, and in the four uninfected CD8 depleted controls. Our data suggests increased monocyte turnover is not linked to CD4 T cell count, is affected by CD8 depletion and rapid CNS disease, but not CD8 lymphocyte depletion alone.

## P30

### The tetracycline antibiotic minocycline prevents the activation of CD14/CD16 monocytes in blood and accumulation in the brain, as well as the development of SIV encephalitis in rhesus macaques

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Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infects the central nervous system (CNS) and often results in a pathological state termed neuroAIDS. Although highly active anti-retroviral therapy (HAART) is generally effective in lowering plasma viral load, neurologic complications in HIV-infected patients are still prominent. Minocycline is a commonly used broad-spectrum tetracycline antibiotic that has immune suppressive effects and has recently been shown to inhibit both HIV and SIV replication. Current research is examining the possible neuroprotective and anti-inflammatory effects of minocycline against HIV/SIV disease progression. In this study, we utilized a rapid disease animal model where 12 rhesus macaques were infected with simian immunodeficiency virus (SIV) and depleted of CD8 T lymphocytes using a chimeric CD8 depleting antibody. Seven animals were treated with minocycline (4mg/kg) daily starting at day 28 post-infection. MR spectroscopy revealed that minocycline reversed neuronal injury by restoring the NAA/Cr ratios. Minocycline was shown to be neuroprotective. Three of the five non-treated animals developed SIV encephalitis (SIVE); however, none of the minocycline treated animals developed SIVE. The pro-inflammatory CD14+ CD16+ monocyte subset was reduced with the treatment of minocycline and there were decreased inflammatory macrophages in the CNS. Minocycline treatment also resulted in a reduction in naive memory CD4+ T cells. We have demonstrated in this study that in SIV-infected rhesus macaques minocycline suppressed monocytes/macrophage activation and inflammatory macrophage accumulation in the CNS. These decreases resulted in inhibition of encephalitis and neurodegeneration associated with neuroAIDS.

## P31

### α7-nicotinic acetylcholine receptors are up-regulated after exposure to HIV-1 gp120: potential implication in the pathogenesis of HIV-1 associated dementia

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Human immunodeficiency virus 1 (HIV-1) is the etiological agent that causes acquired immunodeficiency syndrome (AIDS). Also, arising from this disease, a significant percentage of the affected population can develop severe neurological impairments of the central nervous system (CNS) that lead to dementia and peripheral neuropathy. One of these neurological diseases is know as HIV-1 associated dementia (HAD). The neurophathogenesis of this illness is yet to be characterized since the number of cells that are productively infected is relatively small and consist primarily of macrophages and microglia. However, HAD causes neuronal loss and injury. Various mechanisms have been proposed and implicate the HIV-1 coat protein, gp120 as an important factor mediating neuronal injury in the brain. It has also been shown that α7-nicotinic acetylcholine receptors (α7-nAChRs) might have an involvement in mechanisms preceding neuronal injury. To examine the potential role of α7-nAChRs in the development of HAD, we are using a transgenic mice model expressing the gp120 gene. Using the gp120-transgenic mice line, our laboratory has shown that α7-nAChRs, labeled with the specific α7-nAChRs antagonist α-bungarotoxin, are upregulated in vivo on macrophages and at a central level, as shown by confocal microscopy imaging. The upregulation of the α7 nAChR in vivo could have serious implications in HIV/AIDS ranging from neurodegeneration to inflammation

in HIV-infected patients. The results from these experiments could lead to novel therapeutic strategies that would increase life expectancy and also enhance cognitive deficit and overall quality of life in people living with HIV/AIDS.

## P32

### Human brain microvascular endothelial (HBMVE) cells in PML pathogenesis: Friends or foes?

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Progressive multifocal leukoencephalopathy (PML) caused by JCV is an important cause of morbidity and mortality among immunocompromised individuals. Despite the fact that PML results from active JCV replication in oligodendrocytes in CNS, it is yet unclear how JCV disseminates across the BBB.

Methods: We employed primary HBMVE cells and human brain cortical astrocytes (HBCA), derived from adult brain, to develop an in vitro BBB model and used it to study the possible mechanism(s) of JCV trafficking across the BBB.

Results: HBMVE cells in-vitro did not express alpha 2,3-linked sialic acid but robustly expressed alpha 2,6-linked sialic acid. Neuraminidase treatment reduced alpha 2,6-linked sialic acid expression by more than 80% and significantly reduced JCV genome and transcripts expression in HBMVE cells. Moreover, cell-free infectious JCV virions transmigrated across the in-vitro BBB after replicating in HBMVE cells beginning on day 7 post-inoculation and neuraminidase treatment significantly reduced JCV transmigration. Interestingly, JCV did not alter the BBB permeability and JCV virions were mostly secreted into the upper chamber after replication in HBMVE cells.

Conclusions: Our data suggest that JCV infection of HBMVE cells and transmigration across the BBB is mediated by alpha 2,6-linked sialic acid. While HBMVE cell facilitates it also effectively limits JCV transmigration across the BBB, thereby explaining the low rate of JCV detection in brains from healthy individuals in spite of very high JCV infection rate in the general population.

## P33

### Experimental Herpes Simplex Virus-1 Encephalitis induces Neural Stem Cell Proliferation and Migration

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Neurological deficits are common in patients surviving Herpes Simplex virus encephalitis (HSE), which is mediated in part by immune responses to infection. Previous studies in our laboratory, using a murine model of experimental HSE, have shown that T lymphocytes persist in the infected brain long after viral clearance. This chronic inflammation was associated with both neuropathology and spatial memory deficits in infected animals. Neural stem cells (NSCs) have been shown to respond to inflammatory cues within the brain. NSCs migrate to lesioned areas, differentiate into functional brain cells, and potentially play a role in the reparative process. We hypothesized that NSCs would respond to sustained inflammation ensuing HSE. Increased numbers of actively dividing BrdU-labeled cells were observed in the subventricular zone (SVZ), a known NSC niche, among HSV infected animals compared to uninfected controls. Some BrdU labeled cells in SVZ also immunostained with GFAP, indicating proliferation of type B astrocyte-like stem cells. To further characterize NSC responses in vivo, stem cells derived from luciferase-transgenic mice were transplanted into the SVZ 5 d prior to intranasal HSV-1 infection. Luciferase activity from the transplanted NSCs was found to be 2–4 fold greater among HSV-1 infected animals during the first 3–12 d p.i indicating that proliferative responses begin early during the infection. Interestingly, increased cranial bioluminescence was detected in the caudal third of the brain up to 30 d p.i, suggesting migration of transplanted NSCs. Additional experiments are underway to determine if NSC responses during HSE are shaped by the inflammatory milieu. These studies will provide insights that may facilitate the development of novel therapies for the neurological deficits seen in the wake of viral encephalitis.

## P34

### Subclinical reactivation of JC virus in blood and urine of multiple sclerosis patients treated with natalizumab

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Background: Progressive Multifocal Leukoencephalopathy (PML) has occurred in 6 natalizumab-treated multiple sclerosis (MS) patients. JC virus (JCV), the etiologic agent of PML, infects most adults asymptomatically. We sought to determine whether natalizumab exposure causes subclinical reactivation and neurotropic transformation of JCV.

Methods: We followed 19 natalizumab-treated MS patients over 18 months and measured JCV reactivation by quantified PCR in blood and urine, using the JCV-related polyomavirus BK as control. We determined JCV-specific T-lymphocyte responses by ELISPOT, antibody responses by ELISA and analyzed JCV regulatory region (RR) sequences.

Results: JCV prevalence in urine increased from 19% before, to 63% of patients after 12 months of natalizumab therapy ($p = 0.02$). JCV became detectable in 3/15 (20%) available plasma samples and 9/15 (60%) available PBMC samples after 18 months of treatment ($p = 0.02$). JCV RR sequences in PBMC were similar to those usually found in PML. Conversely, BKV remained stable in urine and was undetectable in blood. The cellular immune response against JCV dropped significantly between 6 and 12 months of treatment, and variation of the cellular immune response over time tended to be greater in individuals who developed JC viremia. None of the patients showed any clinical or radiological sign of PML.

Conclusions: Subclinical JCV reactivation occurs frequently in natalizumab-treated MS patients, and appears to originate first in the kidneys before becoming detectable in the blood, where the virus is predominantly cell-associated. Viral shedding is associated with a transient drop of the cellular immune response against JCV. Molecular monitoring of JCV may allow early detection of natalizumab-treated patients at risk of PML.

## P35

### Neuropsychiatric changes during hepatitis C treatment in patients with HIV and substance use comorbidities

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Introduction: Patients receiving treatment for hepatitis C virus (HCV) infection often complain of neuropsychiatric symptoms, including depressed mood, fatigue, and cognitive dysfunction. At the HIV Neurobehavioral Research Center, we are following the course of neuropsychiatric changes in a group of HCV+ patients receiving treatment with pegylated interferon and Ribavirin at the UCSD Liver Center, San Diego, CA, USA.

Method: Of 88 patients enrolled to date, 34 have reached 6 months on treatment and have complete neuropsychological and psychiatric assessments. Patients were 16 men and 18 women, with a mean (SD) age of 48.5 (8.7) and 12.6 (2.0) years of education. All had detectable HCV viral load at baseline, 79% are genotype 1, and 24% had fibrosis scores of 3 or 4. Ten (30%) were HIV+ and most (85%) had a lifetime history of substance abuse or dependence, including 50% for alcohol and 35% for cocaine, as well as 17% meeting dependence criteria for methamphetamine with use within the last 18 months. None met current criteria for substance use disorders. History of major depressive disorder was present in 44% of the sample. In addition to their routine HCV care, participants received a comprehensive neuropsychological assessment, a neurologically focused medical exam, and assessment of mood status and fatigue.

Results: Mean current depressive symptoms as measured by the Beck Depression Inventory (BDI) increased from 10.2 (6.4) at the pre-treatment baseline, to 14.0 (9.4) ($p < .05$) at 6 months on treatment. The proportion of patients meeting current criteria for MDD increased from 9% to 13% (p, not significant (NS)). Overall levels of fatigue measured by the Multi-Symptom Fatigue Inventory also tended to increase [15.6 (22.4) to 22.4 (25.9), (p, NS)]. Concurrently 24% of patients exhibited global neuropsychological impairment at baseline, compared to 35% at the 6-month time point. Neuropsychological decline was not associated with HIV status, prior history of substance use disorders, or current depressive symptoms. Those with neuropsychological decline ($n = 15$) reported greater overall fatigue at follow-up [32.0 (24.3)] compared to those who improved or had no change ($n = 18$) [14.5 (25.2)] ($p = .03$). This included worsening general complaints of fatigue [11.5 (8.0) vs. 6.8 (8.1), ($p = .05$)], physical fatigue [9.1 (6.7) vs. 4.5 (1.5), ($p = .03$), mental fatigue [7.9 (5.9) vs. 4.8 (6.8), ($p = .090$], and psychological distress [3.5 (5.9) vs. −1.6 (6.9), ($p = .02$)].

Discussion: Although only change in current depressive symptoms and fatigue reached statistical significance in this small sample, there was a trend toward worsening neuropsychological and neuropsychiatric status at 6 months on HCV treatment. Findings corroborate patient reports and clinician

observations of mood, stamina, and cognitive changes during HCV treatment.

## P36
### Rapid Transient Increases in mRNA Levels of Transforming Growth Factor βeta- 1 in Primary Rat Astrocytes by Nef

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Even after the introduction of High Active Antiretroviral Therapy (HAART), motor and cognitive impairments develop in HIV infected people. Nonproductive infection in astrocytes, through the synthesis of viral proteins (including Nef), contributes to this neuropathology. Nef alters cellular signaling pathways through interactions that may depend on myristoylation of its N-terminus. One of these interactions is between calmodulin (CaM) and Nef which promotes the release of cytokines. Our hypothesis is that the interaction of CaM and Nef alters signal transduction which results in the production of proinflammatory molecules. We tested our hypothesis in cultures of Sprague Dawley primary rat astrocytes and examined levels of Transforming Growth factor β-1 (TGF β-1) and Monocyte Chemoattratant Protein (MCP-1). Nef was delivered by transfection and real time RT-PCR was used to measure changes in mRNA levels of cytokines. In addition, astrocytes expressing Nef were infused in 30-day-old Sprague Dawley rat in the hippocampus to see neuroenviromental changes. We noted a TGF β-1 increases in early time points ($<24$hrs) and an increases of MCP-1 in 48 hrs ($p < 0.05$). Moreover, we noted a cell aggregation in rats infused with astrocytes expressing Nef, but not in control rats. In conclusion, this data suggests Nef upregulation of TGF β-1 may regulate downstream chemokines, such as MCP-1, that are important pathological contributors to HIV-Neuropathology. This work provides support that Nef signaling in astrocytes may have a role in causing inflammation and damage in HIV neuropathology. This work is supported by GM082406, GM008239, and RR003050.

## P37
### Role of LGP2, a cytoplasmic RIG-I-like helicase, on neuronal innate immune gene expression during rabies virus infection

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Rabies virus infection of the nervous system (NS) is accompanied by a moderate inflammatory and interferon response that might be important for rabies immunosubversive strategy. This innate immune response in infected neurons may be the result of detection of viral 5′ triphosphate RNA by the cytoplasm helicase RIG-1. LGP2, a RIG-1-like helicase, has been proposed to function as a feedback inhibitor of innate immune signaling triggered by RIG-1. To analyze further the contribution of LGP2 in rabies virus-induced innate immune response, we generated transgenic mice over-expressing human LGP2 (hLGP2) and further compare in such mice and in their normal counterparts, the course of the disease and expression of critical immune mediators. Remarkably, an up-regulation of inflammatory cytokines (IL-6 Rantes, MIP-1gamma, MCP-1, IFN-beta, and IFN-beta-inducible genes such as B7-H1 was observed in wild-type (WT) but not in hLGP2 mice. Thus, hLGP2 acts as an inhibitor of IFN-beta and inflammatory cytokine induction in the NS during rabies. Similar results were obtained in cell culture over-expressing hLGP2. Moreover, preliminary results indicated that hLGP2 mice were able to clear the virus off the brain and survived better than WT mice, suggesting that IFN-beta induction could be important for the successful immunosubversion of the rabies virus in the NS. These data are consistent with a previous observation reporting that TLR3-/- mice- in which IFN-beta and B7-H1 expression were reduced- survived better after a rabies virus challenge than WT mice (Lafon et al, 2008). This work was supported by internal grants of Pasteur Institute, including PTR 186.

## P38
### Gene Expression in the Brain During Virus-Induced Encephalitis

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Reovirus-induced encephalitis is associated with activation of transcription factors, including NF-kB, c-Jun and STAT1. These transcription factors have also been shown to play a role in reovirus-induced neuronal apoptosis and/or disease severity. However, gene expression changes resulting from transcription factor activation in reovirus-infected brains have not been investigated. Microarray analysis of RNA extracted from reovirus-infected, compared to mock-infected, brains at 8 days post

infection (pi) identified 188 probe sets that were differentially regulated with a false detection rate (FDR) of $<2\times10-4$ ($p<1\times10-6$). Multiple probe sets representing individual genes were then combined and genes that were differentially regulated less than 2-fold were discarded. This resulted in a total of 160 differentially regulated individual genes in reovirus-infected, compared to mock-infected brains. A large subset (40) of these genes were interferon-stimulated genes (ISGs) with suggested roles in antiviral defense or apoptosis. Within this group the fold increase in expression ranged from 2–500 and was confirmed by real time PCR. Many of the genes within this group were also up-regulated at 3 days pi ($p<0.002$) indicating that these genes are up-regulated at early times and that up-regulation persists throughout the infection.

## P39
### Regulation of Neuronal ADAM17 by the Chemokine SDF/CXCL12 and its Role in Fractalkine/CX3CL1 Cleavage

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Fractalkine/CX3CL1 is a neuronal chemokine acting as a transmembrane protein or as a soluble factor following cleavage of its extracellular chemokine domain, and has been implicated in the regulation of microglia-induced neurotoxicity. Little is known, however, concerning the specific role of soluble fractalkine in the CNS or the regulation of fractalkine cleavage by neurons. This study identifies SDF-1/CXCL12 (another neuroprotective chemokine) as a positive regulator of fractalkine cleavage from neurons as measured by ELISA, and demonstrates the homeostatic effects of glia on soluble fractalkine accumulation. Furthermore, analogously to non-neuronal cells, constitutive fractalkine cleavage is shown to be mediated predominantly by the metalloproteinase ADAM10, and SDF-1-induced cleavage to rely on an up-regulation of the metalloproteinase ADAM17. This study also identifies excitotoxic NMDA as a short-term inhibitor of alpha-secretase activity and fractalkine cleavage, prior to inducing cell death. Together these data support a novel regulatory mechanism which may contribute to the modulation of neurotoxic microglia responses in vivo, and suggest a unique role of soluble fractalkine in this process.

## P40
### Genetic and functional analysis of HIV-1 Tat derived from central nervous system isolates of patients with HIV-1 associated dementia

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Objectives: HIV-1 penetrates the central nervous system (CNS) and frequently causes HIV associated dementia (HAD) or less severe forms of neurocognitive impairment. While macrophages and microglia are the sites of productive infection in the CNS, astrocyte infection is restricted and non-productive. Nonetheless, astrocytes exhibit dysfunction and undergo apoptosis, contributing to the pathogenesis of HAD. Our recent analysis of the transcriptional activity of long terminal repeats (LTRs) from CNS-derived HIV-1 demonstrated decreased basal transcriptional activity in astrocyte cells compared to lymphoid-derived LTRs from the same patient. These data suggest that unique HIV-1 transcriptional regulatory mechanisms exist in the CNS. Tat is a potent transcriptional activator of the HIV-1 LTR, and has additional roles in the pathogenesis of HAD through neurotoxic and chemo-attractant properties. Understanding the role of Tat in the transcriptional regulation of HIV-1 in the CNS will provide insights into the pathogenesis of HAD. In this study, we investigated Tat sequences from a cohort of matched CNS and lymphoid-derived HIV-1.

Methods: Tat sequences were PCR-amplified from a cohort of seven HAD autopsy subjects with matched CNS-(frontal lobe, cerebral spinal fluid, or spinal cord) and lymphoid-(lymph node, spleen, or PBMC) derived isolates. Multiple, independent PCR products were pooled and cloned into the pTargeT vector (Promega USA). Independent clones were sequenced and subjected to phylogenetic analysis using a neighbor joining algorithm. CNS and lymphoid derived Tat plasmids were co-transfected with a HIV-1 LTR luciferase reporter into the SVG astrocyte cell line and Jurkat T-cell line. Tat-mediated LTR transactivation was assayed by luciferase activity in cell lysates.

Results and Conclusions: Sequence and phylogenetic analyses of CNS and lymphoid derived Tat sequences showed patient specific clustering, with a subset compartmentalised to reflect the tissue of origin. Heterogeneity was observed in the ability of both CNS and lymphoid derived Tat to transactivate the LTR, reflecting sequence changes within functional domains of Tat. Multiple sequence changes were also observed in neurotoxic and chemo-attractant domains, suggesting potential alterations in

neurotoxic and chemo-attractant properties. Taken together, these data suggest the presence of unique Tat sequences within the CNS impacting on transcriptional regulation of HIV-1 in the brain.

## P41
### Cognitive decline over one year among HIV-infected former plasma donors in China

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Objective: To quantify incidence and nature of cognitive decline over one year in a cohort of HIV-infected (HIV+) former plasma donors in China, using Western neuropsychological (NP) tests and normative standards that adjust for practice effect (PE) and other factors affecting test-retest variability.

Methods: We developed norms for change using the NP data from 101 demographically comparable HIV-negative (HIV−) former plasma donors. The NP test battery covered seven cognitive ability domains. We used the multiple regression change score approach with the HIV− sample to derive the norms for change that were then be applied to 192 HIV+ participants. Followup test scores were adjusted for the control group median PE to more precisely classify NP impairment at the second testing.

Results: Among the HIV+ individuals, 27% (N = 53) developed significant cognitive decline. Baseline history of immunosuppression, lower CD4 at followup, lower viral load when on cART, AIDS status, and lower baseline performance in processing speed were associated with cognitive decline. NP decline also was associated with decreased independence in accomplishing activities of daily living. Using NP-impairment scores that were PE-corrected, we found that among the decliners, 41.5% (N = 22) had incident impairment, 38% (N = 20) declined within the impaired range and another 20.7% (N = 11) declined within the normal range.

Conclusion: This study is the first to adapt regression-based norms for NP change in a developing country. Substantial cognitive decline and persistence of NP-impairment despite cART confirm that neurocognitive complications in HIV-infection remain a concern worldwide.

## P42
### HIV-associated neurocognitive impairment in the context undetectable plasma viral load

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Objective: To determine the prevalence of neuropsychological (NP) impairment in the context successful combination antiretroviral therapy (cART), defined as plasma HIV RNA $<50$ copies/ml, in an advanced HIV-infected cohort and to assess which laboratory, clinical and treatment factors are associated with this type of impairment.

Methods: 116 HIV-infected individuals (Stage CDC C3) were enrolled on cART and screened out for major psychiatric confounds including current substance use. All were assessed with standard NP testing covering six cognitive domains.

Results: In this cohort, 51% had undetectable plasma viral load. Demographically corrected NP-impairment reached 18% in undetectable HIV-infected individuals which was not statistically different from 24% in detectable individuals. In comparison with NP-normal and undetectable individuals, NP-impairment in undetectable individuals was associated with shorter duration of current cART and lower pre-morbid abilities, while cART CNS penetration effectiveness was associated with lesser cognitive severity in NP-impaired-undetectable individuals. CD4-T cell count level, depression or anxiety symptoms, and past CNS HIV-related diseases were not explanatory of NP-impairment in undetectable individuals.

Conclusions: This study provides cumulative evidence that despite suppression of systemic viral load, NP-impairment is present in a non-negligible proportion of HIV-infected individuals. Plausible explanations for persistent NP-impairment in virally suppressed individuals are multi-factorial but slow recovery after a new cART initiation seems to be important and perhaps more so when the regimen is less CNS penetrative. Other more speculative factors such as neurodegenerative processes (as well as vascular-related cognitive impairment) facilitated by chronic HIV infection and neurotoxcity require further studies.

## P43
### The effect of age in HIV-associated neurocognitive impairment before and after the era of combination therapy

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Objective: To determine the extent of combined age and HIV effects on cognitive functions in individuals with HIV-associated neurocognitive disorder (HAND).

Methods: Participants were recruited across the pre-combination antiretroviral therapy (cART) era and cART era in Australia where HIV risks factors have remained stable. In the pre-cART cohort, 52 HIV-infected (HIV+) men with HAND were receiving a dual-therapy of zidovudine/didanosine. In the cART cohort, 72 HIV+ men with HAND were receiving at least three ART. 33 HIV negative (HIV-) men in the pre-cART era and 30 in the cART era were also enrolled. Pre-cART cohorts were younger by 10 years on average. All were assessed with a medium size neuropsychological battery targeting HIV-related impairment. The influence of age on HAND was investigated using standard multiple regression on a demographically uncorrected score.

Results: The strength of a negative association between age and cognitive performance was equivalent in both HIV− groups and in the cART HAND group. Unexpectedly increasing age was associated with better cognitive performance in the dual-therapy HAND group likely reflecting suboptimal treatment in this group. In analyses focusing on the subgroup of individuals with HIV-associated dementia (HAD), a trend for a steeper age effect on cognitive impairment was observed only in patients receiving cART.

Discussion: Consistent with previous cross-sectional studies increasing age is associated with greater cognitive deficits in all aged HIV− controls and most middle-aged HAND individuals. Tentative evidence for a compounding HIV and age effect on cognitive deficits in middle-aged demented individuals on cART needs further consideration.

## P44

### Phosphatase activity of a protein from St. John's Wort modulates alcohol-induced neuronal cell injury

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p27SJ is a novel truncated protein isolated from St. John's Wort (Hypericum perforatum) that belongs to an emerging family of DINGG proteins which are related to a prokaryotic phosphate-binding protein superfamily. Previously, we demonstrated the ability of p27SJ to suppress HIV-1 replication via cross-communication with C/EBPβ and other factors. Here we demonstrate that the p27SJ full-length protein purified from the plant, p38SJ, protects neuronal cells from injury induced by ethanol. Furthermore, pre-treatment of neuronal cells with p38SJ diminishes the level of the pro-apoptotic protein BAX and cleavage of caspase 3. In addition, reduced levels Erk1/2 were observed upon treatment of ethanol-exposed cells with p38SJ. Accordingly, we demonstrate that p27SJ exhibits phosphatase activity and that its expression in cells decreases the level of phosphorylated Erk1/2, a key protein of several signaling pathways. Treatment of p27SJ-expressing cells with phosphatase inhibitors including okadaic acid, maintained Erk1/2 in its phosphorylated form, suggesting that dephosphorylation of Erk1/2 is mediated by p27SJ. Expression of p27SJ also affects Erk1/2 downstream regulatory targets such as STAT3 and CREB. Moreover, the level of expression of cyclin A that associates with active ERK1/2 and is regulated by CREB, was modestly reduced in p27SJ-expressing cells. Results from in vitro kinase assays also revealed a noticeable decrease in the activity of cyclin A in cells expressing p27SJ and cell cycle analysis demonstrated dysregulation at S and G2/M phases in cells expressing p27SJ, supporting the notion that a decline in cyclin A activity by p27SJ has a biological impact on cell growth. These observations provide evidence that p27SJ alters the state of Erk1/2 phosphorylation, and impacts several biological events associated with cell growth and function. Supported by a grant awarded by NIH/NIMH to SA.

## P45

### Pur-alpha and HIV-1 Tat affect neuronal differentiation and neurite outgrowth via the Rac/Rho signaling pathway

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The molecular mechanisms whereby viral and host factors collaboratively alter neuronal function, leading to neuronal cell injury, are a key question in the pathogenesis of HIV-1 associated neurological disorders. RhoGTPases and related molecules play an important role in neuronal cell development, including neurite outgrowth, differentiation, axon pathfinding, dendritic spine formation, as well as neuronal cell maintenance. Results from our studies indicate that Tat has the ability to disturb this

pathway by promoting RhoA activation, and by simultaneously blocking activation of another key RhoGTPase, i.e. Rac1, and its partner p21-activated kinase 1 (PAK1), which are important regulators of actin cytoskeletal dynamics, neuronal cell integrity, and neurite outgrowth. More specifically, Tat dysregulates cell signaling resulting in prevention of neurite outgrowth in NT2 cells treated with the RhoA inhibitor, C3 transferase. In addition to promoting the activity of RhoA and inhibiting the activity of Rac1, the basal levels of RhoA and Rac1 proteins are also affected by Tat treatment, i.e. upregulation of RhoA and downregulation of Rac1 protein. The cellular protein, Pur-alpha, is a key binding partner of Tat for directing the regulation of HIV-1 transcription, translation, and viral RNA transport. Results in the Pur-alpha knockout mouse model have revealed a critical role for Pur-alpha during development, particularly in the coordinated development and differentiation of neuronal cells throughout the brain. Our data shows that silencing of Pur-alpha leads to inactivation of Rac1 and stabilization of RhoA, which means that Pur-alpha would promote neurite outgrowth thereby having the opposite effect of Tat treatment on neuronal cells. However, overexpression of Pur-alpha may also lead to neurite retraction indicating that the concentration of Pur-alpha in neuronal cells is critical for neuronal differentiation. In support of this concept, Rac1 and RhoA were found to display aberrant expression and subcellular localization in the Pur-alpha knockout mouse model, suggesting that Pur-alpha is required for proper neuronal outgrowth. Based on these observations, we hypothesize that by disturbing the well-balanced activities of Rac1 and RhoA, Tat interferes directly and/or indirectly via Pur-alpha binding with the function of Rac1 and RhoA signaling pathways resulting in neuronal cell injury in the context of HIV-1. Further study will reveal the molecular basis of Tat's cross-talk with the Rac1 and RhoA pathways in the presence and absence of Pur-alpha and its impact on neuronal cell integrity.

## P46
### Mechanisms of primary axonal damage in a viral model of multiple sclerosis

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Recent studies have demonstrated that significant axonal injury also occurs in MS patients and correlates with neurological dysfunction, but it is not known whether this neuronal damage is a primary disease process, or occurs only secondary to demyelination. In the current studies, neurotropic strains of mouse hepatitis virus (MHV) that induces meningitis, encephalitis, and demyelination in the CNS, an animal model of MS, were used to evaluate mechanisms of axonal injury. The pathogenic properties of genetically engineered isogenic spike protein recombinant demyelinating and non-demyelinating strains of MHV were compared. Studies demonstrate that a demyelinating strain of MHV causes concomitant axonal loss and macrophage-mediated demyelination. The mechanism of axonal loss and demyelination in MHV infection is dependent on successful transport of virus from gray matter to white matter using the MHV host attachment spike glycoprotein. Our data show that axonal loss and demyelination can be independent direct viral cytopathic events, and suggest similar direct axonal damage may occur in MS. These results have important implications for the design of neuroprotective strategies for CNS demyelinating disease, and our model identifies the spike protein as a therapeutic target to prevent axonal transport of neurotropic viruses.

## P47
### Role of Sumoylation in Complement component C3 gene regulation

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The complement system is an integral component of the innate and adaptive immune response in the defense against invading pathogens. However, uncontrolled complement biosynthesis and activation as a result of excessive IL-1b synthesis as observed in patients with acute (stroke, trauma) and chronic neurodegenerative diseases (such as HIV dementia, Alzheimer's disease, Parkinson's disease) can be injurious to host tissues. We have demonstrated earlier that IL-1 beta induces C3 synthesis in astrocytes and monocytes in a C/EBP dependent manner. We now investigated whether IL-1 beta and C/EBP mediated C3 promoter activation is regulated by the enzymes of the SUMO pathway. Our studies demonstrate that overexpression of E3

SUMO ligase, PIASy in astrocytes and monocytes inhibits not only IL-1 beta but also C/EBP-delta and C/EBP-beta isoform LAP mediated C3 promoter activation. On the contrary, overexpression of SUMO protease, SENP1 further enhances the transcriptional response of the C3 promoter by IL-1 beta and C/EBP. Furthermore, site directed mutagenesis of the SUMO recognition motif ''yKXE/D'' in LAP and C/EBP delta increases C3 promoter activity. These observations demonstrate that modification of the transcription factor C/EBP by sumoylation and desumoylation play significant role in C3 gene regulation.

## P48

### Morphine-treatment of human monocyte-derived macrophages induces differential miRNA expression and affects secretion of FGF-2, MCP-2 and IL-6

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HIV-1 infected drug abusers are known to exhibit in some cases an accelerated form of HIV-1 associated dementia. The concomitant effects of HIV-1 infection and drugs of abuse could potentially lead to enhanced neurological dysfunction. Our current understanding of the molecular basis of this dysfunction is limited. HIV-1 infection of the Central Nervous System (CNS) is associated with macrophage infiltration, formation of microglial nodules and multinucleated giant cells, activation of astrocytes and loss of neurons, predominantly in the hippocampus and basal ganglia. HIV-1 productively infects perivascular macrophages and microglial cells. In order to identify putative novel effectors that might mediate the damage afflicted by morphine in the CNS, we utilized miRNA from morphine-treated human monocyte-derived macrophages (h-mdms) in a miRNA array hybridization analysis. In addition, we determined secretion of chemokines, cytokines and growth factors as a result of morphine-treatment. A total of 27 differentially expressed miRNA were identified ($p < 0.01$) of which hsa-miR-15b had maximal increase in expression levels. MCP-2 and IL-6 secretion was induced by morphine. In addition, FGF-2 secretion was decreased as a result of morphine. Of note, FGF-2 transcript was predicted as one of the targets for hsa-miR-15b. Collectively, these observations suggest a potential novel mechanism whereby the blood brain barrier integrity could be compromised, leading to an influx of HIV-1 infected monocytes and enhanced viral transcription as a result of decreased expression of FGF-2, MCP-2 and IL-6, respectively.

## P49

### Novel anti-inflammatory actions of the opioid receptor antagonist, beta-funaltrexamine

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Approximately 10–15% of HIV-1 infected individuals present with HIV-associated encephalitis and HIV dementia, collectively termed neuroAIDS. Increasing evidence suggests that astroglial mediated inflammation contributes to the neuronal damage associated with neuroAIDS. Astrocyte-derived chemokines, including CCL2 and CXCL10, have been implicated as inflammatory molecules involved in neuroAIDS. To model a key aspect of HIV-associated neuroinflammation human astroglial cells are exposed, in vitro, to TNFα and HIV-1 Tat1-72 resulting in the induction CCL2 and CXCL10. We recently found that the opioid receptor antagonist, β-funaltrexamine (β-FNA) inhibits proinflammatory-induced astroglial chemokine expression. The mechanism by which β-FNA inhibits astroglial chemokine expression remains to be determined. Initial findings suggest that β-FNA may exert its anti-inflammatory actions through an opioid receptor independent mechanism. Data further indicate that β-FNA mediated inhibition of chemokine expression may be related to an attenuation of NF-κB activation. Identification of the mechanism governing the anti-inflammatory actions of β-FNA may be instrumental in the development of therapeutic strategies to prevent and treat neuroinflammatory conditions including, HIV-1 neuropathogenesis.

## P50

### Reactivation of latent viral infections in patients receiving natalizumab for the treatment of multiple sclerosis

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Background: Reactivation of JC polyomavirus (JCV) has been reported in patients receiving the α4-integrin monoclonal antibody natalizumab for multiple sclerosis (MS). The development of JCV-induced progressive multifocal leucoencephalopathy (PML) has also been described. We hypothesised that another polyomavirus, BK (BKV), could reactivate and cause disease in this patient group. We report here our findings from an Irish cohort.

Methods: Patients receiving natalizumab therapy for active relapsing remitting multiple sclerosis (RRMS) were enrolled in a study to identify potential predictors of polyomavirus reactivation. Blood and urine samples were collected for JCV and BKV DNA at baseline, monthly for three months, then three monthly thereafter. CD4:CD8 ratios and absolute counts were analysed in peripheral blood at the same intervals, as was renal function. Three-monthly plasma samples were collected and stored for retrospective testing as guided by initial findings.

Results: Fifty-seven (41 female; 16 male) patients with a mean age of 36.1yrs have been treated for a mean of 15 months (range 1–29). Baseline prevalence (in 36 patients studied pre-treatment) for BK viruria was 8.3%. BKV reactivation occurred in 12 (7 female; 5 male) patients (22.2%) after a mean of 11.2 (range 1–23) doses. BK viruria was transient in 10 patients and persistent in two. Persistent viruria was associated with transient viraemia, lasting 1 and 2 months. No evidence for concomitant reactivations of JCV or human herpes virus 5/ cytomegalovirus (CMV) was found. Reactivating BKV subtypes were heterogeneous and no genetic rearrangement in the polyomavirus non-coding control region (NCCR) was detected. CD4 counts fluctuated but remained within normal limits, as did CD4:CD8 ratios. Renal function remained normal.

Conclusions: BKV reactivation occurs with natalizumab therapy: this cohort of patients should be monitored for evidence of renal dysfunction and/or BK viruria where possible.

## P51

### Neuronal subtype specific responses to HIV Transactivator protein, Tat

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Direct neuronal excitation by HIV transactivator protein (Tat) has been demonstrated in numerous experimental systems; however, the selectivity of Tat excitation in neuronal subpopulations remains unknown. The hippocampus is composed of multiple neuronal classes, including glutamatergic pyramidal neurons and GABAergic interneurons. Interneurons represent an extremely diverse neuronal class: in the CA1 region of the hippocampus 16 subtypes have been described based on functional analysis, anatomy, and expression of molecular markers. Previous studies have demonstrated differential expression of NMDA receptor subunits in interneuron subtypes. Because Tat-mediated excitation depends on interactions with NMDA receptor subunits, we are testing the hypothesis that Tat selectively and differentially excites interneuron subpopulations. Whole-cell voltage clamp recordings will be obtained from adult rat hippocampal brain slices. CA1 stratum pyramidale interneurons will be targeted and loaded with biocytin for post hoc immunocytochemical analysis. During whole-cell recording, Tat protein will be locally applied to the dendrites of the recorded interneuron. Both voltage clamp and current clamp modes will be employed to study the membrane currents and their impact on membrane potential, respectively. After recordings, brain slices will be fixed and processed for double label immunohistochemistry for the interneuron markers, cholecystokinin and parvalbumin. Because interneurons play a critical role in normal hippocampal function, the selective functional vulnerability of interneuron subtypes could contribute to cognitive deficits in the absence of overt neuropathology. Supported by the University of Miami Developmental Center for AIDS Research and the Johns Hopkins NIMH Center for Novel Therapeutics.

## P52

### Study of Human Polyomaviruses reactivation in Multiple Sclerosis patients during Natalizumab therapy

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Background: Natalizumab therapy in Multiple Sclerosis has been associated with JC virus (JCV)-induced PML and reactivation of BK virus (BKV) has resulted in BKV-associated nephropathy (PVAN) during other immunosuppressive therapies. As biologic agents aimed at immune system manipulation are currently widely used in the therapy, the risk of infections with latent or acute pathogens continue to be problematic. Since the presence of Human Polyomaviruses DNA in body fluids is thought to represent active viral shedding or replication and since blood testing and urine analysis are less invasive than CerebroSpinal Fluid (CSF) testing, they are an attractive option to reveal some clues about Polyomaviruses reactivation in MS patients on Natalizumab.

Material and Methods: Peripheral blood monocytes (PBMCs), serum and urine from 14 Relapsing Remitting (RR) MS patients subjected to Natalizumab treatment have been monthly collected (mean of follow up: 3; range 1–5) and the genomes of JCV and BKV were searched and quantified by means of specific real time assays. If a clinical specimen was proven positive for the presence of JCV DNA,

molecular characterization of the viral strain was performed.

Results: JCV was found in the PBMCs of 1/14 patients (at time point 4; viral load: 66 copies/ug) and in the urine of 6/14 patients. In particular, JCV reactivation occurred in the urine of 2/14 patients at time points 2 and 3, respectively, whereas JCV viruria decreased in two patients over the time and was stable in two patients. JCV median viral load in the urine was 1.2E+08 (range: 8.45E+05 −9.3E+10). The molecular characterization of the amplified JCV strains revealed the only presence of archetype form and the presence of both genotype 1A (5 patients) and genotype 2B (1 patient). In the urine of one patient the persistent coinfection of JCV and BKV was found.

Conclusions: Genomes of the Human polyomaviruses were not detected in both the PBMCs and sera of RRMS patients, but JCV viruria occurred during Natalizumab therapy. However, so far, the significance of JCV reactivation in the urine remains unclear. Due to the fact that these data are preliminary, we propose regular monitoring for JCV/BKV presence in patients receiving Natalizumab.

## P53

### Human T-Lymphotropic Virus Type I/II Associated with Recurrent Longitudinally Extensive Transverse Myelitis resembling Neuromyelitis Optica. Two case reports

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Purpose: To describe two patients with recurrent longitudinally extensive transverse myelitis (LETM) resembling Neuromyelitis Optica and associated with HTLV- I/II exposure and NMO-IgG antibody in one case.

Background: Neuromyelitis Optica (NMO) is a severe, inflammatory, necrotizing demyelinating disorder of the CNS affecting the optic nerves and long segments of the spinal cord. NMO is a B-cell mediated disease caused by serum autoantibodies (NMO-IgG). In 50–73% of cases, the water channel aquaporin-4 appears to be a specific target for these autoantibodies, suggesting that NMO is a new autoimmune channelopathy. The disease spectrum includes cases of LETM. Half of cases are associated with other autoantibodies such as ANA, and NMO onset may be related to infections and immunizations. HTLV-I is a retrovirus that can cause myelopathy (HAM/TSP) and has been associated with other autoimmune diseases like polymyositis, uveitis and Sjögren syndrome. It has been reported that patients with HAM had a significant dysfunction of the humoral system when compared with asymptomatic HTLV-1 carriers and with seronegative controls. HTLV-II has been associated with spastic ataxia which closely resembles HAM/TSP.

Case #1: 61y/o female, wife of an IV drug abuser, developed Lhermitte's sign followed by rapid onset of tetraparesis in 1999. She had a full functional recovery over three months. In 2006, she had a second episode of transverse myelitis with severe band-like pain at the thoracic level, paraplegia and urinary retention. Neurological exam revealed a T9 sensory level to pain and temperature. MRI of C- and T-spine demonstrated extensive increased T2 signal with gadolinium enhancement from C7 to T4. She had serum NMO IgG, as well as serum and CSF antibody to HTLV-II. Active HTLV-II infection was confirmed by PCR.

Case #2: 51 y/o Jamaican Male who developed severe neck pain with burning and weakness of right hand in September 2003 in NY. MRI of C-spine showed an intramedullary lesion. Systemic corticosteroid treatment led to functional improvement, but in November 2003 he developed numbness of the distal lower extremities followed by a right foot drop. Severe neck pain evolved into a right hemiparesis in December 2003. He became paraplegic and developed urinary retention during admission to another hospital. An MRI of the C-spine revealed a longitudinally extensive (C2–C7) intramedullary, enhancing expansile lesion. On transfer to our institution, about two weeks later, neurological exam showed a prominent right hemiparesis, absence of proprioception in the right upper extremity and in both legs. Gait was very ataxic. The patient had serum antibody to p19 protein on HTLV-I western blot analysis. Serum ANA was positive 1:160.

Discussion: NMO is an autoimmune CNS disorder that causes severe neurological disability. Thus far there is one case in the literature reporting a possible association of NMO and HIV infection. The HTLV-I/II retroviruses are known causal agents of human myelopathy, making the possible association of HTLVI/II with NMO more feasible. Elucidating the mechanisms underlying the immunopathology of NMO in cases associated with neuroviral exposure should contribute to the development of more effective therapies for the optimal management of the affected patients. Patients presenting with LETM should be evaluated for prior or concurrent human retrovirus infection.

## P54

### Local IFNAR Signaling Protects Against Virus Spread Within the Central Nervous System

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Several neurotropic viruses such as vesicular stomatitis virus (VSV) induce peripheral neutralizing immune responses and still can infect cells within the central nervous system (CNS). To address whether local type I interferon receptor (IFNAR) triggering is instrumental in controlling virus replication within the brain, we generated mice with a cell type-specific IFNAR deletion in neuroectodermal cells of the CNS (NesCre+/−IFNARflox/flox). Intranasal VSV infection with 103 plaque-forming units was well tolerated by wild-type (WT) mice, whereas conventional IFNAR-/- mice died within 2–3 days. In contrast, brain-specific NesCre+/− IFNARflox/flox mice survived until day 5–6, then became hemiplegic and died. Terminally sick NesCre+/−IFNARflox/flox mice showed 10 to 100-fold higher virus loads in the brain than IFNAR-/- mice, whereas little or no virus was found in other organs. In WT animals, virus could be re-isolated only from the olfactory bulb until day 6 where also STAT1 activation as a measure of IFNAR triggering was detected. Virus infection was found exclusively in glomerular structures of the olfactory bulb, whereas surrounding cells that showed STAT1 phosphorylation as a measure of IFNAR trigging were free of virus. In conclusion, our data indicate that upon intranasal VSV instillation early and localized IFNAR triggering in the glomerular layer of the olfactory bulb is critically required to prevent viral spread over the whole CNS and thus to confer survival. Currently mice with a specific IFNAR-deletion in olfactory receptor neurons are studied to clarify whether IFNAR triggering of neurons or of other cell types is important.

## P55
### Mechanisms of minocycline: effects on the innate and adaptive immune system during HIV/SIV infection and implications for HIV-associated neurological disease

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Immune connections between the central nervous system and the peripheral immune system are still largely undefined. In the case of HIV infection, new efforts are underway to discern whether the chronic activation of the innate and adaptive immune system in the peripheral organs contributes to CNS disease. Though current HAART therapy effectively reduces viral replication in plasma, up to 30% of patients still succumb to neurological sequelae such as motor/cognitive disorders or dementia. Thus, there remains a need for therapeutic agents that can prevent or lessen the severity of neurological disease in HIV-infected patients. Previous work in the lab using our accelerated pigtail macaque model of HIV CNS disease has shown that minocycline is neuroprotective. We hypothesized that this neuroprotection might be at least partly due to alteration of innate and/or adaptive immune responses. In this study we examined whether minocycline could affect three hallmarks of the immune response to HIV infection: 1) chronic interferon production, 2) hyperactivation of immune cells, and 3) increased sensitivity of T cells to apoptosis. We isolated human PBMCs from the blood of healthy donors and treated them with AT2-MN HIV, which is an aldrithiol-treated MN strain of HIV that can fuse with cell membranes and induce an IFN response but cannot replicate due to cross-linking of nucleocapsid protein. These cells were cotreated with or without 20ug/ml minocycline, which is a dose relevant to clinical usage. Minocycline shut down production of type I and type II interferons in PBMCs, reduced activation of macrophages and T cells as measured by cell size and CCR5 expression, and reduced expression of the pro-apoptotic ligands and receptors PDL1, TRAIL, and PD1 on T cells. Functional analysis to determine whether minocycline reduces T cell apoptosis due to the decrease in PDL1 and TRAIL is underway. These initial findings suggest that minocycline may be able to blunt the impact of HIV infection on the peripheral immune system, which in turn could result in less CNS disease in patients infected with HIV.

## P56
### Morphine and HIV Tat and gp120 alter Toll-like receptor expression in astrocytes

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Nearly one third of individuals living with HIV are abusing opiates, and this drug use has been shown to exacerbate the progression of HIV encephalitis (HIVE). Astrocytes are an important convergence point for morphine and HIV in the brain, where activation of these cells by morphine and the HIV protein Tat in combination, produce synergistic increases in cytokine and chemokine production. These inflammatory response mechanisms are involved in the progression of neurotoxicity in the presence of the virus. Toll-like receptors (TLRs), which are significant mediators of cytokine/chemokine production, are an integral component of the

innate immune response through recognition of conserved viral and bacterial particles-pathogen associated molecular patterns (PAMPS). Recent studies suggest that morphine and other opioids modulate this system as TLR agonists. However, controversy still arises as to whether astrocytes even express TLRs. We hypothesized that morphine affects HIV-induced changes in innate immunity by altering TLR expression in astrocytes. We addressed this hypothesis by first looking at the expression of TLRs in astrocytes, and then began to examine functional activity of these receptors. Primary cultures, highly enriched in astrocytes, were isolated from postnatal day 1–2 ICR mouse striata, propagated in fetal bovine-serum containing medium, and exposed to leucine methyl ester (5mM, 2 hours) to eradicate microglia immediately before treatment with morphine (500nM), Tat (100nM), and/or gp120 (500pM). Western immunoblots, in-cell Westerns and RT-PCR showed that TLRs are expressed by astrocytes and that Tat selectively increases protein and mRNA levels of TLR-2, while morphine suppresses and gp120 augments Tat-induced increases in TLR-2 expression. TLR responsiveness, demonstrated through the release of proinflammatory cytokines and chemokines, revealed that although TLR protein levels were not always affected by HIV proteins and morphine, that these receptors were activated by selective TLR agonists. Co-localization of TLR and glial fibrillary acidic protein immunoreactivity in astrocytes demonstrated (i) heterogeneity in expression among phenotypically distinct subpopulations of astrocytes and showed (ii) that the proportion of astrocytes possessing TLR-2, TLR-3, and TLR-5 immunoreactivity changed dynamically in the presence of HIV proteins. Our results suggest (1) that there is considerable diversity on TLR expression among individual astrocytes, (2) that the patterns and levels of expression of a particular TLR by discreet subsets of astrocytes are plastic and modifiable, and (3) that morphine, HIV Tat, and/or gp120 modulate innate immunity to HIV and potentially other pathogens by selectively modifying TLR expression in astrocytes.

## P57

### Neuropathic Pain Contributes to an Excess of Unemployment and Disability in HIV Infection: the CHARTER study

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Background: Neuropathic pain due to HIV sensory neuropathy (HIV-SN) is believed to cause significant disability, but no reports have evaluated the impact of painful HIV-SN on occupational status and dependence in activities of daily living.

Methods: HIV-SN, neuropathic pain, occupational status and dependence in activities of daily living were assessed in 1,539 HIV-infected individuals enrolled in a prospective, observational study at 6 US sites. HIV-SN was defined as at least one clinical sign in a symmetrical, bilateral pattern on neurological examination. Signs included distal loss of reflexes, vibratory sensation and ability to discriminate sharp from dull. Employment was assessed by using a self-report questionnaire and dependence in instrumental activities of daily living (IADLs) was determined according to a modified Lawton-Brody scale.

Results: Subjects were mostly middle-aged (mean + sd 43.2 + 8.5) men (76.6%) who met 1993 CDC criteria for AIDS (63.0%). Median [IQR] nadir and current CD4 counts were 175 [49–300] and 419 [263–602] cells/uL. 71.1% took combination antiretroviral therapy (cART), 13.6% took neurotoxic dideoxynucleoside antiretrovirals (d-drugs) and 38.2% had prior exposure (median duration, 36 [13–68] months). HIV-SN prevalence was 57% and neuropathic pain was reported by 29% of subjects, ranging from slight (11%) to severe (5%). Increasing numbers of abnormal HIV-SN exam findings were associated with increasing pain severity ($p < .0001$). 74% of subjects were unemployed, and 19% were dependent in activities of daily living. The likelihood of being unemployed was significantly increased among subjects with HIV-SN (OR 1.5 [95% CI: 1.2, 1.9]) or neuropathic pain of any severity (OR 1.8 [1.3, 2.3]), and remained significant after adjusting for current and nadir CD4, cART, d-drug use, plasma viral load (VL), neuropsychological impairment, current major depression and demographic factors such age and education. More severe pain was associated with a significantly greater likelihood of being unemployed. The likelihood of being dependent in IADLs was significantly increased among subjects with HIV-SN (OR 1.4 [1.1–1.8]) or neuropathic pain compared to those without (OR 2.5 [1.9, 3.3]), and remained significant after adjusting for current and nadir CD4, cART, d-drug use, plasma VL, neuropsychological impairment, current major depression and demographic factors. More severe pain was associated with a greater likelihood of dependence in IADLs.

Conclusions: Despite improvements in both the effectiveness and tolerability of antiretroviral regimens, painful HIV-SN remains common and contributes to an excess burden of unemployment and disability in activities of daily living.

## P58
### Risk factors for incident neuropathic pain in HIV infection: The CHARTER Study

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Background: Despite dramatic improvements in overall health among HIV infected individuals on antiretroviral therapy, neurologic morbidity, including neuropathic pain, continues to rise. We hypothesized that asymptomatic peripheral nerve injury, indicated by abnormal distal sensation and reflexes, would predict incident neuropathic pain in HIV. Because previous studies have demonstrated that a history of opioid dependence increases pain susceptibility, we also analyzed past DSM-IV-diagnosed opioid dependence as a risk factor for incident neuropathic pain.

Methods: In a multicenter, prospective, observational study, we assessed 449 pain-free HIV-infected individuals with serial, targeted neurological examinations and clinical interviews.

Results: At baseline, participants were mostly middle-aged (mean 43 years) males (81%) with a median [IQR] CD4 nadir of 181 [50–323] cells/uL and current CD4 437 [278–636]. 229 (51%) had at least one abnormal exam finding consistent with neuropathy and 82 (18%) had a history of opiate dependence or abuse. During a median duration of follow-up of 12 months [IQR 6–18] among 449 HIV+ subjects who were pain-free at baseline, 94 developed neuropathic pain. The hazard for developing incident neuropathic pain was increased (1.97 [95% CI: 1.28–3.03]) for subjects with one or more abnormal neuropathy exam findings at baseline as compared to those without neuropathy. Subjects with 2 or more abnormal signs were at greatest risk (HR 2.51 [95% CI: 1.50–4.19]; $p = 0.0006$), followed by those with only 1 sign (HR 1.65 [95% CI: 1.01–2.70]; $p = 0.045$). Similarly, the hazard was significantly increased (1.85 [95% CI: 1.17–2.92]) for past history of opiate dependence or abuse. Other predictors evaluated including current CD4, nadir CD4, age, d-drug exposure and hepatitis C infection were not significant predictors of incident pain ($p > 0.05$). After controlling for each other in a multivariate proportional hazards model, history of opiate dependence or abuse and neuropathy (at least one abnormal sign) remained significant predictors of incident pain.

Conclusions: Abnormal exam findings and a history of opiate dependence or abuse predict subsequent development of neuropathic pain in HIV. Interventions that reduce the probability of acquiring abnormal neuropathy signs may also prevent the development of neuropathic pain.

## P59
### HIV-1 Vpr's differential pattern of intracellular localization may be associated with the mechanism of Vpr secretion in specific cell populations

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Human immunodeficiency virus type 1 (HIV-1) viral protein R (Vpr) is a virion-associated multi-functional viral regulatory protein that acts within the viral life cycle and a number of cellular pathways. HIV-1 Vpr has been previously shown to mainly accumulate in the nucleus and nuclear envelope in HeLa or 293T kidney cells. These cellular phenotypes, however, have little to do with the overall pathogenesis of HIV-1 infection and disease. To address this gap in knowledge, we have examined HIV-1 Vpr localization patterns in cell lines that represent major cellular targets for HIV-1 infection within peripheral blood, bone marrow, and central nervous system (CNS). For localization studies, two different tags were employed, green fluorescent protein (GFP) and Hemagglutinin (HA). GFP-Vpr, with the tag placed at either the N- or C-terminus, and HA-Vpr, with the tag at the N-terminus, produced two different localization patterns. GFP-tagged Vpr, regardless of the location of the tag, was mainly found in speckles inside the nucleus and to a lesser extent in the nucleoli and nucleolar rim, with a dispersed accumulation in the cytoplasm. On the contrary, the shorter HA tag resulted in a more dense nuclear accumulation, with exclusion from the nucleolus with a defined but scattered presence in the cytoplasm. Additionally, HIV-1 Vpr was also detected in an extracellular virus-free form, which suggests that Vpr may be secreted from selected cell populations. This study may shed new light on the mechanisms associated with Vpr secretion from HIV-1-infected cells, which may lead

to detrimental effects on bystander uninfected cells in response to extracellular virus-free Vpr.

## P60

### Modulation of HIV-1 replication, monocyte/macrophage phenotype, and cytokine gene expression, by tyrosine kinase inhibitors: A novel approach for pharmacotherapeutic intervention in HIV infection and neuroAIDS

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Macrophages play a prominent role in AIDS pathogenesis and the development of HIV encephalitis (HIVE), at least in part, through virus dissemination and the maintenance of viral reservoirs. We have previously examined the role of monocytes/macrophages in HIV-1 induced CNS disease and in patients with HIV infection. Perivascular macrophages and microglia that accumulate in HIVE are CD14+/CD163+/CD16+ and serve as a tissue reservoir of viral infection as determined by HIV-1 p24 immunostaining and co-localization studies. Monocytes with a similar phenotype, CD14+/CD163+/CD16,+ are expanded in circulation in HIV infection, suggesting a role for altered monocyte/macrophage homeostasis in the pathogenesis of AIDS. In a cross-sectional analysis of HIV-1 infected individuals with and without detectable viremia, the percent frequency of this monocyte subset was found to correlate directly with viral load and inversely with CD4 count below a threshold level. Our results, as well as additional studies performed by others groups suggest the potential role for altered monocyte/macrophage homeostasis and immune polarization in AIDS pathogenesis. To begin to explore therapeutics targeting monocyte/macrophage homeostasis in AIDS, we have examined the effects of several tyrosine kinase inhibitors (some of which are FDA approved for human use in other disease settings and targeting distinct molecular pathways) in pre-clinical in vitro studies. Our results demonstrate the differential and concentration dependent effects in reducing the percent frequency of the expanded CD16+ subset in PBMC, the ability to inhibit HIV replication in macrophages, and the modulation of cytokines implicated in the pathogenesis of neuroAIDS. These results suggest novel therapeutic approaches targeting host-molecular pathways for the treatment of AIDS and neuroAIDS.

## P61

### Disruption of Long-Term Potentiation and Reduction of Dendritic Spines in the Hippocampal CA1 Region After Chronic Tat Induction in HIV-1 Transgenic Mice

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HIV-associated cognitive impairment, including memory dysfunction, continues to be a major clinical manifestation of advanced HIV infection in the highly active antiretroviral therapy (HAART) era. To assess whether HIV Tat is responsible for disrupting neuronal plasticity in patients with HIV infection, we used a GFAP-driven, doxycycline (DOX)-inducible Tat transgenic mouse model to examine structural and functional changes in CA1 pyramidal cells. We examined the effects of Tat-induction (~7-day DOX exposure) (1) on long-term potentiation (LTP) of field excitatory postsynaptic potential (fEPSP) at Schaffer collateral fiber-CA1 synapses; and (2) on the number of dendritic spines in Golgi-Kopsch impregnated pyramidal neurons in hippocampal field CA1. Our findings demonstrate that Tat induced suppression of LTP in hippocampal CA1 pyramidal neurons, and the loss of function coincided with increased dendritic pathology and significantly reduced numbers of dendritic spines in CA1 pyramidal neurons. We speculate that the decrease of dendritic spines and the disruption of LTP is interrelated. Collectively, the data provide strong evidence that Tat per se causes neuronal injury and disrupts synaptic plasticity, and that chronic exposure to Tat underlies cognitive dysfunction seen in patients infected with HIV-1. Support: NIDA DA19398.

## P62

### Cell migration through the blood brain barrier (BBB) in feline immunodeficiency virus infection is significantly influenced by the pre-existence of virus and TNF-α within the CNS: studies using an in vitro feline BBB model

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Feline Immunodeficiency Virus (FIV) infection of cats is a model of HIV-1 associated neuropathology. Virus enters the brain early in infection, associated with leukocyte trafficking across the blood-brain barrier. Cytokines, including TNF-alpha, enhance adhesion molecules expression at the BBB. The present study evaluated cell-free FIVGL8 and FIV-infected lymphocyte (Mya-1) migration across an in vitro model of the feline blood-brain barrier and the extent to which this is influenced by TNF-alpha and virus in serum or CNS. Cell-free FIV migrated across the BBB model in statistically insignificant quantities, which were not significantly increased in the presence of TNF-alpha, and BBB tight junctions were not disrupted. In contrast, cell-associated FIV readily crossed the BBB in a similar magnitude to uninfected, activated cells, with neither cell population altering BBB integrity. With scenarios to mimic serum and/or CNS TNF-alpha concentrations, a statistically significant increase in transmigration of both cell populations was observed, and accompanied by a moderate disruption of barrier integrity. Further enhancement of migration occurred with infected cells and TNF-alpha within the brain, and this induced the most significant disruption of BBB tight junctions suggesting that, in vivo, small quantities of virus in the brain with the potential to trigger TNF-alpha production may attract greater viral entry into the CNS. The mechanisms of lymphocyte and virus transmigration were investigated using real-time PCR to quantify ICAM-1, VCAM and TNF-alpha expression on feline brain endothelial cells (FBEC), feline astrocytes and Mya-1 cells exposed to FIVGL8, TNF-alpha or a combination of FIV and TNF-alpha. VCAM expression was enhanced in FBEC cultures exposed to TNF-alpha alone or in combination with FIV, but not with FIV alone. In contrast, ICAM-1 and TNF-alpha expression on FBEC cultures was not altered in response to FIV or TNF-alpha. While Mya-1 cells expressed ICAM-1, VCAM and TNF-alpha, this expression was not enhanced when the cells were exposed to FIV or TNF-alpha. These studies suggest that the synergistic effects of TNF-alpha and FIV on BBB function could be mediated through VCAM, rather than ICAM expression on FBEC, and the mechanism is not the result of further TNF-alpha production.

## P63
## Viral Infection Leads to CD8+ T Cells with Dual Specificities to Virus and Self

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Multiple sclerosis (MS) is an inflammatory demyelinating disease where CD8+ T cells represent a primary component of the inflammatory response comprising the lesion. Viral infections are associated with exacerbations of clinical disease and have been proposed to be triggers for the initial disease. There have been reports that CD4+ T cells isolated from MS patients can recognize not only self myelin epitopes but also viral or microbial determinants. Such data indicate that T cell receptors on CD4+ T cells recognize cross-reactive epitopes and that microbes have the potential of triggering such cross-reactive immune responses through molecular mimicry. Theiler's murine encephalomyelitis virus (TMEV) infection of SJL/J mice leads to a chronic demyelinating disease where CD4+ and CD8+ T cells play a role in the disease. We are investigating the contribution of cross-reactive CD8+ T cells in the inflammatory demyelinating disease of the CNS following this viral infection. We can isolate TMEV specific CD8+ T cells that can kill not only virus infected target cells but uninfected target cells as well. Previously, we were able to isolate CD8+ T cell clones that reacted with the capsid protein(s) of TMEV as well as a self determinant. These dual reactive CD8+ T cell clones when injected into the brain resulted in inflammatory lesions in the brain and spinal cord. We have now produced T cell hybridomas from these T cell clones that have the same specificities (recognizing virus and self) using ELISPOT assays. Following peripheral intravenous adoptive transfer of these T cell hybribomas, mice develop clinical signs very similar to mice with experimental autoimmune encephalomyelitis. About 7–10 days after adoptive transfer, mice develop limp tail followed by hind limb paralysis. CNS pathology includes meningitis and perivascular cuffing which is comprised of T cell hybridomas and host derived inflammatory cells. This is the first example that adoptive transfer of anti-viral CD8+ T cell hybridomas into naïve mice can result in clinical signs and CNS pathology. We are

currently extending our characterization of the viral epitope as well as the host determinant recognized by these anti-viral T cell hybridomas. These CD8+ T cells are likely the CD8 counterpart to the previously described CD4+ T cells isolated from MS patients that can recognize myelin and microbial epitopes. This research is supported by NIH P01AI581501.

## P64
### Differential Effects of HIV-1 Clade B and Clade C Tat Protein on Expression of Pro- and Anti-inflammatory Cytokines by Primary Monocytes

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The existence of multiple subtypes of HIV-1 worldwide has created new challenges to control HIV-1 infection and associated neuropathogenesis. Previous studies indicate a difference in neuropathogenic manifestations and development of Neuro AIDS between HIV-1 clade B and C infected subjects with clade B being more neuropathogenic than clade C. However, the exact mechanism underlying the differences in the neuropathogenesis by both the subtypes remains elusive. Development of NeuroAIDS is associated with a complex interplay between pro-and anti-inflammatory cytokines and chemokines. In the current study, we hypothesize that HIV-1 clade B and C Tat protein exert differential effects on human primary monocytes leading to differential expression of cytokines implicated in neuropathogenesis of HIV-1. Primary human monocytes were treated with clade B and clade C Tat protein, quantitative real time PCR was performed to determine gene expression of pro-inflammatory (IL-6 and TNF-α) and anti-inflammatory cytokines (IL-4 and IL-10). Further, cytokine secretion was measured in culture supernatants by ELISA, whereas the intracellular cytokine expression was detected by flow cytometry. Results indicate that Tat B treated cultures showed significant upregulation of pro-inflammatory cytokines, IL-6 and TNF-α as compared to Tat C treated cultures. On the contrary, anti-inflammatory cytokines, IL-4 and IL-10 expression was found to be significantly higher in Tat C treated compared to Tat B treated cultures. This differential modulation of neuropathogenic molecules by Tat B and Tat C may be correlated with the differences in NeuroAIDS manifestations induced by clade specific infections.

## P65
### HIV Tat Increases NMDAR Surface Expression on Human Neurons

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With the advent of effective therapies, people are living longer with HIV, leading to an increased prevalence of infected individuals developing a wide range of neurological deficits from mild cognitive impairment to frank dementia. Although HIV does not infect neurons, it has been demonstrated that the HIV protein tat plays a major role in mediating neurotoxicity. Recent studies from our laboratory indicate that tat acts on neurons through formation of a signal transduction complex including the NMDA receptor (NMDAR) that activates neuronal nitric oxide synthase, leading to apoptosis in human neurons and astrocytes. Although we have shown that the NMDAR is necessary for tat-induced apoptosis, the role of NMDAR trafficking in this process has not been characterized. This work examines changes in NMDAR surface localization with tat treatment. Surface biotinylation demonstrated an increase in surface expression of the NMDAR 5 minutes and 1.5, 2, and 3 hours following tat treatment. We have shown that tat leads to src kinase activation and tyrosine phosphorylation of the NMDAR, which is known to inhibit clathrin-mediated endocytosis of this receptor. The increased NMDAR surface expression corresponded with a significant decrease in NM DAR association with beta-adaptin, a critical adaptor protein in clathrin-mediated endocytosis, at 60 minutes after tat treatment. In addition to NMDAR tyrosine phosphorylation, this may be mediated by tat activation of PKC and/or PKA, kinases that are known to enhance NMDAR serine phosphorylation and increase trafficking to the membrane. Preliminary studies suggest that tat increases PKC phosphorylation. Our work indicates that tat-induced neuronal apoptosis is facilitated by dysregulation of the NMDAR, which could have important implications in the development of therapeutics for HIV-associated neurocognitive disorders.

## P66
### PML associated adaptive mutations in JC virus (JCV) VP1 protein are involved in viral receptor binding

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Background: PML is a fatal demyelinating disease caused by JCV infection of oligodendrocytes. Although the virus is highly prevalent in humans and resides chronically in peripheral sites following primary infection, PML develops only very rarely and in conditions of immune disorders. Understanding the nature of viral and host factors that play a role in viral pathogenesis is important for developing the strategies to prevent and treat this disease. Since the major JCV capsid protein, VP1, is involved in viral cell entry through binding with sialic acid residues, genetic changes in this region might be might be implicated in neurotropism and neurovirulence. Through analysis of Genbank obtained VP1 sequences from 253 healthy adults and 55 PML patients we showed that certain VP1 mutations are a result of adaptive selection in PML. Thus, the objective of this study was to investigate the presence of VP1 mutation in cerebrospinal fluid (CSF), plasma and urine samples of PML patients and whether these mutations affect VP1 binding in vitro.

Methods and Results: We cloned and sequenced VP1 from CSF of 26 PML patients, for some of the patients we also obtained paired urine (n = 6) or plasma (n = 11) sequences. Analysis of clinical samples showed that almost all CSF clones from each of 24/26 patients carried one PML associated mutation. Same corresponding mutations were recognized in paired plasma but not in paired urine samples. 3D modeling was used to map the location of all PML specific mutations on VP1 structure. All the mutated residues clustered on the VP1 surface within or in immediate proximity to binding site with the sialic acid cell receptor. To examine the effects of PML associated mutations on viral receptor specificity we created viral like particles (VLPs) using wild type and various mutant VP1 molecules and investigated their binding to cells and various carbohydrate receptors using FACS, hemagglutination and ELISA assays. All VLP binding assays revealed that PML associated mutations are responsible for the dramatic change viral receptor specificity.

Conclusions: CSF and plasma but not urine sequences of PML patients carry specific VP1 mutations that may differentially affect JCV binding to target cells in the periphery vs CNS. Overall these findings fit in a model whereby JCV acquires adaptive changes during the transition from its site of asymptomatic replication to the brain thus eventually leading to PML.

## P67

### Tissue-specific sequence alterations in the HIV-1 envelope favoring CCR5-usage contribute to persistence of dual-tropic virus in brain

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Background: Most neurotropic HIV-1 strains are CCR5-restricted, but R5X4 variants have been identified in brain from some infected individuals.

Methods: HIV-1 Envs were cloned from R5X4 primary viruses isolated from brain (n = 6) and spleen (n = 6) of subject MACS1. R5X4 Envs cloned from brain and blood of a second subject were also examined (aBR01, aBL01). Envs were sequenced and analyzed to identify amino acid variants that influence coreceptor usage in fusion assays with wild type and mutant coreceptors. Sensitivity to inhibition by the CCR5 and CXCR4 inhibitors, Maraviroc and AMD3100 respectively, was tested in single-round entry assays. Mutagenesis was performed to determine the biological activity of amino acid variants.

Results: MACS1 brain (M1br) and brain-derived aBR01 Envs were more fusogenic than Envs from matched spleen or blood in cells expressing CD4/CCR5, whereas MACS1 spleen (M1sp) and blood-derived aBL01 Envs were more fusogenic than Envs from matched brain in cells expressing CD4/CXCR4. Entry assays in CD4/CCR5/CXCR4-expressing JC53 cells treated with Maraviroc, AMD3100 or both inhibitors showed brain- and spleen-derived R5X4 Envs exhibited preferential usage of CCR5 and CXCR4 for entry, respectively. Studies using CCR5 and CXCR4 mutants with attenuated coreceptor activity showed that, compared to the spleen/blood-derived Envs, brain-derived Envs had reduced dependence on residues in the CCR5 N-terminus (Y15), ECL1 (H88), and ECL3 regions (E262, F264) for CCR5-mediated fusion. Compared

to brain-derived Envs, spleen/blood-derived Envs had reduced dependence on residues in the CXCR4 N-terminus (Δ4–36) and ECL2 region (R183) for CXCR4-mediated fusion. Sequence analysis identified R306 in the V3 loop of 6/6 M1sp Envs and S306 in 6/6 M1br Envs. Mutagenesis studies showed R/S306 was responsible for preferential CCR5 or CXCR4 usage in JC53 cells and reduced dependence on specific CCR5 or CXCR4 residues that influence coreceptor function by the brain- and spleen-derived R5X4 Envs, respectively. Entry assays with Env mutants in primary PBMC and MDM treated with coreceptor inhibitors showed that the presence of S306 permitted HIV-1 entry via CCR5, whereas R306 conferred a CXCR4-restricted phenotype to spleen-derived R5X4 Envs.

Conclusions: Tissue-specific selection for adaptive changes in the V3 region of gp120 that alter coreceptor usage may enhance the tropism of compartmentalized R5X4 strains for cells expressing CCR5 in brain and CXCR4 in lymphoid tissues.

## P68

### IGF-IR dependent expression of Survivin is required for T-Antigen mediated protection from Apoptosis and proliferation of neural progenitors

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The Insulin-like Growth Factor-1 Receptor (IGF-IR) and the human *polyomavirus* JCV protein, T-Antigen cooperate in the transformation of neuronal precursors in the cerebellum, which may be a contributing factor in the development of brain tumors. Since it is not clear why T-Antigen requires IGF-IR for transformation, we investigated this process in neural progenitors from IGF-IR knockout embryos (ko-IGF-IR) and from wild type non-transgenic littermates (wt-IGF-IR). In contrast to wt-IGF-IR, the brain and dorsal root ganglia of ko-IGF-IR embryos showed low levels of the anti-apoptotic protein Survivin, accompanied by elevated numbers of apoptotic neurons and an earlier differentiation phenotype. In wt-IGF-IR neural progenitors *in vitro*, induction of T-Antigen expression tripled the expression of Survivin, and accelerated cell proliferation. In ko-IGF-IR progenitors induction of T-Antigen failed to increase Survivin, resulting in massive apoptosis. Importantly, ectopic expression of Survivin protected ko-IGF-IR progenitor cells from apoptosis and siRNA inhibition of Survivin activated apoptosis in wt-IGF-IR progenitors expressing T-Antigen. Our results indicate that reactivation of the anti-apoptotic Survivin may be a critical step in JCV T-Antigen induced transformation, which in neural progenitors requires IGF-IR.

## P69

### Differential CaMKII alterations associated with HIV/SIV-induced neuronal damage versus CNS immune activation

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Synaptodendritic damage corresponds with the severity of cognitive impairment in HIV-infected individuals. Ca + 2/calmodulin-dependent kinase-II (CaMKII) plays a central role in induction of long-term post-synaptic modifications following synaptic activation and also may regulate CNS immune responses. Given these roles, CaMKII is an attractive target to explain the deleterious effects of HIV on the CNS. To determine whether HIV and SIV induce alterations in CNS CaMKII expression and/or CaMKII activation (autophosphorylation), we measured alterations in CaMKII expression and activation by quantitative immunoblotting in both an in vitro HIV/neuronal culture model and in vivo in an SIV-infected macaque model of HIV-associated neurological damage. In an established in vitro model system using primary rat hippocampal neuronal cultures exposed to culture supernatants harvested from HIV-1-infected human monocyte-derived macrophages (HIV/MDM), CaMKII activation was significantly decreased in HIV/MDM-exposed rat hippocampal neurons compared to untreated/MDM-exposed neurons ($P = 0.003$). In contrast with HIV/MDM-exposed neuronal cultures, CaMKII activation was increased in HIV-infected human macrophages compared with mock-infected macrophages ($P = 0.035$). Total CaMKII expression was not significantly altered in either HIV/MDM-exposed neurons or in HIV-infected macrophages. In the simian immunodeficiency virus (SIV)/macaque model of HIV CNS disease, a significant decrease in CaMKII activation was detected in the hippocampus and the frontal cortex of infected macaques compared with uninfected control animals ($P = 0.020$). Furthermore, total CaMKII expression in the hippocampus and frontal cortex was inversely correlated with CNS viral load ($P = 0.014$ and 0.002, respectively), while CaMKII activation was not associated with viral load. Our observations in HIV/MDM-exposed

neurons and the SIV/macaque together suggest that alterations in neuronal CaMKII activation may mediate impairment of long-term potentiation (LTP) and cognitive function in HIV/SIV infection. Neuronal CaMKII may be an ideal therapeutic target for synaptic repair and functional recovery to improve neurologic function in HIV-associated cognitive disease.

## P70
### HIV-1 binding induces interferon stimulated genes in primary astrocytes

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Astrocytes are the major cellular component of the central nervous system (CNS) and they play multiple roles in brain development, normal brain function, and CNS responses to pathogens and injury. In the brain of an infected individual, astrocytes are likely exposed to HIV-1 particles, viral proteins, and cytokines produced by virally-infected macrophages and microglia. Although HIV-1 infection occurs in fewer than 1% of astrocytes in the brain, HIV-1 binding alters cellular gene expression of astrocytes and disrupts many essential functions of the cells. The mechanism whereby HIV-1 binding alters astrocyte biology is poorly understood. We are interested in studying downstream pathways that are induced or suppressed by HIV-1 binding of astrocytes. Our recent data indicate that one pathway that is highly induced by HIV-1 binding is the interferon pathway. After a 6 hour exposure of astrocytes to virus, we find upregulated mRNA levels of a number of interferon stimulated genes (ISG) by qPCR analysis. OAS2 mRNA levels are increased by 60 fold, IRF7 mRNA levels by 100 fold, and IFIT1 mRNA levels by over 1000 fold. Blocking studies using KN-93 suggest that the Ca2+/calmodulin-dependent protein kinases (CAMKII) may be one of the downstream components that modulate the interferon response to virus. Based on these results, we believe that there is cross-talk between calcium signaling pathways and the interferon pathway, which leads to HIV-1 induced overexpression of ISG in primary astrocytes. This overexpression of ISG could alter neuroprotective functions of astrocytes and contribute to neurotoxicity in the infected brain.

## P71
### Mechanisms of persistent NF-κB activation by HTLV-I Tax

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HTLV-I Tax inhibits cell death and transforms cells in part by activating the transcription factor NF-κB. Tax is a potent activator of the IKK kinases by interacting with NEMO, however the precise mechanisms of IKK activation by Tax remain elusive. Here, we demonstrate that the Tax interacting protein TAX1BP1 is a critical negative regulator of persistent NF-κB signaling. TAX1BP1 inhibits NF-κB by recruiting the ubiquitin-editing enzyme A20 to ubiquitinated substrates such as RIP1, TRAF6 and NEMO that are targeted for inactivation. Tax potently inhibits the adaptor function of TAX1BP1 thus rendering A20 unable to interact with its substrates in Tax expressing cells. Together, our observations support a model whereby Tax persistently activates NF-κB by two complementary mechanisms: 1) Tax interacts with NEMO to trigger IKK activation, and 2) Tax counteracts the function of the potent NF-κB inhibitors TAX1BP1 and A20.

## P72
### Deregulation of NMDA receptor trafficking by gp120; A lock n overload mechanism of synaptic death

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The trafficking of ionotropic glutamate receptors in the postsynaptic membrane plays critical roles in modulating synaptic strength. We have shown recently that TNFα can induce transient and focal increases of ceramide that promote the surface localization and clustering of NMDA receptors by enhancing the fusogenic properties of receptor-laden vesicles with the plasma membrane. These events were initiated in the order of seconds, terminated within 10 minutes, and were critical for TNFα-induced long-term potentiation. We now provide evidence that the HIV-coat protein gp120 induces long-lasting increases of ceramide that lock NMDA receptors into clusters where sustained high rates of focal calcium flux can destabilize synaptic integrity. Hippocampal neurons were treated with gp120IIIB (250–500 pM) for 6 and 12 h before biochemical and functional analysis. In gp120-treated cultures, ceramide was increased within 6 h and remained elevated for at-least 12 h. In a similar time frame, gp120-induced the clustering of NMDA receptors containing NR1 subunits phosphorylated on serine 896 (NR1S896) into ceramide-rich lipid raft domains (phosphorylation of this site on NR1 is known to be important for surface localization of the receptor). Inhibition of nSMase2 (catalyzes the hydrolysis of sphingomyelin to ceramide) or IP3-mediated calcium release prevented gp120-induced phosphorylation of NR1S896 and clustering of this NMDA receptor subunit into lipid

rafts. Functional studies showed that the fraction of high amplitude NMDA evoked calcium events along dendritic branches was increased by gp120, suggesting that NMDA receptors were redistributed into clusters. A similar increase of NR1S896 was observed in brains of aging mice transgenic for gp120, suggesting that hyperphosphorylation of NR1 may worsen with age in the setting of HIV infection. These findings suggest that by perturbing ceramide metabolism gp120 may lock NMDA receptors into clusters where calcium overload could trigger synaptic degeneration. Supported by MH077542 and AG023471.

## P73

### Fueling neurodegeneration through the abuse of alcohol in the setting of HIV-1 infection

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Alcohol is a common drug of abuse that can worsen the decline of cognitive function in HIV-infected patients. Our current understanding of the effects of alcohol on neurocognitive function, suggests that this protic solvent has complex effects on neural activity that to some degree depend on dose and duration of alcohol use. At low and moderate dose, alcohol may have neuroptorective effects. For example, low dose acute alcohol exposure can protect neurons from the toxic effects of gp120 by depressing the activity of NMDA receptors. However, at high dose (or with chronic overuse) alcohol can exert neurodamaging effects that may involve the generation of reactive oxygen species, or disruptions of endogenous antioxidant defense systems. For instance, high-dose alcohol has been demonstrated to amplify the neurotoxic effects of gp120 and Tat by actions on redox-sensitive inflammatory pathways and downstream events that perturb a variety of calcium flux pathways. There are in addition, effects of alcohol that involve its chemical properties as an organic solvent. Alcohol can remove cholesterol and sphingolipids from neural membranes disrupting the biophysical properties membranes and the receptors imbedded in this matrix. The removal of alcohol soluble sterols and lipids from neuronal membranes causes a rearrangement of GM1 gangliosides and a redistribution of NMDA and AMPA receptors into clusters where agonist-evoked calcium oscillations are enhanced. The addition of gp120 to this alcohol-sensitized system results in a further dysregulation of NMDA and AMPA receptors and synaptic collapse. These findings suggest that alcohol may accelerate neurodegeneration in the setting of HIV infection by actions that involve biophysical alterations to membrane structure and chemical alterations to cellular redox systems. Supported by NIH AA017408 to NJH.

## P74

### Persistence and Progression of HIV-associated Neurocognitive Impairment (NCI) in the Era of Combination Antiretroviral Therapy (CART) and the Role of Comorbidities: The CHARTER Study

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Introduction: CART typically suppresses HIV viral replication in plasma and CSF, but its long-term effects on NCI are unclear. This multi-site study examined the prevalence and predictors of NCI in the CART era, within an HIV+ sample reflective of clinic populations with varying degrees of comorbidity.

Methods: 1555 HIV-infected patients at six university clinics across the US received comprehensive neuromedical and neuropsychological (NP) evaluations. Participants were classified according to severity of their comorbidity (factors which could contribute to, or cause, NCI independent of HIV: minimal, $n = 843$; moderate, $n = 473$; and severe, $n = 239$.

Results: Overall, 53% of the cohort had NCI based on NP testing, with rates increased in groups with greater comorbidity (minimal [39%], moderate [54%] and severe [79%]; all p's $< .01$). Associations between NCI and both markers of disease severity and CART-related viral suppression were found only in the group with minimal comorbidity, such that those on CART who achieved undetectable plasma viral load, and whose CD4 was never below 200 were most cognitively intact. In a 6-month follow-up of 680 patients, each successive comorbidity group had a higher rate of cognitive decline (11% vs. 18% vs. 27%, respectively; $p < .05$).

Conclusions: Despite most being on CART, there was a high prevalence of NCI. At baseline, nadir CD4 and successful viral suppression were related to NCI only in patients without significant comorbidities. However, cognitive decline over time was found to occur more frequently in patients with greater comorbidity. Thus, both HIV and comorbidity

contribute to NCI in the CART era, and CNS comorbidities may increase risk of subsequent HIV-related cognitive decline.

## P75

### HIV-1 Tat antagonizes Wnt/beta-catenin signaling to diminish beta-catenin-induced suppression of HIV replication in the central nervous system

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HIV-associated neurocognitive disorder (HAND) persists despite combination antiretroviral therapy. A better understanding of the underlying mechanisms of HAND is needed for therapeutic intervention. Mounting evidence links decreased levels of β-catenin and neurodegeneration. β-catenin signaling is a pro-survival pathway in neurons and astrocytes. We reported an inverse relationship between β-catenin activity and HIV replication. Specifically, suppressing endogenous β-catenin enhanced HIV replication in astrocytes by three-fold. Given that monocytes and macrophages are critical sources for HIV replication in the CNS, we evaluated the role of β-catenin in modulating HIV replication in these cells. We demonstrate here that monocytes and monocyte-derived macrophages (MDMs) differentially express β-catenin. Using two indicators of β-catenin activity (transfection with TCF/LEF TOPflash construct and intracellular staining for active β-catenin), we show that monocytes have an approximately 4-fold higher endogenous activity of β-catenin than MDMs. Inducing β-catenin in MDMs by lithium treatment suppressed HIV replication by 2-fold ($p < 0.05$) while inhibiting endogenous β-catenin in monocytes by transfection with a dominant negative (DN) mutant for the downstream effector of β-catenin pathway (TCF-4) promoted productive HIV replication by 2-fold ($p < 0.0005$). These findings indicate that endogenous levels of β-catenin contribute to resistance or susceptibility of monocytes and MDMs, respectively, to productive HIV replication. Further, given that the HIV Transactivator of Transcription (Tat) activates glycogen synthase kinase 3-β (GSK-3β), a negative regulator of the β-catenin pathway, we evaluated the impact of Tat on β-catenin in astrocytes. Human astrocytic cell lines (U87MG and U251MG) and primary human astrocytes were left untreated or treated with HIV-1 recombinant Tat1-86 and β-catenin expression was measured 12 hours post-treatment by flow cytometry. Tat at 10ng/ml caused a 20% reduction in hypophosphorylated (active) β-catenin expression in comparison to untreated cultures. Inhibition of β-catenin by transfecting astrocytes with a DN mutant for TCF-4 resulted in a 14% decrease in the anti-apoptotic factor Bcl-XL. These data demonstrate that Tat inhibits β-catenin signaling and suggest that by doing so it may interfere with cell survival. Collectively, our data indicate that b-catenin is a suppressor of HIV in the CNS and demonstrate that HIV has evolved a mechanism (Tat) by which it can diminish β-catenin levels to promote its own propagation.

## P76

### Production of HSV-induced reactive oxygen species by murine microglia

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Production of reactive oxygen species (ROS) in the central nervous system contributes 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. HSV-induced ROS production was not detected in cultures of primary murine astrocytes. Comparison of virus-induced ROS production in microglia from wild-type and TLR2-/- mice indicated that it was largely mediated through TLR2. In our ongoing studies, we are examining ways to modulate virus-induced ROS production and reduce oxidative brain damage through the induction of antioxidants.

## P77

### Analysis of transcription factor binding site sequence variants within the HIV-1 LTR co-selected for throughout HIV-1 disease

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HIV-1 proviral sequence variants are selected for in a manner corresponding with stage of disease, and may be hallmarks of progressive HIV-1 disease. HIV-1 LTR nucleotide variation could result in the production of a conditionally operational viral promoter. Previous examination of sequence variation at C/EBP site I and Sp site III in peripheral blood (PB)-derived LTRs revealed a 3T configuration at C/EBP site I (C-to-T at position 3) and a 5T configuration at Sp site III (C-to-T at position 5), which were found to increase with increased disease severity. The 3T and 5T variants, both low affinity configurations, were observed in 25% and 16% of HIV-1-associated demented patients (HAD) patients, respectively, and suggested that LTR sequence variation may be predictive of progressive immunologic and neurologic HIV-1 disease. Interestingly, further analysis revealed that 100% of 3T LTRs also contained the 5T Sp site III variant, indicating that these variants were co-selected for during late stage disease. We have expanded our studies to include patients from the DrexelMed HIV-1 cohort to determine whether these variants continue to arise in the era of combination anti-retroviral therapy (cART), and to determine if new sequence variants are selected for which exhibit similar phenotypic properties. This analysis has revealed the presence of the 3T and 5T LTR variants. Analyses have demonstrated that the 5T Sp site III co-selected with the 6G C/EBP site I. In addition the 3T C/EBP site I co-selects with the 6C variant of the ATF/CREB binding site. These studies confirm that previously observed genetic variants are found in the DrexelMed cohort and future studies will focus on determining if these variants arise during disease progression as the patients in this cohort are followed longitudinally.

## P78

### Herpes simplex virus type 1, apolipoprotein E, Alzheimer's disease, & antiviral treatment

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The causes of Alzheimer's disease (AD) in non-familial cases, which comprise the vast majority, are unknown. We have implicated herpes simplex virus type 1 (HSV1) as a major factor, discovering that it resides latently in many elderly brains (J. Med. Virol. 1991, et seq.), that in carriers of the type 4 allele of the apolipoprotein E gene it confers a strong risk of AD (Lancet, 1997), and that it reactivates in brain, possibly recurrently (J. Med. Virol., 2005). Further, we linked HSV1 directly to the abnormal features of AD brain, infection causing (1) deposition of intracellular beta amyloid (Abeta), the main component of amyloid plaques, in cultured cells and in mouse brain (Neurosci. Lett., 2007); (2) formation of hyper-phosphorylated tau protein (the main component of neurofibrillary tangles) in cultured cells (J.Alz. Dis., 2009). We recently examined and quantified HSV1 location in relation to amyloid plaques, using in situ PCR to detect viral DNA combined with thioflavin S staining for plaques, or immunohistochemistry for Abeta. Ninety percent of plaques contain viral DNA, and in AD brains, 72% of the viral DNA lies within plaques, and in aged normal subjects, 24% of the viral DNA (J.Pathol., 2009); the difference possibly reflects lesser Abeta production or better clearance in AD brains. This co-localization, considered with the HSV1-induced Abeta deposition, strongly implicates HSV1 in the formation of toxic Abeta products and plaques, substantiating its being a significant aetiological factor in AD. Our data point to the usage of antiviral agents to treat the disease and possibly of vaccination to prevent it. Antiviral agents would inhibit all viral damage, whereas current treatments merely inhibit disease symptoms, whether or not they are actually damaging and, in general, they are ineffectual. We have found that the antiviral agent acyclovir (ACV) greatly reduces HSV1-induced Abeta deposition and AD-like tau phosphorylation in various cell types, substantiating our proposed use of antivirals for AD. Currently, we are examining another antiviral agent which acts by a different mechanism from ACV, and we are investigating if Abeta is produced as an attempted protective mechanism by the cell or, conversely, for the benefit of the virus.

## P79

### Rabies Virus Infection of Dorsal Root Ganglia in Experimental Rabies and in Cultured Adult Mouse Dorsal Root Ganglia Neurons: Selective Vulnerability and Evidence of Oxidative Stress

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Rabies virus infection of dorsal root ganglia (DRG) was studied in an in vivo model of experimental rabies using the challenge virus standard (CVS) strain, and also in vitro in cultured adult mouse DRG neurons. Adult ICR mice were inoculated in the right hindlimb footpad with CVS. Spinal cords and DRG were evaluated at serial time points for histopathological and ultrastructural changes and for biochemical markers of cell death. Light microscopy showed multifocal mononuclear inflammatory cell infiltrates in the sensory ganglia and a

spectrum of degenerative neuronal changes. Ultrastructural changes in gangliocytes included features characteristic of the axotomy response, the appearance of numerous autophagic compartments, and aggregation of intermediate filaments, while the neurons retained relatively intact mitochondria and plasma membranes. Later in the process, there were more extensive degenerative neuronal changes without typical features of either apoptosis or necrosis. The degree of degenerative neuronal changes in gangliocytes contrasts with observations in CNS neurons in experimental rabies. Hence, gangliocytes exhibit selective vulnerability in this mouse model. DRG neurons infected with CVS in vitro show prolonged survival and few morphological changes. Cultured DRG neurons from adult mice infected with CVS showed axonal swellings with immunohistochemical staining for 4-hydroxynonenal (4-HNE). Mitochondrial-based oxidative stress results in lipid peroxidation and formation of adducts of 4-HNE, which modifies the function of key mitochondrial and cytoskeletal proteins leading to the formation of axonal swellings. In vitro DRG neurons are much more permissive to CVS infection, but exhibit evidence of oxidative damage, which we have also observed in CNS neurons in a mouse model. Further study is needed to determine the mechanisms for selective vulnerability of DRG neurons and the role of oxidative stress in the pathogenesis of rabies.

## P80
### Migration of JC virus across the human blood-brain barrier occurs via clathrin-mediated endocytosis

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The JC virus (JCV) is a human polyomavirus found in the majority of the adult population. Infection is asymptomatic in healthy individuals. The virus remains latent in the kidneys and lymphoid tissue. In immune compromised persons, JCV migrates into the brain where it infects both oligodendrocytes and astrocytes, leading to progressive multifocal leukoencephalopathy. The mechanisms involved in viral transmigration from the peripheral circulation into the brain are not understood. We examined the mechanisms involved in brain uptake of the Mad-4 strain of JCV. Exposure to JCV did not result in disruption of the in vitro blood-brain barrier (BBB) model as determined by paracellular permeability of the monolayer to an intravascular marker and measurement of transendothelial electrical resistance. Real time PCR was used to track JCV migration across a monolayer of a human brain endothelial cell line, tHBEC XIII. The virus was transported across the BBB in a time-dependent manner. Several pharmacologic transport inhibitors were investigated for their effects on JCV transmigration in the in vitro BBB. JCV migration was not inhibited by sialic acid, but was significantly reduced by chlorpromazine, an inhibitor of clathrin-mediated endocytosis. Transmission electron microscopy showed vesicles containing JCV in the endothelial cells. In conclusion, JCV does not disrupt the BBB. Instead, it transmigrates across the endothelial cells by clathrin-mediated endocytosis.

## P81
### Trafficking of dendritic cells across blood brain barrier during neuroinflammation

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During multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE), autoreactive T cells invading the central nervous system (CNS) must recognize myelin-derived antigens presented by antigen-presenting cells in order to display effector functions. In this process, the role of dendritic cells (DCs) has emerged but the mechanisms regulating their trafficking into the CNS still need to be characterized. We investigated in vivo live imaging of DC recruitment across the spinal cord white matter microvasculature during EAE by intravital fluorescence videomicroscopy. Immature bone marrow-derived DCs were efficiently recruited into the inflamed spinal cord white matter. However, upon LPS-activation, DC recruitment was dramatically impaired. Immature and LPS-activated DCs also demonstrated differences in their ability to subsequently diapedese through the inflamed microvessel wall and transmigrate into the CNS parenchyma. Blocking α4-integrins did not significantly reduce neither rolling nor capture of immature and LPS-activated DCs to the BBB endothelium but did quite abolish their firm adhesion to the microvasculature, preventing their subsequent transmigration within the CNS parenchyma compared to control. This study supports the notion that during EAE DCs migrate into the CNS, where they display a major role in the perpetuation of autoimmune responses. Therapeutic strategy aiming at blocking α4-integrins with natalizumab in MS, may directly affect DC trafficking into the CNS and thus impair the stimulation and maintenance

of the autoreactive immune response within the CNS during the course of the disease.

## P82
## DC-SIGN as a potential target to block HTLV-1 transmission and infection

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Despite the susceptibility of dendritic cells (DCs) to HTLV-1 infection and the defined role of these cells in disease pathogenesis, the mechanisms of viral binding to DCs have not been fully delineated. Recently, a glucose transporter GLUT-1, heparan sulfate proteoglycans (HSPGs), and neuropilin-1 (NRP-1) were demonstrated to facilitate HTLV-1 entry into T cells. DCs express their own array of antigen receptors, the most important being the DC-specific intercellular adhesion molecule-3 (ICAM-3)-grabbing nonintegrin (DC-SIGN) with respect to retrovirus binding. Consequently, the role of DC-SIGN and other HTLV-1 receptors was analyzed in viral binding, transmission, and productive infection using monocyte-derived DCs (MDDCs), myeloid DCs, and B-cell lines expressing DC-SIGN. The relative expression of DC-SIGN, GLUT-1, HSPGs, and NRP-1 was first examined on both DCs and B-cell lines. Although inhibition of these molecules reduced viral binding, HTLV-1 transmission from DCs to T cells was mediated primarily by DC-SIGN, with some indication of GLUT-1 involvement as well. DC-SIGN was also shown to play a role in the infection of MDDCs as well as model B-cell lines. HTLV-1 infection of MDDCs was also achieved in myeloid DCs following the enhancement of virus-induced interleukin-4 production and subsequent DC-SIGN expression in this cell population. This study represents the first comprehensive analysis of potential HTLV-1 receptors on DCs and strongly suggests that DC-SIGN plays a critical role in HTLV-1 binding, transmission, and infection, thereby providing an attractive target for the development of antiretroviral therapeutics and microbicides.

## P83
## Increased EBV Reactivation in the Cerebrospinal Fluid of Patients with Early Multiple Sclerosis

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Epstein-Barr virus (EBV) has been consistently associated with multiple sclerosis (MS), but whether this virus is a trigger of MS remains undetermined. Recently, EBV-infected B cells recognized by activated CD8+ T cells have been detected in the meninges of autopsied MS patients. In addition, a strong EBV-specific CD8+ T cell response in the blood of patients with MS of recent onset was reported. Here, to further explore the putative relationship between MS and EBV, we assessed the EBV-specific cellular and humoral immune responses in the blood and the cerebrospinal fluid (CSF) of patients with early MS or other neurological diseases, separated into inflammatory (IOND) and non-inflammatory (NIOND) groups. The MS non-associated neurotropic herpesvirus cytomegalovirus (CMV) served as a control. Fifty-eight study subjects were enrolled, including 44 patients (13 with early MS (onset of MS less than one year prior to the assay), 15 with IOND and 16 with NIOND) in the immunological arm of the study. The cellular immune response was investigated using a functional CFSE cytotoxic T lymphocyte (CTL) assay performed with short-term cultured EBV- or CMV-specific effector T cells from the CSF and the blood. The humoral immune response specific for these two viruses was also examined in both the blood and the CSF. The recruitment of a given virus-specific antibody in the CSF as compared to the blood was expressed as antibody indexes (AI). We found that, in the CSF of early MS patients, there was an enrichment in EBV-, but not CMV-specific, CD8+ CTL as compared to the CSF of IOND ($P = 0.003$) and NIOND patients ($P = 0.0009$), as well as compared to paired blood samples ($P = 0.005$). Additionally, relative viral capsid antigen (VCA)-, but not EBV encoded nuclear antigen 1 (EBNA1)- or CMV-specific, AI were increased in the CSF of early MS as compared to IOND ($P = 0.002$) or NIOND patients ($P = 0.008$) and correlated with the EBV-specific CD8+ CTL responses in the CSF ($rs = 0.54$, $P = 0.001$). Fourteen additional patients were enrolled in the virological arm of the study: using semi-nested PCR, EBV-encoded nuclear RNA1 (EBER1)-a transcript expressed during all stages of EBV infection-was detected in the CSF of 2/4 early MS, but only 1/6 IOND and 0/4 NIOND patients. Altogether, our data suggest that a reactivation of EBV, but not CMV, is taking place in the central nervous system of patients with MS of recent onset. These data significantly strengthen the link between EBV and MS and may indicate a triggering role of EBV in this disease. This work was supported by grants from the Swiss National Foundation and from the Swiss Society for Multiple Sclerosis.

## P84
### SUMOylation of Neuronal PINCH during HIV infection of the CNS

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HIV enters the CNS soon after initial infection and again at various times through out the disease course resulting in multiple insults upon cells of brain. Synaptodendritic damage to neurons can occur either directly from viral proteins or indirectly from inflammatory factors released from neighboring infected/ activated glial cells. In some cases, damage has been shown to be at least partially reversible, suggesting the induction of host-mediated repair mechanisms. In this context, we are investigating the role of a protein called PINCH (Particularly Interesting New Cysteine Histidine - rich protein), in neuronal responses to HIV infection of the CNS. PINCH is required to maintain neuronal polarity during development, but is largely silenced in the adult CNS in health. PINCH also mediates the formation of multi-protein complexes at focal adhesion junctions to promote bidirectional signal transduction between the extracellular matrix and intracellular networking pathways. Our previous work shows robust expression of PINCH in neurons in the brains of HIV infected patients as compared to normal controls where PINCH expression is nearly undetectable. Increased immunodetection of PINCH in HIV patients' brains corresponds with neurons that show decreased markers for synaptodendritic complexity. In vitro, in neurons exposed to factors present in the brain during HIV infection such as TNF-a and Tat, increased levels of PINCH protein are detected. In the current study, we found that PINCH levels are regulated in part, post- translationally by SUMOylation. SUMOylation is a post-translational modification by which proteins are 1) stabilized, 2) targeted for sub-cellular localization, and 3) modified to form multi-protein complexes required for signaling. PINCH SUMOylation may allow for stabilization of interactions among PINCH and its binding partners such as ILK and Nck2, both of which are necessary for proper neuronal communication with the extracellular matrix. Understanding the mechanism(s) by which PINCH functions during HIV-associated CNS alterations will provide new insights to limit neurological alterations in HIV.

## P85
### Viral- and myelin-specific cellular immune response in patients treated with natalizumab: a cross-sectional and a longitudinal prospective study

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Introduction: Natalizumab, a monoclonal antibody binding to the alpha4 integrins, is efficient in preventing relapses and progression of disability in multiple sclerosis (MS) patients. However, a total of seven MS patients treated with natalizumab suffered from progressive multifocal leukoencephalopathy (PML), on a total of 53′000 patients (data of March 6, 2009) treated with this drug. PML is a disease affecting immunosuppressed people, which is caused by the polyomavirus JC (JCV). This virus produces a lytic infection of the oligodendrocytes. Yet, natalizumab cannot be considered as a classical immunosuppressant, such as suggested by the fact that no increased incidence of other opportunistic infections was reported with this drug. It has been postulated that, by closing the blood-brain, natalizumab might prevent JCV-specific CD8+ T cells to reach the CNS and perform immune surveillance. Alternatively, it has been suggested that this drug acts by releasing JCV from the bone marrow, one of its site of latency. In this study, we address the question whether there is an increased activity of JCV in the blood of natalizumab-treated MS patients.

Material and Methods: In this prospective longitudinal study, we are following a cohort of 24 MS patients receiving monthly injections of natalizumab. Blood and urine are drawn every one to three months, up to 12 months. As a control group, we follow 16 MS patients treated with IFN-beta. For this control group, there are two time-points: before and $10 \pm 4$ months after treatment onset. We are analysing the viral (JCV-, EBV- and CMV-) as well as the myelin- (MOG-, MOBP-) specific cellular immune responses using proliferation and ELISPOT (IFN-gamma) assays. For JCV, we study the response against VP1, the major capsid protein. For JCV VP1, MOG and MOBP, we use 15-mer peptides overlapping by 10 amino acids, thus eliciting CD4+ as well as CD8+ T cell response. These peptides encompasse the whole sequence of the proteins. For EBV and CMV, we use pools of immunodominant 8- to 10-mer peptides eliciting CD8+ T cells. At the same time-points, using RT-PCR, we determine the presence of JCV DNA coding for the VP1 protein in the PBMC, plasma, and urine.

Results: At the time of writing this abstract, 16 patients have reached the 9-month (T9), and 11 the T12 time-point. We expect that by the ISNV meeting in June 2009, 18 and 14 patients will be at T9 and T12, respectively. Virological and immunological results will be presented.

Conclusions: This ongoing longitudinal prospective study should tell us whether there is an enhanced JCV activity in the peripheral blood of patients on natalizumab. This work is supported by the FNS (PP00B-106716), the Swiss MS Society and a research grant from Biogen Dompé.

## P86

### Neuropsychological profile of patients commencing HAART in Cape Town, South Africa

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Introduction: HIV-Associated Neurocognitive Disorders (HANDs) are now known to be highly prevalent in patients with HIV/AIDS, increasing with greater immunosuppression. Some have suggested that the prevalence and severity of HANDs differ across the various viral clades. Integral to the assessment of HANDs is the appropriate measure of neuropsychological profile. We undertook to evaluate the extent of HIV-associated impairment in a sample of young adults attending 3 primary care clinics in Cape Town, South Africa, where HIV clade C predominates.

Methods: 87 adults between the ages of 18 and 35 were recruited at random from 3 primary care clinics. A first assessment of neuropsychiatric disorders and functional status was followed by a second study visit during which a neuropsychological battery and neuromedical assessment was performed. The battery included measures of learning and memory, psychomotor speed and executive function. We also administered the neuropsychological battery to 20 HIV negative control subjects. The battery was translated into two local languages, Afrikaans and Xhosa, and administered by a first-language technician to those whose first language was either of these.

Results: Patients were on average 29 years of age and had 9.67 years of education, compared to controls who were 27 years of age and had 11.3 years of education respectively. Compared to controls, patients with clade C HIV who are about to commence HAART performed significantly more poorly on tests of learning and memory, psychomotor speed and executive function.

Conclusions: Our results suggest that HIV clade C is associated with significant neurocognitive impairment. Further, the pattern of cognitive impairment in clade C HIV appears consistent with the neuropsychological pattern observed in patients with clade B HIV residing in North America. Overall our results are consistent with findings from other studies of people with clade C HIV, and suggest that this clade, the most prevalent HIV clade in the world, is neurovirulent. Our group is currently collecting laboratory and neuroimaging data (MRI, DTI) on patients with clade C HIV to determine the neural pathways affected by this viral clade.

## P87

### Alcohol Upregulates Serotonin transporter and Monoamine oxidase in Human Dendritic Cells: Possible Implication in Neuroimmune Deregulation

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Alcohol is the most widely abused substance and its chronic consumption causes neurobehavioral disorders, increases susceptibility to infections. Dendritic cells (DC) serve as the first line of defense against infections and they are known to accumulate neurotransmitters like 5-hydroxytryptamine (5-HT). 5-HT is selectively transported into neurons through the serotonin transporter (SERT). SERT also serves as a receptor for psychostimulant recreational drugs. It has been demonstrated that several drugs of abuse such as amphetamine and cocaine inhibit the SERT expression; however, the role of alcohol is yet to be elucidated. We hypothesized that alcohol can modulate SERT and monoamine oxidase A (MAO-A) expression in DC leading to reciprocal downregulation of 5-HT in extracellular medium. DC were treated with different concentrations (0.05% to 0.2%) of alcohol and after 24–72 h period, the DC were processed for SERT and MAO-A expression using Q-PCR and Western blot analysis. In addition, 5-HT concentration in culture supernatant was also quantitated. Our results show that alcohol at 0.1% caused upregulation of SERT and MAO-A in DC as evidenced by Q-PCR and Western blots with a reciprocal downregulation of 5-HT in the culture supernatant. The present study suggests that SERT and MAO-A upregulation by alcohol may lead to decreased concentration of 5-HT in the extracellular medium. Since 5-HT is a major neurotransmitter and an inflammatory mediator, its depletion may cause neurological and immunological dysregulation and alcohol may play a significant role in neuroimmune deregulation.

## P88

### Hemochromatosis (HFE) Gene Polymorphisms and HIV-Associated Brain Pathology at Autopsy: Results of a NeuroAIDS DNA Bank Study

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Background: Neurodegenerative disorders affecting the central nervous system remain serious and debilitating complications of HIV infection in the era of highly active antiretroviral therapy (HAART). They include HIV encephalitis (HIVE), characterized by microglial nodules with formation of multinucleated giant cells in gray and white matter, and vacuolar myelopathy. HIV neurotropism, opportunistic infections, cytokine release by inflammatory cells such as microglia, and HAART toxicity have all been variably implicated in their pathogenesis. We hypothesized that immune-modulating hemochromatosis (HFE) gene variants, previously associated with reduced peripheral neurotoxicity during HAART, may also influence susceptibility to HIVE, the closely related entity of microglial nodule encephalitis (MGNE), and myelopathy.

Methods: Using genomic DNA from a neuropathologically based neuroAIDS DNA Bank, we evaluated associations of HFE G845A and C187G genotypes with HIV brain pathology (HBP, either HIVE or MGNE) and myelopathy in 410 HIV-infected individuals who died with AIDS and underwent autopsy between 1989 and 2006. The distribution of HFE genotypes among 146 HIV-negative autopsy cases was also assessed. HFE genotype associations with HBP were determined by univariate chi-square or Fisher's exact tests and race-stratified logistic regression analyses, adjusting for age and sex.

Results: Genotyping at both HFE loci was successful in 98% of autopsy cases with MassARRAY iPLEX Gold (Sequenom), and in 97% with TaqMan (ABI). HFE genotype frequencies did not differ from expected values based on race/ethnicity and were unrelated to HIV infection. Among 410 HIV-positive decedents (41% non-Hispanic black, 11% Hispanic, 46% non-Hispanic white; 2% other; median age 37 years (range 22–75), HIVE occurred in 113 (27.6%) and MGNE in 22 (5.4%); 275 decedents (67.1%) had no HBP. Non-Hispanic whites with at least one HFE 187G allele were more likely to have MGNE than 187CC homozygotes (18% vs. 6%, respectively, $P = 0.03$), and HBP was somewhat more prevalent in whites with HFE 845GA as compared to 845GG genotype (45% vs. 27%, respectively, $P = 0.07$). Myelopathy was also more common among combined white and Hispanic decedents with HFE 845GA as compared to 845GG genotype (31% vs. 15%, respectively; $P = 0.04$). In multivariate analyses, the HFE 187G allele remained significantly associated with MGNE in non-Hispanic whites [adjusted odds ratio (OR) 3.7; 95% CI 1.1–12.4, $P = 0.03$] and the 845A allele with myelopathy in whites and Hispanics combined (OR 2.7; 95% CI 1.1–6.7, $P = 0.04$). Decedents with either HFE variant were more likely to have HBP (OR 2.0; 95%CI 1.0–3.8, $P = 0.05$).

Conclusions: Based on this autopsy study, common HFE gene variants that impact inflammation and iron transport may influence HBP. These findings should be confirmed and further explored in larger studies, along with the modifying role of HAART.

## P89

### Astrocytes serve as a potential source of cell-free Tax protein during human T cell leukemia virus type 1-associated myelopathy/ tropical spastic paraparesis

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The mechanism of HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) pathogenesis is heavily reliant on the viral transcriptional transactivator protein Tax. The presence of cell-free Tax has been demonstrated in the cerebrospinal fluid of HAM/TSP and the effects of extracellular Tax have been reported in a number of cell types including microglia and neurons. Since astrocytes are one of the primary glial cells within the brain and are known to be infected with HTLV-1, we hypothesized that HTLV-1-infected astrocytes could play a critical role in HAM/TSP pathogenesis by releasing Tax into the extracellular space. In the study reported herein, astrocytes were transduced with lentivirus vector expressing green fluorescent protein labeled Tax (Tax-GFP), and were observed under fluorescent microscopy to determine localization of the protein. Tax was found to be present in high concentrations within cytoplasm with very little or no Tax being found in the nucleus 48 hr post-transduction. Subsequently, both cell lysate and supernatant were collected from the transduced cells and analyzed for the presence of Tax by western immunoblot analysis. Results indicated that Tax is released by astrocytes within 48 hr of transduction, in accordance with visual confirmation of Tax presence in the cytoplasm. A cytokine array analyses was also performed to monitor the changes in selected cytokine levels within astrocytes in response to Tax. The Tax-mediated

induction of proinflammatory cytokines and chemokines was observed indicating activation of astrocytes and alteration in their function. Future experimentation will determine the effect of Tax-transduced astrocytes on neuronal damage to correlate these observations with HAM/TSP pathogenesis.

## P90
### HIV-1 LTR transcription factor binding sites demonstrate altered conservation in drug abuse patients

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The HIV-1 long terminal repeat (LTR) contains several cis-acting elements that specifically bind host transcription factors and thereby modulate the promoter activity of the integrated provirus. Previous studies have demonstrated that a 3T configuration at C/EBP binding site I (C-to-T at position 3) and a 5T configuration at Sp binding site III (C-to-T at position 5) increase in prevalence with disease severity in the peripheral blood, and also correlate with HIV-associated dementia. Drug abuse and its impact on the spread of HIV through injection drug use and needle sharing is only one way drugs have had an impact on the HIV-1 epidemic. More importantly, drugs and alcohol alter HIV disease by altering the activation state of the infected cells and compromising the immune system, thereby affecting viral replication and selection. Drug use can also decrease patient compliance with antiretroviral therapy regimens. Therefore, we characterized the HIV-1 LTR sequence variation in the DrexelMed HIV-1 cohort within twelve known transcription factor binding sites to determine if the use of drugs of abuse can impact the genotypic variants seen within these sites. We have found that, while cocaine use and marijuana use decrease conservation at some sites, they increase conservation at others. Interestingly, the profile of conservation across the binding sites examined is not consistent between cocaine and marijuana users. This suggests that there may be a drug-specific molecular mechanism that accounts for the loss or gain of conservation at specific transcription factor binding sites within the HIV-1 LTR.

## P91
### Neurotrophin Mimetics reverse effects of HIV gp120 on Macrophages and Microglia

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Recent data suggest that neurotrophins, and a new class of small molecule neurotrophin mimetics which activate the p75neurotrophin receptor (p75NTR), may have excellent neuroprotective and anti-inflammatory potential. However little is known about the functions of neurotrophin receptors on macrophages and microglia. In this study, we have assessed the ability of neurotrophin mimetics to reverse the response from human monocyte-derived-macrophages (MDM) and human microglia (MG) treated with gp120. Antibody-based arrays for 120 proteins were used to characterize the response to gp120. Stimulation with gp120 changed the secretory profile of MG and MDM in different ways. Microglia showed increased secretion of IL-6, MCP-1 and RANTES and lower levels of IL-10, IL-8, MIP-1alpha, MIP-1beta, TIMP-1 and TIMP-2. The neurotrophin mimetic, LM11A-31, was able to reverse or partially reverse many of the gp-120 induced changes in protein expression including: GRO, sTNF-RI, Eotaxin-2, LIGHT, MCP-1, and MIP-3a for both MG and MDM. Reversal of changes in IL-1ra, MCP-2, MCP-3 were seen for MG only. Reversal of changes in IL-6R, MIP-1a, MIP-1b, sTNF-RII, TIMP-1 were seen for MDM only. Using FLOW cytometry, we assessed classical (CD197, CD86, CD80, CD16, CD206) and alternate activation marker expression (CD192, CD14, CD163) in response to gp120+ LM11A-31. LM11A-31 reversed many of the responses to gp120 and included decreases in the M1 activation markers CD16 and CD80 as well as decreases in the M2 activation markers CD192 and CD163. The ability of these compounds to reverse effects of gp120, or lower the expression levels of activation markers, on MDM and MG correlated with a reduction in the neurotoxic activity of the conditioned medium. The suppression of MDM and MG activation by LM11A-31 may protect against the earliest stages

of neural dysfunction. However, differences in both M1 and M2 responses of MDM and MG highlight the need to better understand the functions of these cells in order to effectively target anti-inflammatory therapeutics.

## P92

### Innate Immunity Contributes to Seizures and Epilepsy Following Viral Encephalitis

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We have developed a virus infection model for epilepsy. Infection of C57BL/6 (B6) mice with the DA strain (DAV) of Theiler's murine encephalomyelitis virus (TMEV) results in more than 50% of animals developing acute seizures and about half of these mice have spontaneous seizures after a latent period. Hippocampal neurons expressed large amounts of virus antigen and were observed to undergo cell death. These changes within the brain share many similarities with individuals with mesial temporal lobe epilepsy. Virus antigen was cleared by day 14 post infection (pi) and genome by 21 days pi from the brain. Mice were observed to have acute and electrographic seizures between day 3 and 10 pi. Since the seizures started around day 3, we initiated studies to determine the role of the innate immune response in the development of the acute seizures. Minocycline is an antibiotic that has neuroprotective effects as well as mitigating some of the effects of pro-inflammatory cytokines produced early during viral infection. B6 mice were treated with minocycline (2 times a day) and infected with DAV. Minocycline treatment of DAV infected mice decreased the number of mice having seizures by almost 50%. Since minocycline was observed to have an effect, we infected B6 mice deficient in the IL-1 receptor (IL-1RI). There was not a significant difference between wild-type B6 mice and IL-1RI infected mice in the number of mice developing seizures. To confirm this result, we infected MyD88 deficient B6 mice with DAV. Similar results were observed: no decrease in mice having seizures. In contrast, fewer numbers of TNF-alpha RI deficient and IL-6 deficient B6 mice developed seizures following infection with DAV. TNF-alpha and IL-6 mRNA levels were also seen to increase in seized mice. Inflammation, in the form of perivascular infiltrates, the presence of macrophages/activated microglia and gliosis, was greater in seized mice compared to controls. The anti-viral adaptive immune response played little or no role in these acute seizures, since OT-I mice infected with DAV still developed acute seizures which started around day 3 and ended by day 10 pi. Our studies implicate the innate immune response to virus infection, particularly TNF-alpha and IL-6, and concomitant inflammatory changes in the brain as contributing factors to the development of acute seizures. This model is the first infection driven model of mesial temporal lobe epilepsy with hippocampal sclerosis. This research is supported by Citizens United for Research in Epilepsy (CURE), Margolis Foundation and DeLand Foundation.

## P93

### Macrophage-Colony Stimulating Factor Regulation in Mononuclear Cells: Relevant Pathways in HIV Pathogenesis and the development of 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 in HIV infection and correlates directly with viral load and inversely with CD4+ T cell count below a critical threshold. Macrophages and microglia accumulating in CNS tissue in HIV dementia also express this phenotype and 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. M-CSF further induces production of MIP-alpha promoting chemotaxis of uninfected macrophages to sites active replication. HIV infection is also known to induce M-CSF production thus forming a positive feedback loop. While certain cytokines which are elevated in HIV-1 are known to promote M-CSF expression, the exact mechanism of M-CSF upregulation is unknown. Using ELISA based MCSF assays and luciferase reporter assays, we found that IL-6 and TNF-a upregulate M-CSF secretion in macrophages, and influence promoter activity in mononuclear cell lines. We further identified four

putative NFkB and four C/EBP bindings sites within the M-CSF promoter. Our findings using M-CSF promoter constructs mutated at individual NFkB and C/EBP locations suggest that that M-CSF transcription is regulated differentially by the binding of NFkB and C/EBP factors to specific promoter elements.

## P94
### JCV T-antigen mediated transformation of bone marrow-derived mesenchymal stem cells into cancer initiating cells

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Recently, cancer stem cells (CSCs) have been identified within a wide range of tumors, and CSCs are recognized as a deriving force for sustaining tumor growth. Therefore, our understanding of tumorigenesis depends on better understanding of molecular and cellular origins of tumors. Bone marrow-derived stromal cell (MSCs), also referred to as mesenchymal stem cells, have the ability of trafficking and multilineage differentiation into different organs. The polyomavirus, JCV, is a ubiquitous human virus with tropism for the brain which encodes a viral oncoprotein, T-antigen, which has been detected in human cancers and induces a variety of neural tumors in animal models. In this study we demonstrate that normal adult MSCs isolated from the bone marrow undergo neoplastic transformation induced by JCV, early protein, T-antigen. These transformed cells exhibit anchorage independence in culture, and are tumorigenic when transplanted into the flanks of Nude mice as compared to their non-transformed counterparts. Histologically, the tumors are heterogeneous with mesenchymal and neural crest characteristics as evidenced by expression of the neural crest markers p75, SOX-10, and S-100, with populations of tumor cells exhibiting characteristics of primitive neuroectodermal cells. In addition, subsets of T-antigen positive tumor cells exhibit a high proliferation index as detected by Ki-67 labeling, and co-express CD133, a marker which is expressed on CSCs isolated from various human tumors. These results show that MSCs are susceptible to JCV T-antigen induced transformation and develop malignancies resembling tumors of neural crest origin, which contain T-antigen and CD133 positive cells. Further, these studies suggest that JCV associated neuroectodermal tumors can be initiated in mesenchymal stem cells, a globally accessible adult stem cell that reside within different organs. In light of earlier reports on the association of JCV with a broad variety of human tumors, our data suggests that T-antigen transformation of adult stem cells with a multipotent capacity can serve as a possible common origin for some cancers, and offers a novel model for studying the oncogenesis.

## P95
### HIV-1 in the CNS Causes Dopaminergic Deficits in Multiple Brain Regions: Implications in Neurocognition

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Human immunodeficiency virus type 1 (HIV-1) invades the central nervous system (CNS) shortly after infection and becomes localized in varying concentrations in different brain regions. The most recognized vulnerable region to HIV-1 assault is the basal ganglia (BG). It is hypothesized that HIV-1 mediated neuropathogenesis in the subcortical regions causes degeneration of dopaminergic neurons in the substantia nigra, and the loss of dopaminergic terminals in the BG, leading to wide spread deficits in the central dopaminergic activity, and progressive impairment of neurocognitive and motor functions. In the era of highly active antiretroviral therapy (HAART), although, the incidence of HIV-associated dementia (HAD) has decreased, but the neurocognitive and neuropsychological deficits continue to persist after HAART. In this study we investigated the impact of HIV-1 on dopaminergic activity with respect to concentrations of dopamine and homovanillic acid (HVA) in different brain regions of post mortem human brains of HIV-1- and HIV-1+ individuals and their relationship to neurocognitive impairment. We found a wide range of dopamine (DA) and HVA concentration in different brain regions of HIV-1+ as well as HIV-negative cases. In HIV-negative cases, DA concentration was the highest in putamen, caudate, substantia nigra and the basal ganglia. In HIV-1+ cases, there was a significant decrease in DA levels in caudate, nucleus, putamen, globus pallidus, and substantia nigra, compared to that in HIV-negative cases, and a strong correlation was found between DA levels in substantia nigra and other brain regions. Concentration of HVA in HIV-negative cases was also highest in the regions containing high dopamine levels. However, no significant decrease in regional HVA levels was found in HIV-1+ cases. HIV-1 RNA load also ranged widely (non-detectable to log106.9 copies/g tissue) in the same brain regions of HIV-1+ cases. Interestingly, the brain regions having the highest HIV-1 RNA had the maximum decrease in DA levels. Age, gender, ethnicity and postmortem interval were not correlated with decrease in DA levels. HAART treatment was not found to affect the profile of DA, HVA and HIV-1 RNA levels in the

brain regions of HIV-1+ individuals. The majority of HIV-1+individuals had variable degrees of neurocognitive impairments, but no specific relationship was found between the regional DA content and severity of neurocognitive deficits. These findings suggest wide spread deficits in dopamine in the CNS of HIV-1infected cases, and that these deficits may be the result of HIV-1 induced neurodegeneration in the subcortical regions of human brain. Supported by the NIH grant # RO1 NS43982.

## P96

### Susceptibility of oligodendrocytic cell lines to Herpes Simplex Virus type 1

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Oligodendrocytes (OLs) are the myelin-producing cells of the central nervous system (CNS). The myelin sheet is a glycosphingolipid (GSL)- and cholesterol-rich multilamellar insulating layer that surrounds axons in both the central and peripheral nervous systems. We have investigated the susceptibility of three oligodendrocytic cell lines to Herpes Simplex Virus type 1 (HSV-1). Preliminary studies were carried out on KG-1C cell line, finding that these human cells were highly susceptible to HSV-1. Infection of KG-1C cells was characterized by a high level of virus production and a notable progression of the cytopathic effect. After infection, significant shut-off of host mRNA translation took place, correlating with evident synthesis of viral proteins. An examination by electron microscopy of the infected cells showed the presence of large clusters of mitochondria located in the proximity of intracellular HSV-1 particles groups. Herein we also show the HSV-1 infection of other oligodendrocytic cells: the human HOG and the murine Oli-neu cell lines. We have found that these cells are, as described in KG-1C cells, susceptible to HSV-1 infection. In addition, after culturing with differentiation medium, the infection course was delayed, suggesting an inhibitory effect of the differentiation process on HSV-1 infection. Finally, expression and localization of MAL2, a detergent-insoluble lipid rafts protein, was studied. Detection of MAL2 significantly increased after infection and it was colocalized with HSV-1 proteins.

## P97

### Rescue of impaired hippocampal neurogenesis in HIV-gp120 transgenic mice with voluntary running and reversal by detraining

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Impairment of adult neurogenesis in the hippocampus may contribute to memory and cognitive impairment associated with human immunodeficiency virus (HIV) infection. Modes of rescuing hippocampal neurogenesis need to be developed for treating these patients. We examined the effects of voluntary wheel running and detraining on hippocampal neurogenesis in a transgenic mouse model with glial expression of the HIV envelope protein, gp120 (gp120-tg). There was a 35% reduction of BrdU+ cells in the dentate gyrus of these mice ($n=6$) as compared to littermate wild type (wt) mice ($n=4$) (mean $\pm$ SE $= 1795 \pm 247$ BrdU+ cells/mm3 in gp120-tg versus mean $\pm$ SE $= 2725 \pm 441$ BrdU+ cells/mm3 in wt; $p < 0.01$) suggesting a defect of adult hippocampal neurogenesis resulting from a reduction of neural progenitor cell (NPC) proliferation. Double-label immunohistochemistry and confocal analysis showed a 45% reduction in newly generated mature neurons (BrdU+NeuN+) in the dentate gyrus of the gp120-tg mice ($n=5$ each) (mean $\pm$ SE $= 4432 \pm 515$ BrdU+NeuN+ cells/mm3 in gp120-tg versus mean $\pm$ SE $= 8054 \pm 1264$ BrdU+ NeuN+ cells/mm3 in wt; $p < 0.05$). Over the course of ten days, both the wt ($n=4$) and gp120-tg ($n=6$) mice housed in cages with running wheels averaged 5–6 km/day. Running increased NPC proliferation in hippocampi of wt mice by 40% (mean $\pm$ SE $= 4262 \pm 447$ BrdU+ cells/mm3 in wt-run versus mean $\pm$ SE $= 2725 \pm 441$ BrdU+ cells/mm3 wt-no run; $p < 0.001$). Importantly, the same ten day period of voluntary running was sufficient to completely rescue the deficit in NPC proliferation in the dentate gyrus of gp120 mice, increasing NPC proliferation by 54% (mean $\pm$ SE $= 3884 \pm 572$ BrdU+ cells/mm3 in gp120-tg-run versus mean $\pm$ SE $= 1795 \pm 247$ BrdU+ cells/mm3 in gp120-tg no run; $p < 0.001$). Voluntary running for five weeks resulted in a marked increase in the number of newly generated hippocampal neurons in gp120-tg ($n=4$), to levels higher than that of wild type non-running mice ($n=5$) (mean $\pm$ SE $= 16514 \pm 4260$ BrdU+NeuN+ cells/mm3 in gp120-tg-run versus mean $\pm$ SE $= 5897 \pm 798$ BrdU+ NeuN+ cells/mm3 in gp120-tg no run $p < 0.05$). Additionally, we determined whether the effect of voluntary running on cell proliferation could be maintained after exercise cessation. A ten-day period of running followed by ten days of detraining completely reversed the effect of voluntary running. This rebound effect was shown by the group's low cell proliferation compared to the control group (gp120-tg-control mean $\pm$ SE $= 1737 \pm 24$ versus gp 120-tg-detrained group mean $\pm$ SE $= 1579 \pm 78$; $p < 0.05$). These findings demonstrate the importance of sustained voluntary exercise in rescuing NPC

proliferation and generation of new neurons in hippocampi of adult gp120 transgenic mice which may have important therapeutic and life-style implications for patients with HIV-associated neurocognitive impairment.

## P98
### Suppression of neurodegeneration by modulation of glial gene expression: role of interferon regulatory factor 3

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The balance between proinflammatory and immunoregulatory cytokines in the local inflammatory milieu may determine the outcome towards neurodegeneration or neuroprotection. In a number of human neurological diseases, activation of microglia and astrocytes with overproduction of cytotoxic and proinflammatory cytokines may contribute to neuronal dysfunction and neurodegeneration. We have shown that the transcription factor interferon regulatory factor 3 (IRF3) plays a critical role in the innate antiviral immune response against viruses such as HIV and human cytomegalovirus (HCMV) infection of human glia. IRF3 is essential in the glial expression of IFNβ, and potentiates the induction of numerous antiviral genes that are stimulated by IFN (ISGs). Unexpectedly, we observed that overexpression of IRF3 via adenovirus-mediated gene transfer not only increased the expression of IFNs and ISGs but also differentially regulated the expression of many cytokine and chemokine genes. For example, while increasing the expression of IP-10/CXCL10, IL-13, IL-10 receptor and IL-1 receptor antagonist, IRF3 suppressed the expression of others including IL-1α, IL-1β, TNFα, GROα/CXCL1 and IL-8/CXCL8. Inducible nitric oxide synthase (iNOS) expression was also suppressed. In particular, there was a persistent and potent anti-inflammatory effect of IRF3 on the IL-1 axis: while IL-1 and IL-1 receptor (RI) were suppressed, IL-1 receptor antagonist was potently increased. These changes were notable in cells activated by inflammatory cytokines and toll-like receptor ligands (poly I:C and LPS), as well as in unstimulated cells, which showed low level gene induction by adenoviral vector only. Since IL-1 signaling crucially contributes to proinflammatory glial activation and neurotoxicity, these data suggest that IRF3 gene therapy may inhibit neuroinflammation but promote neuroprotection. In addition, IRF3 may also suppress the known immunogenic and inflammatory side effects of the adenoviral vector. The inhibition of IRF3-independent (but NF-κB-dependent) genes by IRF3 is novel and indicates a presence of an as yet undefined cross-talk between the IRF3 and NF-κB pathways. Powerful induction of innate immune response genes in CNS cells in combination with suppression of neurotoxic cytokines makes IRF3 a unique candidate for gene therapy for virus-induced neurological disorders. (Supported by NIH grants RO1MH55477, T32NS 007098 and P30AI051519).

## P99
### NAA factor scores suggest neuronal recovery during antiretroviral therapy: an MRSI study

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Introduction: Magnetic resonance spectroscopy is a useful tool for quantifying and analyzing the metabolic environment of brain, especially in the context of neurodegenerative diseases. Longitudinal magnetic resonance and cognition studies are lacking with respect to the effects of anti-retroviral therapy (ART) use in chronically HIV-infected subjects, especially those with HIV-associated dementia (HAD). Recently we reported factor analysis of magnetic resonance spectroscopic imaging (MRSI) metabolism data from a cross-sectional study of a chronically-infected cohort was found to be able to distinguish subjects based on HIV infection status (a ''choline factor'') and cognitive impairment status (an ''NAA factor''). Choline is an important component of lipid membranes and its levels reflect glial activation. Creatine is an energy marker representing general metabolic activity in the brain. NAA is found almost exclusively in the neurons and is a marker of neuronal integrity. The present study examined the longitudinal effects of initiating a new ART regimen on brain metabolism and cognition.

Methods: Fifty-one chronically infected HIV+ subjects (25 with MSK = 0 or 0.5 and 26 with MSK = 1 or 2) underwent MRSI and subsequent factor analysis before initiating a new ART regimen, and at 3 and 10 months thereafter to explore changes in metabolite concentrations in various brain regions due to changes in ART. Subjects also underwent a battery of neuropsychological testing and blood and CSF samples were obtained. Repeated-measures (RM) ANOVA and matched pairs t-tests were used to assess and isolate changes between time points.

Results: Over the 10 months, subjects demonstrated signs of a positive response to ART: compared to baseline values, CD4+ T cell levels improved and plasma and CSF viral loads were decreased (RM ANOVA: $P = 0.0006$, $P = 3.7 \times 10^{-12}$ and $P = 9.7 \times 10^{-5}$, respectively). NAA factor scores significantly increased from baseline at 3 months and were maintained at 10 months (RM ANOVA: $P = 0.0001$). Neither the choline factor nor the creatine factor scores changed significantly with therapy. MSK scores during this time period did not change ($P = 0.4$). The grooved pegboard non-dominant hand task was the only neuropsychological test to exhibit signs of improvement after a change in ART (RM ANOVA, $P < 0.05$) and did so only after 10 months of therapy ($P = 0.03$).

Conclusions: MRSI and factor analysis can be useful for determining the course of metabolic changes in the brain. The NAA factor, which heavily represents NAA, was found to improve after 3 months of ART, indicating an early reprieve of neuronal dysfunction in all subjects regardless of cognitive impairment. However, ''glial'' metabolism, represented by the choline factor, was not found to change, implying that low-level viral infection, and possibly inflammation/repair mechanisms, may be on-going in the brain after 10 months of therapy. Interestingly, the lack of MSK and cognitive domain (except fine-motor function) improvement reinforces the idea that recovery of cognitive function is much slower than that of the neuronal integrity marker NAA. It is possible that these cognitive domains recover in a specific manner in the context of ART. Further longitudinal studies are required to determine the progression of this recovery.

## P100

### Neuronal metabolism in HIV+ subjects lacking immune control correlates to M-CSF levels in cerebrospinal fluid

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Introduction: Macrophages are possibly the initial cell type infected and are key to the establishment of viral reservoirs such as the brain early during HIV infection. Current theories suggest infected monocyte-derived macrophages from the periphery permeate the blood-brain barrier (BBB) to bring the virus into the brain, which initiates a cascade of neurotoxic events. In lieu of pathology, macrophage colony-stimulating factor (M-CSF), which is responsible for the maturation and survival of monocytes into macrophages, may be a marker of monocyte/macrophage activity within the central nervous system in the context of HIV entry and subsequent neuronal damage. Magnetic resonance spectroscopic imaging (MRSI) allows for non-invasive measurement of N-acetylaspartate (NAA), which is a surrogate marker of neuronal integrity. The present study explores the relationship between cognition, M-CSF levels and neuronal dysfunction in HIV+ subjects who lacked immune control.

Methods: Forty-nine HIV+ subjects (24 with HIV-associated dementia, 25 without) about to begin a new anti-retroviral regimen were evaluated for NAA concentrations in seven brain regions (gray and white matter) using MRSI. Blood and CSF samples were collected to document the lack of immune control over the virus (CD4+ T-cells $< 200$ cells/mL, or viral RNA $> 50{,}000$ copies/mL). Neuropsychological tests were administered. M-CSF ELISAs were performed on CSF samples. Thirty-six of these subjects underwent repeat MRSI and cytokine evaluation at three and 10 months post-therapy change. Repeated-measures ANOVA was used to assess changes over time.

Results: After the therapy change, CD4+ T cell levels improved ($p < 0.002$), while M-CSF ($p = 0.003$), plasma and CSF viral levels of each subject declined ($p = 2 \times 10^{-8}$, $p < 0.0003$, respectively) indicating improvement in the overall health of each subject. Consequently, neuronal dysfunction receded as indicated by a significant rebound in NAA levels, with the significant increases seen in the parietal gray matter, frontal and parietal white matter ($p < 0.02$, $p = 0.001$, $p = 0.001$, respectively). Cognitive status did not improve over the 10 months ($p = 0.5$). At study entry, Spearman rank correlations indicated M-CSF levels were negatively correlated to NAA levels in 6 of the 7 brain regions at baseline (P-value range: 0.01 to $< 0.00004$). The strongest correlations were seen in the thalamus, basal ganglia, and centrum semiovale. This relationship was found to weaken after 3 months of therapy and disappear altogether after 10 months.

Conclusions: These results suggest that NAA is a marker of neuronal dysfunction that is capable of recovery before improvements in cognitive function. These improvements occurred regardless of dementia status, indicating a reprieve from neuronal dysfunction and the control of viral burden. At study entry, higher levels of M-CSF were associated with lower concentrations of NAA, the MRS markers of neuronal integrity. After therapy initiation, this association decreased gradually over time. These data suggest that higher expression of M-CSF may facilitate monocyte/macrophage-dependent neuronal injury across the brain and is controlled with initiation of ART. These data support

the use of MRSI as a means of monitoring treatment efficacy in patients with neuroAIDS.

## P101
### Lower Fibroblast Growth Factor (FGF)-1 Levels in Cerebrospinal Fluid from HIV-infected Methamphetamine Users are Associated with Worse Neuropsychological Performance

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Background: FGFs are expressed in the brain and may play important roles in neuroprotection. FGF-1 is primarily produced by neurons, promotes neuronal survival, and may protect calbindin-immunoreactive interneurons from the neurotoxic effects of HIV-gp120. FGF-2 is produced by astroglial cells and sustains endothelial cell fitness and blood-brain barrier homeostasis. To evaluate the relationships between FGFs and the brain, we measured FGF-1 and FGF-2 in CSF from volunteers differing in HIV status and METH use and compared the results to global neuropsychological (NP) performance.

Methods: 169 volunteers enrolled in a NIDA-funded Program (P01 DA12065) that assessed the impact of HIV and METH use on the nervous system. CSF was obtained by lumbar puncture in all volunteers. FGF-1 and FGF-2 were measured by ELISA. METH dependence was determined by the Composite International Diagnostic Interview. NP performance was determined by a comprehensive battery of standardized tests and was summarized by the Global Deficit Score (GDS). Results were analyzed by standard statistical methods, including t-tests, correlations, and multivariable regression.

Results: Volunteers were mostly middle-aged (mean 43 years), white (71%) men (87%). 93 (55%) were HIV seropositive and 100 (59%) had a history of METH dependence. 69 (41%) were HCV seropositive. HIV and METH were each associated with lower levels of FGF-1 in CSF (HIV: 1.68 vs. 1.84 log10 pg/mL, $p = 0.037$; METH: 1.70 vs. 1.84, $p = 0.068$). Only METH was associated with levels of FGF-2 (0.96 vs. 0.88 log10 pg/mL, $p = 0.02$). Worse GDS values correlated with lower FGF-1 levels and higher FGF-2 levels (FGF-1: $r = -0.20$, $p = 0.01$; FGF-2: $r = 0.14$, $p = 0.07$). Multivariable regression predicting GDS and accounting for HIV, METH, and HCV status confirmed that worse NP performance was associated with lower FGF-1 levels (β = $-0.20$, $p = 0.006$; Model $R2 = 0.07$, $p = 0.01$).

Conclusions: These findings provide in vivo support that HIV and methamphetamine can alter expression of fibroblast growth factors and that these alterations, particularly reductions in FGF-1, may contribute to the neurocognitive abnormalities that are common in these individuals. The investigational human recombinant FGF-1 that has been developed may provide benefits for HIV − and methamphetamine-associated cognitive impairment.

## P102
### Invasion of the CNS by Influenza A Virus Following Intranasal Inoculation Increases the Number of Cytokine-Immunoreactive Neurons in the Olfactory Cortex, Amygdala and Hypothalamus

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Some aspects of the acute phase response (APR) induced by influenza virus infection are regulated by the brain, including changes in body temperature (hypothermia), locomotor activity and sleep. These symptoms are thought to be regulated by cytokines produced in the infected respiratory tract that then act upon the brain. Mice infected intranasally with high dose PR8 influenza virus show virus invasion of the olfactory bulb (OB) within 4 h and become hypothermic within 15 h. Both viral replication intermediates and antigen are expressed in glia within the OB glomerular layer at 10 and 15 h post-infection (PI). To characterize the cytokine response caudal to the OB, we determined the distribution of viral, IL-1beta and TNF-alpha antigens at 10 and 15 h PI in brain regions that have direct and indirect projections from the OB after influenza intranasal inoculation. Using immunohistochemistry we examined the dominant olfactory pathway [piriform cortex (Pir) and olfactory tubercle (Tu)] and an autonomic pathway [basolateral amygdala (BLA), central amygdala (CeA), the medial preoptic (MPO) and arcuate (Arc) nuclei of the hypothalamus]. Virus-IR was not detected beyond the OB outer layers. After live virus inoculation, a significant increase in the number of IL1-IR neurons in the Pir, Tu, CeA, MPO and Arc occurred at 15 h but not at 10 h PI. The number of TNF-IR neurons also significantly increased in the Tu and CeA at 15 h but not at 10 h PI. These results suggest that viral replication in the OB outer layers is followed by activation of cytokine expression in neurons along neuronal pathways from the OB to the temperature-regulating hypothalamic nuclei, and that cytokine-expressing neurons are increased above control levels at the time the animals become sick, but not 5 h before. The correlation of illness onset with increased expression of IL1 in key hypothalamic nuclei suggests that brain cytokine induction by virus invading the OB could play a role in the viral APR. Supported by NIH grants HD36520, NS25378 and NS31453. Leyva-Grado was also supported by the DGAPA-UNAM.

## P103

### Dramatic enhancement of HIV-1 Tat transduction via modulation of endocytic and trafficking pathways using cationic liposomes

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HIV-1 Tat protein can be secreted from infected cells and taken up by other target cells. The property of Tat membrane transduction has been widely exploited for delivering macromolecules into target cells. We investigated methods to efficiently deliver Tat protein to the nucleus and found that it could be dramatically enhanced by a cationic liposome reagent (lipo) in Hela-derived cell lines (HL3T1 and TZM-bl). HIV LTR transactivation was increased >500 fold in HL3T1 cells treated with Tat coupled with lipo. Enhanced membrane binding and some 1–2 μm particles were typically observed in the cells taking up the lipo-coupled Tat-v (fused to a fluorescent protein, venus). Compared to the chloroquine-enhanced transduction, Tat-internalizing pathways were modulated in the lipo-facilitated delivery which involved lipid raft-dependent endocytosis and macropinocytosis as determined by pharmacological inhibitors of endocytosis (MβCD, genistein, etc.) and immuno-colocalization of Tat-v with anti-clathrin1or anti-caveolin1 antibody. Molecules carrying ''+'' or ''−'' charges, including macromolecules (e.g., heparin, polybrene) and small molecules (e.g., spermine) all inhibited the lipo-Tat uptake, but the ''+'' molecules attached to the cell membrane carrying ''−'' charges and the ''−'' molecules bound to the lipo-Tat complex with ''+'' charges. However, the lipo-Tat complexes were very likely formed by hydrophobic bonds linking liposome's lipid to Tat hydrophobic core domain, considering both Tat proteins and the liposome reagents carried repellent ''+'' charges. The synthetic hydrophobic peptide (Tat36-47) could competitively inhibit the uptake of the lipo-coupled Tat and there was no inhibition on the LTR transactivation or HIV-1 replication. In conclusion, cationic liposomes could facilitate Tat protein to traverse cell membrane by modulating endocytic and trafficking pathways and significantly enhanced its delivery to nucleus in a lysosome-independent manner. These observations have important implications for elucidating the mechanism of Tat uptake and trafficking and exploring the intracellular delivery of biomolecules

## P104

### Defining the fitness of HIV-1 LTR quasispecies containing specific core/enhancer region binding site polymorphisms

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The HIV-1 promoter or long terminal repeat (LTR) regulates viral gene expression by interacting with multiple viral and host factors. Studies have shown that specific sequence configurations within CCAAT enhancer binding protein (C/EBP) site I, and transcription factor Sp binding site III of peripheral blood (PB)-derived LTRs from HIV-1-infected patients are preferentially encountered in late stage HIV disease. Specifically, the 3T configuration of C/EBP site I and the 5T configuration of Sp site III were binding site variants found in low frequencies in PB-derived LTRs from patients at early stages, and at relatively high frequencies in patients in late stage disease in sequence analyses performed in the pre-HAART era. Based on gel shift and BIAcore technologies, the C/EBP 3T and Sp 5T binding site configurations have been shown to exhibit a lower binding affinity for C/EBP and Sp1 compared to the consensus subytpe B LTR configuration at these sites, respectively. In recent studies, the HIV-1 3T/5T LTR genotype has now been detected in HIV-1-infected patients in the DrexelMed Cohort and we are now exploring the relative fitness of HIV-1 quasispecies containing these specific sequence alterations. Transient expression analyses have shown that LTRs containing the Sp 5T binding site configuration exhibit a spectrum of basal and Tat-driven functional activities within cells of the T cell and cells of the monocytic lineage. Results also demonstrate this LTR activity to be cell-type specific. These results suggest that the 5T and 3T/5T LTRs may be present in infectious viral quasispecies circulating in the peripheral blood and other compartments during mid- to late-stage HIV disease.

## P105

### Methamphetamine increases HIV-1 viral protein expression in the spleen of the HIV-1 transgenic rat

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The HIV-1 transgenic (HIV-1Tg) rat carries a gag-pol-deleted HIV-1 genome under the control of a HIV-1 viral promoter, and expresses 7 of the 9 HIV-1 genes. The expression of various HIV-1 viral proteins in this transgenic rat appears to mimic the persistent low level of infection in HIV patients stably treated with highly active anti-retroviral therapy (HAART), and is, thus, a useful model for HIV patients given

HAART. Methamphetamine (METH) is a highly addictive stimulant drug that exerts its effects on the neuronal system. METH abuse appears to exacerbate AIDS symptoms and disease progression in HIV infected patients. We used the HIV-1Tg rat to test our hypothesis that METH abuse enhances the expression of HIV-1 viral proteins, such as gp120 and Tat. We examined gp120 and Tat mRNA levels in the spleen (peripheral immune organ), thymus (central immune organ), and prefrontal cortex, striatum, and hypothalamus of the brain of 12–13 wk old male HIV-1Tg rats with and without exposure to a sensitization dosage of METH (2.5 mg/kg i.p.) for 6 d using real time RT-PCR. Compared to the saline control rats, there was a slight but insignificant increase in gp120 and Tat mRNA expression in the prefrontal cortex ($1.30 \pm 0.24$ and $1.08 \pm 0.21$ fold, respectively), striatum ($1.02 \pm 0.15$ and $1.06 \pm 0.15$ fold, respectively), and hypothalamus ($1.05 \pm 0.08$ and $1.18 \pm 0.11$ fold, respectively) of the HIV-1Tg rats, and a slight but insignificant decrease in the thymus ($0.80 \pm 0.06$ and $0.96 \pm 0.12$ fold, respectively) of the HIV-1Tg rats. There was, however, a significant increase in the spleen ($1.59 \pm 0.04$ and $1.57 \pm 0.07$ fold, respectively) of the HIV-1Tg rats compared to control animals. These data indicate that, although METH has little effect on viral protein expression in the brain and central immune organs, it has clear effects on viral protein expression in peripheral immune organs. Further studies are needed to investigate the mechanism by which METH exerts differential effects on central versus peripheral organ systems. (Supported, in part, by DA016149 to SLC.)

## P106
### HIV-1 Infection Dysregulates miRNA Expression in Human Primary Macrophages

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Thirty-three million people worldwide are infected with HIV, the virus which causes AIDS. The characteristic pathology believed to cause HIV-Associated Neurocognitive Disorder (HAND) includes neuronal damage and death, inflammation, elevated levels of the chemokine CCL2 in the Central Nervous System (CNS), and leukocyte infiltration of the CNS. Monocytes/Macrophages are one of the first cells infected during the course of HIV progession towards AIDS; the transmigration of infected cells of this lineage across the Blood Brain Barrier (BBB) is one way in which the virus can enter the brain. HIV Infection induces changes in the gene expression profiles of macrophages, in turn affecting their activity during HIV infection. Although the cellular response of macrophages toward HIV infection has yet to be fully understood, it is hypothesized that the expression profile of small non-coding RNAs, such as microRNAs, might be altered by the virus. MicroRNAs (miRNAs) are a recently discovered class of endogenous, small non-coding RNAs ($\sim$22nt) that control gene expression by directing their target mRNAs for degradation and/or transcriptional repression. MicroRNAs require specialized machinery for their production and affect many cellular functions, including differentiation and hematopoiesis; their dysregulation has been shown to affect the course of many diseases. To date, little is known about the miRNAome of primary human monocyte derived macrophages (macrophages), and the role this machinery plays in the progression of HIV infection. To evaluate the role of miRNAs in HIV infection of macrophages, we confirmed the presence of the key proteins of the miRNA machinery of human primary macrophages. To determine the presence of miRNAs in these cells, we performed miRNA microarrays with both HIV infected and uninfected human primary monocyte derived macrophages. Our preliminary results demonstrated that over 40 miRNAs are differentially regulated during HIV infection of macrophages. These results were confirmed using a second array technique ($N = 4$ separate donors). We have confirmed expression differences in miRNAs of HIV-infected versus uninfected macrophages using quantitative Real Time PCR, and have found differences in miRNAs known to regulate inflammatory proteins that are key to the responses of macrophages to infection. We will use Argonaut immunoprecipitation to purify RNA from RNA-induced silencing complexes (RISC), and align processed mRNA with the miRNAs regulating them. These novel findings will contribute to our understanding of key activities of macrophages during HIV infection: why cells of the monocytic lineage are the first cells infected; why they transmigrate across the BBB more effectively. Ultimately, this research will identify key mechanisms to target for developing therapeutic strategies for the treatment of HAND.

## P107
### Murine leukemia viruses induce characteristic electrophysiological changes in specific neuron subsets: Implications for spongiform neuropathogenesis

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The prototypic neurovirulent murine leukemia virus, CasBrE, induces a fatal paralytic disease in susceptible mice characterized by progressive vacuolar neurodegeneration. Our studies to date have revealed that the viral env gene alone is sufficient for mediating neuropathogenesis, however, the molecular and cellular mechanisms involved remain elusive. Previous ultrastructural studies indicate that CasBrE-induced vacuolation first arises post-synaptically and progresses to include involvement of neuronal and glial cell bodies. This pathologic picture is highly reminiscent of excitotoxicity, however, in vivo have not supported this idea as multiple glutamate antagonists have failed to alter the severity or course of disease. Therefore, in order to identify the specific virus-induced neuronal changes that ultimately become manifest as spongiosis, we undertook a electrophysiologic analysis of the inferior colliculus (IC), an affected midbrain region containing six distinct neuronal subtypes. Specifically, neurons within the central nucleus of the IC were examined by patch clamp analysis in acute brain slice preparations from mice infected with either the highly neurovirulent CasBrE env containing virus, FrCasE, the isogenic non-neurovirulent ecotropic virus Fr57E (differing only in env), or mock-infected controls. Slices were assessed at multiple time points corresponding to before and after the onset of vacuolar changes, and before and after virus spread from the vasculature to proliferating microglia and neural progenitors (NG2 cells). Of the neurons types examined, only the three characterized by calcium-dependent post inhibitory rebound firing (rebound neurons; RNs) were affected by MLV infection. Regardless of whether the mice were infected with FrCasE or Fr57E these cells lost their capacity for rebound firing but maintained their normal depolarization induced firing activity. However, FrCasE infection also induced bursts of spontaneous neuronal firing that was limited to a single subtype of rebound neuron namely the rebound regular cells (RRs). The spontaneous RR firing was associated with slow membrane depolarization that was only eliminated by holding the resting membrane potential below -90mV. Importantly, the initial detection of bursting behavior was temporally coincident with the first appearance of IC vacuolation. NMDA receptor antagonists did not affect any of the virus-induced changes, however, increasing the calcium buffering capacity in the affected neurons was able to restore rebound firing and suppress spontaneous RR firing. These results indicate that CNS expression of MLV envs alters neuronal calcium homeostasis that is likely responsible for the development of spongiosis. These findings have significant implications for understanding the mechanisms of neuropathogenesis induced by unique viral and host proteins. This work was supported by NIH grants NS37614 and DC008120 to WPL and SS respectively.

## P108

### DARPP-32 signaling: A common neuropathogenic pathway in HIV-1 infected drug abusers

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It is well established that opiates like heroin modulate dopaminergic activity via the dopamine transporter (DAT). An association between the DAT1-VNTR (variable number of tandem repeats) polymorphism and dopaminergic activity and neuronal function has been recently reported. The DAT1 genotype may therefore modulate neuronal function via modulation of dopaminergic activity. An important constituent of dopaminergic activities within the brain is the 32 kDa dopamine and adenosine 3′,5′-monophosphate-regulated phosphoprotein (DARPP-32) recognized to be critical to the pathogenesis of drug addiction. Opiates act as cofactors in the pathogenesis of HIV-1 infections. Drug abuse by HIV-1 infected subjects may exacerbate the progression of HIVE as a consequence of the combined effects of HIV-1 induced neurotoxins plus psycho-stimulant drug induced increase in D1 receptor activation and consequent DARPP-32 modulation resulting in dysfunction of the dopaminergic system. We hypothesize that the DARPP-32 signaling pathway may be the central molecular mechanism that integrates the neuropathogenic activities of both HIV-1 infection and the abuse of opiates. Based on this hypothesis we proposed these specific aims. Aim 1: To investigate expression levels of the DAT1 genotype in HIV-1 infected subjects who abuse/do not abuse heroin ($n = 12$ patients /group). Aim 2: To silence DARPP-32 gene expression in vitro in primary human dopaminergic neuronal cells (DAN cells) using a DARPP-32 siRNA and evaluate the effects of heroin (10-7–10-9 M) and/or HIV-1 viral protein (Tat) (10–50ng) treatment on the expression of signal transduction molecules like PP-1 and ERK that are downstream of DARPP-32 in the transfected DAN cells. Our results using QPCR show a significant increase in DAT1-VNTR gene expression (Relative expression ratio $= 0.48 \pm 0.016$; $p = 0.001$) in HIV-1 subjects who abused heroin as compared to those who did not ($0.23 \pm 0.012$). A significant decrease in PP-1 (46%, $p < 0.05$) and ERK (71%,

$p < 0.01$) gene expression was observed in DARPP-32-siRNA transfected DAN cells that were treated with a combination of heroin and HIV-1 tat. These studies highlight the role of dopaminergic signaling pathway in initiating molecular changes that influence neuronal plasticity contributing to the drug addiction process. A better understanding of the neurobiological mechanisms underlying drug addiction and its role in the enhanced neuropathogenesis of HIV-1 will help develop novel therapies for drug addiction.

## P109

### Latent simian varicella virus reactivates in monkeys treated with tacrolimus with or without exposure to irradiation

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Clinical, pathological, immunological and virological features of simian varicella virus (SVV) infection of primates closely parallel human varicella zoster virus (VZV) infection. After natural SVV infection of primates, virus becomes latent in ganglionic neurons and reactivates upon social and environmental stress. Earlier we also demonstrated experimental reactivation of SVV after X-irradiation and treatment with tacrolimus and prednisone. Herein, natural SVV infection was established in 5 Cynomologous and 10 African green monkeys. Four of the Cynomologous monkeys were treated with tacrolimus ($80-300$ μg/kg/day) for 4 months and one was untreated (group 1). Four of the African green monkeys were exposed to a single dose (200 cGy) of X-irradiation (group 2), and 4 others were exposed to X-irradiation and treated with tacrolimus for 4 months (group 3); the remaining 2 African green monkeys served as controls. Zoster rash developed $1-2$ weeks after treatment with tacrolimus in 3 of 4 monkeys in group 1, after X-irradiation in 1 of 4 monkeys in group 2 and 6 weeks after irradiation in all of the 4 monkeys in group 3. Punch biopsies of the skin rash were taken for immunohistochemical analysis. All monkeys were sacrificed $1-4$ months after being immunosuppressed, and SVV glycoproteins gH and L were detected in punch biopsies as well as in sections of lungs from most monkeys. SVV DNA was detected at very low copy number in ganglia from all three groups of monkeys including the controls. RNA specific for SVV ORFs 61, 63 and 9 were detected in ganglia from one immunosuppressed monkey in group 1. SVV glycoproteins gH and gL were detected in multiple ganglia from all immunosuppressed monkeys in all groups, but not in control animals. Our results reveal that immunosuppression by treatment with tacrolimus, with or without X-irradiation reactivates latent SVV to produce zoster.

## P110

### Regulation of MSRV (Multiple Sclerosis-associated retrovirus) and Syncytin-1 by viruses and cytokines

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Multiple sclerosis (MS) is a chronic neuro-inflammatory disease, characterized by demyelination and gliosis, with various degrees of axonal pathology and episodic or progressive neurological disability. The aetiology is unknown, but the immunopathogenic phenomena are thought to be triggered by an environmental (infectious) factor operating on a predisposing genetic background. The most consistent studies for a potential role in the disease exist for some herpesviruses, such as the Epstein Barr virus (EBV) and human herpesvirus-6 (HHV-6), and two elements of the W family of human endogenous retroviruses (HERV-W): MSRV (MS-associated retrovirus, the founder member of the HERV-W family), and syncytin-1, the env product of the replication-incompetent ERVWE-1 HERV-W element. The mechanisms by which these viruses and other potential candidates might initiate, exacerbate, and perpetuate the disease are far from understood. However both MSRVenv and syncytin-1 proteins have pro-inflammatory properties that might be pathogenic, as observed both in vitro and in animal models. In the past we have studied MSRV in MS patients in various temporal and clinical stages of the disease, in follow up evaluations of MS progression and during therapy, as well as in the conversion of patients suffering of optical neuritis to full blown MS. In all cases, the data showed a striking parallelism between MS behaviour and MSRV/HERV-W presence and/or load. By simultaneous detection of both MSRV and HHV-6, we found a direct correlation between MS and MSRV load, while no significant differences from controls were found for HHV-6. As for EBV, epidemiological and immunological studies evidenced altered immune reactivity to EBV in MS patients, and that increased immune reactivity to EBV is an early event in MS. Moreover, in cultured B cell lines EBV activates the transcription of the env gene of another retroele-

ment, i.e. HERV-K18. In search of a connection with MS pathogenesis, we looked for possible triggers of activation of MSRV and syncytin-1, that could be consistent with the above findings. To this end, we monitored the presence and expression of MSRV, syncytin-1, HERV-K18, EBV, HHV-6 in MS blood and brain samples, and performed in vitro experiments on blood cells from patients and control individuals, as well as in astrocytic cells, that had been exposed to MS-related and unrelated viruses (EBV and HIV) and cytokines (TNF$\alpha$, Type I, II and III interferons). Evaluation methods included detection of released and cell-associated sequences and proteins, by selective real time RT-PCR assays, ELISA, flow-cytometry and western blot. Data from patients confirm the correlation between expression of HERV-W elements and in vivo MS status and behaviour. In vitro experiments showed that infection with the EBV herpesvirus stimulates the expression of MSRVenv and syncytin-1; the activation occurs both in blood cells and in astrocytes. The activation of both HERV-W elements is not a generalized phenomenon due to a virus infection, since infection with the HIV retrovirus determines a differential modulation of MSRVenv and syncytin-1 expression. HIV effect on Syncytin-1 is due at least in part to its tat regulatory protein, since it is observed also in astrocytes by transient transfection with cloned syncytin-1 promoter and exposure to tat, that acts on the NF-$\kappa$B binding site present in the promoter. The recently discovered Type III interferon$\lambda$2, that favours the replication of exogenous retroviruses, is able to enhance the expression of both MSRVenv and syncytin-1, thus behaving as a pro-inflammatory cytokine. Totally unknown is whether this interferon plays any role in MS. Combining our findings with those from the literature, one could hypothesize in MS a direct role as pathogenetic effector for HERV-W/MSRV/syncytin-1 in MS, and an indirect effect for EBV, that could increase the expression of HERV-W/MSRV/syncytin-in glial or lymphocytic cells. This interaction between viruses (EBV and HERV-W/MSRV) could establish in MS patients toxic mechanisms against the oligodendrocytes, that induce inflammation, demyelination and axonal damage. Work supported partly by grants from Ministero Università e Ricerca PRIN 2007 and Regione Autonoma Sardegna.

## P111

### Natural Host Genetic Resistance to SIV CNS Disease: A Neuroprotective MHC Class I Allele in Pigtailed Macaques

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HIV frequently causes neurologic impairment even with anti-retroviral treatment. Although associations between MHC class I allele expression and the syndrome AIDS have been reported, the role specific MHC class I alleles play in restricting development of HIV-induced neurologic disease has not been well characterized. This study examined the relationship between expression of pigtailed macaque MHC class I alleles and development of SIV central nervous system (CNS) disease using a well-characterized simian immunodeficiency (SIV)/pigtailed macaque model. In 80 SIV-infected macaques, the odds of developing CNS disease (SIV encephalitis) were six times higher for animals that did not express the MHC class I allele Mane-A*10 ($P < 0.001$). Correspondingly, animals that expressed the Mane-A*10 allele had significantly lower amounts of activated macrophages (measured by CD68 immunostaining), SIV RNA, and neuronal dysfunction in the CNS than Mane-A*10 negative animals ($P < 0.001$). Mane-A*10 positive animals with the highest CNS viral burdens contained SIV gag escape mutants at the Mane-A*10-restricted KP9 epitope in the CNS whereas wild type KP9 sequences dominated in the brain of Mane-A*10 negative animals with comparable CNS viral burdens. These findings demonstrate that MHC class I alleles can play major neuroprotective roles in lentiviral-induced CNS disease. These studies are supported by NIH RR019995.

## P112

### Role of dendritic cells in priming cellular immune responses during HTLV-1-associated neuroinflammatory disease

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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+ 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 the

priming of anti-Tax CTL response under both in vitro and in vivo conditions. We investigated two routes (direct verus indirect) of Tax presentation using live virus and CD4+ T-cell lines (MT-2, an HTLV-1-infected cell line, and C8166, an HTLV-1-mutated line that only expresses Tax), respectively. 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.

## P113

### Polychromatic FACS analyses of the phenotypic and functional characteristics of dendritic cells and T cells in a cohort of HTLV-1-infected samples from the Jamaican region

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The debilitating neurodegenerative disease, HAM/TSP, is characterized by a robust immune response including the oligoclonal expansion of CD8+ cytotoxic T lymphocytes (CTLs) specific for the viral oncoprotein Tax. However, the specific underlying mechanism resulting in this disease process is currently unknown. A high proviral load is a significant risk factor for the development of HAM/TSP. Furthermore; the antiviral efficacy of a CTL response is known to be affected by many factors including the efficiency of epitope processing and presentation. Since dendritic cells (DCs) are the most potent antigen presenting cells, understanding their status in HTLV-1-associated autoimmune neuropathogenesis is critical. Therefore, we have standardized a new DC cocktail that contains 11 different markers related to DC functions utilizing a BD LSR II polychromatic flow cytometer. In addition we have included a pre-standardized 14-color T cell polyfunctional cocktail to investigate the immune activation status of T cells in HTLV-1-infected clinical samples obtained from a Jamaican cohort. Our current polychromatic DC and T cell ex vivo analyses have enabled us to begin to investigate differences among three individual groups: seronegative controls, asymptomatic carriers, and HAM/TSP patients from the same geographical region. The results of these studies are being statistically analyzed in reference to the matched HTLV-1 proviral loads as well as Tax mRNA levels. Collectively, these investigations possess great potential to shed light on the current understanding of the immunopathogenesis of HTLV-1-induced neuroinflammatory disease and to similar diseases of other etiologies.

## P114

### Profound differences encoded by various Creutzfeldt-Jakob Disease (CJD) and scrapie infectious agents are conserved in normal mice and monotypic murine cultures

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Our laboratory has developed parallel animal and tissue culture models to facilitate detailed comparison of diverse transmissible encephalopathy (TSE) agents. Understanding the origin and spread of individual agents is essential for their diagnosis and prevention. We have been successful in serially passaging many different TSE agents in normal mice expressing standard prion protein (PrP). Transmissions include multiple isolates of human sporadic CJD (sCJD), unique Japanese CJD isolates, variant CJD (vCJD), an agent linked to epidemic bovine infections (BSE), New Guinea kuru, and various established sheep scrapie agents, e.g., 22L scrapie and the Chandler (RML) agent. We also were able to infect normal mice with the highly cloned mutant 263K hamster scrapie agent selected for low pathogenicity in mice. All these agent strains were additionally propagated in neuronal GT1 cell cultures, and all display clear differences from each other. BSE from cows is identical to vCJD, but unlike any other CJD isolate. Although vCJD replicated in human brain for >5years, it failed infect PrP humanized mice but infected normal mice. We also found that kuru is clearly a distinct geographic agent that is different than sCJD. In cultured cells one can evaluate each agent in a highly controlled single cell type. Most TSE culture models, as well as transgenic mouse models, support only one or a few agents, limiting the appreciation of profound and stable TSE agent differences. TSE agents preserve their fundamental identities in various species and culture cell types regardless of variant host PrPs. PrP appears to be a required receptor for the TSE infectious particle, and a large body of data indicate this particle probably contains a strain-encoding (viral) nucleic acid. The presumed spontaneous

generation of infectious PrP has not yet been observed, and PrP itself has been unable to reproduce significant infectivity. Since several TSE agents can replicate to high brain-like levels in GT1 cultures, unlike other culture models, it is feasible to use these cultures for better molecular characterization of the infectious agent. To verify agent recoveries, without long and expensive animal assays, we developed a rapid and reliable infectivity assay using GT1 cells. Infectious particles in cultured cells, as in brain, can be separated from many host proteins including various visible forms of PrP. More purified infectious particle preparations may be used to discover viral antigens as well as intrinsic agent nucleic acids.

## P115
### Gene-targeted deletion of E2F1 evokes similar physiological and behavioral deficits to HIV-Associated Dementia

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Several cell cycle proteins have been identified that are involved with the neuroimflammatory and neurotoxic pathways of HIV-Associated Dementia (HAD). One of these cell cycle proteins, E2F1, also plays an important role during generation and replacement of neurons in the adult brain as evidenced by a substantial reduction of adult neurogenesis (ANG) in the olfactory bulb (OB) and dentate gyrus (DG) of E2F1 knockout mice (KO). A relatively new and exciting hypothesis has emerged that proposes that the pathologies and neurologic effects of neurodegenerative diseases, including HAD, could arise from the loss of ANG. Physiological, morphological, and behavioral studies suggest ANG plays a substantial role in the maintenance, plasticity and function of the OB and hippocampus, hence it is important to note that HAD pathology involves olfactory and memory deficits. We therefore hypothesized that KO mice may develop deficits in olfactory ability and memory. In fact, six separate behavioral tests demonstrate that KO mice display a strikingly similar sequence pattern of age-dependent olfactory-, memory-, and anxiety-related behavioral deficits to that of HAD. Wild-type (WT) and KO mice of five age ranges were subjected to general anosmia, odor habituation, short- and long-term object memory paradigms, as well as classical light/dark box and marble burying tests for anxiety. During general anosmia testing, KO mice displayed a threefold increase in retrieval time of a scented object versus WT mice, suggesting that age-dependent deficits in olfaction and/or motivation arise. KO mice older than P40 also lose the ability to discriminate between octanols varying by a single carbon, indicating a second type of olfactory deficit. Additionally, all WT age groups demonstrated adequate short- and long-term memory, whereas KO mice object memory recognition deteriorated over time, failing tests at P180 and beyond. Interestingly, E2F1 KO mice displayed incessant digging during tests, so marble burying and light/dark box (LDB) behavior were assayed to determine anxiety levels. E2F1 KO mice buried significantly more marbles than WT mice in all age groups, and spent significantly less time in the light chamber of the LDB in 4/5 age groups. Lastly, KO mice displayed a significant reduction of post-synaptic density 95 (PSD-95) expression in the hippocampus, consistent with previous reports of PSD-95 loss during the dieased state. Thus, KO mice display resemble clinical behavioral changes seen in HAD patients, along with synaptic scaffolding abnormalities.

## P116
### Spi-B Binding to Target Sequences within the JC Virus Regulatory Region

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JC virus (JCV) is the etiological agent of the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy that occurs in approximately 5% of AIDS patients, and is an emerging threat for those undergoing immunomodulatory therapies for diseases like Multiple Sclerosis. JCV undergoes a lytic infection in the brain and a latent infection in cells of the immune system. JCV latency is associated with hematopoietic progenitors in the bone marrow and mature B cells in circulation. JCV gene expression is tightly regulated through usage of cellular transcription factors. Because JCV latency is associated with cells undergoing hematopoietic development, it is probable that lymphoid specific transcription factors regulate JCV gene expression in these cells. Spi-B is expressed at high levels in developing and mature B cells, and is involved in differentiation and maturation of B cells. Nine Spi-B binding sites have been identified on the prototype, including MAD1 and MAD4, and archetype JCV regulatory regions. Five sites are present in all three viral sequences. Three of the potential sites were bound by proteins expressed in a hematopoietic precursor cell line (KG1a), a mature B cell line (BJAB) and progenitor derived astrocytes in electromobility shift assays. One of the potential sites was bound only by proteins expressed in progenitor derived astrocytes. Importantly, two of the Spi-B sites, specific to the MAD1 and MAD4 sequences, are located adjacent to TATA boxes on the viral promoter, which have been shown to be essential for

expression of early and late viral genes. Interestingly, Spi-B binds the TATA binding protein (TBP) and potentiates expression from a variety of promoters. Cooperation of Spi-B and TBP on the TATA boxes on the JCV promoter is an attractive model for reactivation from latency in developing B cells. Future studies will determine the functional consequence of Spi-B binding on the JCV regulatory region on the outcome of JCV infection in cells of the brain and immune system.

## P117

### Genetic deletion of CCR5 reduces alteration of CNS gene expression and prevents neuronal injury, but not astrocytosis, induced by HIV-1 gp120 in a transgenic mouse model expressing the viral envelope protein in the brain

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We studied the potential role of the CCR5 chemokine coreceptor for human immunodeficiency virus-1 (HIV-1) in brain injury that is initiated by HIV-1 infection. In conjunction with CD4, HIV-1 coreceptors CCR5 and CXCR4 interact with the envelope protein gp120 of HIV-1. AIDS patients and mice expressing HIV-1 gp120 as transgene in the brain (gp120tg) share several hallmarks of HIV-1 neuropathology including the reduced number of neurites and synapses and pronounced astrocytosis in comparison to uninfected individuals and wild type (wt) controls, respectively. The loss of neuronal processes and synapses, contrary to astrocytosis, correlates well with neurocognitive impairment in AIDS patients and is reflected by a diminished immunoreactivity of the neuropil for microtubule-associated protein (MAP)-2 and Synaptophysin, respectively. Astrocytosis is reflected by increased immunoreactivity for glial fibrillary acidic protein (GFAP). For the present in vivo study we crossed HIV gp120tg mice with CCR5KO mice. Microarray analysis of brain tissue from 6 month-old mice revealed that HIV-1 gp120 caused differential expression of 867 genes in the presence of CCR5 but of only 51 genes in the absence of the HIV coreceptor. Differentially expressed genes in CCR5wt/gp120tg mice suggested alterations in the function of the nervous system, immune response, cell trafficking, endocrine system, metabolism and cell death pathways compared to non-transgenic controls. Gene expression between CCR5KO/gp120tg and non-transgenic CCR5 KO control mice differed mostly for GFAP and three factors of the innate immune system. Bioinformatic analysis linked 106 of the differentially expressed genes to neurological, 42 to metabolic, 32 to endocrine, 35 to inflammatory disorders and 127 genes to cell death mechanisms in CCR5wt/gp120tg mice. In contrast, for CCR5KO/gp120tg mice only 15 of the differentially expressed genes were implicated in neurological, 8 in metabolic, 7 in endocrine, and 9 in inflammatory disorders. Also, 9 genes indicated changes in cell death mechanisms. Interestingly, a preliminary database analysis showed that numerous genes differentially expressed in CCR5wt/gp120tg mice have also been reported for the brains of neurocognitively impaired AIDS patients and SIV-infected non-human primates, including the chemokines CCL2/MCP-1 and CXCL10/IP-10. In a separate approach, we estimated the percentage of MAP-2 or Synaptophysin positive neuropil by quantitative fluorescence and deconvolution microscopy in sagittal brain sections of 6 month-old gp120tg mice both expressing and lacking CCR5 using CCR5KO and wild type animals as control. CCR5wt/gp120tg mice displayed a significant reduction in the percentage of MAP-2 positive neuropil and Synaptophysin immuno-reactivity in comparison to all of the other three genotypes. Surprisingly, quantification of GFAP immunofluorescence revealed that astrocytosis occurred in brains of gp120tg animals regardless of CCR5-deficiency. Altogether, we propose that in our in vivo model CCR5 is required for the HIV-1 gp120 transgene to cause damage to neuronal processes and synapses, but not to initiate astrocytosis. Importantly, our findings suggest that astrocytosis is not necessarily associated with neuronal damage. Furthermore, gene expression analysis indicates that brain injury induced by HIV gp120 in the presence of CCR5 involves not only immune, degenerative and cell death mechanisms but also metabolic, endocrine and developmental pathways. Finally, our observation of a requirement of CCR5 for neuronal damage is consistent with the coreceptor usage of most HIV-1 strains previously isolated from brains of neuroAIDS patients. Supported by NIH grant R01 NS050621 (to M.K.).

## P118

### Influence of Apolipoprotein E Genotype on Maturing Human Fetal Neurons Exposed to HIV-1

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HIV-1-associated neurocognitive illness may be caused by neuronal synaptodendritic injury. Apolipoprotein E genotype has been reported to influence susceptibility to the neurotoxic effects of HIV-1 proteins. The CNS may respond to neuronal injury by differentiating new neurons from resident human neuroepithelial progenitor cells (NEP), which differentiate into neuronal lineage-committed precursors

and eventually into terminally-differentiated neurons. When exposed to HIV-1, NEP show decreased neuronal microtubule or neurofilament antigens, suggesting that HIV-1 exposure can impair neurogenesis. Our hypothesis that apolipoprotein E influences the susceptibility of human neuronal precursors to HIV-1. To test this, human NEP cultures of known apolipoprotein E genotype were continuously exposed to HIV-1 during differentiation. Cultures of human fetal NEP were prepared from individual fetal specimens, thereby ensuring that all cells in individual cultures expressed the same apolipoprotein E genotype. NEP cultured as ''neurospheres'' were differentiated into a mixed population of astrocytes and neurons. Differentiation medium contained DMEM/F12 with 2.5% fetal bovine serum. Apolipoprotein E genotyping was performed on total cell DNA extracted from neurospheres. Cells with genotypes E2/E3, E3/E3, E3/E4, or E4/E4 were assayed. Replicate ''virus-exposed'' cultures were incubated in differentiation medium containing diluted macrophage-tropic HIV-1(SF128A) or lymphotropic HIV-1(SF2). ''Mock-exposed'' control cultures contained diluted conditioned medium from mock-infected peripheral blood mononuclear cells (PBMC). Replicate cultures were harvested after 22 days of incubation for quantitative immunoblotting to assay neuronal antigen levels, using alpha-tubulin as an internal standard. Neuronal antigens included microtubule -III-tubulin and the 68kDa neurofilament protein. Glial fibrillary acidic protein (GFAP) from astrocytes was also measured for comparison. At day 22, -III-tubulin levels in both SF2- and SF128A-exposed cells with the apolipoprotein E3/E4 genotype were decreased by 40% compared to levels in the corresponding mock-exposed control cells. There was no significant decrease in β-III-tubulin levels in virus-exposed cells with the E2/E3 or E3/E3 genotypes. The difference between E3/E4 ($N = 4$) and E3/E3 ($N = 8$) in SF2-exposed cultures was significant (t-test, $p = 0.02$). Surprisingly, β-III-tubulin levels in SF2-exposed cells with the E4/E4 genotype ($N = 2$) were increased by 45% compared to the levels in the corresponding mock-exposed control cells. A similar result was obtained with neuronal NF-L antigen. NF-L levels were decreased by 25% in virus-exposed cells with the E3/E4 genotype compared to the levels in the corresponding mock-exposed control cells. Similar to β-III-tubulin, NF-L levels in SF2-exposed cells with the E4/E4 genotype ($N = 2$) were increased by 25% compared to mock-exposed control cells. The difference between E3/E4 ($N = 4$) and E3/E3 ($N = 8$) in SF2-exposed cultures was significant (t-test, $p = 0.05$ for SF2). Levels of GFAP did not vary significantly with virus exposure among all apolipoprotein E genotypes, with the exception of a 10% decrease seen with SF128A-exposed cells with the E3/E4 genotype. These data suggest that the presence of two apolipoprotein E3 alleles associates with a relative protection of neuronal microtubule and neurofilament antigens against HIV-1-related toxicity in maturing human fetal neurons. The combination of E3 with E4 allele associates with decreased neuronal antigen expression during HIV-1 exposure for both microtubule and neurofilament neuronal antigens. Neuronal structural antigen expression is essential to neuronal synaptodendritic development in response to injury. Thus apolipoprotein E genotype could influence susceptibility to neurocognitive illness in HIV-1 infection. Supported by the Department of Veterans Affairs Merit Review program.

## P119

### Human Organotypic Slice Cultures to Model Neurogenesis in HIV-1 Infection

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Human Immunodeficiency Virus type 1 (HIV-1) infection of the human brain is a chronic inflammatory disease that is particularly difficult to model in vitro, given the species specificity of the virus, and the multiple cellular interactions involved in neuropathogenesis. Brain slice cultures grown on semipermeable membranes can retain the multi-cellular composition and spatial organization of specific brain regions, and arguably can preserve synaptodendritic complexity and the character of the neurogenic microenvironment. In a published report of a human cell culture model using hippocampal slice cultures, human neuroepithelial progenitor cell (NEP) proliferation in the slice cultures was reversibly inhibited by exposure to HIV-1 envelope proteins. We have recently developed slice cultures from human fetal brainstem or telencephalon for the study of neurogenesis and neuronal survival in the setting of HIV-1 exposure or infection. Slice cultures were prepared from human fetal brainstem or telencephalon of 60-110 days gestation. Separate cultures were prepared from individual fetal tissue specimens. Transverse slices from 200 to 400 m thickness were cut with a tissue slicer fitted with a micrometer. Slices were transferred to and cultured on Millicell–CM organotypic culture plate inserts (Millipore Corp). In order to generate autologous NEP for inoculation onto slice cultures, remaining brain fragments from the same fetal specimen were mechanically dissociated to prepare NEP cell suspensions (''neurospheres''). Neural progenitor maintenance medium sustained healthy neurosphere as well as slice cultures for up to one month, as judged by propidium iodide staining for cell death. To detect the proliferation and migration of NEP inoculated onto slice cultures, neurospheres were infected with a lentiviral vector expressing green

fluorescent protein (GFP). To examine the effect of HIV-1 exposure, slice cultures were incubated for 21 days in medium containing 2.5% fetal bovine serum and dilutions of conditioned medium from mock-infected peripheral blood mononuclear cells (PBMC) or from PBMC infected by lymphotropic HIV-1(SF2) or macrophage-tropic HIV-1(SF128A). Virus concentration was 44ng p24 antigen per slice. When cultured slices were fixed and immunostained, regions of nestin expression were readily detected, particularly along the midline of slices from brainstem. Cell processes expressing glial fibrillary acidic protein (GFAP), a marker for astrocytes, radiated out from slice regions enriched in nestin. When GFP-labeled neurospheres were inoculated directly onto slices, GFP-labeled cells with extensive processes migrated outward from the inoculation site, though the processes tended to coalesce towards slice regions enriched for nestin expression. Migration of GFP-labeled cell processes was greater in cultures incubated in neural progenitor maintenance medium compared to standard DMEM/F12 cell culture medium supplemented with fetal bovine serum. Slice cultures were also incubated with conditioned medium from either mock-infected or HIV-1-infected PBMCs. Mock- or virus-exposed slices still supported extensive migration of GFP-labeled cell processes when these slices were inoculated with GFP-labeled neurospheres. Our preliminary data indicate that slice cultures from human fetal brainstem or telencephalon support the differentiation of NEP. When inoculated onto slice cultures, NEP give rise to highly processed cells that migrate extensively into the slice, with some preference for slice regions enriched in nestin. This suggests that the slice cultures provide a neurogenic environment, and that appears to be true even during prolonged incubation in HIV-1-containing culture medium. Supported by the Developmental Center for AIDS Research (D-CFAR), Miller School of Medicine, University of Miami.

## P120

### HIV-1 Exposure Impairs Neurotrophin Responsiveness in Maturing Human Fetal Neurons

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HIV-1-associated neurological illness may be caused by neuronal synaptodendritic injury. The brain may counter this by activating neuroprotective cell signaling pathways and by differentiating new neurons from resident neuroepithelial progenitor cell (NEP) populations. These mechanisms will only be effective if neurons-at-risk for HIV-induced injury respond to neurotrophins. In this study, differentiating human NEP cultures that were continuously exposed to HIV-1 showed relatively decreased neuronal proliferation or activation of MAPK signaling when ''challenged'' with added neurotrophins. Human fetal NEP were differentiated into a mixed population of astrocytes and neurons in DMEM/F12 medium with 2.5% fetal bovine serum. Replicate ''virus-exposed'' cultures were incubated in differentiation medium containing diluted macrophage-tropic HIV-1(SF128A) or lymphotropic HIV-1(SF2). ''Mock-exposed'' cultures were incubated in differentiation medium containing diluted conditioned medium from mock-infected peripheral blood mononuclear cells (PBMC). ''Untreated'' cultures contained differentiation medium only. To ''challenge'' cultures for neurotrophin responsiveness, nerve growth factor (NGF) or neurotrophin-3 (NT3) was added at day 15 of incubation. Replicate cultures were then harvested after 60 minutes for phosphorylated cell signaling kinases, or after 5 days for neuronal cell counts and antigen levels. Without neurotrophin challenge, neuronal cell numbers or neuronal β-III-tubulin levels were lower in untreated compared to mock- or virus-exposed cultures. After neurotrophin challenge, neuronal cell numbers increased 3-fold and β-III-tubulin levels increased 25–60% in untreated cultures, but there was little or no increase in these measures in mock- or virus-exposed cultures. After neurotrophin challenge, fold-activation of ERK isoforms was 50% higher in untreated compared to mock- or virus-exposed cultures, but signaling kinases associated with stress related pathways were preferentially phosphorylated in virus-exposed cultures. Maturing neurons subjected to HIV-1-exposure may have higher basal levels of neurotrophin-mediated activity, but more limited capacity to respond to exogenous neurotrophin challenge. Supported by the Department of Veterans Affairs Merit Review program.

## P121

### Stabilizing intracellular calcium regulation with novel neurotrophin mimetics provides potent neuroprotection against gp120-induced macrophage toxins

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Cognitive motor deficits resulting from HIV infection are thought to be due, in large part, to the actions of macrophage and microglial secretory products which damage and disable neurons. Early indices of neuronal damage include beading and pruning of dendrites and are followed, in severe

cases, by neuron death. These processes can be reproduced in vitro by challenging neurons with supernatant from gp120-stimulated macrophages. The macrophage products cause an acute elevation of intracellular calcium followed by a long plateau and a large delayed calcium rise. The calcium plateau and delayed rise correlated with a reduction in the rate of calcium clearance from the cell and preceded a reduction in the mitochondrial membrane pootential. Dendritic beading, dendrite retraction and neuronal swelling appeared during the delayed calcium rise. Glutamate concentrations present in the macrophage supernatant failed to reproduce the effects on calcium and the acute calcium rise could be dissociated from the late destabilization indicating that the pathogenesis is not driven by an early excitotoxic-like event. Treatment with nanomolar concentrations of the nonpeptide neurotrophin mimetic, LM11A-31, provided strong neuroprotection, accelerated calcium recovery and greatly suppressed the late calcium destabilization. This stabilization of calcium homeostasis most likely contributes to the potent neuroprotection by this novel compound. Supported by NIMH MH079726.

## P122
### HIV Programs Autophagy-dependent Cell Death in Neuro-Glial cells

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HIV infection in the brain leads to encephalitis and neurological complications including motor and cognitive impairment, and HIV-associated dementia (HAD). It is known that HIV mediated neuron drop out occurs indirectly either via inflammatory products or viral proteins. We sought to investigate the mechanism of HIV-mediated neuro-glial toxicity using wild type HIV, mutant-HIV and supernatants collected from HIV-infected supernatants from macrophages. Cytokine profile in Bioplex array on CSF from HIV-demented compared to HIV-infected non-demented patients (n = 7), showed five upregulated signature cytokines (IFN-α, IL-6, IP-10, PDGF and MCP-1). The supernatants from HFA after 48 hrs of treatment with either wild type virus particles or viral integrase defective particles were also investigated in parallel. We further investigated whether viral particles could induce CD40/CD40L on astrocytes to implicate their role in initiating inflammatory response. Flow cytometric analysis revealed about 16% basal CD40 expression but not CD40L, which could not be induced further upon treatment with high concentration of viral particles, IL-6 or IFN-α or in combinations with virus. Remarkably, HIV particles either from wild type or mutated viruses or supernatants collected from HIV-infected macrophages induced neuro-glial (neurons and astrocytes) toxicity. Toxicity was robustly attenuated either via CXCR4-receptor blocker AMD3100 or blocking HIV envelope via soluble CD4 or NMDA-R blocker. Heat treatment ablated the toxicity imparted by virus particles but was unaffected in supernatants from infected macrophages. Also, TNF-α significantly augmented the direct virus mediated toxicity that could not be completely attenuated by CXCR4 blocker. This suggests that viral envelope may be conferring neuro-glial toxicity which can be amplified by TNF-α or some other proinflammatory cytokines downstream. HIV-infection also suppresses autophagy, a cellular innate defense system to clear unwanted, altered or harmful structures from the interior of the cell. To implicate its role, we used inhibitors 3-MA & Bafilomycin-A with HIV virus. Autophagy inhibitors in combination with virus elevated HIV-induced neuro-glial toxicity but protected neuro-glial cells when autophagy inducer Rapamycin was used. It is concluded that HIV infection in the brain induces proinflammatory response and neuro-glial toxicity via viral components and inflammatory molecules involving autophagy.

## P123
### Toll like receptor 3 (TLR3) plays a major role in the formation of rabies virus Negri bodies and in rabies virus neuroinvasiveness

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Human neurons express the innate immune response receptor, Toll-like receptor-3 (TLR3). TLR3 levels are increased in pathological conditions such as brain virus infection. Here, we further investigated the production, cellular localisation and function of neuronal TLR3 during neuronotropic rabies virus (RABV) infection in human neuronal cells. Following RABV infection, TLR3 is not only present in endosomes, as observed in the absence of infection, but also in detergent-resistant perinuclear inclusion bodies. As well as TLR3, these inclusion bodies contain the viral genome and viral proteins (N and P but not G). The size and composition of inclusion bodies and the absence of a surrounding membrane, as shown by electron microscopy, suggest they correspond to the previously described Negri Bodies (NBs). NBs are not formed in the absence of TLR3 and TLR3-/- mice — in which brain tissue was less severely infected — had a

better survival rate than WT mice. These observations demonstrate that TLR3 is a major molecule involved in the spatial arrangement of RABV-induced NBs and viral replication. This study shows how viruses can exploit cellular proteins and compartmentalisation for their own benefit.

## P124

### A subset of seroindeterminate patients have detectable antibodies to HTLV-I GAG and Tax as defined by a novel luciferase immunoprecipitation system (LIPS)

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Human T-lymphotropic virus type I (HTLV-I) is an exogenous retrovirus, which infects between 15 and 20 million people worldwide. Currently, donated blood is screened by ELISA using disrupted whole virus. Repeatedly reactive sera undergo a confirmatory western blot, which uses antigens derived from a mixture of whole virus lysate and recombinant HTLV-I and HTLV-II envelope proteins. Studies have estimated the seroprevalence of confirmed HTLV infection among blood donors in the United States at 0.01%, in Tawain at 0.06% and in Argentina at 0.046%. However, it is noteworthy that there is another significant subset of samples that demonstrate partial WB reactivity without fulfilling the criteria to be classified as WB seropositive; these samples are considered seroindeterminate and may go undetected in an initial HTLV-I ELISA screen. For this reason, it is imperative to understand the clinical significance of seroindeterminate samples, which to date, remain controversial. We identified 17 seroindeterminate individuals referred to the neurological clinic of the Neuroimmunology Branch, National Institute of the Neurologic Disorders and Stroke (NINDS). Serum samples from seroindeterminate patients were analyzed by ELISA and HTLV-I/II western blot. In addition, PBMC samples (when available) were screened for HTLV-I tax sequences by real-time PCR to obtain viral load determinations. While a subset (5 of 13) of seroindeterminate samples demonstrated low levels of HTLV-I tax reactivity at the threshold of sensitivity of the assay, the majority (8 of 13) were HTLV-I tax negative. These observations were supported by use of a novel serological platform called luciferase immunoprecipitation system (LIPS) to study the HTLV-I antibody reactivity of sera collected from HTLV-I seroindeterminate patients, patients with HAM/TSP, asymptomatic HTLV-I infected individuals and uninfected controls. LIPS technology provides a means for rapid and quantifiable analysis of antibody responses to lysates expressing a variety of renilla-tagged viral proteins. The results indicate that LIPS could distinguish between HTLV-I infected and uninfected samples with a 100% sensitivity and 100% specificity on the basis of detecting positive anti-Gag antibodies. LIPS analysis of anti-tax and anti-ENV antibodies identified significantly elevated responses in HAM/TSP patients compared to asymptomatic HTLV-I infected patients. Subsequent analysis of the seroindeterminate samples showed that 47% of seroindeterminate patients in our cohort demonstrate positive anti-HTLV-I gag reactivity and 12% have antibodies to HTLV-I tax. Importantly, we were able to amplify HTLV-I tax sequences by PCR from one of the seroindeterminate patients positive for anti-tax antibodies by LIPS. None of the samples demonstrated HTLV-I ENV reactivity by LIPS although attempts are currently underway to clone HTLV env sequences from seroindeterminate samples. These LIPS results demonstrate HTLV-I antibody responses can be detected in sera from subsets of HTLV-I/II WB seroindeterminate individuals and support the hypothesis that HTLV-I/II WB seroindeterminate banding patterns may in some cases represent prior exposure to prototype HTLV-I and persistent infection with low-levels of HTLV-I.

## P125

### Minocycline's immunomodulatory effects are partly due to calcium chelation

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Minocycline is a second generation, semi-synthetic tetracycline derivative that is commonly used for the treatment of acne and rheumatoid arthritis. We have demonstrated that it reduces the severity of SIV-associated encephalitis and suppresses macrophage/microglial activation, T cell activation, and SIV replication. Minocycline's protective effects have been linked to a wide range of actions including reducing cytochrome c release from the mitochondria, blunting macrophage and T cell activation, reducing harmful MAPK activation, and

chelating calcium; however, a single, unifying mechanism of action has remained elusive. We hypothesize that minocycline acts by interfering with actions that are upstream of many shared pathways. Here we examine minocycline's effect on calcium signaling and potential to interfere with lipid raft clustering, both of which are critical to T cell activation. We first investigated the effect of minocycline on calcium transients in primary T cells. Bead selected CD4+ cells were blasted then incubated for 24h with or without minocycline. Cells were then loaded with Fura-2, a calcium sensitive dye and stimulated with ionomycin, a calcium ionophore, or gp120 and calcium transients were measured over time. Minocycline diminished calcium transients induced by both stimuli, confirming that this is a potential mechanism for blunting T cell activation. To help define the importance of calcium chelation in this process, we used a non-chelating analog of minocycline, called 12S-hydroxy-1,12-pyrazolinominocycline (PMIN). We activated peripheral blood lymphocytes with CD3/CD28 alone or in the presence of minocycline or PMIN and examined effects on the activation markers CD25 and CCR5 in CD4+ and CD8+ T cells. PMIN inhibited T cell activation, but to a lesser degree than minocycline. This suggests that minocycline's immunomodulatory actions are not due to calcium chelation alone. Given that tetracyclines localize to membranes, especially at sites of high calcium concentrations, we hypothesized that minocycline may interfere with plasma membrane fluidity and potentially lipid raft clustering. Taking advantage of the natural fluorescence of minocycline in the presence of metal cations, we found that minocycline localized to plasma membranes in CEM $\times$ 174 cells, with minocycline often localizing to one pole of the cells. We currently are defining minocycline's subcellular distribution using coimmunofluorescence with compartmental markers.

## P126
### Is HIV-1 C Less Pathogenic To Human Neural Precursor Cells?

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More than half of new HIV-1 infections in world and nearly 95% of the HIV infections in India are caused by clade C. Human neural precursor cells (hNPCs) are important cells of central nervous system as they are responsible for neurogenesis in aged and developing brain. Impairment of this property of hNPCs has significant implications in the neuropathogenesis of HIV-1, especially in pediatric neuro AIDS cases. Recently HIV-1 particles are reported in nestin-positive cells however it is not clear whether presence of HIV-1 in neural precursor cells contributes to neuropathogenesis. To check the infectivity of HIV-1 clade B and clade C virus, we used hNPCs and observed that replication/infection of clade C virus is earlier than clade B as observed by p24 levels. Human neurospheres derived from hNPCs were used to investigate the effect of HIV-1 B and C on proliferation and differentiation. Proliferation of hNPCs decreases by clade B virus, whereas HIV clade C virus did not effect proliferation that efficiently. Further we observed that the ability of hNPCs to differentiate into Tuj-1 positive neuron was also significantly impaired following infection by HIV B virus, though not so by HIV C virus, indicating the clade specific effect of HIV on properties of neural precursor cells. In quest to investigate the mechanism of HIV induced impairment of hNPCs properties in detail, we used HIV Tat protein as well. These findings provide new insights into HIV neuropathogenesis involving hNPCs. (Study was supported by research grants (BT/PR6838/Med/14/881/2005 And BT/PR6615/Med/14/857/2005) from Department of Biotechnology, New Delhi, India. The research fellowship to MM from NBRC is highly appreciated).

## P127
### Neuronal factor analysis of proton MR spectroscopic imaging data in HIV infection: Regional patterns of involvement and relationship to cognitive status

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Introduction: Magnetic resonance spectroscopy (MRS) provides detailed metabolic information about the neuronal dysfunction and glial metabolism in many neurodegenerative diseases. Many single voxel research studies in neuroAIDS have been undertaken, but few have looked at more than three representative regions at a time. Magnetic resonance spectroscopic imaging (MRSI) provides data on brain metabolism from many regions both quickly and simultaneously. However, a complication in using MRSI lies in the large number of variables generated in its analysis and the need for statistical correction, which may be compensated by non-traditional forms of analysis such as factor analysis. In the present study, factor analysis was applied to MRSI data from a large cohort of

HIV+ and seronegative control subjects, with the aims of 1) determining regional metabolism changes induced by HIV infection, 2) comparing these changes to individual neuropsychological evaluations, and 3) comparing them to clinical and immunologic markers for HAD.

Methods: Seventy-four chronically infected HIV+ (34 with MSK = 1 or 2 and 40 with MSK = 0 or 0.5) and 20 seronegative, healthy control subjects underwent MRSI. N-acetylaspartate (NAA), choline (Cho) and creatine (Cr) concentrations were calculated in seven brain regions: parietal and frontal cortical gray matter, parietal and frontal white matter, centrum semiovale, basal ganglia, and thalamus. Blood and cerebrospinal fluid (CSF) samples from HIV+ subjects were obtained for viral and immunologic marker quantification. ANOVA and t-tests were used to determine the factor scores relationships to HIV infection and dementia status. Spearman rank coefficients measured the degree of correlation between variables.

Results: Factor analysis was performed on the 21 variables from the MRSI experiment, thus producing 3 factors. Examination of the loadings in each factor indicated major contributions due to a specific brain metabolite. Thus, one factor was named a ''choline factor,'' an ''NAA factor,'' and a ''creatine factor''. Subject's scores generated from the choline factor were capable of differentiating between HIV- and HIV+ subjects, indicated higher choline levels in those who are HIV+ across all deep gray matter and white matter regions (ANOVA, $p = 0.0002$). The creatine factor indicated elevations in creatine across white matter regions in those who were HIV+, but this was only a trend (ANOVA, $p = 0.09$). The NAA factor was capable of differentiating between those who had cognitive impairment and those without (ANOVA, $p = 0.02$) with lower NAA scores resulting in subjects with cognitive impairment. This factor is heavily weighted towards NAA concentrations in white matter regions, and implies these regions may be more sensitive in distinguishing those with dementia and those without. Subjects with lower NAA factor scores also had worse scores on both psychomotor and executive function testing.

Conclusions: This is an extensive report of regional variations in brain metabolism using absolute concentrations from HIV+ subjects. These results validate the importance of early white matter involvement in HAD, and (although not a longitudinal study) support the model of early glial cell proliferation (Cho and possible Cr elevations) in HIV infection, and later neuronal dysfunction (NAA decrease) associated with dementia. Through factor analysis, metabolite patterns can reveal differences between HIV status and severity of HIV-associated cognitive impairment, and provides information on the spatial distribution of metabolic changes within these subjects.

## P128
## Establishment of a human fetal brain progenitor-derived oligodendrocyte culture system and its use for study of JC virus infection

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Cultures of human neural progenitor cells are capable of proliferating while maintaining the potential to give rise to the major neural phenotypes: neurons, astrocytes and oligodendrocytes. We previously demonstrated that human fetal brain multipotential progenitor cells (progenitors) can be differentiated into progenitor-derived-neurons (PDN) and progenitor-derived-astrocytes (PDA) (Messam et al., 2003). We have now expanded this cell culture model to direct progenitors in an oligodendrocytic lineage, resulting in progenitor-derived-oligodendrocytes (PDO). Successful differentiation into this lineage was demonstrated by the coordinated expression of specific markers such as A2B5, O4, GalC (galactocerebroside) and MBPs (myelin basic proteins). This cell culture model was developed in part as a unique tool to study the cellular tropism of the human polyomavirus, JCV, which is restricted in the brain to multiply within glial cells, particularly the oligodendrocyte resulting in the demyelinating disease progressive multifocal leukoencephalopathy (PML). Unexpectedly, and in sharp contrast to PDA which are highly susceptible to JC virus (JCV) infection, PDO exposed to JCV showed only low levels of infection. Because the extent of JCV receptor expression could not account for the level of susceptibility to infection, we tested the cellular regulation of viral transcription known to be a critical determinant supporting astrocytic infection. The DNA binding protein, nuclear factor I class X (NFI-X) is highly expressed in cells susceptible to JCV infection. In directed differentiation from progenitors to PDO, NFI-X expression did not rise to the same levels seen in PDA and JCV multiplication was lower. The use of this cell model to study viral gene regulation in glial cells highlights differences between oligodendrocytes derived from early stages of

development compared with oligodendrocytes in the adult brain.

## P129

### Motor Disinhibition Is More Common Among Neuropsychologically Impaired HCV+ Persons than Comparable HIV+ Individuals or Methamphetamine Abusers

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Background: Hepatitis C Virus (HCV), human immunodeficiency virus (HIV) and Methamphetamine Abuse/Dependence (METH+) produce motor impairments. We hypothesized that impairments on certain neuropsychological tests would be associated with motor disinhibition and that these associations may point to a common underlying pathophysiology.

Method: Force steadiness (FS), the ability to maintain steady levels of isometric hand muscle force, was used as a measure of motor disinhibition in the following groups: HCV+ (n = 35), METH+ (n = 20), HIV+ (n = 35), HCV+/METH+ (n = 12), HCV+/HIV+ (n = 8), METH+/HIV+ (n = 23), HCV+/METH+/HIV+ (n = 14). We examined the association with three cognitive domains likely to show variation across the various risk groups: Speed of Information Processing (SIP), Motor Skills (MS), and Executive Functions (EF).

Results: Examining persons who were and were not SIP impaired showed that only HCV+ individuals had worse motor disinhibition scores as compared to non-SIP impaired HCV+ individuals. Comparing those who were and were not MS impaired revealed worse motor disinhibition in the HCV+ group and the METH+/HCV+ and METH+/HIV+ groups, but not METH+ or HIV+ groups. No differences were found among individuals who were or were not impaired on EF.

Conclusion: HCV may exert a stronger motor disinhibition effect among SIP and MS impaired individuals than HIV or METH whereas a "double-hit" is needed to observe a motor disinhibition effect in METH or HIV. Findings suggest that motor disinhibition is more common among HCV individuals than HIV and METH, and that the cognitive and motor impairments observed in HCV may share a common pathophysiology.

## P130

### Persistent HIV in the Central Nervous System During Treatment is Associated with Worse Antiretroviral Therapy Penetration and Cognitive Impairment

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Background: Cognitive impairment can occur or persist during antiretroviral therapy (ART). Explanations include comorbidities, neurotoxic ART, persistent neuroinflammation, or persistent HIV replication in central nervous system (CNS). This analysis assessed whether low levels of HIV in cerebrospinal fluid (CSF) were associated with inter-individual differences in ART regimens and neuropsychological (NP) performance.

Methods: 329 participants (pts) were selected from the CHARTER cohort because they were taking ART, and had HIV RNA levels below 50 c/mL with an ultrasensitive assay (Roche Amplicor) in CSF and blood. Paired CSF and blood plasma (PL) specimens that were obtained within 1 hour of each other were assayed with a more sensitive assay, a modified version of the NucliSens EasyQ (bioMerieux) assay capable of qualitatively detecting HIV at 2 c/mL. To determine the stability of low-level HIV in CSF, a follow-up specimen from 61 pts was assayed (median duration between visits, 7.0 months). Penetration of ART into the CNS was estimated by CNS Penetration-Effectiveness (CPE) ranks. NP performance was summarized by the Global Deficit Score (GDS), a validated method which integrates relevant information about 7 NP performance domains.

Results: Pts were mostly non-white (55%), middle-aged (mean 45 yr) men (76%) with AIDS (75%) who were HCV seronegative (68%). Median duration of the current ART regimen was 15 months. Median CD4 count was 467/μL. By the more sensitive assay, 136 (41%) had detectable HIV in CSF and 216 (66%) had detectable HIV in plasma. Detectable HIV in CSF was associated with worse CPE scores (mean 1.49 vs. 1.70, d = 0.29, p = 0.009) and detectable HIV in PL (71% pts with HIV detected in CSF vs. 62% undetected, p = 0.077). Other demographic or disease characteristics were not found in association. Of this group of pts, 39 (28%) had detectable HIV in CSF but not in PL, had worse global deficit scores (GDS) (0.63 vs. 0.37, p = 0.012), and were particularly likely to have at least moderate global impairment (GDS greater than 0.93, 28% vs. 8%, p = 0.005). Multivariate analyses identified that worse global deficit scores were associated with

detectable HIV in CSF but not in PL (B = 0.13, p = 0.007), HCV seropositivity, shorter durations of ART, and ethnicity other than white (model R2 = 0.29, p < 0.0001). In the subgroup with a follow-up CSF specimen assayed, CSF values became undetectable in 18 (30%) pts although remained detectable in the other 43 (70%). Transition from detectable to undetectable was associated with higher CD4 counts at the second visit (46% of undetectable pts had CD4 > 500 vs. 12% in detectable, p = 0.09), and HCV seropositivity (57% vs. 24%, p = 0.04), but not CPE scores or improved global NP performance.

Conclusions: Despite having achieved virologic suppression in CSF and plasma by the Roche ultrasensitive assay, we found that ART-treated individuals frequently (41%) had low, detectable levels of HIV in CSF, and this was associated with less penetrant ART. At least a quarter of these individuals (28%) had persistent HIV in CSF, but not in PL, and this was linked to the presence of worse neurocognitive functioning. People living with HIV may have cognitive impairment as a result of ART that is incompletely effective in the CNS.

## P131

### Excessive CXCL2 production and neutrophil infiltration following cytomegalovirus brain infection of IL-10-deficient mice

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While wild-type mice control murine cytomegalovirus (MCMV) brain infection, the infection is lethal to animals deficient in interleukin (IL)-10. This increased mortality is associated with elevated levels of proinflammatory cytokines and chemokines in the central nervous system. Here, we report that MCMV-infected IL-10 knockout (KO) mice displayed a marked increase in CD45(hi)Ly-6G(+)MHCII(−) neutrophil infiltration into the infected brain when compared to wild-type animals, as early as 3 d p.i. Elevated levels of microglial cell activation, determined by upregulation of MHC class II, were also observed in IL-10 KO animals. Separation of cells isolated from wild-type murine brain tissue into distinct populations using FACS, along with subsequent quantitative RT real-time PCR, showed that brain-infiltrating CD45(hi)/CD11b(low) cells were the source of IL-10 within the brain. The brains of IL-10 knockout and wild-type mice were then analyzed for presence of CD4(+)CD25(+)FoxP3(+) cells (5 d p.i.). Compared to wild-type mice, the brains of IL-10 KO animals were found to have reduced Treg cell infiltration. Interestingly, adoptive transfer of both CD4(+)CD25(+) and CD4(+)CD25(−) cell populations from wild-type mice into IL-10 KO animals protected them from the lethal effect of viral brain infection. Experiments are currently underway to isolate Tregs from FoxP3-GFP transgenic mice and assess their effect on MCMV brain infection-induced microglial activation, CXCL2 expression, and neutrophil infiltration through additional adoptive transfer studies.

## P132

### Promyelocytic leukemia nuclear bodies localize adjacent to the VZV genome during early infection and are disrupted during late infection

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Varicella zoster virus (VZV) is a human, neurotropic alphaherpesvirus. Primary infection results in chickenpox after which virus establishes latency in cranial nerve, dorsal root and autonomic ganglia. In association with a decline in cell-mediated immunity to VZV, virus can reactivate and cause shingles, postherpetic neuralgia, stroke, spinal cord disease and vision loss. VZV causes a productive infection in non-neuronal cells, but becomes latent in neurons. The mechanisms that determine productive versus latent infection are unclear, but likely involve cell type-specific interaction of viral proteins and host cell antiviral defense mechanisms. Promyelocytic leukemia nuclear bodies (PMLNBs) are constitutively expressed in the nucleus of cells and have antiviral properties via viral transcriptional repression. We hypothesize that the outcome of VZV infection (lysis vs. latency and subsequent reactivation) is the result of cell type-specific interactions of VZV proteins with the host cell's antiviral PML-NBs. Since the interaction of PML-NBs and VZV has not been well-characterized, in this study we examined uninfected MeWo cells and MeWo cells infected with cell-free VZV 6 and 24 hours post-infection. Cells were fixed and dual stained with primary antibodies against VZV 29 protein to detect the VZV genome and against PML to detect the PML-NBs followed by fluorescently-labeled secondary antibodies then examined by confocal microscopy. At 6 hours post-infection, PML-NBs are seen surrounding the VZV DNA replication compartments, with an average of 4.9 +/− 2.4 PML-NBs per infected cell. At 24 hours post-infection, there is a significant decrease or no PML-NBs present in VZV-infected cells with an average of 0.9 +/− 0.5 PML-NBs per infected cell. Uninfected cells at six and 24 hours post-infection have an average of 7.4 +/− 3.9 and 7.7 +/-4.3 PML-NBs respectively, diffusely distributed throughout the nucleus, without a

significant difference in PML abundance between the two timepoints. These results demonstrate colocalization of PML-NBs with the VZV genome during early virus infection and VZV infection-mediated disruption of PML-NBs during later infection.

## P133
### CNS-Immune Reconstitution Inflammatory Syndrome (IRIS): Good, Bad or Ugly

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Prior to the HIV era, hypersensitivity reactions had been well described in HIV-seronegative patients treated for bacterial meningitis, tuberculous meningitis, secondary syphilis and lepromatous leprosy. These patients showed clinical deterioration following antimicrobial therapy. Adjunctive steroid therapy is an accepted, but untested, mode of intervention for this phenomenon. In the post-antiretroviral era, a similar phenomenon, IRIS, may occur in HIV-infected patients responding to antiretrovirals either in the absence of opportunistic infections, or more commonly with PML or cryptococcal meningitis. Rare cases of IRIS with VZV or EBV encephalitis and candida meningitis have also been described. PML-IRIS may occur in patients with autoimmune diseases when immune suppressive therapy is stopped due to occurance of PML. Because antimicrobial therapies are insufficient or unavailable to irradicate the infection, cellular immune responses are necessary for controlling the infections particularly HIV and PML. However, these host cellular immune responses may trigger hyperactive T cell responses that lead to pathogenesis of IRIS. Unfortunately, these cellular immune responses in the form of IRIS may be fulminant and lead to severe disability or death. In a retrospective study (1992–2008) at Johns Hopkins University we recorded 109 patients with PML. 22 patients developed IRIS and 15/22 (68%) developed either severe disability or died. Histopathological evaluation of CNS-IRIS shows massive infiltration of CD8 cells in the brain. We determined the mechanism of T cell activation by HIV-Tat protein. The production of Tat is not generally reduced even after successful antiretroviral therapy once proviral DNA has been formed. that activated T cells can cause neuronal injury and We found that enzymes released from cytotoxic granules from activated T cells can cause neuronal injury. In particular granzyme B initiates neurotoxicity via interactions with a Gi protein coupled receptor and activation of voltage gated potassium channels. Chloroquine can block T cell activation and clofazamine can block these potassium channels suggesting novel modes of intervention might be possible for IRIS that do not require treatment with steroids.

## P134
### The chemokine CXCL12 regulates expression of the NMDA receptor subunit NR2B via a Histone Deacetylase - dependent pathway

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The chemokine CXCL12 (also known as SDF-1) is constitutively expressed in the central nervous system and plays important roles in regulation of neuronal/glial function in developing and adult brain. Furthermore, in vitro and in vivo studies suggest that activation of CXCR4, the specific CXCL12 receptor, is involved in neuronal survival under normal and pathological conditions, such as neuroAIDS. We have previously demonstrated that stimulation of CXCR4 by the chemokine promotes activation of neuronal survival pathways and protects neurons from excitotoxicity, including NMDA-induced cell death. NMDA receptor (NMDAR) activation is required for physiological neuronal function. However, over-activation can lead to excessive rise in intracellular calcium leading to cell death. Recent studies suggest that receptor subtype and localization contribute differently to neuronal survival. Some data suggest that NR2A receptors play a neuroprotective role while NR2B-containing channels lead to neuronal death. NR2B transcriptional regulation has been shown to be mediated by the retinoblastoma protein (Rb). Our previous work indicates that CXCL12 up-regulates Rb and supports neuronal survival. Therefore, we hypothesized that CXCL12 may act as epigenetic regulator, which mediates NR2B transcriptional repression to protect neurons from NMDA neurotoxicity. To test this hypothesis, we asked whether CXCL12 was required during NMDA treatment to protect neurons or if a CXCL12 pretreatment was sufficient to prevent cell death induced by a following NMDA treatment. Rat primary cortical neurons were pre-treated with CXCL12 (20nM) for 24hr and then exposed to NMDA (20 min, 100uM; in Mg free solution with 15uM glycine) or vehicle (same solution without NMDA). Neuronal survival was evaluated the next day using a combination of vital/nuclear dyes. These experiments showed a significant reduction in NMDA-induced cell death in neurons treated with CXCL12. Moreover, Western Blot and RT-PCR analysis reveals a reduction in NR2B protein and mRNA in CXCL12 treated neurons, whereas no changes in NR1 or NR2A were observed. Similarly, $Ca2+$-imaging studies indicate that the CXCL12 pretreatment also reduces the intracellular calcium increase caused by NMDA. This effect is abolished

by co-treatment with the CXCR4 inhibitor AMD3100 (100ng/ml). Preliminary studies with RO-256981 (NMDAR antagonist that preferentially blocks NR2B-containing channels) further support the role of NR2B in mediating CXCL12 effect. To determine if CXCL12-induced changes in gene repression involve epigenetic regulation, experiments were conducted with the histone deacetylase (HDAC) inhibitor trichostatin A. These experiments indicate that blocking HDAC function prevents the effects of CXCL12 on NR2B protein expression. Overall, these findings suggest that CXCL12/CXCR4 may protect neurons from excitotoxicity by repressing transcription of the NMDA receptor 2B subunit.

## P135
### Morphine and gp120 modulation of macrophage phagocytosis and bactericidal effect

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Opiate use and abuse has been known to suppress a number of immune responses and, therefore, have been postulated to serve as cofactors in the progression of HIV-1 infection. According to latest CDC statistics, since the HIV epidemic began, injection drug use has directly and indirectly accounted for more than one-third (36%) of AIDS cases in the United States. The high frequency of bacterial sepsis observed in HIV-positive patients has been shown to be in part due to impairment of innate immunity, specifically macrophage function. Impairment of macrophage functions such as phagocytosis and bacterial killing ultimately results in increased dissemination of bacteria to other vital organs such as the brain. Our goal is to better understand effects of opioids on innate immunity in presence of HIV-1 envelope protein gp120. Our results indicate that chronic morphine treatment in vitro and in vivo modulates Fc-gamma receptor mediated phagocytosis and bacterial killing in murine macrophages. In addition, in cells treated with morphine and gp120 this effect is further exacerbated. Using fluorescence microscopy and fluorometry, we show decreased internalization of opsonized FITC tagged E. coli bacterial particles as well as opsonized live GFP tagged E. coli following chronic morphine treatment. Microscopic analysis shows that chronic morphine leads to decrease pseudopodia and phagosomal cup formation indicating morphine's modulation of actin polymerization via small GTP-ases such as Cdc42, Rac and Rho. Forskolin induced elevation of cAMP levels in the macrophage cell line led to suppression of Fc-gamma receptor mediated phagocytosis in morphine and vehicle treated cells. In addition, H89 induced inhibition of PKA resulted in reversal of morphine induced phagocytosis of opsonized bacteria. Together these data indicate that chronic morphine inhibits phagocytosis via a cAMP and PKA dependant pathway, ultimately affecting actin polymerization. Ongoing studies will further elucidate the mechanism of morphine and gp120 induced decrease in bacterial internalization and their role in modulation of actin polymerization.

## P136
### Activation of astrocyte-elevated gene-1 (AEG-1) by JCV T-antigen in glioblastoma

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Recent studies have reported the detection of the human neurotropic virus, JCV, in a significant population of brain tumors, including glioblastomas. 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 -catenin and IRS-1, play a role in the oncogenic function of JCV T-antigen. Here we demonstrate an induced level of expression of astrocyte-elevated gene-1 (AEG-1) in T-antigen-expressing glioblastomas compared to those that show no association with JCV. AEG-1 has the capacity to promote anchorage-independent growth and cooperates with Ha-ras in malignant transformation. Results from transcription studies revealed the ability of T-antigen to stimulate promoter activity of AEG-1 in astrocytes. Of interest is our finding that AEG-1 expression levels remain constant during lytic infection with JCV. This observation suggests that expression of T-antigen in the absence of the viral infection cycle, an event that is seen in T-antigen-positive human tumors as well as in transgenic mouse tumor models, promotes the expression of AEG-1 in astrocytes. These observations point to the possible involvement of T-antigen via activation of AEG-1 in the spread of tumors. Studies are in progress to identify the molecular pathways involved in the regulation of AEG-1 by T-antigen and the biological importance of AEG-1 activation in tumor pathogenesis.

## P137
### Transcriptional regulation of the chemokine co-receptor CCR5 by the cAMP/PKA/CREB pathway

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The cyclic adenosine monophosphate (cAMP)-dependent signaling pathway directs the expression of several genes involved in diverse neuroendocrine, immune, metabolic, and developmental pathways. The primary effectors of this pathway are members of the cAMP response element binding (CREB) family of transcription factors, in particular the CREB-1 and cAMP response element modulator (CREM). Both these genes encode alternative splice variants that serve as activators or repressors in a context- and position-specific manner. Although the beta-chemokine receptor CC chemokine receptor 5 (CCR5) has been identified on progenitor cells in the bone marrow, the regulatory mechanisms orchestrating its expression are not fully understood. Previous reports have identified putative cAMP response elements in the CCR5 promoter and have described a suppressive role for cAMP in CCR5 expression. In this study, the CD34+CD4+CCR5+ human bone marrow progenitor cell line TF-1 was used to investigate the detailed kinetics of CCR5 transcription in response to the elevation of intracellular cAMP levels and the underlying molecular events. We hypothesize that CCR5 transcription follows an asymmetrical sinusoidal pattern in TF-1 cells that parallels a protein kinase A-dependent alternating change in the ratio of activator pCREB-1-alpha,delta to repressor pCREM-alpha,beta isoforms. However, elevated CCR5 mRNA levels do not correlate with enhancement in infectivity with respect to the R5 human immunodeficiency virus type 1 (HIV-1) strain, although there is an increase in X4-utilizing virus. These results lend critical insight into the precise mechanism governing the cAMP-CCR5 axis in progenitor cells and pose interesting questions regarding its functional role in HIV-1 infection.

## P138
### Altering SIV Infection in Monocyte Derived Macrophages by siRNA Mediated Knockdown of PCNA

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Increased expression of PCNA has been documented in CNS macrophages in the perivascular space and nodular lesions in rhesus macaques productively infected with Simian Immunodeficiency Virus (SIV). Notably, PCNA positivity has been significantly correlated with the presence of SIV RNA in the same cell. Additional studies have reported that all HIV-1 p24 positive cells express PCNA in tissue from patients with HIV encephalitis. In both studies, PCNA positive cells are lacking in other markers of proliferation, e.g. Ki67, suggesting PCNA may be active in DNA damage repair as a specific result of viral infection or via indirect mechanisms. Our work indicates that PCNA expression is upregulated in CD14+ monocytes cultured in vitro in the presence of Macrophage Colony Stimulating Factor (M-CSF). Additionally, PCNA is associated with increased longevity and susceptibility to viral infection in macrophages relative to undifferentiated monocytes. The role of PCNA in SIV infection is currently being investigated by utilizing RNAi mediated knockdown of PCNA in cultured monocyte derived macrophages from Rhesus macaques. Macrophages transfected with in vitro synthesized siRNA constructs show a 30–50% reduction in mRNA expression by quantitative PCR with a commensurate decrease in protein levels, as determined by western blot, relative to a non-silencing siRNA construct. Knockdown of PCNA prior to SIV infection did not significantly alter the percentage of infected cells as determined by SIVp28 immunohistochemistry. However, viral replication increased $88 \pm 28\%$ in cells expressing low levels of PCNA. Prelimiary experiments suggest that cells with low PCNA show decreased viability in response to transfection or viral infection. Our current studies seek to explore possible cytoprotective roles for PCNA and to elucidate the means by which PCNA alters viral replication.

## P139
### HIV-1 Tat Induces Socs3 Expression in Macrophages and Microglia: Implications for HIV-associated Dementia

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HIV-1 invades the central nervous system (CNS) early following systemic infection, but typically does not lead to the cognitive disruptions characteristic of HIV Associated Dementia (HAD) until late in disease progression. This phenomenon is due, in part, to the initial ability of the innate immune system to effectively suppress viral replication in the brain. Interferon (IFN)-α, a cytokine that is readily expressed in the CNS in response to viral pathogens, has been shown to play a dominant role in this immune response by inhibiting HIV replication in macrophages, which are the most important source of productive HIV infection in the brain. However, the mechanism by which HIV eventually overcomes IFN-α's antiviral effect is unknown. Here, we show that the HIV-1 protein transactivator of transcription (Tat) induces the expression of Suppressor Of Cytokine Signaling (SOCS) 3 in CNS-relevant immune cells, such as macrophages

and microglia. Studies indicate that HIV-1 Tat induces SOCS3 expression directly through a transcriptional mechanism which is NF-κB-dependent. Although the role of SOCS3 in the HIV-infected brain is unclear, SOCS proteins have been shown to be potent inhibitors of IFN signaling. We demonstrate that HIV-1 Tat treated macrophages produce a diminished response to IFN-α when compared to untreated cells as measured by subsequent activation of STAT proteins. This response can be produced by SOCS3 overexpression alone and is abolished by NF-κB inhibitors which prevent production of SOCS3. Furthermore, in a well described SIV macaque model of HAD, increased SOCS3 expression in the CNS correlates with onset of disease. These studies suggest that HIV-1 Tat-induced SOCS3 expression in immune cells of the CNS may contribute to the diminished antiviral response to IFN-α and promote progression toward HAD.

## P140

### Activation of the endoplasmic reticulum stress response in neurons, astrocytes, and macrophage/microglia and their role in toxicity of therapeutic combinations of antiretroviral drugs

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Highly Active Anti-Retroviral Therapy (HAART) has appreciably decreased mortality and morbidity among HIV-positive patients. However, the prevalence of HIV-associated dementia (HAD) and minor cognitive diagnoses in HIV-positive patients is increasing. The side effects of antiretrovirals include neurotoxicity in the peripheral nervous system that can be modeled in cultured dorsal root ganglion neurons. However, the contribution of HAART to HIV-associated neurocognitive disorders is unknown. We have observed that individual antiretroviral drugs, in particular 10μm or greater concentrations of the HIV protease inhibitors (PI) ritonavir and saquinavir, are neurotoxic in primary rat cortical cultures exposed to the drugs for 8 days. Furthermore, drugs such as the PI, indinavir (25μm), and the nucleoside reverse transcriptase inhibitor (NRTI), stavudine (25μm), do not induce neuronal damage when applied to cultures. However, they become neurotoxic within 48 hr when added to the cultures in a combination recommended in HAART (NRTI + PI + low dose ritonavir boost). Mechanisms of antiretroviral drug toxicity reported in non-neural cell types are dependent on the class of drug and involve, among other things, alterations in protein metabolism and mitochondrial damage-induced generation of reactive oxygen species (ROS). These mechanisms, as well as HAART drugs themselves, can initiate the endoplasmic reticulum (ER) stress response, a multi-pronged pro-survival signaling pathway that may become deleterious if chronically activated. We have previously reported an increase in a downstream mediator of the ER stress response, BiP, in neurons and astrocytes in the mid-frontal cortex of patients with HAD or mild cognitive and motor dysfunction (MCMD). As all of these patients were on HAART prior to death, we examined the levels of BiP and other ER stress response mediators in CNS cultures following treatment with antiretroviral drugs. We found that, following drug treatments, BiP and ATF4 were increased in both primary cortical cultures and cultures of purified astrocytes. Similar to the neurotoxicity results, the increases in BiP were greatly enhanced when drugs were added in various combinations. In addition, BiP was increased in cultures of human monocyte derived macrophages (MDM) treated with antiretroviral drugs. An understanding of the cellular mechanisms triggered by the different classes of HAART drugs, in both neurons and glia, is crucial for guiding the design of new drugs, as well as for the assembly of combinational antiretroviral therapies that will minimize neurological impact. Supported by R01 NS056885 (KJS) and T32 AI-07632-06 (MGW).

## P141

### STAT1 dependent activation of STAT5 in peripheral blood of HTLV-I-associated myelopathy/tropical spastic paraparesis

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Immune activation is a key feature of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic inflammatory disorder of the central nervous system that affects a subset of individuals infected with HTLV-I. A number of pro-inflammatory cytokines including interleukins-2 and -15 (IL-2 and IL-15) and interferon-gamma (IFN-g) have been implicated in the immune activation of HAM/TSP. We examined STAT (signal transduction and activator of transcription) phosphorylation events in cultured peripheral blood mononuclear cells (PBMC) from subjects with HAM/TSP to characterize endogenous cytokine signaling events in HAM/TSP. STAT1, STAT5 and STAT6 activation, but not STAT3 or STAT4 activation, were detected as early as 4 hours of culture in HTLV-I infected PBMC, and peaked at 16-20 hours of culture, corresponding to the peak of viral protein (Tax) expression. The addition of anti-Tac and MiKβ1 antibodies to block IL-2/IL-15 receptor

signaling abrogated STAT5 activation, and the addition of anti-IFN-g and anti-INF-gRI antibodies blocked STAT1 activation. Inhibition of STAT1 activation by IFN-g blocking antibodies led to significant reduction in STAT5 activation, but the inhibition of STAT5 activation by IL-2/IL-15 receptor antagonist antibodies had no effect on STAT1 activation. The addition of STAT1 pathway activator 2-NP enhanced STAT5 activation, further supporting a causal interaction between STAT1 and STAT5 activation in HAM/TSP. The presence of secretory inhibitor Brefeldin A in the culture abolished all STAT activation, and abolished intracellular production of IL-2 but not IFN-g, suggesting that the upregulation of IFN-g may be a primary event in the immune activation of HAM/TSP. The absence of secretory inhibtion for the first 12 hours of culture restored intracellular IL-2 production, further supporting a down-stream role of the IL-2 pathway in the immune activation associated with HAM/TSP. These results suggest that STAT1 dependent STAT5 activation may be a feature of immune activation in HAM/TSP, where upregualtion of IFN-g and the subsequent activation of STAT1 pathway exerts a causal influence on STAT5 activation.

## P142

### Nonstructural proteins of Theiler□fs murine encephalomyelitis virus (TMEV), leader (L) and L*, influence apoptotic cell death

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TMEV belongs to the genus Cardiovirus of the family Picornaviridae. TMEV strains are divided into two subgroups on the basis of their different biological activities. GDVII strain and other members of the GDVII subgroup cause acute and fatal polioencephalomyelitis in mice. In contrast, DA strain and other members of the TO or DA subgroup persist in the spinal cords of susceptible mouse strains and cause demyelination. The leader (L) protein of TMEV is a non-structural protein encoded at the most N-terminus of the polyprotein. When the sequences of GDVII and DA strains are compared, L protein shows markedly lower homology at the amino acid level (86%) than any of the other viral proteins (>92%). Another non-structural protein, L*, is translated out-of-frame with the polyprotein from an alternative AUG. L* protein is only synthesized in DA subgroup strains since the L* AUG is substituted by an ACG in GDVII subgroup strains. L and L* are thought to be key proteins regulating TMEV biological activities, but their functions remain relatively unknown. In order to investigate the function of L, DA and GDVII L was transiently expressed in BHK-21 cells. In addition, DA L was transiently expressed in L*/BHK-21 cells, which constitutively express L* protein. Cell viability was examined by trypan blue exclusion test at 24 to 72 h after transfection. Cells were analyzed 24 h after transfection by flow cytometer using Annexin V/7-amino-actinomysin D (7-ADD) and by Western blot using antibodies specific for caspase-3 and poly (ADP-ribose) polymerase (PARP). Expression of DA L decreased cell viability and increased the number of apoptotic cells (Annexin V-positive and 7-ADD-negative cells). Western blot analysis showed the cleavage of caspase-3 and PARP. L*/BHK-21 cells were resistant to cell death induced by L. The inhibition of cleavage of PARP and the increase in the number of apoptotic cells were also observed. These results suggest that the interaction between L and L* regulates the apoptosis of macrophages, a major site of virus persistence, and contributes to TMEV subgroup-specific biological activities.

## P143

### Role of JC virus Late Regulatory Agnoprotein in Virion Biogenesis

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Posttranslational modifications including phosphorylation are known to play critical roles in the activity of many viral and eukaryotic regulatory proteins. We and others have previously reported that the late regulatory agnoprotein of human polyomaviruses, JC virus (JCV) and BK virus (BKV) is phosphorylated by a serine and threonine kinase, protein kinase C (PKC) and this phosphorylation is critical for its function. When the PKC phosphorylation sites of agnoprotein (Ser7, Ser11 and Thr21) were singly (Thr21 to Ala) or combinatorially (Ser7 and Ser11 to Ala or Ser7, Ser11 and Thr21 to Ala) converted into Ala, neither of the phosphorylation mutants was able to sustain viral infection cycle, but the expression of each mutant was detectable by Western blotting during the first cycle of infection. These observations suggest that phosphorylation mutants were either defective in assembly of infectious particles or unable to release infectious viral particles in to media, and therefore, the subsequent infection cycle cannot proceed. To address these questions, we further analyzed the mutants by viral release assays and electron microscopy (EM) studies. The results revealed that although mutant viral particles can be efficiently released from the infected cells and are morphologically indistinguishable from those of WT, they were deficient in DNA content, suggesting that

agnoprotein plays an important role in JCV virion biogenesis, either through regulating the interaction between viral DNA and capsid proteins (VP2/VP3) or by mediating the process of encapsidation of viral DNA into the viral capsids. To address these questions, we have initially examined the interaction of agnoprotein with VP2/VP3 and mapped the interaction domains of those proteins with agnoprotein. Interestingly, results showed that agnoprotein strongly interacts with the DNA binding domains of VP2 and VP3; supporting the notion that agnoprotein may be involved in the encapsidation process of JCV virions. We will further characterize this interaction between agnoprotein and capsid proteins to delineate the role of agnoprotein in JCV biogenesis. Collectively, results from such experiments may provide an opportunity to design effective therapeutic strategies to limit JCV propagation in infected individuals.

## P144

### Modulation of surface CXCR4 by μ-opioid agonist DAMGO reduces HIV-1 replication in TF-1 human bone marrow progenitor cells

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Several studies now suggest that opioids modulate innate, humoral, and cell-mediated immunological processes. There have been reports supporting both protective as well as exacerbating effects of mu-opioids in the final outcome of human/simian AIDS. In vitro studies have shown that under chronic exposure to mu-opioid receptor ligands like morphine there is increased replication of X4-utilizing HIV-1 strains in cells of the monocyte-macrophage lineage. CD34+/CD38− progenitor cells within the bone marrow are refractile to HIV-1 infection, probably due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5. We have utilized the human CD34+/CD38+ TF-1 erythromyeloid progenitor cell line to study the effects of the mu-opioid-specific agonist DAMGO on cell surface expression of the HIV-1 co-receptor CXCR4 and concomitant HIV-1 susceptibility. These studies have identified the presence of the mu-opioid receptor-1 isoform on TF-1 cells. Flow cytometry assays exhibit a downregulation of CXCR4 subsequent to DAMGO treatment of TF-1 cells. In line with these observations, DAMGO reduced viral replication in these cells. This suggests a cell-type specific role for mu-opioids in modulating co-receptor expression and viral replication. Current studies are underway to define the molecular mechanisms underlying these observations.

## P145

### Specific inflammatory profiles and HIV viral load in the cerebrospinal fluid (CSF) that correlate with AIDS-associated opportunistic infections of the central nervous system (CNS)

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Background: Opportunistic infections (OI) of the CNS are a frequent cause of mortality among AIDS patients in developing countries. The challenge of their diagnosis and treatment is magnified by the lack of a clear understanding of the pathogenic processes. CNS-OI disease may result from HIV-induced systemic immunosupression or from immunological impairments unique to the CNS environment. Histopathological findings in CNS-OI suggest differential inflammatory patterns associated with specific OI. Therefore the assessment of such immune response may provide clues about pathological processes that facilitate the identification of specific OI. The main goal of this study was to identify specific cytokine/chemokine profiles in the CSF during neuroAIDS-OI.

Material and Methods: We conducted a cross-sectional study of the profiles of cytokines/chemokines and HIV viral load in the CSF of 62 recently diagnosed patients with neuroAIDS and the four common OI: cryptococcal meningitis (CM), toxoplasmosis (TE), tuberculosis (TBM) and Neurosyphilis (NS). We also obtained profiles in 20 control patients with new diagnosis of AIDS without CNS-OI that included 10 cases of neurological complications (HIV-associated dementia or HIV-associated myelopathy or stroke) and 10 cases without CNS disease (AIDS control group). The study was approved by the IRB and written consent was obtained from all patients or closest relatives. A multiplexed flow cytometric bead assay was used for the measurement of 22 cytokine/chemokines in paired CSF/serum samples (Beadlyte® Human 22-plex, Millipore®). HIV-1 RNA was quantified in CSF and plasma samples by COBAS ®Ampliprep/COBAS TaqMan HIV-1 assay. Non-parametric tests were used to evaluate differences between the values of cytokines/chemokines or the HIV RNA titers from OIs and non OIs groups. All tests were 2-sided and significance was considered when $P < 0.05$.

Results: No differences were seen in serum profiling of the 22 cytokines/chemokines in all AIDS cases independent of the CNS disease status. In contrast, specific patterns of cytokine expression

in CSF were seen in patients with specific OIs. Significant differences were found in several of the inflammatory markers evaluated in the CSF or in their index of intrathecal production (CSF/serum ratio > 2) between OIs. A differential CSF expression of IFNg, IL-1b, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, IL-12, TNF-a, IL-8, MIP-1a and IP-10 was detected between OI groups. In particular, TBM and CM cases had a distinct intrathecal profile of cytokine/chemokine expression as compared with TE or NS cases. Patients with TE or NS expressed significantly fewer cytokines in the CSF. Despite of similar CSF cytokine/chemokine profiles in the meningitis cases, levels of IL-12p40, IFN-g and TNF-a were significantly higher in TBM as compared with CM. Interestingly, MCP-1 and IL-1a were the most consistently increased markers that showed intrathecal production in all AIDS cases even in the absence of neurological disease. Mean CSF HIV viral load was higher in the OIs group as compared with controls (5.0 log vs. 2.9 log; $P = 0.002$). Furthermore, CSF viral load distinguished CM or TBM from TE and NS cases. Patients with meningitis had more viral copies in the CSF than TE (5.6 log vs. 4.4 log; $P = 0.013$).

Conclusion: Patients with neuroAIDS-OI exhibit particular profiles of cytokines/chemokines expression in the CSF that facilitate their identification. These preliminary data in the assessment of neuroinflammatory markers in CSF of patients with neuroAIDS OIs indicate that subsets of cytokines/chemokines and HIV viral load within CNS are elevated and may be relevant in AIDS neuropathogenesis.

## P146

### HIV-1 Nef mediated induction of MCP-1 and cumulative effect of methamphetamine in astrocyte

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Both methamphetamine and human immunodeficiency virus (HIV-1) are known to induce neuroinflammation and thereby might be playing an important role in dementia. Recent studies have shown that methamphetamine causes increase in intensity of virus replication. Furthermore, HIV prevalence has been reported to be higher among methamphetamine users compared to that among non users. Likewise, increased level of monocyte chemoattractant protein-1 (MCP-1) has also been implicated in neuroinflammation in many diseases including HIV/AIDS. In this study we report that both methamphetamine and HIV-1 Nef cause increased production of MCP-1 in astrocytes. A three-day treatment of astrocytes with 500 uM methamphetamine caused $10.79 + 5.6$ fold higher expression of MCP-1 RNA which could be partially abrogated ($50 + 4.24\%$) by use of metabotropic glutamate receptor 5 (mGluR5)antagonist 2-methyl-6-(phenylethynyl)pyridine (MPEP). Likewise, transfection of astrocytes with HIV-1 Nef caused $3.3 + 0.1$ fold higher expression of MCP-1 RNA which could be almost completely abrogated by use of HIV-1 Nef specific siRNA. There results clearly suggest that MCP-1 over-expression could be a possible mechanism by which methamphetamine causes neuroinflammation. Experiments are underway to determine whether methamphetamine causes synergistic activity with HIV-1 Nef.

## P147

### Altered Structural Conformation of the V3 Region between CSF Isolates from HAD and Non-HAD Individuals: Implications for Co-Receptor Usage

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Background: 20% of untreated HIV-1 patients will develop HIV associated dementia (HAD). This has led to the hypothesis that specific strains of HIV are responsible for HAD pathogenesis. The HIV-1 Env glycoprotein binds to CD4 and secondary chemokine receptors to allow viral entry into the cell. In the brain, this is predominantly CCR5. Specific sequences within the V3 region of gp120 influence neurotoxicity. We therefore analysed V3 sequences from the CSF of individuals with/without HAD for amino acid substitutions which could cause conformational changes and therefore influence HAD pathogenesis.

Methods: 10 individuals with HAD and 8 without HAD were examined. All individuals were in AIDS stages of HIV-1 infection. PCR was performed to amplify the V3 region from CSF and products were cloned into pGEM-72Zf(+). Clones were sequenced using ABI dye terminator sequencing. DNA sequences were aligned using ClustalW software and the V3 loop charge was calculated. Homology modelling was used to examine structural differences in the V3 loop by grafting HAD and non-HAD V3 sequences onto the V3 loop- containing gp120 crystal structure of the JRFL neurotropic isolate.

Results: In general all clones had R5-like amino acid sequences regardless of the presence or absence of HAD. However, 8/10 HAD clones had V3 loop charge $< 4$ whereas 4/8 of the non-HAD clones were $> 4$. This is consistent with R5-like isolates. Thirdly, homology modelling of V3 loops from the HAD individuals showed distinct differences in folding towards the CCR5 binding site.

Conclusions: These results suggest HAD isolates despite being R5-like sequences are more likely to have an increased efficiency for CCR5. This has direct relevance to CCR5 based antiretroviral therapy.

## P148

### Mechanism for involvement of S-100β in HIV associated dementia

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Introduction: The astrocyte is thought to be important in HIV associated dementia (HAD) pathogenesis putatively because HIV-1 can infect astrocytes leading to a compromise of their physiological detoxifying and neuronal support functions. Confirmatory in vivo data supporting astrocyte involvement are emerging. Currently, the only widely available marker of the astrocyte is the protein S-100β. Given that quinolinic acid (QUIN) has an important role in the monocyte/macrophage arm of HIV neuropathogenesis we hypothesized that QUIN would have a similarly important role in the astrocyte arm of neuropathogenesis.

Methods: First the significance of S-100β was assessed by examining autopsied brain tissue from patients with differing HAD stages and progression rates. Secondly, potential modulatory factors for S-100β production were evaluated. In vitro experiments with S-100β stimulated macrophages and QUIN stimulated astrocytes were performed to assess the relationship between QUIN and S-100β. For comparison, IFNγ and TNFα stimulated astrocytes were also assessed for S-100β production. Additionally, the role of HIV-1 was evaluated by using different HIV-1 strains to infect an astrocytic cell line (SVG). Finally, to determine which region of the HIV-1 genome is responsible for stimulating S-100β production, SVG cells were infected with different HIV-1 constructs and S-100β production was measured in the supernatants.

Results: Individuals with rapidly progressing HAD and severe HAD had higher levels of staining for S-100β than individuals with slowly progressing HAD. Macrophages stimulated with S-100β produced neurotoxic concentrations of QUIN at 48 h ($1416 \pm 546$nM/l) compared with unstimulated macrophages $287 \pm 20$nM/l QUIN at 48 h ($p = 0.003$). Astrocytes stimulated with QUIN produced $0.95 \pm 0.08$ μg/L of S-100β at 48 h (vs $0.74 \pm 0.023$ μg/L for controls $p = 0.00240$. Astrocytes produced $0.90 \pm 0.04$ and $0.88 \pm 0.13$ μg/L of S-100β when stimulated with IFN-γ and TNF-α (vs controls $0.74 \pm 0.023$ μg/L $p = 0.0004$). SVG cells infected with various HIV-1 strains produced a maximum of $1.82 \pm 0.23$ μg/L of S-100β (vs untreated SVG cells $0.38 \pm 0.09$ μg/L, $p = 0.03$) and there were significant differences between HIV-1 ADA and both 89.6 and YU-2 ($p = 0.01$ and 0.02 respectively). Finally, SVG culture supernatants infected with different HIV-1 constructs produced up to $2.33 \pm 0.07$ μg/L S-100β and the difference between the negative control and NL4.3-RRE was significant ($p = < 0.0001$) Conclusions: These results further support the importance of the astrocyte in HIV neuropathogenesis. Moreover, they underline that S-100β is an important neurotoxin and marker in HAD.

## P149

### Proteomic Profiling of Cerebrospinal Fluid Reveals Potential Neuropathogenic Changes in neuroAIDS

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Predicting, diagnosing and treating HIV-1-associated neurocognitive disorder (neuroAIDS), which occurs in approximately one-third of infected individuals, have all been hindered by the lack of useful biomarkers. In quest for such biomarkers for neuroAIDS and other neurodegenerative conditions, protein profiling of biofluids especially the cerebrospinal fluid (CSF), has garnered immense interest as well as feasibility due to rapid advances in mass spectrometry. We have performed a proteomic examination of CSF in a nonhuman primate model for neuroAIDS. We developed a simple and efficient liquid chromatography coupled mass spectrometry based proteomics approach that utilizes small amounts of CSF, and show its efficacy in identifying proteins such as alpha-1-antitrypsin, complement C3, hemopexin, IgM heavy chain and plasminogen, whose expression is increased with CNS disease. Furthermore, we find that the increase in CSF proteins is linked to increased expression of their genes in the brain parenchyma, revealing that the CSF alterations identified reflect changes in the brain itself and not merely leakage of the blood-brain or blood-CSF barriers. We have now increased the sensitivity of the technique in order to apply it to

the proteomic analysis of the CSF in clinical specimens. The ability to obtain measurements from individual samples instead of pools, combined with this optimized technique, allows for the examination of diagnostic as well as mechanistic biomakers in neuroAIDS and other CNS disorders. Supported by NIH grants MH073490, MH062261 and DA026146.

## P150
### ICAM-5 modulates chemokines production in CNS during the course of herpes simplex virus type 1 infection

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Herpes simplex virus infection can cause herpes simplex encephalitis (HSE). Although the event is rare, the potential dirt consequences due to high mortality rate and the sequlae in majority of the recovery individuals have caught the attention of the public health. In addition, increasing reports have associated several neurological disorders, such as bipolar behavior and Alzheimer disease, with herpes simplex virus type 1 (HSV-1) infection. Although chemokines have been suggested to have a role in HSE, the exact mechanisms by which how these chemokines are modulating are largely unknown. Recently, we have identified a unique HSV-1 gene product, UOL, contributing to virulence (death of animals due to encephalitis) in experimental infected mice. To understand the host factors involving in the process, a Bacteria Two Hybrid system was used to screen mouse brain cDNA library with UOL as the bait. A binding partner, intercellular adhesion molecule 5 (ICAM-5, telencephalin), was found. The ICAM-5 has been suggested to play an immune modulator role in CNS. Viral load in brains was not different in mice ocularly infected with HSV-1 deleted UOL virus (ΔUOL) compared with wild type virus. In contrast, higher infiltrating immune cells was observed in brains of mice infected with ΔUOL than with wild type virus. Interestingly, the levels of ICAM-5 did not alter in brains of mice infected with ΔUOL while the levels of ICAM-5 were gradually reduced during the peak infection periods, notably on day 5 and day 7, in wild type infected mouse brains. Additionally, cytokine and chemokine arrays revealed that though slightly fluctuation levels of cytokines except interferon gamma, which was higher in wild type virus infected mouse brains, were observed in both infected mouse brains, chemokines were dramatically higher, such as CXCL10, CCL2, TIMP-1 and RANTES, in brains of mice infected with wild type, while little or no changes in ΔUOL infected mouse brains. Our results suggested that ICAM-5 plays a critical role in modulating chemokines production in immune cells infiltrating into the CNS in HSV-1 infection.

## P151
### Alcohol abuse and mouse models of HIV-1 neuropathogenesis

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Neuroinflammatory conditions (like human immunodeficiency virus-1 encephalitis, HIVE) are associated with oxidative stress and inflammatory brain injury, and excessive alcohol use can exacerbate tissue damage. Using a murine model of HIVE, we investigated the effects of alcohol abuse on the clearance of virus-infected macrophages and neuroinflammation. Severe combined immunodeficient mice were reconstituted with human lymphocytes, and encephalitis was induced by intracranial injection of HIV-1-infected monocyte-derived macrophages (HIV-1+ MDM). Animals were fed an ethanol-containing diet beginning 2 weeks before lymphocyte engraftment. Ethanol did not change lymphocyte engraftment. Alcohol-mediated immune modulation in ethanol-fed mice manifested by a significant diminution of CD8/interferon-gamma-positive T lymphocytes, increased viremia, and diminished expression of immunoproteasomes. While both groups showed similar amounts of CD8 T-lymphocyte infiltration in brain areas containing HIV-1+ MDM, ethanol-fed mice featured doubled amount of HIV-1+ MDM in the brain as compared to controls. Ethanol-exposed mice featured enhanced microglial reaction and enhanced oxidative stress. Alcohol exposure impaired immune responses (increased viremia, decreased immunoproteasome levels, and prevented efficient elimination of HIV-1+ MDM) and enhanced neuroinflammation in HIVE mice. We also demonstrated that alcohol exposure increased permeability of blood brain barrier in vivo suggesting additional mechanism of alcohol-induced neurodegeneration. Thus, alcohol abuse could be a co-factor in progression of HIV-1 infection of the brain.

## P152
### Hypoxia inducible factor-1 alpha (HIF-1α) activation of the JCV promoter. Role in the pathogenesis of Progressive Multifocal Leukoencephalopathy

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Activation of viral promoter transcription is a crucial event in the life cycle of several viruses. Hypoxia inducible factor-1 (HIF-1α) is an inducible transcription factor whose activity is dependent on environmental conditions, most notably oxygen levels and cellular stress. HIF-1α has been implicated in the pathogenesis of several viruses, including HIV-1, HHV-8 and RSV. Under hypoxic conditions or oxidative stress, HIF-1α becomes stable and translocates to the nucleus where it modulates gene transcription. The objective of the present study was to investigate a possible role for HIF-1α in the activation of JCV. Glial cell cultures infected with JCV demonstrated a significant increase in the levels of HIF-1α, in where it is located to the nucleus. Immunohistochemical studies corroborated upregulation of HIF-1α in JCV infected oligodendrocytes and astrocytes in clinical samples of PML compared with normal glial cells from the same samples in which HIF-1α expression is weak. CAT assays performed in co-transfected glial cells demonstrated activation of the JCV early promoter in the presence of HIF-1α. This activation was potentiated in the presence of Smad3 and Smad4. Finally, chromatin immunoprecipitation assays demonstrated the binding of HIF-1α to the JCV control region. These results suggest a role for HIF-1α in the activation of JCV; understanding of this pathway may lead to the development of more effective therapies for PML, thus far an incurable disease.

## P153
### Role of Ferritin Heavy Chain and opiates in neuroAIDS

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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 are still undefined and controversial findings have been reported. One of the unresolved issues relates to the interaction between opioid receptors and the HIV co-receptors, the chemokine receptors CCR5 and CXCR4, which are abundantly expressed in both the immune and nervous system. These receptors belong to the superfamily of G-protein coupled receptors and primarily signal through Gi/Go activation. 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 examining expression of MOR, CXCR4/pCXCR4, and FHC in brain tissue samples from control subjects and HIV-infected individuals (including opiate users). The goal is to gather initial evidence about cellular localization of FHC in the human brain (with respect to MOR and CXCR4) and about CXCR4 activation in correlation to neurological impairment. Furthermore, the effect of morphine on expression of the FHC receptor TIM-2 on neuronal and glial cells is also being investigated. Preliminary data suggest that a deficit in CXCR4 function is associated with over-expression of FHC expression in neuroAIDS patients.

## P154
### Fatigue is Associated with Learning and Memory Difficulties Among People Infected with the Hepatitis C Virus (HCV)

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Introduction: Individuals with HCV often complain of fatigue and cognitive deficits. The present study examines the relationship between fatigue and cognitive abilities among HCV+ persons many of whom have additional comorbidities including HIV co-infection and past methamphetamine abuse/dependence.

Method: We examined 31 HCV+ men and 30 HCV+ women with the Fatigue Severity Scale, an instrument developed to assess disabling fatigue and also administered a neuropsychological battery that assessed 7 cognitive domains. Mean age was 48.4 (range 22 to 66), the mean (log) HCV RNA was 5.9 (range 4.2 to 7.3), and the AST-to-Platelet Ratio Index (APRI) mean was 0.85 (range 0.1 to 5.9).

Nineteen were HIV seropositive and 27 met criteria for past methamphetamine abuse/dependence.

Results: Although severity of fatigue was not associated with a summary neuropsychological score, severity of fatigue was negatively associated with learning and recall (learning: $\rho = -0.36$, $p = 0.004$; recall: $\rho = -0.35$, $p = 0.005$). No other domain scores were correlated with fatigue. In terms of HCV disease characteristics, neither HCV RNA nor APRI were significantly correlated with severity of fatigue. HIV serostatus was not associated with severity of fatigue. In terms of psychiatric disorders, neither lifetime Major Depressive Disorder nor lifetime methamphetamine abuse/dependence was associated with severity of fatigue (all p's $> 0.10$).

Discussion: Severity of fatigue appears to be associated with worse learning and recall performance among HCV+ individuals. Further assessment is necessary to determine whether fatigue and learning/memory difficulties are the result of a common HCV-induced pathway or whether the two may derive independently.

## P155

### Modeling HTLV-1 infection and cellular immune response in HLA-A*0201 transgenic mice

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HTLV-1 is associated with two immunologically distinct diseases: HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T-cell leukemia (ATL). To date, the lack of a suitable small animal model has hindered in vivo investigations for HTLV-1-associated diseases particularly those related to the immunopathogenesis of the neuroinflammatory disease HAM/TSP. Part of problem has been the inability of HTLV-1 envelope to fuse with murine cells. Therefore, a chimeric HTLV-1 viral plasmid was used in which the HTLV-1 envelope gene was replaced with that of Moloney murine leukemia virus to facilitate fusion between the virus and murine cells. The chimeric HTLV-1 and Tax (viral transcriptional transactivating protein) expressing plasmids were co-transfected into HEK-293T cells, which were then injected intraperitoneally into the 129Sv and C57BL/6-HLA A2.1 transgenic mice. Utilizing the HTLV-1 gag gene primers and Gag PCR product as probe, viral integration could be detected in the mice splenocytes by nested PCR and Southern blot hybridization at selected time points. Infection was also confirmed by real time PCR and indicated by a clear reduction in the threshold cycle (Ct) number for gag amplification in the infected mice over control. In addition, the generation of a strong cell-mediated immune response was verified by flow cytometry analyses for an increase in the number of IFN-γ-producing CD8+ T cells. To evaluate the role of dendritic cells (DCs) in the onset and progression of HTLV-1 pathogenesis, CD11c-DTR transgenic mice were also used in parallel that can be selectively depleted of the conventional DCs. These results highlight the use of a chimeric HTLV-1 virus in the context of a mouse model that offers the advantage of being inbred with the availability of multiple transgenic lines to perform immunologic studies.

## P156

### Combined antiretroviral therapy (CART) results in decreased astrocytosis and reversal of neuronal injury in SIV-infected CD8-depleted rhesus macaques

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Introduction: With the advent of effective antiretroviral therapy (ART), the prevalence of severe neurological deficits in patients infected with HIV has decreased markedly. However, minor cognitive motor dysfunction and mild AIDS dementia are still common, and as patients live longer the prevalence of neuroAIDS is again increasing. Because many antiretroviral agents do not cross the BBB, it has been suggested that the brain may serve as a proviral reservoir in HIV patients. A previous study by our group demonstrated that combination antiretroviral therapy (CART) initiated at 4 weeks post infection (wpi) for four weeks with non-CNS-penetrating agents resulted in a near-complete reversal of N-acetylaspartate/creatine, a reliable marker of neuronal injury using in vivo proton magnetic resonance spectroscopy and prevented the development of SIVE. Here, we report quantitative immunohistochemistry (IHC) results on these CART treated animals and compared those results to IHC markers of untreated animals sacrificed at 4 wpi (the time of treatment initiation) and to uninfected CD8-depleted animals.

Methods: Twelve rhesus macaques were CD8+ T-lymphocyte depleted by treatment with anti-CD8 antibody administered at 6, 8 and 12 days post

infection (dpi) in order to accelerate AIDS progression. Eight animals were inoculated with SIVmac251 and 4 of these animals were treated with CART (PMPA and RCV) beginning at 4 wpi for 4 weeks. Four SIV+ animals were euthanized at 4 wpi, the other 8 animals were euthanized at 8 wpi. Brain tissue from the parietal cortex was harvested for quantitative neuropathology. The degree of reactive astrogliosis was assessed with monoclonal anti-glial fibrillary acidic protein (GFAP). The integrity of the synapses and dendrites was evaluated with monoclonal antibodies against synaptophysin (SYN) and microtubule-associated protein-2 (MAP-2), respectively. The number of cortical neurons was quantified by using stereologic evaluation.

Results: There is a statistically significant difference in GFAP levels among the three cohorts ($P = 0.0004$, ANOVA). Least-means squared t-tests showed that untreated SIV+ animals euthanized at 4 wpi had 56% higher GFAP levels compared to uninfected controls ($p = 0.0001$) and 32% higher compared to CART animals ($p = 0.0019$). There was also a slight 19% increase in GFAP in the CART animals compared to uninfected controls ($p = 0.04$). MAP-2 changes were significant across the three cohorts ($p = 0.03$, ANOVA), with 48% decrease in untreated SIV+ animals compared to uninfected controls ($p = 0.01$), and 34% lower levels in untreated SIV+ animals compared to CART treated animals (trend $p = 0.09$). SYN differed across the three cohorts ($p = 0.03$, ANOVA), with highest levels in the uninfected animals, followed by CART animals (16% lower), and then untreated animals (30% lower). However, significant differences between CART and untreated animals were not observed ($p = 0.19$). Neuronal counts were significantly different across the three cohorts ($p = 0.027$, ANOVA), with 30% lower counts in untreated SIV+ animals compared to uninfected animals ($p = 0.01$), and a 25% lower counts compared to CART treated animals ($p = 0.03$).

Conclusions: Combined antiretroviral using drugs that do not cross the BBB reduces reactive astrocytosis and improves neuronal integrity in addition to normalizing brain metabolism as revealed by 1H MR spectroscopy in the SIV infected accelerated neuroAIDS macaque model.

## P157

### Brain Creatine Elevation and NAA Reduction detected by 3T 1H MR Spectroscopy Indicates Neuronal Dysfunction in the Setting of Enhanced Glial High Energy Metabolism in a macaque model of neuroAIDS

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Introduction: In vivo proton magnetic resonance spectroscopy (1H MRS) has emerged as one of the most informative methods for the study of neuroAIDS, as it provides biomarkers to assess disease progression and to monitor treatment. N-acetylaspartate (NAA) is localized within neurons and its decline in neuroAIDS as measured by MRS is established as a sensitive indicator of neuronal injury/loss. Changes in the creatine (Cr) resonance as a result of neuroAIDS are poorly understood. Increases in Cr levels have been reported in antiretroviral-naïve HIV patients. We have now established that Cr rises with SIV infection in the macaque brain, and we propose that in neuroAIDS creatine may be a useful biomarker to monitor gliotic/inflammatory processes in the brain.

Methods: Ten rhesus macaques were inoculated with SIVmac251 and their CD8+ T-lymphocytes were depleted with anti-CD8 antibodies at 6, 8, and 12 days post inoculation (dpi) to accelerate AIDS progression. Single voxel MRS was performed two times pre-infection, and biweekly until sacrifice at 4 weeks post infection (wpi) (4 animals), 6 wpi (2 animals) or 8 wpi (4 animals), using a Siemens 3.0 Tesla system. Voxels were placed in the parietal cortex (PC), frontal cortex (FC), basal ganglia (BG) and white matter semiovale (WM). Metabolite concentrations (NAA, Cr, choline (Cho) and myo-Inositol (MI) were determined using LCModel. Quantitative measurements of glial fibrillary acidic protein (GFAP) were obtained from frontal cortex in six of these animals.

Results: Two animals euthanized at 6 and 8 wpi developed SIV encephalitis, defined as accumulation of macrophages and MNGC. Most animals had gliosis and cortical neuronal degeneration. NAA/Cr was found to be significantly decreased in all 4 brain regions measured (PC, FC and WM $p < 0.001$, BG $p = 0.004$). When measuring NAA and Cr changes with respect to tissue water as the internal standard, we observed a decrease in NAA concentration (WM $p = 0.03$) indicative of neuronal injury. Interestingly, we found an increase in Cr with disease progression (PC $p = 0.01$, WM $p = 0.013$, BG $p = 0.07$). Choline increased at 2 wpi (PC, FC, WM $p < 0.001$, BG $p = 0.002$), then decreased to baseline values or below. With further disease progression, Cho increased once more at 8 wpi. (PC and WM $p = 0.003$). MI and MI/Cr show complex regional variations over time with increases at 2 and 4 weeks and then

normalization to baseline values. Histopathologic examinations revealed intense astrogliosis in the FC detected by increased GFAP levels compared to levels of uninfected control animals ($p = 0.0008$).

Discussion: Changes in total creatine are associated with altered energy metabolism. It is believed that the virus enters the brain through infected monocytes that later differentiate into macrophages. During this process of monocytic cell infiltration, astrocyte and microglial activation and proliferation, a high metabolic demand may explain an increase in Cr. NAA decreases as a result of neuronal injury, and thus a decline in Cr would be expected in these cells. Our observations are explained if the expected decline in neuronal Cr is overcompensated by Cr elevations produced by the metabolic rates within activated astrocytes and microglia. Our hypothesis of creatine elevation as a biomarker for astrocytic and microglial activation is supported by further studies in which elevated Cr decreases with antiretroviral therapy in macaques and patients. These combinations of decreased NAA and increased Cr make the NAA/Cr ratio detected by MRS a sensitive marker for brain disease status.

## P158

### 1H MR Spectroscopy reveals neuroprotection by minocycline in an accelerated rhesus macaque model of neuroAIDS

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Introduction: There is a consensus that HIV enters the CNS primarily through the trafficking of virally infected/activated monocytes. This influx of infected, activated monocytes excites astrocytosis and microgliois. This reaction, accompanied by the production of neurotoxic substances by activated glia as well as infected macrophages, induces neuronal cell injury and death by apoptosis. The goal of this study was to investigate minocycline (MN) as a neuroprotective agent for neuroAIDS. Minocycline has been tested in a variety of neuronal diseases with advantageous effects against inflammation, microglial activation, apoptotic cell death, and viral production. However, some studies have found inconclusive data regarding MN's neuroprotective effects and some have even reported a harmful effect on patients with ALS. The focus of our study was to serially assess neuronal health in an accelerated macaque model of neuroAIDS by measurement of N-acetylaspartate/creatine (NAA/Cr), a reliable marker of neuronal injury using in vivo proton magnetic resonance spectroscopy (MRS).

Methods: Eight rhesus macaques were infected with SIVmac251 and treated with the anti-CD8 antibody cM-T807 to deplete CD8 T lymphocytes at 6, 8 and 12 days post inoculation (dpi) in order to accelerate AIDS progression. Four of these animals received daily treatments of minocycline (4 mg/kg/day) starting 4 weeks post inoculation (wpi) for four weeks. Animals were examined with MRI and MRS (3.0 T TIM Trio Siemens) 2-3 times before and biweekly after SIV infection until 8 weeks post infection. Single voxel 1H MR spectra were acquired from the parietal cortex (FC) and frontal cortex (FC), basal ganglia (BG) and white matter centrum semiovale (WM). NAA/Cr was quantified by LCModel. Repeated measured (RM) ANOVA along with Holm's t tests was performed to reveal temporal changes due to infection and treatment within the cohorts. Student's t-tests between the cohorts' metabolite changes were performed Results: SIV infection and CD8 depletion in the untreated animals resulted in a rapid decline in NAA/Cr levels in all four brain regions measured (PC $-15\%$ $p < 0.0001$, FC $-20\%$ $p < 0.0001$, BG $-13\%$ $p = 0.004$, WM $-17\%$ $p < 0.0001$) indicative of neuronal injury. Furthermore, two of these animals developed encephalitis, defined as accumulation of macrophages and microglial nodules. The animals selected for treatment also demonstrated declines in NAA/Cr in all regions at 4 wpi. After 4 weeks of MN treatment, the brain NAA/Cr levels in these animals were close to pre-infection values in all regions (PC $-6\%$ $p = 0.18$, FC $-5\%$ $p = 0.57$, BG $-3\%$ $p = 0.62$ and WM $-5\%$ $p = 0.13$). None of the 4 MN treated animals developed SIVE.

Conclusion: Our results suggest that MN is an effective neuroprotective agent in this nonhuman primate model of accelerated neuroAIDS. Brain measurements of NAA/Cr is a sensitive biomarker of neuronal health. NAA/Cr correlates highly with synaptophysin during early neuronal injury, and with other molecular markers of neuronal integrity with progression of neuronal injury. The accelerated macaque neuroAIDS model produces a profound decline in brain NAA/Cr by 8 wpi which was prevented by MN treatment starting at 4 wpi. Furthermore, MN prevented the development SIVE. Ongoing studies to elucidate minocycline's mechanisms of action include confirmation of neuroprotection by quantitative neurohistopathology, its affect on astrocytosis, microgliosis and viral analysis in the blood, CSF and brain tissues.

## P159

### Rat organotypic Slice Cultures and Neuronal Survival with HIV-1 Exposure

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Human Immunodeficiency Virus type 1 (HIV-1) infection of the human brain is a chronic inflammatory disease. HIV-1 infection of the brain is particularly difficult to model in vitro, given the species specificity of the virus, and the multiple cellular interactions involved in neuropathogenesis. Organotypic hippocampal cultures retain the intrinsic synaptic connections; preserve synaptodendritic complexity and the character of the neurogenic microenvironment. In the current study, we tested the hypothesis that HIV-1 exposure affects neuronal survival within the hippocampus ''neurogenic zone'' in rat hippocampal slice cultures. Organotypic hippocampus slice cultures were prepared from Sprague-Dawley neonatal rats 9–11days of age. Slices were incubated for 21 days in medium containing 2.5% fetal bovine serum and dilutions of conditioned medium from mock-infected peripheral blood mononuclear cells (PBMC) or from PBMC infected by lymphotropic HIV-1(SF2) or macrophage-tropic HIV-1(SF128A). Slices exposed to conditioned medium from mock-infected PBMC (''mock-exposed'') were controls for slices exposed to HIV-1-containing medium (''virus-exposed''). Virus concentration was 44ng p24 antigen per slice. At the end of treatment, slices were fixed and immunostained for NeuN antigen to detect the pyramidal neurons of the hippocampal cell layer. Each immunostained slice culture was examined with an Olympus microscope fitted with epifluoresence optics. Microscopic fields were recorded digitally and NeuN-positive cells were counted using Sigma Scan Pro image analysis software. Results demonstrated presence of NeuN-positive cells in hippocampus, including virus-exposed and mock-exposed slices. NeuN-positive cells in the CA1 region were counted and normalized to length. SF128A exposure resulted in approximately a 40% decrease in NeuN cells as compared to mock exposure. SF2 exposure did not decrease NeuN cells in the CA1 region. However, when NeuN-positive cells were counted in CA1, 2 and 3 regions, and normalized to length, an effect was seen with both SF2 and SF128A exposure. SF128A exposure resulted in approximately a 60% decrease in NeuN cells as compared to mock exposure. SF2 exposure resulted in approximately a 45% decrease in NeuN cells as compared to mock exposure. These data indicate that SF128A exposure leads to a decrease in NeuN cells in CA1 alone and further decrease in NeuN cells in all CA regions. SF2 exposure does not lead to a decrease in NeuN cells in CA1 but does produce a decrease in all CA regions, probably predominantly in the CA3 region. Based on these results we conclude that the macrophage-tropic HIV-1 strain SF128A was more neurovirulent for rat hippocampal neurons and was not selective for CA regions; in contrast, lymphotropic strain SF2 was more selective for CA3.

## P160

### Two functional C/EBP sites in the SIVmac239 LTR mediate transcriptional activation and IFN-mediated suppression of LTR activity in primary macrophages

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Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infected cells invade the CNS early in acute infection; however, HIV-associated cognitive disorders do not manifest until late in the disease process. HIV/SIV replication is downregulated in the CNS after acute infection in large part due to the innate immune responses, particularly type I IFN responses (IFN). Using an accelerated and consistent SIV/macaque model for HIV/AIDS and CNS disease, we have demonstrated that while SIV DNA levels in the brain remain constant, SIV RNA levels peak at day 10 post-inoculation (p.i.) and are downregulated by day 14 p.i., suggesting that control of viral replication occurs, in part, at the transcriptional level. We have shown that downregulation is associated with the production of IFN in the brain. IFN treatment of macrophages has been shown to inhibit ongoing, active HIV/SIV replication by inducing CCAAT/enhancer binding protein (C/EBP) dominant negative protein, LIP (liver-enriched transcriptional inhibitory protein) through alternative translation of C/EBP mRNA. C/EBP and C/EBP binding sites in the HIV-1 LTR are crucial for HIV-1 replication in monocyte/macrophages and for the ability of IFN to inhibit ongoing, active HIV replication in these cells. Here we examined two C/EBP sites, JC1 ($-100$bp) and DS1 ($+134$bp), located within the minimal region for transcriptional activation of the SIVmac239 LTR in primary macrophages. Both the JC1 and DS1 C/EBP sites bind C/EBP and LIP, but are functionally different. Specifically, JC1 but not DS1 is important for transcriptional activation of the SIV LTR in primary macrophages. In contrast, either JC1 or DS1 is sufficient to mediate IFN-induced downregulation of LTR activity in these cells. Competition EMSAs demonstrated that C/EBP binds to the JC1 site with 2 fold higher affinity than the DS1 site while LIP binds both sites with equal affinity, thus suggesting that the differential affinity of the two isoforms for the JC1 and DS1 C/EBP sites may contribute to the differential regulation of the

SIV LTR. Taken together, these results along with our previous studies demonstrate that the JC1 and DS1 C/EBP sites may play a crucial role in establishing latency and persistence in macrophages in the brain.

## P161
### Protective role of IGF-I - FOXO3a signaling axis in high glucose-mediated accumulation of reactive oxygen species (ROS) and neuronal damage

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High glucose in the blood alters cellular redox status through the accumulation of reactive oxygen species (ROS) in different organs including CNS. In HIV patients anti-retroviral medications often lead to multiple metabolic abnormalities including development of insulin resistance, and type 2 diabetes mellitus. Since the mammalian homolog of the Forkhead family of transcription factors, FOXO3a, controls ROS metabolism, and the activity Forkhed proteins can be modulated by IGF-I, we asked whether FOXO3a contributes to IGF-I–mediated protection of differentiated neurons from high glucose (HG). Our results indicate that high glucose (25mM applied for 16 hours), elevated accumulation of ROS in differentiated PC12 neuron-like cells and in primary cultures of rat cortical neurons. We found that IGF-I significantly lowered ROS accumulation and increased stability of neuronal processes in neuronal cultures exposed to HG. This accumulation of ROS in HG was accompanied by translocation of FOXO3a to the nucleus, loss of mitochondrial potential and gradual retraction of neuronal processes. Protective effects of IGF-I were accompanied by FOXO3a nuclear export, decreased FOXO3a transcriptional activity, and by changes in mRNA levels for FOXO3a transcriptional targets: pro-apoptotic Bim and antioxidant MnSOD. Clinical relevance of these findings are supported by the detection of nuclear FOXO3a in TUNEL positive cortical neurons from clinical samples of HIV encephalitis (HIVE), and suggest potential neuroprotective role of IGF-I against ROS–mediated neuronal damage.

## P162
### Unsuppressed HIV replication elicits an interferon-alpha-induced phenotype in CD14+ monocytes

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HIV infection alters leukocyte gene expression and dysregulates the innate immune system. Chronic activation of the immune system is a defining feature of infection and is considered critical for pathogenesis. As potential candidates for immune disruption, we evaluated three HIV-related peripheral factors, interferon (IFN)-alpha, IFN-gamma and lipopolysaccharide, which have been implicated in various studies. To characterize the in vivo HIV-induced impact on gene expression, CD14+ monocytes from 12 HIV seronegative controls, 22 HIV-seropositive subjects with low viral loads (LVL, $<$10,000 RNA copies/ml) and 22 HIV seropositive subjects with high viral loads (HVL, $>$10,000 RNA/copies/ml) were analyzed using high-density microarrays. For comparison, microarray analysis was obtained from HIV seronegative monocytes treated with IFN-alpha, IFN-gamma or lipopolysaccharide (LPS) for 48h. The results of this cross-sectional study showed that subjects with viral loads above 10,000 RNA copies/ml exhibited an activated monocyte phenotype. Characterization of the gene expression pattern by Gene Ontology reveals an ongoing immune response to viral infection including inflammation and chemotaxis. Gene expression analysis of in vitro-treated HIV seronegative monocytes with IFN-alpha, IFN-gamma or LPS indicated that IFN alpha most accurately recapitulated the HIV HVL profile. There was no detectable LPS-induced gene expression signature even in those HIV subjects with the highest LPS plasma concentration (Pyrogene assay) and sCD14 levels (ELISA). In summary, the gene expression profile in monocytes from subjects with viremia is predominantly due to IFN-alpha, while subjects with LVL have a non-activated phenotype. In monocytes, there was no discernible expression profile linked to LPS exposure.

## P163
### Drugs of Abuse and HIV-1 replication in Normal Human Astrocytes (NHA)

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Galectins are a family of -galactoside binding lectins that regulate cell to cell and cell to matrix interactions, cell adhesion, and cell signaling. In macrophage and peripheral blood mononuclear cells (PBMC), galectin-1 acts as a soluble adhesion molecule enhancing human immunodeficiency

virus (HIV-1) infectivity and replication. It is well established that drug abuse is a significant risk factor for contracting HIV-1 infection and its progression to HIV-1 associated encephalopathy (HIVE). In the central nervous system, astrocytes maintain a homeostatic environment and modulate the expression of various immunoregulatory proteins. We observed that treatment of normal human astrocytes (NHA) with the drug of abuse, methamphetamine, increased gene and protein expression for galectin-1 in a dose dependent manner as determined by quantitative real-time PCR and western blot analyses, respectively. Furthermore, methamphetamine treatment enhanced HIV-1 infectivity of NHA as determined by p24 antigen ELISA. Treatment of NHA with methamphetamine in combination with recombinant galectin-1 caused an additive effect on HIV-1 replication. RNA interference (RNAi) using small interfering RNA (siRNA) directed against galectin-1 complexed with gold nanorods reduced the expression of galectin-1 RNA and protein and prevented the effects of methamphetamine on HIV-1 replication in NHA. The mechanism underlying the association of HIVE with drug abuse may be mediated by the increased expression of galectin-1 which, in turn, enhances HIV-1 binding to target cells. Our findings may lead to novel strategies for preventing HIVE in high risk, drug abusing populations.

## P164
### The Role of Cell Adhesion Molecules in HIV-1 Neuropathology

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Neurocognitive impairment associated with HIV-1 is increasingly presenting as a devastating consequence of infection. Cell adhesion molecules are involved in maintenance of the blood-brain barrier (BBB), leukocyte transmigration into the CNS, and neuronal synaptic integrity; all of which are disrupted in HIV-1 infection. We hypothesize that HIV-1 infection, viral proteins, and chemokine dysregulation collectively disrupt adhesion molecule-mediated cellular signaling and binding interactions such that leukocytes gain enhanced entry into the CNS, the BBB is disrupted, and neuronal function is compromised. CCL2, the most potent chemotactic factor for monocytes, is of particular interest because it is elevated in the CNS of HIV-1-infected individuals and its levels correlate with CNS pathology and neurocognitive impairment. To traverse the BBB, leukocytes must disrupt adherens junction (AJ) complexes. This is partially accomplished through endothelial intracellular signaling. We demonstrate that in response to CCL2, endothelial junctional integrity is transiently disrupted. Endothelial cells retract in a Src-dependent manner and this is associated with focal adhesion kinase activity, VE-cadherin and beta-catenin phosphorylation, and disassembly of the AJ. PECAM-1 acts as a beta-catenin sink, sequestering beta-catenin at the membrane during AJ disassembly. Akt phosphorylates GSK-3beta, reducing its kinase activity and serine phosphorylation of beta-catenin. Combined, the activities of PECAM-1 and Akt reduce the cytoplasmic, nuclear, and cytoskeletal localization and proteosomal targeting of beta-catenin, facilitating a rapid reconstitution of the AJ. PrPc is an adhesion molecule ubiquitously expressed in the CNS which contributes to structural support, BBB maintenance, and leukocyte transmigration. We found that CNS tissue from cognitively impaired HIV-1-infected individuals demonstrate pronounced PrPc expression as compared to uninfected individuals. We also found that CCL2 induces shedding of PrPc from neurons, astrocytes, and endothelial cells. Furthermore, soluble PrPc (sPrPc) is elevated in the sera of SIV-infected macaques, a primate model of HIV-1 infection. We speculate that sPrPc may alter BBB integrity, activate astrocytes, and disrupt NMDA receptor signaling. We have also found that PrPc is downregulated in HIV-1 infected macrophages, suggesting potential impact on function. Further studies are directed at evaluating the use of PrPc as a potential biomarker of HIV-1 CNS pathology and at understanding the functional consequence of PrPc dysregulation.

## P165
### Comparative Immunovirological Study between Buprenorphine and Methadone Using Human Lymphocytes and Glial Cells

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Background and Significance: Buprenorphine (BUP) and methadone (MTD) are mu-opioid receptor agonists widely used for detoxification, short- and long-term maintenance treatment of heroin addiction. Since the emerging and frequent use of these drugs in the HIV-1 population, it is significant to appreciate how these drugs impact the HIV-1 replication at the cellular and molecular level. Previous studies indicate that methadone enhances HIV-1 replication and suppresses cellular function. Unlike MTD, no study has investigated the effect of BUP on HIV-1 replication and cellular function. Previous observa-

tions raise a major concern regarding the possible consequences of MTD that could affect HIV-1 infection especially in the pathogenesis of HIV-associated neurological disorders and perhaps BUP could be a good substitute of MTD in the therapy of HIV-infected recovering drug addicts.

Methods: In this study, we investigated whether MTD or BUP potentiates HIV-1 replication in human lymphocytes and glial cells using quantitative RT-PCR. In addition, we also measure the production of pro- and anti-inflammatory cytokines/chemokines using cytometric bead array (CBA).

Results: We have shown that both MTD and BUP enhanced HIV-1 replication in both lymphocytes and glial cells. This enhancement effect was associated with alterations in the production of both pro- and anti-inflammatory cytokines/chemokines in these cells. Following pre-exposure with mu-opioid receptor antagonists such as nalthrexone (NALT) and naloxone (NALX), HIV-1 replication was not affected suggesting that MTD and BUP may be eliciting its cellular and molecular mechanism through the mu-opioid receptor.

Conclusions: These findings provide a cellular mechanism that supports the notion that both MTD and BUP have a co-factor role in the development of HIV-associated neurological disorders. This work was supported by NIH-RCMI Grant # 2G12RR03035.

## P166
### CCL8/MCP-2 is a target for mir-146a in HIV-1 infected human microglial cells

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MicroRNA-mediated regulation of gene expression appears to be involved in a variety of cellular processes including development, differentiation, proliferation and apoptosis. Mir-146a is thought to be involved in the regulation of the innate immune response and its expression is increased in tissues associated with chronic inflammation. Among the predicted gene targets for mir-146a, the chemokine CCL8/MCP-2 is a ligand for the CCR5 chemokine receptor and a potent inhibitor of CD4/CCR5-mediated HIV-1 entry and replication. In the present study, we have monitored changes in the expression of mir-146a in primary human fetal microglial cells upon infection with HIV-1 and found increased expression of mir-146a. We further show that CCL8/MCP-2 is a target for mir-146a in HIV-1 infected microglia, as overexpression of mir-146a prevented HIV-induced secretion of MCP-2 chemokine. The clinical relevance of our findings was evaluated in HIVE brain samples in which decreased levels of MCP-2 and increased levels of mir-146a were observed, suggesting a role for mir-146a in the maintenance of HIV-mediated chronic inflammation of the brain.

## P167
### Effects of Vitamin A Deficiency and Morphine on Cortical and Hippocampal Parvalbumin+ Neurons in the HIV-1 Transgenic Rat

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Background: Vitamin A (VA) deficiency has been associated with progressive HIV-1 infection. In addition, studies suggest opioid abuse may be associated with a more frequent occurrence and a greater severity of neurocognitive impairment in individuals with HIV-1 infection. To examine the potential role of these factors in the pathogenesis of HIV-related cognitive and motor impairment, we examined the effects of VA deficiency and morphine on total and parvalbumin+ (parv+) neurons in cerebral cortex and hippocampus from Tg and Wt rats.

Methods: 3–6 month old specific pathogen free Tg and age-matched WT Fisher 344/NHsd control rats were used for the studies. The rat were born to females administered a rodent maintenance diet which contained either 400,000 IU/kg of retinyl palmitate, the major dietary form of VA, or the same diet mix formulated minus retinyl palmitate. The offspring were then maintained on the same diet as the dam. Tg and Wt rats on either the normal or VA deficient diet were then implanted with either a placebo pellet or a pellet containing 37.5 mg of morphine sulfate. Seven days later the rats were sacrificed and perfused with paraformaldehyde and then formalin fixed, paraffin embedded sections of brain were stained with NeuN antibody, to determine total neuronal numbers, and for parvalbumin+ neuronal subsets. NeuN+ and parv+ mean neuronal counts in replicate uniform areas were then determined in frontal cortex and in hippocampus and the region-specific counts were compared for the Tg and Wt rats fed either the normal or VA deficient diet and implanted with either the placebo or the morphine pellet.

Results: Mean cortical and hippocampal NeuN+ cell counts were similar for all Tg and Wt groups. For parv+ neurons, mean numbers in cortex were similar for untreated Wt and Tg rats on the normal diet. However, compared to the animals in these groups mean numbers were lower for Wt and Tg rats treated with morphine and the VA deficient diet. In

hippocampus, morphine treated VA deficient Wt rats had lower mean parv+ neurons than untreated Wt rats on the normal diet. Also, untreated Tg rats on either diet had mean parv+ hippocampal neuronal counts that were lower than the Wt control rats whereas morphine treated VA deficient rat mean parv+ cell counts were no different thank counts for untreated Wt controls on the normal diet.

Conclusion: Tg and Wt rat total neuronal numbers were not affected by VA deficiency or by morphine treatment. In contrast, the VA deficient diet and morphine were associated with changes in parv+ neuronal numbers in both cortex and in hippocampus with some opposite effects observed for these two brain regions. Parv+ neurons express gamma amino butyric acid and play an important role in memory and motor control. These neurons also express mu opioid receptor. Therefore, understanding the mechanisms that underlie these obseved effects on parv+ neurons in the Tg model may provide important information information related to the pathogenesis of neurological impairment in HIV infected drug users. Supported by R01DA021556 and R01DA015311.

## P168

### Vitamin A Deficiency and Behavioral Abnormalities in the HIV-1 Transgenic Rat

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Background: The HIV Tg rat model incorporates a non-infectious viral genome that is under similar regulatory control mechanisms in vivo that exist with natural infection. In humans vitamin A (VA) deficiency has been associated with progressive systemic HIV disease. The effects on of VA deficiency on the development of behavioral abnormalities with HIV infection have not been previously described.

Methods: 3-6 month old Fisher 344/NHsd wild type (Wt) and transgenic (Tg) rats maintained on either a normal (VA+) or a VA deficient (VA-) diet were examined for 1) open field activity (total horizontal distance, vertical activity, and stereotypy) in a plexiglass chamber using a 16 beam Digiscan Activity Monitoring System; 2) on a rotarod, with assessments performed at baseline and on days 4 and 17 of the testing, and; 3) on an elevated plus maze with quantitation of the number of entries into the open and closed sections of the apparatus and the time spent in each section at baseline and on days 2 and 20. Groupwise differences in the raw means in the times for the various tasks were examined for the animal groups using analysis of variance. Differences between group pairs were analyzed using t-tests with, statistical significance determined after correction for multiple comparisons.

Results: 1) Open field activity: Statistically significant differences in horizontal, vertical, and stereotypical activity were noted for the four groups of rats. WtVA- rats showed higher levels of horizontal and vertical activity as compared to all other groups. TgVA+ rats showed significantly greater and WTVA- rats showed borderline increased vertical activity as compared to TgVA- rats. 2) Rotatod test: At baseline and on day 4 the groups of rats showed no differences in ability to remain on the rotating rod. On day 17 the TgVA- rats spent significantly less time of the rod than the WtVA+ and WtVA- rats. 3. Plus maze: Differences between the groups were noted for number of closed entries at baseline and for closed entry times at day 20. Only the WtVA+ rats showed avoidance of the open space at any of the time points.

Conclusions: Behavioral abnormalities can be demonstrated by these tests in the HIV-1 Tg rat. Specific deficits in motor learning may also occur as a result of the presence of the transgene in association with the VA deficient state. Supported by R01DA021556 and R01DA15311.

## P169

### Concerns with use of reverse genetics technology for identifying molecular determinants of virus neurotoxicity

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Prior to the advent of mumps vaccines, mumps virus was a leading case of viral meningoencephalitis in developed countries. Mumps virus attenuation has proven difficult and many live attenuated mumps vaccines have retained residual neurotoxicity, causing aseptic meningitis in vaccinees. New approaches to determine the molecular basis of virus virulence include mutagenesis of cDNA clones of viruses, followed by rescue and testing of the new variants. Using this methodology, we identified several mutations within the mumps virus genome that appeared to result in either increased neurotoxicity or in neuroattenuation. Surprisingly, when we repeated the rescue of virus from the same cDNA plasmids, we found, in several instances, significant differences in neurotoxicity/neuroattenuation between the first and second viral rescues. Comparison of the complete nucleotide sequences of virus pairs independently derived from the same cDNA plasmids confirmed the presence of the engineered mutations, but also revealed the evolution of unique spontaneous mutations. Further characterization of

the virus pairs revealed differences in proportions of expressed viral proteins as well as differences in particle-to-plaque forming unit ratios. Thus, in some instances, the observed phenotypic changes were not due to the engineered mutations per se, but rather to unexpected mutations that arose during the rescue process or to altered levels of virus protein expression and/or the differences in the relative proportion of defective particles. Notably, our virus stocks were prepared after a single passage on Vero cells following transfection. We present this data as a caution to those using ''reverse genetics'' techniques and wish to highlight the importance of thoroughly characterizing virus stocks prepared from cDNA plasmids to assure absence of potentially confounding mutations or quality attributes.

## P170
### HIV-Tat Protein Forms an Immune Complex, Binds the NMDA Receptor, and Prevents Receptor Activation and Excitotoxicity: Implications for HIV Neurocognitive Dysfunction

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Viral proteins, such as Tat, the HIV transactivator protein, have been implicated as agents of neurotoxicity via multiple mechanisms, including effects on glutamate receptors. In addition to their role in adaptive immunity, antibodies may complex with foreign proteins, disrupting deleterious cascades. Antibodies may hinder interaction of a toxic agent with a receptor, such as Tat with the glutamate receptor. To evaluate the ability of the immune response against Tat to modulate neurotoxicity at glutamate receptors, we coincubated HIV-1 Tat protein with a monoclonal antibody (mAb) against the N-terminal of Tat, and with NMDA and AMPA agonists, in rat hippocampal slice cultures and mixed human fetal neuron cultures. We measured neurotoxicity via propidium iodide staining and via mitochondrial membrane potential. We tested the specificity of observed effects, using an mAb against the C-terminal of Tat, and mutant and nitrosylated forms of Tat. We used immunoprepitation experiments to demonstrate the interaction between Tat, NMDA receptor, and anti-Tat antibody. Using the known structures of Tat and NMDA receptors, we developed a model of their interactions. The N-terminal mAb attenuated the neurotoxicity caused by Tat alone ($p < 0.005$) in dissociated cultures and hippocampal slice cultures. The Tat-antibody complex attenuated NMDA toxicity ($p < 0.05$), as effectively as kynurenic acid, a glutamate receptor antagonist, but did not significantly affect kainate mediated excitotoxicity. Neither Tat nor antibody alone blocked the excitotoxic effect, nor did an unrelated antigen-antibody combination. The combination of C-terminal mAb and Tat was also protective against NMDA toxicity ($p < 0.001$). However, modifications to Tat which prevent interaction of Tat with the NMDA receptor prevented the protective effect of the mAbs. Host immune responses may influence host susceptibility to effects of viral proteins, modulating HIV complications, such as onset of HIV dementia. These observations provide rationale for development of vaccine or monoclonal antibody therapies targeting HIV Tat for prevention of HIV neurocognitive dysfunction.

## P171
### Toll-like receptors are critical for protection against West Nile virus infection

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Objective: We evaluated the role of MyD88 and TRIF, two key adaptor molecules of Toll-like receptors (TLR) signaling, and TLR3,4,7 and 9 in WNV susceptibility and spread into the brain.

Background: West Nile virus (WNV) is a neurotropic flavivirus that emerged in the US in 1999. WNV, now endemic throughout North America, has become a frequent global cause of viral encephalitis. WNV infection is usually asymptomatic; however, a few % of individuals develop neuroinvasive disease with meningoencephalitis. TLRs play an essential role in initiating innate immune responses by sensing non-self components. Whether TLR signaling pathways are required for host defense against WNV infection has not been fully explored.

Design/Methods: 8-12 week old C57BL6/J and MyD88/TRIF double knockout (dKO), TLR3-/-, TLR4-/-, TLR7-/- and CpG1(TLR9-/-) mice were infected intraperitoneally with $1\times10^7$ pfu of WNV, Eg101 strain. Survival, viral load, cytokine and chemokine production and brain pathology were studied in infected mice.

Results: Ablation of TLR signaling through genetic deletion of MyD88 and TRIF resulted in significantly decreased survival following WNV infection. MyD88/TRIF dKO mice showed increased viral load in the brain and within associated lymphoid tissues. Serum TNFa and IL-6 production were significantly reduced in MyD88/TRIF dKO mice. RNA expression

of TNFa, IL-6, IFNa, MIP1a, MIP1b, and MCP5 were decreased in the brains of WNV infected MyD88/TRIF dKO mice in early infection. Brain-infiltrating macrophages were more abundant in infected MyD 88/TRIF dKO mice. However, these cells failed to up-regulate MHC Class II, suggesting a defective activation pathway. TLR3-/- and TLR7-/- mice were more susceptible than WT mice to WNV infection; however, there were no significant difference in susceptibility of TLR4-/- and CpG1 mice to WNV infection.

Conclusions: TLR signaling is required for protection from WNV infection. Therapeutic treatment with TLR ligands would boost the immune responses in WNV vaccines. Supported by: Study supported by: NIH Grant AI059619.

## P172

### Post-translational regulation of Bcl-3 expression by human T cell leukemia virus type 1 Tax and its effects on Bcl-3 overexpression and growth of HTLV-1-infected T-cells

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Bcl-3 is a member of IκB family that regulates genes involved in cell proliferation and apoptosis. Recent reports indicated that Bcl-3 is overexpressed in HTLV-1-infected T cell lines via HTLV-1 Tax mediated transactivation of an intronic NFκB binding site within the Bcl-3 gene, and Bcl-3 acts as a negative regulator of viral transcription from the HTLV-1 LTR. However, the role of Bcl-3 in cellular signal transduction pathways and the growth of HTLV-1-infected T cells have not yet been reported. In this study, we found that the lentiviral transduction of short hairpin (sh) RNA against Bcl-3 inhibited the growth of HTLV-1-infected T-cells. Although the phosphatidylinositol-3 kinase inhibitor reduced Bcl-3 expression in all the HTLV-1-infected T cell lines tested, inactivation of glycogen synthase kinase-3 (GSK-3), which is an effector kinase of the PI3K pathway, restored Bcl-3 expression only in Tax-negative but not in Tax-positive T-cell lines. Co-immunoprecipitation experiments revealed that Tax interacts with Bcl-3 in HTLV-1-infected T cells through both ankyrin repeat domain and N- and C-terminus domains. By using a luciferase assay, we found that N- and C-terminus domains are indispensable for the suppressive activity of Bcl-3 in the regulation of both NFκB and LTR-mediated transcription. These results indicate that the constitutive expression of Bcl-3 in HTLV-1-infected T cells is regulated by both transcriptional and post-translational mechanisms, and is involved in the enhanced cell growth of HTLV-1-infected T cells.

## P173

### Magnetic Nanocarrier Drug Delivery System for Targeting AZTTP and CTOP to Brain

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Last decade has witnessed a great entangled epidemic of opiate abuse and HIV-1 infection. Opiates act synergistically with HIV proteins to potentiate the HIV-related neurotoxicity that leads to development of NeuroAIDS. Currently no effective treatment exists for NeuroAIDS, which is mainly attributed to the impenetrability of therapeutic molecules across the blood brain barrier (BBB). Recently, nanoparticle based drug delivery systems have shown increasing potential for targeting drugs to the brain. In the current study, we hypothesize that magnetically guided nanocarrier bound to 5′-triphosphate AZT (AZTTP) and CTOP (D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2; μ opioid receptor antagonist) would deliver drugs to the brain under the influence of an external magnetic field. In normal circumstances, free AZTTP and CTOP do not cross the BBB. Our preliminary results indicate that AZTTP and CTOP efficiently bind to the magnetic nanoparticles (MNP). Further, MNP bound AZTTP is able to exert anti-HIV activity comparable to free AZTTP in HIV-infected PBMCs as assessed by p24 antigen quantification and HIV-LTR amplification. Similarly, MNP bound CTOP prevents morphine induced apoptosis in PBMCs. Thus, binding of AZTTP and CTOP to MNP has no effects on their biological activities. The proposed magnetic nanocarrier is anticipated to deliver the drug across the BBB, thereby simultaneously reduce NeuroAIDS and opiate addiction in HIV infected subjects who are opiate users. These results will be presented and discussed.

## P174

### Neurotrophins differently regulate glutamate transport in macrophages and astrocytes

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Glutamate and related compounds may act as excitotoxins and participate in neuronal damage in a

variety of neurological disorders including neuro-AIDS. Neurotrophins are involved in CNS development, and are now recognized for their actions in mature CNS and pathology, including interferences with glutamate signalling. Indeed, recent studies showed that neurotrophins protect neurons against glutamate excitotoxicity, whereas glutamate induces BDNF expression in neurons. Likewise, neurotrophin signalling and inflammation may influence each other. NGF is over expressed in activated perivascular macrophages in HIV encephalitis; it helps activated monocyte response to SDF1 attraction but limits the one of infected monocytes. Astrocytes are key cells in glutamate homeostasis, a function that macrophages and microglia also support under inflammatory conditions, where astrocyte function is disturbed. We therefore evaluated in vitro the effects of neurotrophins on glutamate metabolism in human macrophages, embryonic and adult astrocytes. Our results revealed different neurotrophin response profiles related to cell type and development stage. Macrophages have their glutamate clearance capacity decreased by one half by NT3 while embryonic astrocytes have their glutamate uptake capacity shortly (24h) reduced by one fifth by every neurotrophins. Alternatively, adult astrocytes have their glutamate uptake capacity greatly increased by BDNF, associated with increased EAAT1 gene expression. Surprisingly, both cell types have unchanged intracellular glutathione content under neurotrophin stimulation. These data suggest that BDNF expression may constitute an adaptive response to extracellular glutamate in adult astrocytes and therefore a potential way to protect against excitotoxicity in the mature brain. They also suggest fine regulation of GSH permitting to maintain constant levels despite regulated glutamate uptake by EAAT.

## P175
### Epstein-Barr virus is not present in multiple sclerosis brain

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Abstract Objective: Epstein-Barr virus (EBV), a ubiquitous virus that becomes latent in human B cells, has been considered a risk factor for the development of multiple sclerosis (MS). We searched for EBV in MS brain and in B-lymphocytes and plasma cells of MS cerebrospinal fluid (CSF), and for evidence of an intrathecal anti-EBV humoral immune response in MS CSF.

Methods: Nested non-quantitative real-time PCR was used to detect endogenous and EBV-specific transcripts in MS brain and in single B-lymphocytes and plasma cells of MS CSF. Immunocytochemistry and immunoblotting were used to detect the ability of MS CSF as well as recombinant antibodies (rAbs) prepared from clonally expanded plasma cells in MS CSF to bind to EBV-infected cells. Intrathecal anti-EBV antibody synthesis was measured by an enzyme-linked immunosorbent assay (ELISA).

Results: EBV-specific transcripts were not found in perivascular B cell infiltrates of active MS plaques from subjects with relapsing remitting MS or with secondary progressive MS, or in B-lymphocytes and plasma cells in MS CSF. The frequency of intrathecal anti-EBV antibody synthesis in MS patients did not differ from that found in patients with other inflammatory diseases of the brain. While the CSF of all MS patients bound EBV antigens, rAbs generated from clonally expanded plasma cells of the same patients did not react with EBV.

Conclusions: EBV-infected B-lymphocytes and plasma cells were not detected in MS brain plaques or CSF. rAbs prepared from clonally expanded plasma cells in MS CSF were not directed against EBV. Intrathecal synthesis of anti-EBV antibodies was not exclusive to MS patients. Overall, our studies do not reveal the presence of EBV in MS brain or an intrathecal EBV-specific antibody response in patients with MS.

## P176
### JC Virus Small Tumor Antigen Promotes Cell Cycle Progression and Induces Phosphorylation of Akt and Gsk3-Beta

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JC virus (JCV) is a human DNA tumor virus within the polyomavirus family. Oncogenic potential of JCV has been demonstrated in experimental animals including monkeys, hamsters and mice. Recent data indicate that JCV genome was also detected in a variety of human tumors suggesting that it may also be involved in the induction of some of the human tumors. Cell transformation studies using the polyomaviruses as model systems have provided many insights into the pathways involved in spontaneously arising cancers. JCV encodes two oncoproteins, the large T antigen (LT-Ag) and small t antigen (Sm t-Ag) and both of which play critical roles in tumor induction. LT-Ag has been previously shown to target a number of host regulatory pathways including pRb and p53; however the mechanism by which JCV Sm t-Ag contributes to such a process remains largely unknown. Several studies with SV40 have demonstrated that LT-Ag and Sm t-Ag, when together, sporadically induce tumors in a

rodent system. However when Sm t-Ag is deleted from the genetic background, tumors primary occur in highly proliferative tissues, such as lymphoid organs, suggesting that Sm t-Ag plays a critical role in tumor induction in nonproliferating tissues in the same model system. More importantly, the essential role of Sm t-Ag in cell transformation was demonstrated when observed that LT-Ag alone is not sufficient to fully transform human cells. In other words, LT-Ag requires the biologic activity of Sm t-Ag for this process. To understand the role of Sm t-Ag in cell transformation, we initially examined its effect on cell cycle progression. Comparison of the cell cycle profiles of Sm t-Ag positive cells with that of controls demonstrated that Sm t-Ag positive populations enter S phase significantly earlier than controls when cells are released from G0/G1 arrest. In parallel, examination of the cell cycle stage specific expression profiles of selected cyclins and cyclin-dependent kinases, particularly those active in G1/S and G2/M transition states demonstrated that the rate of the appearance of cyclin E, cyclin B, and cyclin E-dependent kinase 2 (Cdk2) in a given time point substantially increased in Sm t-Ag positive cells compared to controls. Moreover, analysis of the mechanism(s) by which Sm t-Ag influences cell cycle progression demonstrated that it targets one of the important serin/threonine phosphatases, (PP2A) in cell and diminishes its activity. Sm t-Ag was also found to activate the downstream effector molecules of one of the major proliferation pathways {the mammalian target of rapamycin (mTOR)} in cell through the hyperphosphorylation Akt and GSK. We are currently in the process of testing the requirement of Sm t-Ag in cell transformation using soft agar and nude mice assays using different human cell types.

## P177
### The cellular splicing factor, SF2/ASF, is a negative regulator of JC virus transcription and replication in glial cells

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The human neurotropic virus, JCV, is the etiologic agent of demyelinating disease of the CNS, progressive multifocal leukoencephalopathy (PML), seen primarily in immunocompromised individuals, most notably AIDS patients. JCV replicates almost exclusively in glial cells and its promoter sequence, which has tissue-specific characteristics, tightly modulates expression of the viral genome in appropriate cell types and immunoconditions through communication with cellular factors. Here we identified the splicing factor, SF2/ASF, as a potential regulator of JCV as its overexpression in glial cells suppresses viral gene expression and replication. Unexpectedly, down-regulation of JCV by SF2/ASF is mediated at the transcriptional, but not RNA processing stage, thus ascribing a new role for SF2/ASF in the control of promoter activity. SF2/ASF suppresses both viral early and late gene transcription by direct association with a specific DNA motif within the viral bi-directional promoter. Further mapping studies revealed that the RRM1 domain of SF2/ASF is responsible for the observed negative regulatory effect. In light of previous studies showing the ability of Tat to stimulate the JCV genome, our results show the capacity of SF2/ASF to impede Tat function upon JCV replication. These observations open a new avenue for investigating the mechanisms involved in reactivation of the JCV genome in glial cells during the course of immunosuppression and suggest a novel approach for suppressing JCV replication in humans. Supported by grants awarded by NIH/NINDS to KK.

## P178
### Generation and characterization of JCV-permissive cell lines produced between glioblastoma and primary human fetal astrocytes

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JC virus (JCV) is a human neurotropic polyomavirus whose replication in the central nervous system induces the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). JCV particles have been primarily detected in oligodendrocytes and astrocytes of the brains of patients with PML and in the laboratory its propagation is limited to primary cultures of human fetal glial cells. Here, we report the development of a new cell culture system created by fusion of primary human fetal astrocytes with the human glioblastoma cell line, U-87MG. The new hybrid cell line obtained from this fusion has the capacity to efficiently support expression of JCV and replication of viral DNA in vitro up to 16 passages. This cell line can serve as a reliable culture system to study the biology of JCV host cell interaction, determine the mechanisms involved in cell type specific replication of JCV, and provide a convenient cell culture system for high throughput screening of anti-viral agents. Supported by grants awarded by NIH/NINDS to KK.

## P179
### Herpes simplex virus infection of microglial cells triggers oxidative stress responses and damage via Toll-like receptor 2

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Microglia are the principle regulators of neuroinflammation within the central nervous system. Using a murine model of herpes simplex virus (HSV)-1 encephalitis, we have determined that induction of proinflammatory mediators in response to viral brain infection is largely mediated through a Toll-like receptor 2 (TLR2)-dependent mechanism. Published studies have shown that, like other inflammatory mediators, reactive oxygen species and reactive nitrogen species are generated during viral brain infection. It is increasingly clear that reactive oxygen species are responsible for facilitating secondary tissue damage, such as lipid peroxidation and isoprostane production, during and subsequent to HSV brain infection. In this study we show that TLR2 mediates microglial cell-induced oxidative damage in neuronal cultures. Using 8-isoprostane as a marker for lipid peroxidation, we found that HSV-infected microglia from TLR2-/- mice produce less neuronal oxidative damage when added to mixed brain cell cultures. These effects are likely due to the aberrant production of pro-oxidative and anti-oxidative enzymes in TLR2-/- mice. Real-time PCR amplification of cultured microglial mRNA from wild-type mice show the sequential upregulation of pro-oxidative enzymes (NADPH oxidase, inducible nitric oxide synthase) at 24 hours post infection followed by the induction of anti-oxidative enzymes (heme oxygenase-1, glutathione peroxidase-1, superoxide dismutase-1) at 48 hours post infection. Cultured TLR2-/- microglia lack both the early production of pro-oxidants and the subsequent increase in anti-oxidants. We are currently using lentiviral vector-mediated overexpression as well as shRNA knockdown of heme oxygenase-1 to determine if this oxidative damage can be modulated via anti-oxidant gene expression in the brain.

## P180
### HIV-1 Tat Protein Attenuates Proliferation and Differentiation of Human Neural Precursor Cells

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Human immunodeficiency virus (HIV-1) and viral proteins produced by the virus affect neuronal survival, dendritic arbor, function of mature neurons and neuron-glial cell interactions, which culminate in dementia like symptoms. Recently presence of HIV-1 has been demonstrated in regions where neurogenesis occurs in adult as well as pediatric human brain. However, little is known about the effect of HIV or its viral proteins on human neural precursor cells (hNPCs) that may rescue or replenish the damaged neuronal pool. We investigated effect of the HIV-1 transactivating protein (Tat) on cell survival, growth, proliferation, as well as differentiation potential of fetal brain derived human neural precursor cells. Using a human neurosphere cell culture system, we observed that HIV-1 viral protein Tat severely affects the proliferation of hNPCs as assessed by decrease in their size, lower incorporation of BrdU and Ki-67 staining in the developing neurospheres, and modulate other events that have implication in neurogenesis. Our data suggests that HIV Tat mediated changes in properties of human neural stem cells are mediated via MAPK pathway, particularly the pERK1/2 and attenuation of cyclin D1. We believe our study provide new insights into cellular and molecular mechanisms of neurogenesis in neuroAIDS patients. (This study was supported by research grants (BT/PR6838/Med/14/881/2005 And BT/PR 6615/Med/14/857/2005) from Department of Biotechnology, New Delhi, India, to Dr. Pankaj Seth).

## P181
### Establishment of clonal cell lines containing specific genetic alterations for the functional analysis of the HIV-1 LTR within the context of 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 has 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. A series of stably transfected cell lines were developed utilizing bone marrow progenitor, T, and monocytic cell lines (TF-1, Jurkat, and U-937, respectively) to explore the LTR phenotype associated with these genotypic changes from an integrated 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 effectively examine the site of LTR integration within the genome, clones were derived from each population of cells. These clones have been

examined under basal as well as with chemical and cytokine treatment and Tat transactivation. Results have shown that non-LTR expressing clones cannot be induced to express under any circumstances examined, where as expressing clones can be induced by chemical and cytokine treatment and Tat-mediated transactivation. To date, results demonstrate that the site of LTR integration 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.

## P182

### A novel approach for virus inactivation: Role in vaccine development

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In this study we describe a novel method of virus inactivation using a photoactive compound that preserves the immunogens present on the virus surface. This strategy aims to inactivate viruses while preserving the virus structural integrity and immunogenic properties, thus overcoming the limitations associated with conventional inactivation methods. Venezuelan equine encephalitis virus (VEEV) is an enveloped virus in family togaviridae. VEEV is a human pathogen and may cause encephalitis and death in young, old and immunocompromised individuals. VEEV has also been weaponized making it a potential bio-terror/bio-threat agent. Currently, there is no licensed vaccine for prophylaxis against VEEV. Encephalomyocarditis virus (EMCV), a picornavirus, was used as model virus to study inactivation of non-enveloped virus. Hydrophobic photoactive compound, 1, 5 iodonaphthyl-azide (INA) was used to inactivate VEEV and EMCV. Suckling mice infected with inactivated VEEV by either intraperitoneal or intracranial route did not exhibit any sign of disease. These mice developed normally as compared to mice infected with non-inactivated VEEV which died with in 48hr post infection. Transfection of cells with RNA isolated from INA-inactivated VEEV did not result in active virus replication and immunization with INA-inactivated VEEV induced vigorous antibody response and protected mice from subsequent challenge with virulent VEEV. These results suggest that INA inactivation of viruses is an efficient method of inactivation for both enveloped and non enveloped viruses and may be explored for developing vaccine candidates of human and livestock importance.

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## P183

### Dynamics of evolution of HIV-1 Rev binding host factors with their predicted role in HIV associated neuropathogenesis

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Introduction: HIV-1 Rev protein, an important post-transcriptional regulator of Human Immunodeficiency Virus-1 (HIV-1) life cycle has been shown to be associated with neuropathogenesis. Altered Rev activity due to Rev-host protein interactions has been correlated to differential HIV replication in brain cells; however, the exact mechanism of this phenomenon is still not clear. Objective: We aimed to analyze the patterns of evolution of three well known HIV-1 Rev interacting proteins (reported to be associated with HIV-1 replication in brain cells) viz., Dead-Box Helicase protein (DDX-1 helicase), Nucleophosmin and Rev-binding protein (RBP) by correlating their sequence and structural evolutionary analyses approaches across different mammalian species.

Methods: DDX-1 helicase, Nucleophosmin and RBP representing three different aspects of Rev-host protein interaction in brain cells were selected following extensive literature analysis and information gathered through HIV protein interaction database (NCBI). Each protein sequence cluster representing sequence homologues was selected from Uniref database at both 50% (using Uniref 50) and 90% (using Uniref 90) level of similarity. Only clusters showing maximum number of species from Eutheria were recognised. Sequences were aligned by standard Clustal X2 Gonnet PAM250 matrix. Solved 3D structures matching the sequences were obtained from RCSB PDB at an expectation value $<3e60$. Sequence and Structure information for each protein were fed to Evolutionary Trace (ET) server. Calculation yielded rank scores for each amino acids of the sequence which represented the correlation between sequence variations in an alignment and evolutionary divergences. Also, sequence conservation scores were obtained (using empirical Bayesian approach) and plotted on the PDB derived structure using the combination of Consurf server and Chimera Extensible Molecular Analysis System. An overview of codon sequence evolution was also attempted by estimating the ratio of non-synonymous (amino acid altering) to synonymous (silent) substitutions (the Ka/Ks ratio) which is a measure of positive and purifying selection at each amino acid site. M8 Bayesian model incorporated in Selecton

server was employed for the former. Finally, the evolution patterns were studied for their role in effecting protein interactions by analysing binding pockets, interaction hot spots, docking with small ligands and relative entropy measures (using combination of different algorithms such as protein dossier, pocket binder, ASTRO fold and others).

Results: This study revealed occurrence of a number of positively selected sites in DDX-1 helicase ($P > 0.93$) and number of conserved residues in Nucleophosmin and RBP. These residues in DDX-1 helicase are mainly at the N-terminus but some scattered amino acids indicative of positive selection were also located elsewhere. Mostly conserved regions in other two proteins indicated a case of purifying selection. Our study revealed that DDX1 helicase contains a potential cluster of residues that can modulate host defence activities in human brain cells (and hence altered HIV-1 Rev activity) following positive Darwinian selection. Loss of positive selection signatures in Nucleophosmin and RBP could suggest that these proteins may play an indirect role in antiviral strategies and may affect some additional brain specific host proteins down the pathway that they are part of.

Conclusion: Role of host factors in eliciting this mechanism would be interesting to find and could have potential in identification of new drug targets against HIV. Since, the main reservoir for HIV-1 in brain are non dividing cells and most of the regions of Rev are believed to be conserved, the ability of host proteins to undergo variation appears to be the important factor contributing to altered virus replication in this body compartment. A combination of in silico and lab based experimental approach could help in better understanding the interaction of host factors and HIV-1 proteins in neuropathogenesis.

## P184

### Dynamics of HIV-1 Rev binding host factor structure and evolution profiles and their importance in HIV associated Neuropathogenesis

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Correlation of HIV-1 Rev binding host factor structure and evolution profiles and their importance in HIV associated Neuropathogenesis HIV-1 Rev protein, an important posttranscriptional regulator of Human Immunodeficiency Virus-1 (HIV-1) life cycle is known to be associated with Neuropathogenesis. Altered Rev activity due to Rev-host protein interactions observed in brain cells has been correlated to differential HIV replication in brain cells. However, the exact mechanism of this Rev dependent pathway is still far from clear. Role of host factors in eliciting this mechanism would be interesting to find and could have potential in identification of new drug targets against HIV. We aimed to analyze the patterns of protein evolution of 3 HIV-1 Rev interacting proteins (believed to be associated with HIV-1 replication in brain cells) by correlating the sequence and structural evolutionary analysis approaches. Three proteins namely DDX1 helicase, Nucleophosmin and RBP representing three different aspects of Rev-host protein interaction in brain cells were selected following extensive literature analysis and information gathered through HIV protein interaction database (NCBI). Each protein sequence cluster representing sequence homologues was selected from Uniref database at both 50% (using uinref 50) and 90% (using uniref 90) level of similarity. Only clusters showing maximum number of species from Eutheria were recognised. Sequences were aligned by standard Clustal X2 Gonnet PAM250 matrix. Solved 3D structures matching the sequences were obtained from RCSB PDB at an expectation value $< 3e60$. Sequence and Structure information for each protein were fed to Evolutionary Trace (ET) server. Calculation yielded rank scores for each amino acids of the sequence which represented the correlation between sequence variations in an alignment and evolutionary divergences. Also, sequence conservation scores were obtained (using empirical Bayesian approach) and plotted on the PDB derived structure using the combination of Consurf server and Chimera Extensible Molecular Analysis System. An overview of codon sequence evolution was also attempted by estimating the ratio of non-synonymous (amino acid altering) to synonymous (silent) substitutions (the Ka/Ks ratio) which is a measure of positive and purifying selection at each amino acid site. M8 bayesian model incorporated in Selecton server was employed for the former. For clearer picture we also utilised different models which use different Ka/Ks rates at different sites of the sequence. Finally, the evolution patterns were studied for their role in effecting protein interactions by analysing binding pockets, interaction hot spots, docking with small ligands and relative entropy measures (using combination of softwares such as protein dossier, pocket binder, ASTRO fold etc). This study revealed occurrence of number of positively selected sites in DDX helicase ($P > 0.93$) and number of conserved residues in Nucleophosmin and RBP. These residues in DDX helicase are mainly at the N-terminus but some scattered amino acids indicative of positive selection were also located elsewhere. Mostly conserved regions in other two proteins indicated a case of purifying selection. Our study revealed that DDX helicase contains a potential cluster of residues that can modulate host defence activities in human brain cells (and hence altered HIV-1 Rev activity) following positive Darwinian selection. Failure to detect any positive selection signatures in Nucleophosmin and RBP

could suggest that these proteins may play an indirect role in antiviral strategies and they may affect some additional brain specific host proteins down the pathway that they are part of. Based on these studies we have initiated an experimental two hybrid approach to study protein-protein interactions between Rev and host factors derived from human brain. We are also extending these evolutionary studies to other brain specific host proteins. Also, we are attempting to predict theoretical models for some brain derived host proteins and to study Rev-host proteins interactions through docking and lab based yeast two hybrid approaches.

## P185

### A20 downregulates NFκB signaling by disrupting E2:E3 ubiquitin enzyme complexes

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A20 is a critical negative regulator of the transcription factor nuclear factor κB (NFκB) in inflammatory signal transduction pathways. A20 has been proposed to function as a ubiquitin-editing enzyme to inactivate RIP1 downstream of tumor necrosis factor receptor 1 (TNFR1) by first cleaving lysine 63-linked polyubiquitin chain and then conjugating lysine 48-linked polyubiquitin chains. However, it is unclear if A20 uses a similar mechanism to inhibit toll-like receptor (TLR) and interleukin-1 (IL-1R) pathways that function independently of RIP1. Here, we demonstrate that A20 disrupted the binding of the E3 ligase TRAF6 with its cognate E2 enzyme Ubc13. A20, together with the regulatory molecule TAX1BP1, interacted with Ubc13 and triggered its ubiquitination and proteasom-dependent degradation. The A20 DUB activity was dispensable for disruption of E2:E3 complex and Ubc13 degradation. Furthermore, the HTLV-1 Tax oncop NFκB rotein prevented A20-mediated recognition and degradation of Ubc13 thus leading to persistent NFκB activation. Our results suggest that A20 mainly targets activated E2:E3 ubiquitin enzyme complexes to inhibits NFκB.

## P186

### Expansion of monocyte/macrophage subsets correlates with the development of AIDS and SIVE

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The activation and expansion of monocyte/macrophage subsets with HIV and SIV infection is recognized as a critical parameter of AIDS pathogenesis and perhaps a marker for the development of neuropathogenesis. In this study, we used a CD8 T lymphocyte depletion and SIVmac251 infection model of rapid and severe CNS disease to study dynamic changes in monocyte/macrophages after infection, and the development of AIDS. To define monocyte subsets within whole blood, we gated on forward-versus side-scatter dot plots and then selected for the HLA-DR positive cell population. Next, we investigated the absolute cell number and relative percentages of monocyte subsets (CD14+ CD16−, CD14+CD16+, and CD14−,CD16+). Lastly, we examined expression levels of CD68, CX3CR1, CCR2, CCR8, CD11b, CD163, CD64 and CD44v6 within total monocyte/macrophages and within monocyte subsets. Almost immediately after viral infection (as early as day five), we see an increase in the absolute number and relative percentage of CD14+ and CD14+CD16+ monocytes. In addition, CD11b, CD44v6, CCR2, CD163 expression increased on total monocytes. Further, within subsets of monocytes there was a decreased expression of Mac387 and a shift from the CD14+ to the CD14+ CD16+ cells, representative of an accumulation or maturation of CD68 monocyte/macrophages. Four out of the five CD8 lymphocyte depleted, SIVmac251 infected animals developed AIDS and two developed SIVE. Thus, an expansion of monocytes/macrophages in blood correlates with the rate of pathogenesis.

## P187

### Persistent West Nile virus associated with a neurological sequela in hamsters identified by motor unit number estimation

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To investigate the hypothesis that neurological sequelae are associated with persistent West Nile virus (WNV) and neuropathology, we developed an electrophysiological motor unit number estimation (MUNE) assay to measure the health of motor neurons temporally in hamsters. The MUNE assay was successful in identifying chronic neuropathology in the spinal cords of infected hamsters. MUNE was suppressed at day 9 to 92 in hamsters injected subcutaneously with WNV, thereby establishing that a long-term neurological sequela does occur in the hamster model. MUNE suppression at day 10 correlated with the loss of neuronal function as

indicated by reduced choline acetyltransferase-staining ($R2 = 0.91$). Between days 10 and 26, some α-motor neurons had died, but further neuronal death was not detected beyond day 26. MUNE correlated with disease phenotype, because the lowest MUNE values were detected in paralyzed limbs. Persistent WNV RNA and foci of WNV envelope-positive cells were identified in the central nervous system (CNS) of all hamsters tested from 28 to 86 days. WNV positive staining co-localized with the neuropathology, which suggested that persistent WNV or its products contributed to neuropathogenesis. These results established that persistent WNV product or its proteins cause dysfunction, that WNV is associated with chronic neuropathological lesions, and that this neurological sequela is effectively detected by MUNE. In as much as WNV-infected humans can also experience a poliomyelitis-like disease where motor neurons are damaged, MUNE may also be a sensitive clinical or therapeutic marker for those patients.

## P188

### Prevalence of cognitive disorders in HIV+ patients with long-term suppression of viremia

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Background: HAART has contributed to decrease the HIV-related mortality and morbidity. However, the prevalence of HIV-associated neurocognitive disorders (HAND) seems to have increased. The aim of this study was to determine the prevalence of cognitive complaint and of HAND in a cohort of aviremic HIV+ patients in the South-western part of Switzerland.

Design/Methods: Two hundred HIV+ patients who had (1) undetectable HIV RNA concentrations in the plasma for >3 months, (2) no history of major opportunistic infection of the CNS in the past three years, (3) no current use of IV drugs and (4) no signs of major depression according to the DSM-IV criteria, answered a questionnaire designed to elicit cognitive complaints. Cognitive functions of a subset of HIV+ patients with or without cognitive complaints were assessed using the HIV Dementia scale (HDS) and a battery of neuropsychological tests evaluating the sub-cortical functions. Cognitive impairment was defined according to the revised diagnostic criteria for HAND. Non-parametric tests were used for statistics and a Bonferroni corrected standard p level of $p < 0.002$ was applied for multiple comparisons.

Results: The prevalence of cognitive complaints was 27% (54 patients) among the 200 questioned patients. At the time of writing this abstract, cognitive functions of 50 complaining and 28 non-complaining aviremic patients had been assessed with the HDS and the full neuropsychological battery. The prevalence of HAND producing at least mild interference in daily functioning (mild neurocognitive disorders [MND] or HIV-associated dementia [HAD]) was 44% (34/78 patients) in the group who underwent neuropsychological testing. Objective evidences of HAND were more frequent in complaining than in non-complaining patients ($p < 0.001$). Using a ROC curve, a cut-off of 13 on the HDS was found to have a sensitivity of 74% and a specificity of 71% ($p = 0.001$) for the diagnosis of HAND. A trend for lower CNS Penetrating-Effectiveness scores for HAART in patients with MND or HAD as compared to the others was present ($1.5 \pm 0.6$ vs. $1.9 \pm 0.6$; $p = 0.006$ [Bonferroni correction]).

Conclusions/Relevance: So far, our results suggest that (1) the prevalence of HAND is high in HIV+ patients with a long-term suppression of viremia, and (2) cognitive complaints expressed by aviremic HIV+ patients should be carefully investigated as they correlate with objective evidences of cognitive decline in a neuropsychological testing. HAART with a high CNS penetrating-effectiveness may contribute to prevent HAND. Funding: Swiss HIV Cohort Study.

## P189

### Cannabinoid effects on striatal and prefrontal cortex neurogenesis and gliogenesis in rats with Borna Disease

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Borna Disease Virus infection in rats produces deficits in striatal and prefrontal cortex function, expressed as abnormal movements and disinhibited behaviors. Consequences of this chronic viral encephalitis may be mitigated by finding ways to support CNS neurogenesis during infection. The present study explored the vulnerability of striatal and medial prefrontal cortex neurogenesis and gliogenesis to BDV infection, and the effects of systemic cannabinoid administration on cytogenesis in these structures. In cannabinoid-treated BD rats,

there was increased survival of BrdU+ cells and a change of phenotype of surviving cells. The main effect was on gliogenesis, with differential regulation of oligodendroglia and astrocytes. The greatest change in endocannabinoids was anandamide reduction in the striatum of BD rats treated with the cannabinoid agonist WIN55,212. This result may signify a neuromodulatory effect of WIN toward reduction of glutamatergic drive in striatal circuits, with net effect the protection of new cells. Our findings demonstrate that striatal and prefrontal cortex neuro- and gliogenesis are susceptible to infection and can be partially restored by cannabinoid treatment.

## P190

### Genetically Modified CD34+ Hematopoietic Stem Cells Contribute to Turnover of Brain Perivascular Macrophages in Long-term Repopulated Primates

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Deciphering the turnover of brain perivascular macrophages is of importance because of their crucial role in the development of neuroAIDS. Perivascular macrophages in humans and non-human primates are major targets of productive infection by human immunodeficiency (HIV) and simian immunodeficiency (SIV) viruses. Brain perivascular macrophage precursors are likely targets either directly infected or affected by HIV/SIV infection in bone marrow and/or blood and are thus potential targets of gene therapy approaches to make them resistant to infection. We took advantage of a non-human primate animal model of autologous hematopoietic stem cells (HSCS) transplantation to study the ontogeny of perivascular macrophages of rhesus macaques. Using five animals whose CD34+ cells were transduced with SIV- (n = 4) or HIV-based vectors (n = 1) constructed to express EGFP, we investigated the contribution of EGFP+ CD34+ HSC in the repopulation of myeloid cells in blood, lymphoid tissues and the CNS. We show that EGFP+ cells derived from rhesus macaque CD34+ HSC give rise to monocytes and dendritic cells in blood and exclusively perivascular cells in the CNS four years post transplantation. The EGFP+ cells detected in the CNS are located exclusively in the vicinity of large and small blood vessels. Depending on the animal and the CNS region analyzed, between 25% and 85% of EGFP+ cells express CD163 and up to 70% are CD68+. The location, morphology and phenotype of the EGFP+ cells detected in the CNS were consistent with CNS perivascular macrophages. This study reports the detection of long-term EGFP expressing brain perivascular macrophages following transplantation of rhesus macaques with autologous EGFP-expressing CD34+ HSC. Because perivascular macrophages are significant targets of productive HIV/SIV infection in the brain, these observations point to important HSC as targets of HIV/SIV infection and potentially gene therapy.

## P191

### Establishment of a Homogeneous Clonal Cell Line That Supports Persistent Infection with the Human Polyomavirus JC

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A cell line permissive to persistent infection with the human polyomavirus JC (JCV) was established by single cell cloning from a parental culture of SVG cells (1). SVG are a heterogeneous population of human neuroglial cells characterized by rapid growth, and susceptibility to infection by JCV. SVG were derived by transfection of an origin of DNA replication defective mutant SV40 plasmid into a culture of primary human fetal brain cells. Clonal cell lines were achieved from single-cell-cloning in 96-well cell culture plates by serial dilution. Single cell origin was confirmed after plating and before clonal growth by microscopic examination. Eight clones were successfully expanded, then characterized by morphology and secretion of neurotrophic factors BDNF and NGF and the cytokine VEGF using ELISA. Clones determined to be unique were exposed to purified JC virion particles, and assayed for infection at day 14 by in situ DNA hybridization for viral DNA replication, hemagluttination assay for virion maturation, and immunoflourescence for virion proteins. All clones were shown to support JCV multiplication at varying levels. One cell line, P10B1, was selected as a candidate for future in vitro investigations based on its high level of JCV susceptibility and ability to maintain infection regardless of passage. While retaining the rapid growth characteristic of SVG cells, P10B1 cells confer the advantage of homogeneity, stability, increased JCV susceptibility, and enhanced ability to maintain JCV infection over the parental SVG. Such a cell line can serve as a

vehicle for quantifying anti JCV drug effects. 1. Major, E.O. et. al. (1985) Proc. Natl. Acad. Sci. USA. 82, 1257-1261.

## P192

### New Therapeutics Development 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 and identify neuroprotective compounds relevant to HIV-associated neurocognitive disorders (HAND). We have screened more than 2000 compounds for protective efficacy against oxidative stress-mediated neurodegeneration and have identified a group of natural products, called limonoids, as potential neuroprotectants. Numerous limonoid compounds were then extensively evaluated as protectants against oxidative stress-, Tat- and gp 120-mediated neurotoxicity as measured by changes in mitochondrial potential and neuronal cell death. These compounds displayed concentration-dependent neuroprotection, with half maximal potency of about 100 nM. The limonoids also prevent glial cell activation and importantly at least one compound within this class down regulates HIV replication, likely through actions on host proteins which has a distinct advantage since it would be less likely for viral resistance to develop. One agent, limonin, was orally bioavailable and was distributed into the brain at concentrations greater than 100 nanomolar following a single oral administration of the compound. Thus, our data suggest that limonoids may comprise a novel adjunctive neuroprotective therapy to treat HAND. Supported by P30MH075673.

## P193

### The Role of Insulin-like Growth Factor 2 Receptor (IGF2R) in HIV-associated Neurocognitive Disorders

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Insulin-like Growth Factor 2 Receptor (also called cation-independent mannose-6-phosphate receptor: MPRci) binds the specific ligand insulin-like growth factor 2 (IGF2), as well as mannose-6-phosphate (M6P)-containing enzymes/molecules. During development, IGF2R plays an essential role in degrading extracellular IGF2, preventing its binding and signaling through the insulin-like growth factor 1 receptor (IGF1R) and insulin receptor. IGF2R has been implicated in several disorders including cancer (a possible tumor suppressor gene), but since IGF2R KO mice are embryonic lethal, the postnatal function of this receptor in the whole organism is largely unknown. Neurons constitutively express high levels of IGF2R, but expression and function in glial cells has not been studied. Furthermore, a systematic study of the expression and function of IGF2R in the CNS or the immune system, specifically in macrophage-lineage cells, has not been performed. We now show that IGF2R is robustly upregulated in activated microglia in HIV encephalitis, and that IGF2R functions to promote HIV expression and HIV-induced microglial chemokine interferon-gamma inducible protein 10 (IP-10) production. Together, these data indicate that IGF2R expression is highly regulated in the brain, specifically in brain macrophages and that IGF2R is involved in HIV infection and inflammatory activation of microglia, supporting a potential role for IGF2R in the pathogenesis of HIV-associated neurocognitive disorders. (Supported by K01 NIH MH94705, R01 MH55477, U01 MH083501, P30 AI05 1519, and Einstein CFAR pilot project).

## P194

### Demonstration of rabies viral antigen in optic pathway

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Rabies viral infection continues to be a fatal epizootic viral infection in Asian subcontinent. This infection manifests clinically either as hydrophobic form or paralytic, occasionally progressing to other form terminally. During the evolution, the viral antigen is found in the neurons recapitulating the neuroanatomical projections and connections indicating transaxonal spread. Role of astrocytic component is not stressed though noted. Though the involvement of amygdala and limbic cortex is recognized, it is not known whether the visual and auditory pathways participate in the stimulus transmission to brain, clinically manifesting as anxiety, aggressive behavior and hydro/aerophobia. In the

present study we examined the neural tissue from optic pathway (retina, Optic nerve, Lateral geniculate body and striate cortex) collected at autopsy from 8 cases of human rabies encephalitis. The sections were immunostained with polyclonal antibody to rabies nucleoprotein and GFAP (monoclonal) for glial elements. The age of the patients ranged from 7 yrs to 70 years (6 males, 2 females). The incubation period varied from 1 month to 2 years, and the duration of clinical symptoms was 3 to 17 days, once the symptoms have manifested. One case (25yr,M) manifested hydrophobic form and the rest had paralytic rabies. The striate cortex was consistently involved in all the cases essentially involving middle and lower neuronal layers with dendritic spread. With increase in incubation period the antigen density and compact viral inclusions are noted, while with shorter incubation period the viral antigen is noted as fine stippling. In the lateral geniculate body, both magnocellular and parvocellular cells are labeled, though asymmetric, with wide dendritic spread. The inflammatory component was minimal. In the optic nerve the viral antigen of variable density was noted in the axons. The conspicuous feature was vacoulation and antigen load in astrocytes and oligodendroglia. In the retina the plexiform layer was edematous. The ganglion cells had antigen progressing from diffuse staining, fine stippling to dense bodies marginally correlating with the incubation period. The unmyelinated axons in lamina cribrosa and plexiform layer were labeled in two cases, reflecting the transaxonal spread to the ganglion layer and granular layer. In two cases the retinal cones were labeled, sparing the rods. Occasional Muller cells also had the viral antigen. The ciliary nerves in the globe are not labeled. The spread of virus in the visual pathway involving the retina could play a role in clinical manifestations. The strong labeling of astrocytes and oligodendroglia could be facilitating the spread of virus, similar to herpes simplex virus. This is the initial step to understand the participation of visual pathway in the evolution of clinical manifestations in cases of human rabies.

## P195

### Neuroanatomical localization of rabies viral antigen in human brain and its correlation with incubation period. Does it explain the pathobiology of the disease?

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Despite extensive research, the pathogenesis and evolution of neuropathological lesions in rabies viral encephalitis in relation to incubation period and the clinical phenotypes remains unclear, To address some of the lacunae, we studied the pattern and degree of rabies related pathology in 40 human brains collected at autopsy from cases of rabies encephalitis at our center from south India. In this presentation, we document the pattern of histopathological lesions and neuroanatomical distribution of rabies viral antigen in the brain ($n = 10$) and correlated the degree of the changes with incubation period (IP). Large paraffin sections from various neuroanatomical areas were immunostained by standard indirect immunoperoxidase method using in-house polyclonal antirabies nucleoprotein antibody raised in rabbits. The pathological features at three time points in incubation period were analyzed (short IP-15 days to 3 months, Intermediate IP-3 to 12 months and long IP-1-4 years). At short IP, perivascular lymphocytic cuffing was mild and restricted to brain stem and basal ganglia. Moderate degree of CD68 positive microglial response was noted in thalamus, hypothalamus and brainstem, while it was minimal in frontal and temporal cortex. In the intermediate group, perivascular inflammation was noted in hypothalamus and brain stem, whereas the microglial reaction along with neuronaphagia extended to supratentorial structures. With longer IP, the degree of microglial response was enhanced, forming microglial nodules around neurons with neuronophagia diffusely in the brain. The intraneuronal Negri body density and size increased with progression in incubation period. Similarly the rabies viral antigen load also increased, paralleling the duration of incubation period. The limbic structures were consistently involved at all incubation periods, with considerable viral load. The maximal concentration of rabies viral nucleoprotein was observed in thalamus, hypothalamus, caudate nucleus, substantia nigra, raphe nuclei, reticular formation, the vagal and hypoglossal nuclei irrespective of the duration of incubation period. With increase in duration of incubation period the viral antigen was observed in various cranial nerve nuclei and extending to critical areas. In the case with the longest IP of four years, high concentrations of rabies viral antigen load diffusely involved almost all neuroanatomical areas. The antigen distribution pattern was essentially similar whether the subject was vaccinated or not, except for more florid microglial response and perivascular lymphocytic cuffing and neuronophagia in the vaccinated subjects, reflecting the participation of immunopathology in the final outcome.

## P196

### Is death in rabies related to specific neuroanatomical pathology? – A study in human, canine and rodent brains

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Rabies retains the distinction of being the infectious disease with the highest case fatality ratio. In spite of many studies in the area of research pertaining to rabies neuropathogenesis numerous questions remains unanswered, especially the pathomechanism of lethality is not known. Under natural conditions the clinical disease does not usually manifest for a varying periods and varying from host to host. The precise location of the virus during that period and its form remains amongst the most puzzling and intriguing aspect of rabies neuropathogenesis. Considering the above facts we tried to probe for consistent neuropathology in the brain during natural infection of humans and canines and experimental infection in mice by virulent street virus and laboratory CVS strain. In humans and canines following terminal natural infection it is expected that the viral antigen would be global and may not give insight into the progression of the disease. To address this limitation, in a rodent model virulent lab strain of virus was inoculated into mice peripherally and the animal brains were collected at different time points and were examined and compared to the viral load in human and canine brain. The sections from the nervous tissue from human (n = 10), canines (n = 10) and mice (n = 6) was immunostained for rabies viral antigen by immunoperoxidase method and the neuroanatomical distribution was mapped. In all the brains, Human, canine and mice there was consistent involvement of thalamus, raphe nuclei and reticular formation in brain stem and vagal nuclei correlating with fatality. In mice, temporally there was a caudocranial spread of the virus from the peripheral nerve, sensory ganglia, spinal cord, brain stem and thalamus. The cerebellar and cerebral involvement was diffuse and did not appear to correlate with mortality. The critical neuroanatomical areas were found involved only terminally in the mice leading to death and could be applicable to humans and canines.

## P197

### Neuroimaging in HIV Infection and Alcoholism Comorbidity: Synergistic White Matter Damage

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Alcoholism comorbidity is highly prevalent in individuals infected with human immunodeficiency virus (HIV) and can exacerbate HIV infection by acting as an immunosuppressant and interfering with potential positive effects of anti-retroviral therapy. Each condition is known to affect brain structure and function, and when they co-occur may operate synergistically. To examine this possibility, we initiated a study using structural magnetic resonance imaging (MR) of brain macrostructure, MR spectroscopic imaging (MRSI), and diffusion tensor imaging (DTI) of tissue microstructure together with neuropsychological testing in men and women with HIV infection alone, alcoholism alone, HIV infection and alcoholism combined, and non-affected controls. Analysis of MRI data revealed a graded pattern of modest enlargement of the total ventricular system and callosal thinning across the three diagnostic groups. However, substantially greater volume abnormalities were present in individuals with a history of an AIDS-defining event or low CD4+ T cell counts irrespective of alcoholism comorbidity, and the effect of HIV severity was disproportionately exacerbated by alcoholism comorbidity. Callosal microstructure, indexed with quantitative fiber tracking, showed a similar graded pattern, with the greatest disruption of white matter in the AIDS+alcoholism group that was also predictive of speed and dexterity of upper and lower motor control. MR spectroscopic imaging focusing on parietal-occipital cortex indicated that only the comorbid HIV+alcoholism group was affected, exhibiting a significant deficit in N-acetyl aspartate, a marker of living neurons. Although neither HIV infection nor alcoholism alone resulted in such abnormalities, each disease carried a liability that put affected individuals at a heightened risk of neuronal compromise when the diseases were compounded. The high prevalence of alcoholism in HIV-infected individuals and the interfering effect of alcohol on HIV pharmacological response and therapy compliance underscore the need to recognize the independent and synergistic contributions of each condition to impair brain structure and function. Support: National Institute on Alcohol Abuse and Alcoholism (AA017347, AA012999, AA017923).

## P198

### Neuropsychological impairment persists regardless of viral suppression and in the absence of monocyte activation

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Neuropsychological (NP) impairments in HIV-infected individuals remain high despite the introduction of highly active antiretroviral therapy (HAART). We sought to determine whether or not a monocyte gene expression profile with other peripheral factors could be used as biomarkers for neuropsychological impairment among HIV-infected individuals. Forty-four male HIV seropositive subjects (HIV+) and 11 HIV seronegative controls (HIV-) had NP testing and blood drawn for monocyte gene expression analysis. Study subjects were also assessed for CD4 cell counts, viral load, ApoE genotype, plasma lipopolysaccharide (LPS) and other clinical variables. NP scores were normalized to age, gender and education. Twenty-one of 44 (48%) HIV+ individuals showed abnormal NP testing results (=1.5 SD from normal) in 2 or more NP domains. There was no correlation between NP impairment and levels of education, age, ethnicity, plasma viral load, plasma LPS, CD4 cell count, CD4 nadir or years of infection. Also, there was no correlation between ApoE4 genotype and NP performance. However, greater years of infection had diminished visual learning performance. Lower CD4 counts had impaired attention/working memory and verbal learning ability. Lower CD4 nadir correlated with worse fine motor skills in the non-dominant hand. Overall, HIV+ individuals had deficits in attention/working memory, verbal learning, and information processing speed compared to HIV- controls. Peripheral blood monocyte gene expression arrays showed distinct profiles based on viral load. No peripheral monocyte gene profile correlated with NP performance suggesting that HAART calms monocyte activation. We hypothesize that NP impairment results from a lingering HIV reservoir and its' consequences in the brain.

## P199

### Association between the Catechol-O-Methyltransferase (COMT) gene variant, Val158Met, and motor deficits in HIV+ patients

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Motor function deficits are highly prevalent in the symptomatic disease stages of HIV/AIDS. Studies have reported abnormal dopaminergic tone in HIV, which may contribute to these deficits. A polymorphism of the catechol-O-methyltransferase (COMT) gene, Val158Met (rs4680), is associated with altered DA metabolism in the prefrontal cortex (PFC) where the Met allele impairs DA breakdown thereby increasing synaptic DA. This, in turn, may lead to alterations in dopaminergic tone in striatum, and contribute to abnormal motor function in at-risk disease states. We examined the relationship between the Val158Met polymorphism and motor function, as measured by the HIV-Dementia Motor Scale (HDMS), in an HIV+ adult population. Participants included 203 subjects (87 African Americans, 56 Caucasians, 59 Hispanics) with late stage HIV-AIDS from the Manhattan HIV Brain Bank Study (62 females, 141 males). Val158Met minor allele frequency varied significantly between African Americans (0.25) and Caucasians (0.52), with intermediate value in Hispanics (.44). Compared to the non-motor impaired, an increased prevalence of motor impaired patients was associated with presence of the Met allele in Caucasians ($P < .0001$) and Hispanics ($P = .02$). In Hispanics, decreased risk of severe motor impairment was associated with the Met/Val genotype compared to Met/Met ($P = .007$). In Caucasians, the Val/Val genotype was marginally associated with decreased odds of motor impairment compared to Met/Met ($P = .056$). No significant associations were found among African Americans. These findings suggest an ethnicity-specific relationship between the Met allele and increased motor impairment risk in HIV. This is the first study to demonstrate a genetic association for HIV-associated motor abnormalities, and supports theories that alterations in dopaminergic tone are central to the generation of neuroAIDS disorders.

## P200

### Viroporin activity of JCV agnoprotein

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Most non-enveloped viruses exit their host cells following cell lysis, which involves breakdown of the cell membrane and which presumably results from increased plasma membrane permeability. JC virus (JCV) the causative agent of Progressive Multifocal Leukoencephalopathy belongs to the family of Polyomaviruses, which have non-enveloped virions. It has been suggested that extracellular release of the

progeny virions of Polyomaviruses occurs when cells disintegrate or rupture; however, the molecular mechanism(s) employed by JCV to induce cell lysis and facilitate virion release remain elusive. Viroporins are a group of proteins that participate in the promotion of release of viral particles from cells, and interact with cellular membranes modifying permeability. These proteins are not essential for the replication of viruses, but their presence enhances virus growth. The genome of JCV encodes six major proteins, including agnoprotein which is a small auxiliary protein. Previous studies from our and other laboratories indicated that agnoprotein plays an important role in the propagation of JCV, though not fully understood. Here, we demonstrate that JCV small integral membrane protein, agnoprotein acts as a □gviroporin□h that is small viral polypeptides, interacts with cell membranes, and increases their permeability to ions and other low-molecular-weight compounds.

## P201
### HIV-1 gp120 enhances β- and γ-secretase activity in human neuroblastoma cells

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We are now in our third decade since the discovery of HIV and the second decade of antiretroviral therapy (ART) to combat infection with HIV. The success of ART has increased the lifespan of many people infected with HIV and therein created an aging population infected with HIV. This scenario has increased the likelihood that viral and host products associated with HIV-infection may interact with age-associated factors to accelerate the onset or progression of age-associated neurodegenerative conditions. There is accumulating evidence that suggests brain deposition of pathogenic forms of amyloid-β (Aβ) is accelerated in HIV-infected subjects. However, the mechanism of enhanced Aβ deposition in the setting of HIV is still controversial and the mechanisms poorly understood. In this study we sought to determine if the HIV-coat protein gp120 could enhance the formation of Aβ by deregulating APP processing. Human neuroblastoma cells (SH-SY5Y) stably overexpressing human APP or untransfected cells were treated with gp120 (IIIB or CM) for 6 - 18 hours before measurement of α, β, and γ-secretase activities. Neither form of gp120 modified α-secretase activity in any of the cells tested. Both gp120IIIB and gp120CM increased β-secretase activity in APP expressing cells and untransfected cells in a time and dose-dependent manner. Gp120CM, but not gp120IIIB, increased γ-secretase activity in untransfected and APP expressing cells in a time and dose-dependent manner. These data suggest that gp120 may promote aberrant amyloid processing by enhancing β- and/or γ-secretase activities.

## P202
### Alteration of Gene Regulatory Networks Through MicroRNAs in Frontal Cortex of HIV-Infected Individuals

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Background: MicroRNAs are small, non-coding RNAs that regulate gene networks, helping control cell function and phenotype. MicroRNAs are recognized as vital players in CNS patterning, function, and disease. Past studies focused on expression levels of coding mRNAs in the brain of HIV-infected individuals. Because microRNAs affect the abundance and downstream functions of mRNAs, it is important to understand both mRNA and miRNA changes concurrently. We report the first genome-wide microRNA profile in the HIV-infected human frontal cortex. We present methods to integrate mRNA expression with microRNA expression data in a Target Bias Analysis by determining the probability that the number of target-genes of dysregulated miRNAs would be dysregulated in HIV infection versus expected by chance.

Methods: We used Affymetrix arrays for comparing gene expression in frontal cortex from 6 HIV-infected males (no cognitive impairment or encephalitis) and 6 age-matched controls at the mRNA level. We pooled equivalent RNA samples and utilized Applied Biosystems PCR-based array to assess a panel of 379 microRNAs.

Results: Target bias analysis indicated that microRNAs clustered into four types: A) Those with many dysregulated mRNA targets of less stringent significance, B) Fewer dysregulated target-genes of highly stringent significance, C) spectrum from non-bias to combinations of A and B. The dysregulated miRNAs clustered on Chromosomes 14, 17, 19, and X. Those miRNAs that affect many genes may be important "circuits" in gene regulatory networks pertinent to effects of HIV infection in CNS. These may be functional and diagnostic markers of neurologic disease in HIV-infected patients.

## P203

### HIV-1 Clade B and C Tat differentially Induces Indoleamine-2, 3-Dioxygenase (IDO) and Serotonin in Immature Dendritic Cells (IDC): Implications for Neuro-AIDS

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HIV-1 is commonly associated with immune dysfunctions and suppression of antigen presenting cells resulting in immune alterations which could lead to impaired neuronal functions, such as Neuro-AIDS. The neurotoxic factor, kynurenine and the rate limiting enzyme, Indoleamine-2, 3-Dioxygenase (IDO) and serotonin may play a role in tryptophan deficiency and behavioral disorders in Neuro-AIDS. HIV-1 transactivator regulatory protein (Tat) is known to play a major role in immune dysfunction. Previous studies suggest that HIV-1 B and C clades differentially manifest neuronal dysfunctions in infected host and the present study examine the effect of HIV-1 B and C clades derived Tat on IDO, serotonin gene expression and protein modification by dentridic cells as studied by Q-PCR and Western blot. The intracellular IDO expression, IDO enzyme activity, and the levels of kynurenine were also measured. Results indicate that HIV-1 clade B Tat upregulates IDO and down regulates serotonin gene and protein expression and simultaneously upregulated kynurenine level as compared to HIV-1 clade C Tat. These studies suggest that HIV-1 B and C Tat protein may play a differential role in the neuropathogenesis of HAD/MDC in neuroAIDS.

## P204

### Persistence of Poliovirus Type 1 Genome in Patients with the Post-Polio Syndrome

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Decades after being hit by poliovirus (PV), 40–70% of polio survivors develop the ''post-polio syndrome'' (PPS), a progressive condition characterized by chronic fatigue, pain, new muscular weakness, cold intolerance. The etiology and pathogenesis of PPS are undefined. Literature data suggest that PV genome fragments may persist for decades in the central nervous system of affected patients. Using molecular tests, tissue culture studies, and immunofluorescence of infected cells, low-level PV infectivity and genome fragments have been detected in 27/29 PPS patients aged 50 to 75 years (CSF and peripheral blood leukocytes). No virus was detected in negative controls (CSF from 12 adult patients with non-infectious, non-neoplastic pathology, and peripheral blood leukocytes from 18 healthy blood donors). In a few patients, PV genome fragments have also been detected in saliva and urine samples, as well as in primary cultures of skeletal muscle, peripheral nerve, and duodenal mucosa cells. PV genome fragments were present at extremely low levels, thus making whole genome sequencing impossible. Partial sequencing of the 5′UTR, VP1, and 3D (RNApol) regions indicated that amplicons from PV-positive patients were compatible with reference sequences of PV-1. Extensive mutations/deletions were detected in the 5′UTR and VP1 regions. Immunofluorescence and WB with PV-specific mAbs showed that capsid proteins were produced at low levels in primary cultures of muscle and peripheral nerve cells as well as in cell lines infected with samples of PPS patients. These data indicate that PV genome fragments can persist for several decades in polio survivors. The data do not provide a pathogenetic link between virus persistence and PPS development. However, the highly sensitive molecular tools now made available can contribute to detect and characterize PV strains in these patients, with the aim of better defining PPS pathogenesis. The financial support of Post-Polio Health International (St. Louis, MO) is gratefully acknowledged.

## P205

### Neuroprotective and Neurorestorative Effects of Fluconazole against HIV proteins and mitochondrial toxins

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Currently there is no effective neuroprotective treatment for cognitive impairment in human immunodeficiency virus (HIV)-infected patients who are on effective antiretroviral therapy. We screened nearly 2000 FDA approved and natural compounds against several screening assays and found the triazole drug, fluconazole (Diflucan®, Pfizer) to be neuroprotective. In mixed neuronal cultures, fluconazole (500 nM–1 M) 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. With 1M fluconazole nearly complete protection was noted against both toxins. When treated with 3NP

(incremental dose of 20 mg/kg, 40 mg/kg and 60 mg/kg for 5 days), 14-week old C57B/6 mice developed lesions in the basal ganglia. 3-NP lesioned mice pretreated with fluconazole (10 mg/kg i.p.) had significant protection against 3NP toxicity, as quantitated by cresyl violet staining and anti-DARRP32 immunostaining of medium spiny neurons in the striatum ($p < 0.01$). Mice chronically expressing gp120 displayed 40% less proliferation of neural progenitor cells (NPC) in the dentate gyrus, while the gp120 mice treated with fluconazole demonstrated no decrease, but rather increased NPC proliferation ($p < 0.05$). Brain levels of fluconazole were measured by mass spectrometry and found to exceed levels needed for neuroprotection. Fluconazole (10uM) also decreased HIV-Tat induced LTR transactivation in the U373-CXCR4 astrocytic cell line ($p < 0.01$). To determine if a neuroprotective structure-activity relationship exists for fluconazole, we are currently testing a number of fluconazole analogs with bioisosteric replacements. In addition, we synthesized a fluconazole analog containing a phosphate ester group, These derivatives are being evaluated to determine whether potential neuroprotective activities are distinct from the antifungal properties of the compound. These data indicate that fluconazole protects vulnerable neuronal populations from oxidative stress- and HIV protein mediated toxicities in vitro and in vivo,. It provides both neuroprotective and neurorestorative properties and hence may be a novel therapeutic agent for treatment of HIV patients with neurocognitive disorders.

## P206

### NF-κB is an important mediator of the neurotoxic effects of HIV-1 Tat protein and morphine on astrocyte cells

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Disease progression and neurodegeneration associated with Human Immunodeficiency Virus (HIV-1) infection can be worsened by opioid drug abuse. Tat protein through cytokine upregulation may play a fundamental role in neuropathogenesis. We want to demonstrate the relationship between morphine and HIV-1 Tat in cytokine production. In addition, we want to address if the transcription factor NFκB is involved in the cytokine dysregulation. Cytokine expression changes were measured when astrocyte cell lines producing Tat were exposed to morphine. NFκB-p65 subunit immunofluorescence localization and protein isolation were performed. HIV-1 Tat and morphine independently increase astrocyte mRNA and protein levels of pro-inflammatory cytokines; IL-6 and IL-8. In contrast, Tat and morphine combined diminish IL-6 and IL-8 protein release. Astrocyte cells endogenously producing Tat and exposed to morphine showed reduce NFκB p65 protein presence in cell cytoplasm. NFκB-p65 fluorescent signal was re-localized from the cytoplasm to the nucleus in the presence of Tat alone as well as with Tat and morphine co-treatment. NFκB-p65 siRNA knockdown diminished IL-6 and IL-8 cytokine protein secretion. The NFκB nuclear localization indicates that the transcription factor may activate IL-6 and IL-8 gene transcription when cells are exposed to Tat and morphine. NFκB subunit knockdown showed that Tat and morphine cytokine release is p65 dependent. Our results indicate that NFκB can be an essential component in the pro-inflammatory cytokine secretion triggered by Tat and morphine, resulting in neurodegeneration. Supported by F31 DA 023718-01A1, P20 RR-016470, NIH/NIGMS GM008239, and G12RR003050.

## P207

### Identification of Lymphotropic Polyomavirus in Peripheral Blood from Patients with Leukoencephalopathies and Healthy Individuals

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Background: Lymphotropic Polyomavirus (LPV) was first isolated from a B-lymphoblastoid cell line of an African green monkey. This virus shares some characteristics with human polyomaviruses, but it is antigenically distinct from Simian Virus 40 (SV40), BK Virus (BKV) and JC Virus (JCV). Seroepidemiological studies revealed that many human sera had strong reactions in the presence of LPV antigens, and, recently, the viral genome was amplified in the peripheral blood from patients affected with HIV-related leukoencephalopathies.

Methods: the LPV, JCV and BKV genomes were searched by means of real time PCR targeting the VP1 (LPV, BKV) and the large T-Antigen (JCV) coding regions in peripheral blood samples collected from 83 HIV+ patients and 105 Healthy Controls (HC). The HIV+ group was composed of 11 patients with Progressive Multifocal Leukoencephalopathy (PML), 16 patients with Not Determined JCV-negative Leukoencephalopathy (NDLE), 11 patients with Other Neurological Disease (OND) and 45 patients without Neurological Disease (NND). The aim of the study was to define the distribution of LPV in human specimens, to compare it to the distribution of the other human Polyomaviruses BKV and JCV, and to define its possible association with HIV-related leukoencephalopathies.

Results: The LPV genome was detected in 6 out of 83 HIV+ patients (7,2%; 1 PML, 2 NDLE, 3 NND), with a median viral load of 1,41E+01 copies/ug and in 5 out of 105 HC (4,7%), with a median viral load of 4,70E+01 copies/ug. The JCV and BKV genomes were detected in 13 out of 83 HIV+ patients (15,7%; 2 PML, 2 NDLE, 3 OND, 6 NND) and 17 out of 68 HIV+ patients (25%; 1 PML, 3 NDLE, 2 OND, 11 NND), respectively. The median viral loads of JCV and BKV were 1,48E+02 copies/ug and 3,82E+01 copies/ug. As for the control group, JCV and BKV were detected in 2 out of 105 HC (1,9%, median viral load: 1,06E+03 copies/ug) and in 12 out of 105 HC (11,4%; median viral load: 4,15E+01 copies/ug), respectively.

Conclusions: The amplification of LPV genome from human peripheral blood confirms the possibility of LPV-like infections in human subjects. LPV seems to be distributed in the human population with a slightly lower frequency than BKV and JCV. LPV DNA was amplified from patients affected by leukoencephalopathies but also from patients with NND and from HC, with similar viral load, therefore, the results do not support the hypothesis of an association between LPV infection and the development of HIV-related leukoencephalopathies. However, given their high similarity, it is possible that LPV, as well as BKV and JCV, could establish latency in humans and cause disease only in rare circumstances.

## P208

### Possible neuroprotection by a red wine component, resveratrol, in CNS neurotropic virus infection

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Theiler□fs murine encephalomyelitis virus (TMEV) is divided into two subgroups. The highly neurovirulent GDVII strain produces an acute fatal polioencephalomyelitis with axonal degeneration in mice, whereas the attenuated DA strain produces chronic demyelination and axonal degeneration with virus persistence preceded by an acute infection. This chronic DA virus infection is a viral model for multiple sclerosis (MS). While axonal degeneration in the central nervous system (CNS) occurs in human neurotropic virus infection and MS, there is no efficient treatment targeting axonal preservation. In Wallerian degeneration slow (Wld) mice, axonal degeneration is delayed due to an increased nicotinamide adenine dinucleotide (NAD) biosynthetic enzyme (Nmnat) activity. SIRT1 is the downstream effector of the increased Nmnat activity. Preservation of axons in Wld mice is beneficial in experimental autoimmune encephalomyelitis (EAE), an autoimmune model for MS. Resveratrol, a natural polyphenol compound of red wine, has been tested clinically and experimentally as a potential therapy for neurological diseases, such as Alzheimer□fs diseases and stroke, since it can cross the blood-brain barrier. Resveratrol can enhance SIRT1 activity, which confers axonal preservation. Resveratrol also exhibits anti-inflammatory and anti-viral activities. We tested whether resveratrol could be therapeutic, possibly by limiting axonal damage in TMEV infection. Female SJL/J mice were infected with GDVII or DA virus. Infected mice were fed a diet containing 0.04% resveratrol (20 mg/kg/day) or a control diet during the entire course of GDVII virus infection or during the acute stage (days 0 to 14) or the chronic stage (days 21 to 35) of DA virus infection. In mice infected with a low dose (10 PFU) of GDVII virus, resveratrol treatment reduced mortality (40%), compared with control mice (75%). Similarly, during the acute stage of DA virus infection, the resveratrol-treated mice showed more weight gain than control mice at 2 weeks post infection ($P < 0.001$, by ANOVA). CNS tissues and spleen mononuclear cells were harvested 5 weeks after DA virus infection. The mice treated during the chronic stage tended to have higher clinical and pathological scores than control mice, although they did not reach statistical significance. There were no significant differences in viral persistence in the CNS or lymphoproliferative responses to virus among groups. We speculate that reduced mortality in resveratrol treated mice in GDVII virus infection as well as significant weight gain during the acute stage of DA virus infection in the early resveratrol treatment group could be due to a neural protection property of resveratrol. Since degenerated axons do not regenerate in the CNS, axonal degeneration results in permanent clinical disability in neurotropic virus infection and MS. Thus, potential axonal sparing activity by resveratrol could be of great benefit. This work was supported by the National Institutes of Health (NIH), R21NS059724, and the National Multiple Sclerosis Society (NMSS) Pilot Research Award PP1499.

## P209

### High sensitivity of JCV T-antigen expressing medulloblastoma cells to fenofibrate and its association with the inhibition of IGF-IR signaling axis and metabolic crisis

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Human polyomavirus JC (JCV) is well known to trigger cellular transformation in vitro, and is highly suspected in the development of some tumors in humans. The most prominent polyomavirus oncogenic protein, large transforming antigen (JCV T-antigen), has immortalizing and transforming properties in vitro, and is tumorigenic in experimental animals. We have demonstrated a strong inhibition of medulloblastoma growth by fenofibrate, which was accompanied by the attenuation of multiple signaling branches from the IGF-IR. Fenofibrate is a potent agonist of peroxisome proliferator activated receptor alpha (PPARalpha), and has been recently considered as a candidate for tumor chemoprevention. Fenofibrate has low systemic toxicity, and is commonly used as a lipid lowering drug by the mechanism of transctiptional upregulation of genes involved in fatty acid beta-oxidation, and inhibition of glycolysis. We have demonstrated elevated expression of PPARalpha in medulloblastoma clinical samples and in medulloblastoma cell lines. Importantly, JCV T-antigen expressing medulloblastoma cells demonstrated much higher sensitivity to fenofibrate, and responded to the treatment by perinuclear accumulation of peroxisomens, cell cycle arrest, massive apoptosis at later time points, and the attenuation of tumor formation in experimental animals. These strong cytotoxic effects of fenofibrate were associated with fenofibrate–mediated loss of mitochondrial potential evaluated as a percentage of cells with depolarized mitochondrial membranes. In comparison to the untreated BsB8 cells (JCV T-antigen positive), in which 15.9% of the cells had depolarized mitochondria, 53.7% lost their mitochondrial potential within first 24 hrs of incubation with 25microM fenofibrate. Interestingly, Bs1a cells (JCV T-antigen negative) responded to the fenofibrate treatment by only a modest loss of mitochondrial potential. We found that among untreated Bs-1a cells, 4.9% were characterized by depolarized mitochondria. This cell fraction increased only up to 16.4% after the fenofibrate treatment, indicating partial mitochondria resistance to fenofibrate in medulloblastoma cells lacking JCV T-antigen. The substantial loss (53.7%) of mitochondrial potential in medulloblastoma cells expressing JCV T-antigen (BsB8) was accompanied by almost 4-fold increase in the ADP/ATP ratio at 48 hrs following the fenofibrate treatment, suggesting severe energy deficit associated with insufficient ATP production by the oxidative phosphorylation. The same ADP/ATP experiment performed in Bs1a cells indicated only 1.4-fold increase in ADP/ATP ratio in fenofibrate treated cells indicating that JCV T-antigen expressing cells are much more sensitive to the fenofibrate –mediated metabolic crisis.

## P210

### Human herpesvirus 6 antibodies in multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system of unknown origin, characterized by demyelinated white matter plaques. Epidemiological and migratory studies already 40 years ago suggested that environmental factors participate to the pathogenesis of the disease. The most promising candidates are herpesviruses, Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6).If virus is the cause of the disease, oligoclonal bands (OCBs) should contain antibodies against the suspected virus. We studied HHV-6 antibodies in MS serum and cerebrospinal fluid. Furthermore, HHV-6 CSF positive cases were investigated for the presence of HHV-6 specific OCBs. HHV-6 antibodies in serum and CSF were detected using IFA. The presence of OCBs was studied using isoelectric focusing. HHV-6 specific OCBs were identified by blotting the bands to nitrocellulose membrane pre-coated with HHV-6 antigen. The prevalence of HHV-6 antibodies in serum in MS was 100% compared to 70% prevalence in controls. The mean titers were significantly higher in MS vs. controls. In addition, HHV-6 antibodies were detected in CSF of the patients with MS, but not in controls, and two patients with highest HHV-6 IgG titers in their CSF revealed to have HHV-6 specific oligoclonal bands present in CSF but not in serum. OCBs in these cases appeared specific to HHV-6 and did not react with HSV-1 used as control antigen. HHV-6 specific oligoclonal bands can be detected in a subset of MS patients. Together with previous published results our studies further emphasize the role of HHV-6 in the pathogenesis of MS in a subset of patients with MS.

## P211

### Antiepileptic drug use in HIV infection: clinical outcomes and immune effects

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Background: Antiepileptic drugs (AEDs) are widely prescribed to HIV/AIDS patients receiving antiretroviral therapies (ARTs) although the spectrum of AED use and their adverse effects remain uncertain. Here we investigated AED use and their effects within a centralized HIV community clinic and in vitro effect of AEDs on T cell proliferation and viral replication.

Methods: All patients receiving AEDs at the Southern Alberta (HIV) Clinic, Calgary, were identified from 2001 to 2007. Clinical characteristics, laboratory findings, and demographic features were analyzed together with AED indication, type and cumulative dosing. In patients on stable ART regimens, the effects of AEDs on virologic and immunologic parameters were analyzed. Cultured HIV-infected and uninfected T cells were assessed in terms of viral replication and proliferation with exposure to the therapeutic concentration of gabapentin, phenytoin and valproate to determine the direct effect of AEDs on T cells and viral replication.

Results: 166 AED-treated patients were identified within a total 1345 HIV/AIDS patients. AED indications included peripheral neuropathy/neuropathic pain (60%), seizure/epilepsy (24%), mood disorder (13%), movement disorder (2%) and other (0.05%). AED types included: calcium (gabapentin/pregabalin) (45%) and sodium (phenytoin, carbamazepine, lamotrigine) (32%) channel blockers, valproate (16%), and Other (7%). Although the overall frequency of abnormal liver function tests was 16% and cumulative dosages of AEDs was found to be high, liver or virologic failure was not observed. Among patients on stable ART regimens CD4+ T cell counts rose over 12 months in groups receiving either sodium and calcium channel blockers ($p < 0.05$). In vitro proliferation assay showed that valproate suppressed proliferation of HIV-infected and uninfected T cells whereas gabapentin and phenytoin did not affect T cell proliferation. In contrast, none of the AEDs exerted effects on viral production in terms of reverse transcriptase activity Conclusions: AEDs were prescribed for multiple indications without major adverse effects in this population. Immune status in patients receiving sodium or calcium channel blocking drugs was improved despite large cumulative AED doses.

## P212
## HCV co-infection potentiates HIV neuropathogenesis by augmenting neuronal apoptosis

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Introduction: Hepatitis C virus (HCV) RNA and proteins are detected in post-mortem brains and spinal cords indicating productive infection of the central nervous system. The impact of HIV/HCV co-infection on neuropathogenesis remains unclear. We compared the occurrence of neurological disorders in both HIV mono-infected and HCV co-infected persons and investigated the effects of HCV Core protein on neuronal viability alone and with the HIV Vpr protein.

Methods: Retrospective analysis of demographic and clinical variables was performed for all adult HIV-infected patients diagnosed with neurological disorders during the 1998-2008 period in the two centralized clinics providing HIV care in Alberta, Canada. The viability of human fetal neurons exposed to HCV Core protein with and without concurrent HIV Vpr exposure was measured by beta-tubulin immunoreactivity and DAPI staining.

Results: Of 459 HIV-infected patients affected with neurological disorders, 26.1% were seropositive for HCV. Most HIV patients with HCV co-infection (75%) had a history of intravenous drug abuse and there was a higher percentage of First Nations (Aboriginal, Inuit and Metis) in the co-infected group. Co-infection with HCV was associated with an increased mortality rate ($p < 0.05$). The HIV/HCV-infected patients showed a greater reduction in CD4+ T cell levels from the time of HIV-1 seropositivity to nadir levels and the closest time to first neurologic diagnosis ($p < 0.05$). HIV/HCV-infected patients had a higher prevalence of seizures (26.7% vs. 17.4%, $p < 0.05$) and greater severity in HIV-associated neurocognitive disorder, which was associated with severe immunosuppression. HCV Core protein modulated neuronal membrane currents and caused apoptosis compared with control protein ($p < 0.05$). These effects were potentiated by the presence of Vpr protein at subtoxic concentrations ($p < 0.05$).

Conclusion: HCV infection in HIV-infected persons increased the neurologic disease burden, possibly

through the additive neurotoxic effects of HCV- and HIV-encoded proteins. These findings underscore the adverse impact of HCV infection on HIV disease course.

## P213
### Proteomic fingerprints in CSF and blood of HIV-infected patients with neurocognitive disorders

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A biosignature for human immunodeficiency virus type one (HIV-1) associated neurocognitive disorders (HAND) remains elusive. Disease diagnosis can be made only by exclusion of opportunistic infections and malignancies. In attempts to uncover biosignatures for HAND and provide new insight into disease diagnosis and treatment our laboratories initiated a comprehensive bench to beside proteomics research program. During the past 6 years, we have used proteomic approach to study proteomes in clinical samples. To facilitate analysis of low abundance proteins, we removed the 6 most abundant proteins from CSF samples and 12 most abundant proteins from serum samples. Most of our analyses were performed employing 2-dimensional electrophoresis (2DE) with Difference Gel Electrophoresis (DIGE). More recently we used a combination of SELDI-TOF, weak cation exchange (WCX) chromatography and 1-dimensional electrophoresis (1DE). Protein identification has been performed using nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS). In concurrent experiments, we investigated a secretome of HIV-1 monocyte derived macrophages (MDM). The rationale for these analyses was to link function of viral reservoir cells in the brain to the proteome of CSF and blood. Within the CSF, proteins including complement C3 and its fragments, neuronal cell adhesion molecule (NrCAM), cystatin C, vitamin D binding protein, clusterin, gelsolin, procollagen C-endopeptidase enhancer); and in serum/plasma, complement C3, ceruloplasmin, afamin, prealbumin and gelsolin; were differentially expressed in HAND. Initially, our primary validation method was quantitative western-blot analysis and we are now applying a mass spectrometry-based technique (Multiple Reaction Monitoring (MRM)). These results, while preliminary, provide insights into potential biosignatures for HAND.

## P214
### Cellular microRNAs and Interferon beta regulation during SIV infection of the CNS

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Interferon beta production is an inaugural event in the innate immune response to lentiviral infection of the central nervous system, resulting in the activation of important antiviral signaling cascades. During SIV infection of macaques in our rapid and consistent model of HIV encephalitis, we have observed viral entry into the CNS within four days of infection, accompanied by a marked IFN beta RNA response during acute infection. IFN beta levels then decline as SIV enters its latent phase. The critical role of IFN beta to the innate antiviral response combined with potential inflammatory damage associated with long-term activation suggest that IFN beta is likely subject to fine-tuned cellular regulation. In the 1990s, control of IFNb at the transcriptional and message stability levels were established. Here, we present evidence to support the hypothesis that IFN beta is also regulated at the translational level by microRNAs (miRNAs), approximately 22-nt small RNAs recently implicated in translational repression of many cellular mRNAs. We show that several features of the IFN beta mRNA 3′ untranslated region make the transcript a candidate for miRNA regulation. Overlapping prediction algorithms were used to predict 3′ UTR binding sites for known miRNAs. These miRNAs were then filtered on the basis of expression and differential regulation during 1)monocyte to macrophage differentiation and 2)treatment of macrophages with IFN beta. Four remaining candidate miRNAs were studied, and we show that they decrease production of a reporter gene through the IFN beta 3′ UTR and also exert effects on the level of total and secreted IFN beta protein in primary macrophages. These results demonstrate, for the first time, the regulation by miRNAs of IFN beta, a cytokine of central importance during acute retroviral infection and the development of CNS disease.

## P215
### Increased Depression and Neurocognitive Dysfunction in Aging HIV-seropositive Women despite higher CD4 cell counts

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Background: HIV infection is considered a chronic disease since HIV-seropositive patients on antiretroviral treatment are living longer. Aging is associated with several physiological changes such as altered immune function, decrease neuropsychological (NP) performance, and increase neurodegenerative disorders. Aging women have the added effect of marked sex hormonal changes during menopause. Aging in HIV-seropositive patients is associated with increased HIV-associated neurocognitive disorders (HAND), glucose intolerance, and cerebrovascular diseases. Therefore, it is critical to understand the effects of aging in HIV infection especially in women.

Methods: The Hispanic/Latino Longitudinal HIV-seropositive women cohort (HLL-HWC) evaluates women longitudinally (every 6 mo) and characterizes their viral immune profile and NP performance. Since 2002, 153 women have been enrolled, 93 (61%) women were 40 years and older and 61 (40%) women were followed for two visits or more. The objective of this study was to compare the viral immune profile and the NP performance between younger and older HIV-seropositive women. Data from the HLL-HWC repository was analyzed according to their socio-demographic factors, viral immune profile, CSF and plasma MCP-1, and HAND. A matched seronegative control group ($n = 24$) was used to determined NP z-scores. We divided the cohort into those aged 40-49 years old ($n = 80$) and those 50 years and older ($n = 13$). A total of 263 evaluations were analyzed (232 from 40-49 years and 31 from $>50$ years).

Results: Women $>50$ years presented more depressive symptoms as determined by the Beck's Depression Index ($p = 0.005$), higher nadir ($p = 0.009$) and visit ($p = 0.01$) CD4 cell count, and increased CSF MCP-1 levels ($p = 0.047$). Neuropsychologically, they performed worse (NPZ $p = 0.01$) especially on psychomotor speed ($p < 0.001$) and verbal memory domains ($p = 0.02$).

Conclusion: HIV-seropositive women in the 6th decade of their life show increased depression and neuropsychological impairment which is associated with higher MCP-1 levels despite higher CD4 lymphocyte cells. Further studies are needed to better understand the synergistic effects of aging, HIV infection, and long-term treatment. Supported by NIH grants, S11NS46278, U54NS43011, P20rr11126.

## P216
### C/EBP-beta regulates the JCV early and late promoters

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The human neurotropic polyomavirus, JCV, is the etiologic agent of the fatal central nervous system demyelinating disease, progressive multifocal leukoencephalopathy. PML is mainly associated with the severe immunosuppression of advanced HIV/AIDS and is the direct result of virus multiplication in oligodendrocytes. The viral genome of JCV is divided in 3 parts: early region, late region and non-coding control region (NCCR). The JCV NCCR regulates expression of JC viral proteins and contains regulatory elements including an NF-kappaB (p65) binding site which is shown to be involved in transcriptional activation. We have found that C/EBPbeta, especially the LIP isoform, inhibits basal and NF-kappaB-stimulated transcription of the early and late promoter activity of JCV via the kappaB site. Using both CAT and Luciferase assays, we show that C/EBP-beta LIP downregulates p65-stimulated transcription. The measurement of CAT activity after cloning wild type NF-kappaB site and its mutant version into a heterologous promoter confirmed that the inhibitory role of C/EBP-beta LIP isoform is mediated by the kappaB site. Taken together, these findings show a new inhibitory role of C/EBP-beta LIP in the regulation of JCV. The interplay between these two transcriptional factors may be important in controlling the balance between latency and reactivation of JCV infection, which leads to PML. The proinflammatory cytokines in the brain may play regulatory role in balance between activation and latency.

## P217
### Structural and Functional Distinctions among Tat Proteins of Different HIV-1 Clades

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There are several major clades of HIV-1 subtype M, the subtype most predominant throughout the world. Clade B is most common in North America and Europe, and functional characterization has been done using primarily recombinant proteins from this clade. Clades C, D and A/E infect the majority of people in different parts of Africa and Asia. Although certain proteins, notably Vif, are highly conserved among clades, others, such as Env

and Tat, differ considerably, leading to the notion that there may be significant differences in transmission and pathology. We have cloned Tat proteins (72aa) from clades B, C, D and E for expression in human cells and for purification from bacteria. We have also cloned two additional amino acid sequence variants of clade C Tat and one of clade E Tat. Aa's 22 through 37 of clade B Tat contain 7 C residues, certain of which are known to be essential for HIV-1 LTR transactivation. C22 is essential, and it is conserved in all functional Tat proteins. A C22 to G mutation abrogates effects of Tat on LTR transactivation, JC viral (JCV) gene expression, JCV DNA replication, and binding to cellular proteins Pur-alpha and Cyclin T1/Cdk9. In contrast, C31, which is mutated to S in clade C Tat, is not essential for these functions. Mutation of clade C C27 to Y reverses the ability of the Tat protein to transactivate. This mutation is predicted to greatly alter Tat conformation. One clade E variant has two aa inserted into an 11 aa motif believed to be important for cellular uptake of Tat. Experiments are in progress to determine abilities of different clade Tat proteins to enter glial cells, to activate JCV promoter activities and to enter and function in vaginal and cervical epithelial cells. Results will help shed light on differences in pathology and neurological consequences of HIV-1 clades throughout the world.

## P218
### Alcohol perturbs AMPA receptor localization by disrupting the structure of neuronal plasma membranes

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Chronic alcohol abuse is often associated with cognitive deficits and can lead to a dementing illness that is associated with frontal lobe dysfunction. Neuropathology of chronic alcohol abusers includes patchy neuronal loss, dendritic pruning and axonal truncation. HIV-infected subjects who abuse alcohol exhibit deficits in cognitive tasks that assess frontal lobe function compared with HIV-infected subjects who do not abuse alcohol. These findings suggest that alcohol abuse may hasten cognitive decline in HIV-infected subjects. Although some studies have shown that alcohol can accentuate the toxic effects of viral proteins, the exact mechanisms of these effects have not been identified. Here we provide evidence that alcohol impairs neuronal function by modifying the structure of membranes to induce a mislocalization of AMPA receptors. Hippocampal neurons were treated with ethanol (0.03–0.3%) for 2 min to 12 h and we measured sphingolipid and sterol content. Ethanol extracted lipids from neuronal membranes within 2 min, with the following efficiency: Cholesterol > ceramide > sphingomyelin. Each of these lipid products recovered to approximately 50% of baseline within 12 h., suggesting that new synthesis was required to restore the lipids extracted by ethanol. Based on evidence that cholesterol and ceramide are enriched in specialized membrane domains known as lipid rafts, and data that these domains are important for the surface expression of AMPA-type glutamate receptors, we next determined if ethanol disrupted the location of AMPA receptors. At baseline, $46 \pm 6\%$ of neuronal GluR1 localized with GM1 (a gaglioside enriched in lipid rafts) and was distributed along the length of dendritic branches. A 2 min treatment with ethanol caused a collapse of GM1 into aggregates and increased the colocalization of GluR1 with GM1 to $61 \pm 8\%$. Thus, ethanol disrupted the composition and organization of membrane lipids and re-distributed AMPA receptors to create areas of high receptor density, interspersed by regions with a paucity of AMPA receptors. In functional studies we found that a 2 min ethanol exposure (0.1%) followed by AMPA (10 M) applied during ethanol wash-out increased the amplitude of focal calcium bursts in regions of concentrated AMPA receptor density, with no apparent change in the frequency of calcium bursts. Thus, alcohol abuse may contribute to neuronal dysfunction in HIV-infected subjects by perturbing plasma membrane structure and mislocalizing calcium permeable AMPA receptors. Supported by NIH grant R01AA017408.

## P219
### HIV-1 increases STAT1 transcriptional activity and induces blood-brain barrier dysfunction via a PI3K/AKT- and MEK-dependent phosphorylation of STAT1 and STAT3

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How neuroinflammation affect signaling pathways leading to human blood-brain barrier (BBB) dysfunction during HIV infection is incompletely understood. We previously demonstrated that the signal transducers and activators of transcription-1 (STAT1) signaling play an integral role in HIV-1 induced BBB damage and is relevant to viral neuropathogenesis. Here, we report that HIV-1 infection activates both STAT1 and STAT3 at serine-727 (S727) in human brain microvascular endothelial cells (HBMEC), and induced promoter activity of the interferon-stimulated response element (ISRE)/interferon γ-activated sequence (GAS).

The STAT1 inhibitor, fludarabine (FLUD), diminished HIV-1 induced ISRE/GAS promoter activity in HBMEC. Furthermore, FLUD, the mitogen activated protein kinase kinase (MEK) inhibitor (PD98059) and the phosphoinositide-3-kinase (PI3K) inhibitor (LY294002) prevented HIV-induced activation and expression of STAT1 and STAT3, significantly diminished HIV-1-induced ISRE/GAS promoter activity, decreased HIV-1-induced down-regulation of the tight junction proteins ZO-1 and ZO-2, and diminished HIV-1-induced monocyte adhesion and transendothelial migration. In addition, HIV-1 infection did not phosphorylate janus kinases (JAK), but induced activation of the phosphoinositide-dependent kinase-1 (PDK1), the serine-theronine protein kinase (AKT), both downstream effectors of PI3K, and the p44/42 MAPK. These data suggests a crosstalk between STAT1, MEK and PI3K pathways in HIV-1-induced BBB dysfunction.

## P220
### The elusive role of CCL2 in HIV neuropathogenesis: Migrating from chemotaxis to neuroprotection

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CCL2, also known as monocyte chemoattractant protein-1 (MCP-1), plays a critical role in leukocyte recruitment and activation. In the present study we identify an additional role for CCL2 that of neuroprotection against HIV-1 Tat toxicity in rat primary midbrain neurons. Furthermore, we report the involvement of transient receptor potential canonical (TRPC) channels in CCL2-mediated neuroprotection. TRPC are Ca2+-permeable, nonselective cation channels with a variety of physiological functions. Blockage of TRPC channels resulted in suppression of both CCL2-mediated neuroprotection and intracellular Ca2+ elevations. Parallel but distinct ERK/CREB and Akt/NF-κB pathways were involved in the CCL2-mediated neuroprotection. Blocking TRPC channels and specific down-regulation of TRPC channels 1 and 5 resulted in suppression of CCL2-induced ERK/CREB activation, but not Akt/NF-κB activation. In vivo relevance of these findings was further corroborated in wild type and CCR2 knockout mice. In the wild type but not CCR2 knockout mice, exogenous CCL2 exerted neuroprotection against intrastriatal injection of HIV-1 Tat. These findings clearly demonstrate a novel role of TRPC channels in the protection of neurons against Tat through the CCL2/CCR2 axis.

## P221
### Human herpesvirus associated with smell loss may reflect infection of resident olfactory glial cells (olfactory ensheathing cells (OEC)

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Viruses have been implicated in the development of neurodegenerative diseases such as Alzheimer's, Parkinson's diseases and Multiple Sclerosis. The presence of smell loss and olfactory bulb pathology has been demonstrated in the early stages of Alzheimer's and Parkinson's diseases. In addition, magnetic resonance imaging studies have demonstrated correlation of white matter lesions in the olfactory bulb of MS patients associated with smell dysfunction. It is, therefore, of interest to examine whether virus infection could be associated with smell dysfunction in these neurologic disorders. Since, CNS infection of the human herpesvirus-6 (HHV-6) has been linked to a wide variety of neurologic disorders including encephalitis, mesial temporal sclerosis, and multiple sclerosis, we examined the prevalence of HHV-6 detection in nasal mucosa, saliva, serum, and urine of 120 patients with smell loss. Our results demonstrated that >30% of the patients with post-infectious smell dysfunction had HHV-6 DNA in their nasal mucus and/or saliva but not serum or plasma. In contrast, none of the patients with traumatic head injury associated smell loss had detectable HHV-6 DNA. We will extend these observations to individuals with apparently normal smell function including healthy normal control nasal mucosa and patients with multiple sclerosis to determine the extent and specificity of HHV-6 infection. As these results suggested that a herpesvirus infection maybe, in part, associated with smell loss in a subset of patients, we investigated whether this virus can infect cells within the olfactory system. Olfactory ensheathing cells (OEC) are specialized glial cells that are found only in the olfactory system that retain exceptional plasticity and support olfactory neurogenesis. We will present immunohistochemical data characterizing these cells and will demonstrate that these cells are highly permissive for HHV-6 infection. Our results demonstrate that, like other CNS glia, OECs are readily infectable by HHV-6 suggesting a mechanism whereby a human herpesvirus may exploit the olfactory system to gain access to into the CNS by infection of unique resident olfactory glial cells.

## P222

### Reactivation of Human Herpesvirus after Natalizumab Treatment in Patients with Multiple Sclerosis

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Background: Natalizumab is an α4-integrin antagonist effective at modulating migration of inflammatory cell infiltrates into the central nervous system (CNS) and has been shown to have clinical benefits as a therapy in treating Multiple Sclerosis (MS). Mechanistically, it has been suggested that Natalizumab may decrease the migration of auto-reactive pathogenic T cells into the brain. However, global inhibition of inflammatory response into the CNS may be associated with reduced immune surveillance of normal, healthy responses that may result in reactivation of latent viruses present in the CNS. Indeed, while Phase II clinical trials demonstrated benefits of Natalizumab in reducing CNS inflammation, treatment was unexpectedly associated with development of Progressive Multifocal Leukoencephalopathy (PML) in small subsets of treated patients. PML is an opportunistic infection caused by reactivation of JC polyomavirus. It is possible that JC virus reactivation may be associated with reduced immune surveillance subsequent to Natalizumab treatment. As there are many neurotropic viruses that are capable of targeting and invading the CNS, it is reasonable to hypothesize that the viral reactivation in the brain post-therapy may not be exclusively associated with JC virus. The two human herpesviruses that have garnered significant attention in the etiology of MS are EBV and the human herpesvirus-6 (HHV-6). Therefore, it is of interest to examine the association of Natalizumab treatment with reactivation of these agents.

Methods: Sera and CSF from MS patients treated with Natalizumab and non- Natalizumab treated patients were examined for evidence of herpesvirus antibodies using a novel quantitative electrochemiluminescence assay developed in our laboratory, initially for HHV-6 and more recently for EBV using cell lysates from an EBV transformed marmoset B-cell line (B95-8) as antigens to detect specific EBV IgG and IgM. In addition, presence of herpesvirus DNA sequences was evaluated by PCR techniques.

Results: We have recently reported that HHV-6 DNA sequences were specifically detected in a subset of Natalizumab treated MS patients but not in controls. In addition, significantly elevated anti-HHV-6-IgG titers were observed in Natalizumab treated MS patients ($p = 0.04$) compared to un-treated MS patients. To test if these observations were specific for HHV-6, we will extend these observations to EBV using the same ECL ELISA platform designed for HHV-6. This assay has been shown to be specific for EBV in which robust anti-EBV IgG titers were demonstrated in human sera.

Conclusion: In this small cohort of MS patients, our results suggested that Natalizumab plays a role in reducing CNS immune surveillance and contribute to herpesvirus reactivations.

## P223

### Cytomegalovirus associated neuroAIDS is an infrequent Opportunistic Infection in severely Imunosupressed patients in Colombia

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Background: Cytomegalovirus (CMV)-associated neurological complications are frequent AIDS-defining conditions in HIV infected patients. However, the diagnosis of CMV infection of the brain or the spinal cord represents a major challenge and most of the time a presumptive diagnosis is based on clinical signs, neuroimaging and virological markers. Detection of CMV DNA in the cerebrospinal fluid (CSF) is a useful tool to confirm the diagnosis of CMV-induced CNS disease. However, viral DNA in the CSF may also represent a marker of advanced immunodeficiency and enhanced systemic CMV replication rather than CMV infection of the CNS. To determine the CMV DNA and viral load status in the CSF during HIV/AIDS advanced disease, we evaluated free non-cell associated CMV DNA in the CSF of AIDS patients with and without CNS opportunistic infections (OIs).

Materials and Methods: In a prospective clinical study of opportunistic infections in neuroAIDS, CMV DNA was assessed by a sensitive nested PCR (analytical sensitivity = 1 copy) in CSF samples from 92 patients with AIDS-associated CNS complications (OIs = 82 and non-OIs = 10) and 10 AIDS patients without neurological disease. The OIs cases included three patients with clinical and neuroimaging findings suggestive of CMV-associated neurological disease and the rest were diagnosed with other OIs. CMV DNA positive cases by nested PCR (including the 3 cases of CMV related neurological diseases) and 15 additional cases with OIs (5 cases of Cryptoccocal meningitis [CM], 5 cases of Toxoplasma encephalitis [TE] and 5 cases of tuberculous meningitis [TBM]) were re- evaluated by real time PCR (Analytical sensitivity 100 copies/ml) to quantify the CMV viral load in CSF/serum in paired samples.

Results: Only five of the 102 AIDS cases examined (4.1%) were positive for CMV DNA in the CSF. All three suspected clinical cases of CMV were positive while the other two positive samples corresponded to a case with a bacterial meningitis (Listeria monocytogenes) and a Toxoplasmic encephalitis case. Only the 5 patients that were CMV positive by nested PCR assay in the CSF were also positive by the quantitative real time PCR assay test. CSF and serum samples were both positive with viral loads between 2.3–6.3 Log/mL, a finding that suggests that CMV DNA in the blood is an indicator of CMV systemic reativation and is correlated with its detection in the CSF. However, CMV viral load was higher in the CSF compared with the serum levels only in one case of CMV arachnoiditis and in the case of Toxoplasmic encephalitis. None of the samples from the additional cases of CM, TE or TBM which tested CMV negative in the qualitative PCR were positive for CMV DNA when re-evaluated by the quantitative Real time PCR assay.

Conclusion: Detection of cell-free CMV DNA or active CMV replication in the CSF or blood is a non frequent event in HIV advanced disease or NeuroAIDS opportunistic infections despite of the profound systemic immunosupression. Therefore, CMV DNA presence in the CSF represent a good virological marker of active CMV-related CNS disease in AIDS patients. Because CMV DNA detection in the CSF was always accompanied by detection of CMV in the serum, the finding of CMV DNA in serum suggest that serum testing of CMV DNA may serve as a surrogate marker of active replication of CMV in the CNS in AIDS patients.

## P224
### RNA Helicase DDX3 but not RanBP2 is critically involved in HIV Rev-function in Astrocytes

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HIV infects cells of the central nervous system (CNS) and causes a severe disease known as HIV dementia. HIV mainly infects microglia and macrophages; however, astrocytes, the most abundant cell type in CNS, are limitedly infected. Several studies have shown that low-level of HIV replication in astrocytes is due to the inefficient nucleocytoplasmic Rev-shuttling, which then promotes retention of Rev-dependent HIV-mRNAs (unspliced or partially spliced) in nucleus. In this study, we sought to elucidate the molecular mechanism underlying the inefficient Rev-function in astrocytes. Rev-function was verified by Rev-reporters in astrocytes using either VSV-pseudotyped lentiviral vector expressing Rev or VSV-HIV to express Rev in a most efficient manner. We profiled astrocytes for dead box RNA helicases—DDX1 and DXX3 implicated in Rev-function, in combination with RANBBP2. On western blotting, DDX1 and RANBP2 were found to be equally expressed in astrocytes and non-astrocyte cell types, but DDX3, a nucleocytoplasmic shutting protein which is critical in normal functioning of Rev, was barely detectable in astrocytes compared to other cell types. Further, to implicate DDX3 in rev-functioning, we ablated endogenous DDX3 by antisense DDX3 in astrocytes. DDX3 ablation resulted in blockage of Rev-function and subsequently curtailed HIV-replication, which was reversed by over-expression of wild type DDX3. We have also investigated whether RanBP2, a component of nuclear pore complex that spans the nuclear envelope, regulates nucleocytoplasmic HIV-mRNA trafficking. Using RNAi method, we knocked down RanBP2 in astrocytes and found that Rev-function was unaffected in RanBP2 deficient astrocytes.

Conclusion: Our study suggests that the DDX3 is expressed critically low (natural) but enough for full Rev-function in astrocytes and RanBP2 may not be involved in Rev-function. Hence, it is concluded that HIV regulatory protein Rev performs its nucleocytoplasmic HIV-mRNA transportation efficiently in astrocytes.

## P225
### Interferon Lambda Inhibits Herpes Simplex Virus Type 1 Replication in Human Neuronal Cells

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Herpes simplex virus type I (HSV-1) can establish latent infection in the nervous system. Although type I interferons (IFN-alpha and -beta) and type II interferon (IFN-gamma) have the ability to inhibit HSV-1 infection, the anti-HSV-1 of interferon lambda (IFN-lambda), a novel member of IFN family, remains to be determined. We thus investigated whether IFN-lambda has the ability to inhibit HSV-1 infection of human neuronal cells. Human neuronal cells (NT2-N and CHP212 cells) expressed IFN-lambda receptor (IL-10R) at both mRNA and protein levels. When exposed to HSV-1, these cells became infected. However, HSV-1 infection of human neurons was significantly suppressed by IFN-lambda treatment as evidenced by the reduced HSV-1 DNA synthesis and expression of HSV-1 antigens. This IFN-lambda-mediated inhibition of HSV-1 was specific as the antibody to IL-10R could partially block the IFN-lambda action. Our further investigation showed that IFN-lambda had the ability to activate the expression of Toll-like receptors 3 and 9 (TLR3 and 9) as well as IFN regulatory factor 7 (IRF7), leading to upregulation of endogenous IFN-alpha expression in the neuronal cells. These findings

provide the first experimental evidence that IFN-lambda may have therapeutic potential for treatment of HSV-1 infection in the central nervous system.

## P226

### Activation of Toll-like Receptors Inhibits Herpes Simplex Virus-1 Infection of Human Nueronal Cells

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Toll-like receptors (TLRs) play an essential role in initiating intracellular type I interferon (IFN)-mediated innate immunity against viral infections. We examined whether human neuronal cells (primary human neurons, NT2-N and CHP-212) express TLRs and mount type I IFN-mediated innate immunity against herpes simplex virus-1 (HSV-1) infection. Human neuronal cells expressed TLR family members 1–10 and IFN-alpha/beta. The activation of TLR3 or TLR8 by double-stranded RNA (poly I:C) or single-stranded RNA (ssRNA) induced IFN-alpha/beta expression. In addition, HSV-1 infection of human neuronal cells induced IFN-alpha expression. Investigation of the mechanisms showed that poly I:C treatment enhanced the expression of IFN regulatory factors (IRFs) 1, 5, 7 and 9, and ssRNA treatment induced the expression of IRFs 1 and 5 in human neuronal cells. Importantly, the activation of TLR3 and TLR8 by poly I:C and ssRNA prior to HSV-1 infection decreased the susceptibility of the neuronal cells to infection. These observations indicate that human neuronal cells possess intracellular TLR-mediated innate immune protection against HSV-1 infection.