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P1

Replication independent functions of Tat may contribute to hpTau

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Even though HIV does not infect neurons, extracellular Tat is internalized by neurons, localizes to the nucleus and can interact with numerous neuronal proteins to impact a variety of cellular processes. The serine/threonine-protein phosphatase PP1 (PP1) is among the numerous host proteins to which Tat can bind. PP1-alpha contributes to the regulation of AKT and GSK3-beta activity and can de-phosphorylate the microtubule binding protein Tau at residues associated with tauopathy. PP1-alpha's activity is regulated in part by its interaction with the PINCH protein. Previous studies report the robust expression of PINCH in the neurons of HIV patients, whereas, PINCH is nearly undetectable in healthy adult brain. Given the significant level of overlap among PINCH, PP1-alpha and kinases with hyperphosphorylation of Tau (hpTau) and Tat, we hypothesized that replication independent functions of Tat may contribute to hpTau. To assess the potential importance of Tat in the PP1-alpha/PINCH pathway in neurons, we investigated these pathways in HIV patient brains, in HIV Tat transgenic mice and in neurons in vitro. Our results show that PP1-alpha levels are increased and co-localize in the nucleus of neurons in HIVE patients compared to HIV+ patients with out encephalitis. PINCH and PP1-alpha are also increased in the brains of Tat transgenic mice. Our in vitro studies indicate that Tat-mediated changes in PINCH-PP1-alpha and downstream AKT-GSK3-beta signaling are responsible in part for hpTau formation in HIV CNS disease.

P2

Astrocytes Activation Induced by a Neuroadapted Dengue Virus Strain

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Little is known about the cellular and molecular mechanisms involved in neurological symptoms during dengue infection. It seems that astrocyte cell activation could play an important role, as occurred in other neurotropic flavivirus infections. The aim of this work was to study the in vitro response of astrocytes during an infection with a neuroadapted dengue virus strain (D4MB-6). Primary astrocytes cell cultures obtained from 7-day-old Balb/C mice with 95 % of purity were inoculated at MOI:1 with dengue virus serotype 4 (DENV-4) or D4MB-6 during 24, 48, or 72 h. Then, the cells were processed using immunofluorescence assay (IFI) to evaluate the expression of GFAP, and DENV envelope protein. The results showed that the astrocytes were not susceptible to the infection with neither of the evaluated virus (infection <1.0 %). However, the astrocytes changed their morphology, showing long and numerous cytoplasmic extensions mainly in the D4MB-6 infected cultures, suggesting a glial activation

process. It was also observed a higher proportion of cell mitosis at 24 h and chromatin condensation at 72 h in infected cultures. TUNEL assay did not demonstrate an increase in apoptotic cells regarding infected and non-infected cultures. Astrocyte proliferation was only corroborated by MTT assay at 48 h in D4MB-6 with an increase of 10 % in infected cultures but there were not changes in mitochondrial metabolic rate measured by resazurine assay. Concomitantly D4MB-6 infection induced a 50-fold increase in GFAP transcripts at 24 hpi with an evident raise in protein fluorescence intensity. We could suggest that astrocytes cells are activated by the neuroadapted virus, producing a response, leading to physio- or morphological nervous system damage. Future studies regarding this topic should be conducted.

P3

HIV Infection and Immortalization of Freshly Isolated Human Microglial Cells

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During HAART, microglial cells become a reservoir of HIV in brain. However, reliable methodologies and cellular models to study molecular events involved in HIV infection of microglia and in establishment/emergence of latency are practically non-existent. Fresh CNS cortical tissue from patients undergoing brain surgery was dissociated with the Neural Tissue Dissociation Kit (Miltenyi Biotec), and CD11b+cells cultured for 5 days prior to infection with replication competent R5 HIV (AD8gNef-GFP) particles. After virus removal, cells were insulted with Raltegravir, followed by treatment with TNF-alpha. Fixed/permeabilized cells were stained for actin and nucleus, and Deltavision® pictures were taken to detect HIV expression (GFP), stained actin, and nuclei. Primary microglial cells were also infected with VSVG SV40-puromycin virus by spinoculation to induce immortalization. Transformed cells were infected with VSVG-GFP-HIVs, and latency tested with TNF-alpha, LPS, or IL-1beta. The yield of CD11b+cells from brain tissue was between 1,300 and 3,000 cells per mg. Observational analysis indicated that these cells had a typical ramified morphology. Incubation with R5 HIVs resulted in HIV expression 7 days post-viral exposure in a fraction of cells. Exposure of primary microglia to SV40 virus resulted in cell expansion 6 to 8 weeks post-immortalization. A CD11b+clonal population with ramified morphology was infected with VSVG-GFP-HIV and monitored for HIV expression. Our initial studies indicate that HIV expression is induced in by typical pro-inflammatory stimuli. We show here that freshly isolated human microglia from brain can be productively infected with HIV, suggesting a key role of HIV-infected resident cells in HIV-related neurologic disorders. Primary microglia can also be immortalized and infected with pseudo-HIVs to study molecular events associated with HIV entry and emergence from latency.

P4

Detection of Human T-cell Lymphotropic Virus Type I proteins in exosomes from HAM/TSP patient CSF by novel nanotrap technology

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There has been increasing evidence for the role of microvesicles (MV) in CNS inflammation and viral disease. HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neuroinflammatory disease that affects a subset of virus-infected infected individuals. This disorder is immunopathologically mediated since virus-specific immune cells can be found in both the CSF and in the CNS of patients although HTLV-I virus has been difficult to isolate in CNS resident cells. The possibility of transfer of viral proteins via MV from viral reservoirs to uninfected cells in the absence of virus is an intriguing mechanism by which this might occur. Recently, HTLV-1 tax proteins have been shown in exosomes (EX), a type of MV, from HTLV-1 infected cell lines (Jaworski et al., 2014). Therefore, we examined if similar MVs were present in the CNS of HAM/TSP patients, and if these MVs also carried HTLV-1 proteins and genes. Using a novel nanotrap (NT) technology, exosomes containing HTLV-1 tax were successfully isolated specifically from HTLV-1 infected cell lines. Moreover, CSF from HAM/TSP patients but not from HTLV-1 seronegative multiple sclerosis (MS) patients, also demonstrated exosomes that were HTLV-I tax positive by Western blot. These results suggest the possibility that HTLV-1 proteins present in virus-free CSF can be a potential source of antigen in an inflammatory neuropathological disease. Given the implication that regulatory T cells (Tregs) and other immune subsets are prolific producers of exosomes (Okoye et al., 2014), we are investigating the production of MVs and exosomes in ex vivo culture of HAM/TSP peripheral blood mononuclear cells (PBMCs) and virally-transduced normal donor (ND) PBMCs after the introduction of HTLV-1 genes.

P5

HIV-1 Tat genetic polymorphisms associated with neurocognitive impairment impact NMDA receptor docking

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The current studies seek to identify and characterize genetic sequence variation within HIV-1 Tat on the basis of neurocognitive impairment and anatomical source, at the nucleotide, codon, and amino acid level. HIV-1 Tat sequences were obtained from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from autopsied brain tissue regions obtained from the National NeuroAIDS Tissue Consortium (NNTC). Sequences acquired from the Drexel Medicine CARES Cohort were amplified from PBMCs, while NNTC samples were amplified from six regions of the brain in addition to the spleen. Tat nucleotide sequences were translated and aligned to the HXB2 HIV-1 reference genome in order to compare sequence similarity across anatomical compartments and degree of neurocognitive impairment. Multiple sequence alignments were evaluated for evidence of episodic positive selection using a mixed effects model of evolution (MEME) and evolutionary conservation using the bioinformatics tool ConSurf in order to elucidate positions of structural and functional importance. These phylogenetic analyses were followed by an in-depth evaluation of the amino acid diversity at each position, with special consideration towards residues with altered side chain chemical properties. Overall, this study identified altered amino acid usage within multiple domains of Tat using a Euclidean distance metric when comparing between impaired and non-impaired individuals and PBMC and brain tissue. Of greatest interest were the variants C30S and C31R, which were enriched in brain tissue and non-impaired individuals, respectively. These polymorphisms were computationally modeled for docking with the NMDA-receptor and the P-TEFb complex. Overall, these analyses have resulted in the identification of distinct patterns of amino acid usage in relation to neurocognitive impairment and CNS compartmentalization, which may contribute to the excitotoxicity of Tat and HIV-1 neuropathogenesis. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785, and R01 NS089435.

P6

Plasma MicroRNA profiling predicts HIV-Associated Neurocognitive Disorder

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Background: HIV-associated neurocognitive disorder (HAND) is common, affecting 30–50 % of HIV-infected patients despite the availability of effective antiretroviral therapy. The development of HAND is influenced by several factors including altered host and viral gene expression. Host-encoded microRNAs (miRNAs) regulate both host and viral gene expression. Thus, host miRNAs could contribute to the pathogenesis of HAND but also serve as biomarkers of diagnosis and prognosis as well as indicators of underlying disease mechanisms of HAND. Herein, we investigated plasma microRNA profiles among HIV/AIDS patients with and without HAND. Methods: Plasma microRNAs was measured in age and sex-matched HAND (n=22) or nonHAND (n=25) patients (Cohort 1) by array hybridization (Affymetrix 3.0 miRNA genechip). Two software packages (Affymetrix Expression Console and Gene Spring) were used to normalize data and determine differentially expressed miRNAs. The median of each probeset in the HAND or nonHAND was calculated after normalization and differentially expressed miRNAs were identified. A second cohort (Cohort 2) consisting of prospectively recruited age- and sex-matched HAND (n=12) and nonHAND (n=12) patients was used to validate the miRNA profile in Cohort 1. Results: Analyses of comparative expression identified 13 miRNAs in Cohort 1 that were up-regulated with a fold change (FC) of greater than 2 in the HAND group compared to the nonHAND group with one or both computational tools (p<0.05). Analysis of Cohort 2 confirmed up-regulation of 3 miRNAs identified in Cohort 1. In a univariate

logistic regression analysis education level, CD4 and nadir CD4 T cell levels and these three miRNAs predicted HAND status based on p-values and odd ratios. Prediction of HAND status by the individual miRNAs was more robust than that of CD4 nadir CD4 T cell levels. These miRNAs target brain development and apoptosis genes. Conclusions: Our findings revealed differential expression of three cell plasma-derived miRNAs in HAND versus nonHAND patients. These results suggest that plasma miRNAs might be used as biomarkers for HAND and also provide insights into the underlying disease mechanisms.

P7
HIV protein gp120 enters neurons by a dynamin-dependent endocytosis

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The neuronal dysfunction and loss caused by the HIV envelope protein gp120 in rodents are similar to those observed in HIV positive subjects with dementia. Therefore, gp120 has been used to study the molecular and cellular mechanisms underlying HIV-mediated neurotoxicity. Recombinant T-tropic gp120 or viral-derived gp120 is endocytosed by neurons and axonally transported toward cell bodies. These events occur within 30 min of exposure to gp120. Neurons internalizing gp120 undergo axonal retraction within 6 h before showing signs of apoptotic cell bodies. Thus, the endocytotic process appears to be crucial for gp120-mediated cell death. Endocytosis, which is also important for HIV entry, is a dynamin-dependent process, and is crucial for the internalization of CXCR4, the main co-receptor for T tropic gp120. Thus, we have used a dominant-negative dynamin mutant (K44A) to obtain further evidence that gp120 is endocytosed. K44A reduced significantly gp120-mediated endocytosis of CXCR4 as well as intracellular gp120 immunoreactivity in neurons, supporting the hypothesis that gp120 is internalized by a mechanism that involve dynamin-1. To examine whether endocytosis of gp120 is crucial for its neurotoxic effect, we blocked endocytosis by dynasore, a small molecule inhibitor of the dynamin GTP-ase activity. We have found that dynasore blocks both gp120 internalization as well as neurotoxicity. Our data suggest that cellular factors involved in membrane/receptor trafficking are responsible for gp120 endocytic entry and toxicity.

P8
Understanding the pathways for Varicella Zoster Virus latency and reactivation

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Varicella Zoster Virus (VZV) is a neurotropic alphaherpes virus. The primary infection by VZV is chicken pox (Varicella), followed by the virus going dormant (latent) in the sensory, cranial and autonomic ganglia along the entire neuraxis. Viral reactivation, mainly in the elderly, can cause zoster or shingles, which can also result in postherpetic neuralgia, meningoencephalitis (inflammation of the brain), myelopathy (spinal cord infection) and sometimes blindness. We are currently investigating the molecular mechanisms responsible for establishment of viral latency, and reactivation. Techniques such as qPCR and next generation sequencing are currently being employed to monitor viral DNA replication and transcription in the latently infected human ganglia. Understanding the state of viral transcription in these latently infected ganglia will subsequently help us design therapeutic agents against viral reactivation.

P9
MEMRI Assessment of Neuroadaptations Caused by Chronic Nicotine Exposure in Rats

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Introduction: Tobacco smoking is a major public health problem. Smokers, who attempt to quit find maintenance of long-term abstinence difficult and experience relapse. Successful treatment strategies for smoking cessation are limited. A major reason limiting the development of effective smoking cessation strategies is the lack of understanding of neuroadaptations following chronic exposure to nicotine. Therefore, it is essential to understand the neural mechanisms underpinning nicotine dependence. We used Manganese-enhanced MRI (MEMRI) to assess the neuronal responses to chronic nicotine exposure in mesolimbic brain regions. Methods: Male Wistar rats received continuous nicotine infusion (3.0 mg/kg/day) for 7 days using mini-pumps implanted

subcutaneously. A control group of rats received PBS infusion. MEMRI was performed at 24 h after the termination of nicotine/PBS infusion. $MnCl_2$ solution (50 mM) was injected daily for 4 days i.p. on days 4–7 of nicotine/PBS infusion with a daily dose of 60 mg/kg. MRI data were acquired 24 h after the last $MnCl_2$ administration on Bruker Bioscan 7 Tesla/21 cm scanner. T1-wt images were calibrated using T1 values calculated from T1 mapping scan to minimize the effect of MRI system variations on MRI signal. T1-wt images were then registered for pixel-to-pixel t tests between PBS controls and nicotine infused rats. ROI analysis was performed to compare signal intensity on prefrontal cortex, thalamus and striatum. Results: The ROI analysis showed significant increase in signal intensity on striatum (p=0.037) and thalamus (p=0.038) in nicotine infused rats compared to controls. Prefrontal cortex showed the trend of signal increase (p=0.068) in nicotine infused rats. Discussion: The brain regions showing neuronal activation are in the mesolimbic pathway and consistent with previous in vivo studies. MEMRI signal enhancements demonstrate neuronal adaptations associated with nicotine addiction in rodents that reflect the clinical manifestations as they are seen in humans.

P10

Interferon-gamma prolongs survival of varicella zoster virus-infected human neurons in vitro

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In vitro infection of human neurons with VZV at low multiplicity of infection does not result in cell death within 14 days post-infection, despite production of infectious virus. We showed that a cytopathic effect (CPE) ultimately developed in VZV-infected neurons by 28 dpi but the CPE, as well as viral DNA accumulation, VZV transcription and virus production were all inhibited by interferon gamma.

P11

Interactive effects of HIV-1 and morphine on the proliferation of primary hNPCs: role of μ-opioid receptor (MOR) splice variants

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Opiate drug abuse has been shown to exacerbate neuroAIDS, presumably through effects mediated by μ-opioid receptors (MOR) expressed by CNS cells. To date, 21 human MOR splice variants have been identified, but little is known about their specific roles, if any, in MOR-related signaling. We previously demonstrated selective expression of MOR-1 and MOR-1 K splice variants in primary CNS cells and in HIV-1 infected brain samples, suggesting their differential roles in HIV-mediated neuropathology. Neural progenitor cells (NPCs), the precursors of all CNS neurons and glia, were found to express MOR and MOR-1 K, and are vulnerable to HIV-1/Tat and morphine interactions. Current studies investigated 1) the interactive effects of HIV-1 and morphine on the proliferation/differentiation/survival of primary human NPCs (hNPCs) and, 2) whether HIV-1 regulated the MOR splice variants produced by these cells. As a primary hNPC model, we have developed a Sox2- and nestin-enriched hNPCs culture derived from 10-week fetal brain tissues. Treatment with supernatant from HIV-infected PBMCs (HIVsup) at various concentrations (5.0–500 pg/mL HIV p24) significantly decreased the percentage of BrdU+ hNPCs at 12–48 h. Morphine co-exposure further enhanced the HIVsup effect on hNPCs. In addition, HIVsup treatment (50, 500 pg/mL HIV p24), in combination with morphine, also significantly increased hNPC doubling time from 43.57±2.52 h to 55.92±3.17 h. By propidium Iodide flow cytometry and Dead RED staining analysis, no evidence of cell death was observed in hNPCs exposed to all levels of HIVsup±morphine. Furthermore, the differentiation of hNPCs was influenced by 12 days exposure to HIVsup±morphine in appropriate differentiation medium. Lastly, we found that MOR-1 and MOR-1 K were selectively up-regulated in immortalized and primary hNPCs exposed to HIVsup. Our findings provide initial evidence of HIV-morphine interaction in primary hNPCs and suggest that specific MOR splice variants may respond differently to HIV-1. Supported by DA024461.

P12

Human Polyomavirus JC replication in multiple sclerosis patients treated with Natalizumab: viral markers as tools for an early PML diagnosis?

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Background: The human polyomavirus JC (JCV) is the aetiological agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML). PML onset represents a growing concern about the safety of Natalizumab, a biological drug used to treat multiple sclerosis. The precise mechanism by which this medication may have facilitated the PML pathogenesis is a matter of debate. On these bases, JCV infection monitoring, the possible noncoding control region (NCCR) variations and the genotyping analysis of the Viral Protein 1 (VP1) were investigated. Materials and methods: JCV DNA was investigated in biological samples collected at the enrollment (t0) and every 4 months (t1, t2, t3) for 1 year and in the second year of treatment (t4, t5), using a quantitative PCR. PCR products corresponding to JCV NCCR and VP1 were sequenced. JCV-specific antibodies were assessed by STRATIFY JCV® in serum at t0 and t3. Results: After 1 year of Natalizumab, a significant association was found between patients showing viruria and antibodies in the serum respect to those patients with no JCV-specific antibodies. Conversely, at t4 the viremia was significantly prevalent in comparison to viruria. Regarding NCCR, sequencing revealed the presence of 4 rearranged structures in peripheral blood mononuclear cells belonging to patients treated with 12 Natalizumab infusions and STRATIFY JCV® positive at t3. In particular, two of them were compatible with the neurotropic variant found in a PML patient. Finally, VP1 sequence analysis showed the prevalence of the genotypes 1A, 1B, and 4. Conclusions: In conclusion, for a more accurate PML risk stratification, testing JC viruria seems to be useful to identify patients who harbor JCV with an undetectable specific humoral immune response. It may also be important to study NCCR rearrangements since they could generate neuro-invasive viral variants increasing the risk of PML onset.

P13
Epigenetic regulation of polyomavirus JC involves acetylation of specific lysine residues in NF-kappaB p65

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by neurotropic polyomavirus, JC (JCV), a virus that causes lytic infection of CNS glial cells. After primary infection, JCV is controlled by the immune system but virus persists asymptomatically. Rarely when immune function is impaired, it can re-emerge and cause PML. The mechanisms of JCV persistence and reactivation are not well understood but our earlier work implicated epigenetic control by protein acetylation since histone deactelylase inhibitors such as trichostatin A (TSA) strongly stimulate JCV transcription. Since both TNF-alpha and TSA activate JCV transcription via the same unique NF-kappaB site in the JCV control region, we investigated a role for acetylation of NF-kappaB in JCV regulation. A site-directed mutagenesis strategy was employed targeting the known lysine acetylation sites of NF-kappaB p65: K218, K221 and K310. We individually mutated each lysine to arginine, which cannot be acetylated and retains a positive charge like lysine. K218R and K221R were impaired transactivation of JCV early promoter transcription either alone or combined with TSA treatment or coexpression of acetyltransferase transcriptional coactivator p300 but K310R was largely without effect. Mutation of lysine to glutamine gives mutants with a negative charge like acetyl-lysine. However, K218Q and K221Q showed impaired activity and only K310Q showed enhanced transactivation. NF-kappaB acetylation can regulate several aspects of the process of activation including complex formation with IkappaB in the cytoplasm, translocation to the nucleus and DNA binding and transcriptional activation. Cell fractionation studies revealed that the mutants had no defect in translocation to nucleus whereas gel shift studies revealed reduced binding to the JCV NF-kappaB site. Thus acetylation regulates NF-kappaB p65 activity at the level of DNA binding and transcriptional activation.

P14
Degradation of Polyomavirus JC T-antigen by Stress Involves the LIP Isoform of C/EBPbeta

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Endoplasmic reticulum (ER) stress is caused by the accumulation of misfolded or unfolded proteins in the lumen of the endoplasmic reticulum. CCAAT/enhancer binding proteins are one of the cellular proteins whose expression is upregulated during ER stress. Previously, we have identified C/EBPbeta isoforms, especially LIP, as a negative regulator of polyomavirus JC (JCV), the causative agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML). Here, we show that the induction of ER stress by thapsigargin increase the expression of endogenous LIP and the degradation of JCV T-antigen in a JCV-transgenic mouse tumor cell line. Our results also revealed that overexpression of LIP significantly reduced the level of T-Ag and this effect is reversed upon siRNA-mediated silencing of LIP. Immunoprecipitation/Western blot experiments indicated that LIP interacts with T-antigen directly. Treatment of cells that overexpress LIP with MG115, a proteasome inhibitor, partially rescued LIP-mediated degradation of T-antigen. Our observations point to a role of LIP in ER stress regulation of T-antigen stability and may open a new avenue to study host-virus interaction during ER stress.

P15
Microglial cells infection with a neuroadapted dengue virus strain D4MB-6

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Microglial cells are the main source of inflammatory mediators and cytotoxic molecules associated with nervous tissue damage in many pathological processes including viral infections. The aim of this study was to evaluate the susceptibility to infection of microglial cells with a neuroadapted Dengue virus (DENV) strain named D4MB-6. For this purpose, microglial primary cultures were purified from 3-days old Balb/C mice brains using two previously reported protocols with some modifications. These protocols allowed us to obtain a mixed culture (astroglia / microglia) which was treated with a diluted trypsin solution to remove astrocytes, promoting adhesion of microglial cells, characterised by the typical phenotype of large nuclei and numerous elongated branches. To confirm the culture purity, cells were processed by indirect immunofluorescence (IFI) using antibodies to detect specific microglial markers (OX2R, CD11b and Iba-1). Ninety five percent of cells were positive for each one of the markers, which allowed us to confirm a high purity culture. Then, the cells were inoculated with the D4MB-6 virus and processed by IFI to detect E protein of virus at three times post-infection (24, 48, and 72 h). We found that 65 % of microglial cells were reactive for the viral protein at 24 and 48 h p.i., and this value increased to 77 % at 72 h p.i.. Additionally, we observed cell morphology variations like widened cytoplasms and shortened branches, suggesting cell activation. These preliminary results will allow us to characterize the microglia response to infection and to assess their role in neuronal damage induced by the D4MB-6 strain.

P16
Quantitative Detection of Human Herpesvirus-6 with Digital Droplet PCR in a Patient with Post-Transplant HHV-6 Encephalitis

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Objective: In this study, we investigated the relationship between quantitative human herpesvirus-6 (HHV-6) viral load and anatomical distribution using digital droplet PCR in a patient with HHV-6 encephalitis. Background: HHV-6 is a ubiquitous virus with a seroprevalence approaching 100 % in the general population; it also causes Roseola. Rarely this virus reactivates in immunocompromised individuals and can cause a severe limbic encephalitis characterized by seizures and personality changes. Diagnosis is typically made through evidence of CNS dysfunction in the presence of positive HHV-6 PCR in the CSF. However, PCR detection of HHV-6 is not well-standardized, which can be problematic in accurate diagnosis. In this study, a third-generation PCR technique, digital droplet PCR, was used for the accurate and quantitative detection in a patient with post-transplant HHV-6 encephalitis who subsequently went to autopsy. Design/Methods: Various

samples from different body compartments were collected from a patient with post-transplant HHV-6 encephalitis at multiple time points and at autopsy. DdPCR was performed on these samples for the detection and quantification of HHV-6 viral DNA. Results: High viral levels of HHV-6B were detected in blood, CSF, and bronchoalveolar lavage fluid at or shortly after time of diagnosis; these DNA levels decreased with treatment. However, at autopsy, very high levels of HHV-6B DNA were detected in the brain, particularly in the mesial structures such as the hippocampus and basal ganglia. Conclusions: HHV-6 viral DNA levels in the periphery or CSF may not correlate with ongoing active infection in the CNS, as there may be very low levels in the periphery, yet very high levels in brain tissue. In active HHV-6 infection, this virus is found in higher levels in the limbic and midline structures, which may correlate with clinical symptoms. DdPCR is a very useful tool in the accurate and precise quantification of HHV-6 viral DNA.

P17
Pilot Study of Raltegravir, an Integrase Inhibitor, in HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis

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HAM/TSP is a progressive myelopathy occurring in up to 3 % of HTLV-1 infected subjects. A high proviral load (PVL) is the main risk factor for HAM/TSP, as the risk of disease rises exponentially once the PVL exceeds 1 %. Currently there is no effective treatment for HAM/TSP. There is evidence that active HTLV-1 replication, through the retroviral life cycle with new virus integration, occurs in vivo and contributes to the total HTLV-1 PVL. Recently it was shown that Raltegravir could inhibit cell-free and cell-to-cell transmission of HTLV-1 in vitro. Given the substantial clinical experience in HIV-1 infection and its excellent safety profile, this agent is an attractive therapeutic option for patients with HAM/TSP. In this pilot study, we wish to determine the effects of Raltegravir, a clinically approved HIV-1 integrase inhibitor, on HTLV-1 PVL, viral gene expression, and immunologic markers in the peripheral blood and cerebrospinal fluid (CSF) in patients with HAM/TSP. In this 15 months single arm, open label pilot clinical trial, 16 subjects with HAM/TSP will receive Raltegravir 400 mg orally twice daily in an initial 6 months treatment phase, followed by a 9-month post treatment phase. To date, 11 patients have been enrolled, 6 have completed study drug, and 2 patients have completed the study. PVLs are assessed using droplet digital PCR from the PBMCs at 3 month periods, and from the CSF at baseline and at 6 months. While an overall downward trend was observed in PVLs after treatment, this did not reach statistical significance, and more samples are warranted. Additionally, preliminary immunologic studies in 2 patients who completed the trial have shown alterations in CD4+CD25+ T cells and in antibody secreting B cells in the CSF.

P18
Epigenetic Control of Transcriptional Changes Implicated in Defective Learning and Memory in HIV-infected Mice and Prevention by Valproic Acid Treatment

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Despite the success of antiretroviral therapy (ART) in largely preventing AIDS and dementia, many individuals on ART manifest milder neurocognitive diseases (HAND) that impair daily functions and worsen with age. There is no animal model to study mild HAND. We have shown that chimeric HIV (EcoHIV) can infect immunocompetent mice, enter and persist in the brain, replicate in microglia, and under some conditions induce extensive neuroinflammation. Here we report that EcoHIV-infected mice suffer symptomatic neurocognitive impairment (NCI) shown by impaired learning and memory in radial arm water mazes. NCI persisted in HIV-infected mice for 6 months and was absent from mice infected with MLV, the donor of the mouse tropic envelope in EcoHIV. Systems biology approaches were used to begin to investigate the neurobiological basis of HIV-NCI in mice. Genome-wide gene expression profiles of brain tissues from behaviorally impaired mice were compared with profiles of patients with HAND, revealing common dysregulation of pathways

controlling neuronal functions involved in learning and memory. Potential epigenetic control of these functions was broadly assessed by chromatin immunoprecipitation with H3K9m3 antibody, sequencing, and identification of promoters regulated by histone methylation. We found high correlations between chromatin hypermethylation and transcriptional dysregulation for key genes associated with synaptodendritic functions and, by extension, memory, including CaMK2a and NRGN. Reduced CaMK2a and NRGN proteins in brain tissues of EcoHIV-infected mice were confirmed by Western blotting. Immunohistochemical analysis of MAP2 revealed diffuse dendritic damage throughout the mid- and deep cortex, white matter, and putamen similar to the damage observed in patients with HAND. Treatment of infected mice with valproic acid prevented both NCI and down-regulation of selected synaptic genes and hypermethylation of their promoters, without altering HIV burdens in the brain. These findings establish EcoHIV-infected mice as a model for molecular and functional brain studies in HAND. MH083627, DA017618, DA037611

P19
Anti-viral activity of DAS181 against the human polyomaviruses JC and BK

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Background: The human polyomaviruses, JC virus (JCV) and BK virus (BKV), are ubiquitous in human populations and do not cause disease in healthy individuals. In immunosuppressed patients, active JCV infection in the central nervous system causes progressive multifocal leukoencephalopathy in immunosuppressed patients; whereas BKV causes nephropathy, hemorrhagic cystitis, and ureteral stenosis in kidney and bone marrow transplant patients. Currently, no treatment is available. Both BKV and JCV bind to sialic acid receptors for entry into cells. DAS181, a small molecule blocker of sialic acid receptor, is currently used in clinical trial to block parainfluenza and influenza entry. We hypothesize that DAS181 will prevent BKV and JCV from entering host cells and reduce viral replication. Methods: Cells were treated with a range of concentrations of DAS181 (0.1 nM to 1,000 nM) 1 day prior to infection. The human kidney epithelial cell line CCD1105 was infected with BKV and the astro-glial cell line, SVG-A, was infected with JCV separately at a MOI of 0.1. Infected cells were identified by immunofluorescent staining of the capsid protein VP1. Total DNA was extracted and viral DNA quantified by realtime PCR. Results: Immunofluorescent staining for BKV VP1 showed that DAS181 treatment significantly reduced BKV infection, with the largest effect observed in cells 2 days post-infection when treated with 10 nM of DAS181. Unexpectedly, DAS181 treatment did not influence the quantity of viral DNA in cells or supernatant. Likewise, a reduction of number of cells expressing JCV VP1 by immunofluorescent staining was also observed in JCV infected cells treated with DAS181. However, JCV viral load remained constant in cells and supernatant after treatment with DAS181. Conclusions: DAS181 treatment decreased the number of cells expressing BKV and JCV viral capsid proteins after in vitro infection. DAS181 may have the potential to block JCV and BKV entry into cells.

P20
HIV Tat-induced miR-9 released from astrocyte extracellular vesicles contributes to microglial migration: Implications for HAND

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Increased microglial activation and migration are contributing hallmark features of HIV-associated neurological disorders (HANDs). HIV-1 Tat protein is toxic for various CNS cells, while also playing a role in inducing microglial migration. Mechanisms by which Tat mediates its effects are just beginning to emerge. miRNA-mediated regulation of disease pathogenesis represents an evolving area of research that has ramifications for identification of potential therapeutic targets for various neurodegenerative disorders for which currently there exists no cure. The highly conserved brain enriched miR-9 plays critical roles in neurogenesis as well as axonal extension. Its role in microglial migration, however, remains poorly understood. The goal of the current study was to examine how HIV Tat mediates induction and release of miR-9, which in turn, regulates the cross-talk between astrocytes and microglia, thereby contributing to disease pathogenesis. Our findings demonstrated that HIV Tat exposure resulted in increased induction/release of miR-9 in the EVs isolated from astrocytes. MiR-9-enriched EVs, were in turn, taken up by the microglia, resulting in increased migration of these latter cells, as evidenced by increased migration in Boyden chambers. Treatment of microglia with Dotap liposomal formulations containing miR-9, resulted in increased microglial migration and reciprocally, formulations containing anti-miR-9 failed to mediate migration. Mir-9 mediated migration of microglia involved downregulated expression of the key target protein,

monocyte chemotactic protein-induced protein 1 and downstream signaling via the β-catenin pathway. In vivo validation of these findings further confirmed the role of miR-9. These studies are likely to reveal new mechanism(s) and regulatory strategies in the paracrine-mediated regulation of miRNAs with relevance to microglia dysfunction. EV-loaded miRNAs could thus be developed as a potential therapeutic strategy for HAND (Grant support: MH062261)

P21
Antiretroviral Concentrations in Brain Tissue are Similar to or Exceed Those in CSF

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Background: Limited distribution of antiretroviral therapy (ART) drugs into the central nervous system (CNS) can result in lower drug concentrations in CSF than in blood but drug concentrations in CSF may not accurately reflect those in brain tissue. The objective of this analysis was to measure ART drug concentrations in human brain tissue. Methods: 9 HIV+ adults were evaluated in the California NeuroAIDS Tissue Consortium (CNTN) within 6 months of death; reported taking ART at that antemortem visit; and had detectable concentrations of at least one ART drug in serum at autopsy. Autopsies were performed within 30 h of death. Brain tissue was collected and stored at −80 °C. Concentrations of 6 ART drugs were measured in 3 brain tissue regions [globus pallidus (GP), cortical gray matter (CGM), white matter (WM)] by high performance liquid chromatography-mass spectrometry with a lower limit of quantitation of 25 ng/mL. Results: Subjects were mostly men (82 %) with a mean age of 40.4 (SD 5.0). The most common cause of death was pneumonia. Concentrations of ATV, EFV, FTC, and 3TC were similar to published concentrations of these drugs in CSF but concentrations of TDF were higher than reported values in CSF. LPV concentrations in brain tissue were also higher than reported in CSF but only in WM. Drug concentrations appeared to vary by brain region: Across all drugs, concentrations were lower in CGM than in the other two regions (p=0.01). Conclusions: This is the first analysis of ART drug concentrations in human brain tissue. Concentrations of most drugs in this small analysis were similar to reported concentrations in CSF but TDF had higher concentrations than expected based on CSF reports. Regional variation in ART drug concentrations may be important for antiviral efficacy and toxicity.

P22
Associations between monocyte biomarkers and successful cognitive aging in HIV+ older adults

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Older adults living with HIV are a growing population that experience excess morbidity and mortality compared to the general population. Persistent immune activation and aberrant levels of inflammation appear to be critical for this difference. In this study, a cohort of 79 HIV+ and 38 HIV- older (≥50 years) persons was examined. Mean age was 58.4 years (SD=6.1, range of 50–79) in HIV+ adults and 59.3 years (SD=6.8, range of 50–79) in HIV- adults. All subjects completed comprehensive, standardized neurocognitive and activities of daily living (ADLs) assessments using methods sensitive and specific to HIV-associated neurocognitive disorders (HAND) and a self-report of everyday functioning. Biomarkers associated with macrophage immune activation including, soluble CD163 (sCD163), soluble CD14 (sCD14) and osteopontin (OPN), were measured in plasma. Successful cognitive aging (SCA) was defined as the absence of the following: 1) neurocognitive impairment, 2) major depressive disorder and 3) dependence in instrumental ADLs. SCA was identified in 61 % of the HIV- adults and 37 % of HIV+ adults (p<0.05). HIV+ subjects had higher sCD163 (p<0.05), sCD14 (p<0.001) and OPN (p<0.05) levels in the plasma than HIV- subjects. When groups were stratified by SCA and HIV status, sCD163 and sCD14 were higher in non-SCA HIV+ subjects than in SCA HIV- subjects (p<0.05, p<0.05). OPN was significantly higher in non-SCA HIV+ subjects compared to SCA HIV+ subjects (p<0.05). These data suggest that monocyte/macrophage specific sCD163, sCD14, and OPN are elevated in HIV+ adults who do not

exhibit successful cognitive aging. These findings stress the potential role of chronic monocyte/macrophage activation in "accelerated aging" during HIV disease and unsuccessful cognitive aging.

P23

The Neuroprotective Effect of Osteopontin on Cultured Neurons

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HIV propagation in the brain impairs neuronal function and is at the origin of a spectrum of motor, behavioral, and cognitive deficits, collectively known as HIV-associated neurocognitive disorders (HAND). A better understanding of the molecular mechanisms involved in the development of cognitive impairment and the host response is needed to develop new therapies. Osteopontin is a proinflammatory cytokine that is significantly elevated in HAND. Our recent findings show that OPN is expressed in several cells in the CNS including neurons. To test the hypothesis that OPN plays a protective role in HAND we used differentiated SHSY5Y human neuroblastoma cells to test the impact of OPN on HIV-Env mediated neurocytotoxicity. Low concentrations of OPN were able to block Env-induced cell death, however, at higher levels, its ability to block neurotoxicity was lost. OPN alone did not induce cell death. Unexpectedly, we observed that neurons exposed to CCR5 Envs and particularly those from clade B possessed significantly longer axons than cells treated with HIV IIIB Env, which binds to the X4 coreceptor. Axons were also longer in neuronal cultures exposed to OPN and the negative impact of IIIB Env on axonal length was reversed in the presence of OPN. These results suggest that OPN at low levels may serve a protective function for the host by blocking neurotoxicity and promoting axonal growth.

P24

The Role of Thymic Reconstitution in the Outcome of AIDS-Related PML

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Background: A cytotoxic T-cell response against JC virus (JCV) is associated with survival from progressive multifocal leukoencephalopathy (PML). The determinants of the development of an effective JCV-specific cellular immune response in Human Immunodeficiency Virus (HIV)-infected PML patients are yet to be elucidated. Methods: We enrolled 22 patients with acquired immunodeficiency syndrome (AIDS)-related PML. Within 5 weeks of PML onset, all patients were started on combined antiretroviral therapy (cART) which effectively inhibited HIV replication. We collected blood specimens 1–4 months and 6–9 months after PML onset. To assess thymic T-cell output we quantified signal-joint T-cell receptor rearrangement excision circles (TREC) in blood via polymerase chain reaction. We used intracellular cytokine staining (ICS) to characterize JCV-specific T-cell responses in blood. Results: Fifteen patients survived for more than 1 year after PML onset (PML-survivors) and 7 patients died within 1 year of PML onset (PML-progressors). PML-survivors displayed significantly higher TREC levels (median=3,001, range=931–12,201 cps/μg of peripheral blood mononuclear cell DNA), compared to PML-progressors (median=undetectable, range=undetectable-1,507) upon initial testing (p=0.0007) and upon repeat testing (PML-survivors median=3,039, range=1,895-13,815 and PML-progressors median=152, range=undetectable-1,836, p=0.0004). Initial TREC levels correlated with detectable CD4+ and CD8+ T-cell responses against JCV (p=0.023 and p=0.045 respectively). Lastly, thymic T-cell output above 2,000 TREC was strongly associated with survival from PML (p=0.009, hazard ratio=0.041, 95 % confidence interval=0.01–0.531). Conclusions: In the setting of cART, thymopoiesis enhances the development of CD4+ and CD8+ JCV-specific T-cell responses and leads to improved survival from AIDS-related PML. Estimating thymic output in blood bears prognostic value in AIDS-related PML and can help identify patients who might benefit from thymus-stimulating immunotherapies.

P25

Endolysosome-dependent mechanisms in HIV-1 Tat induced neuronal injury

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HIV-1 Tat continues to be implicated as an important pathogenic factor of HIV-1 associated neurocognitive disorder (HAND). Our recent findings that HIV-1 Tat elevates endolysosome pH, alters the structure and function of

neuronal endolysosomes, disrupts synaptic integrity, and increases amyloidogenesis suggest that de-acidification of endolysosomes may play may an early and important role in the pathogenesis of HAND and the development of Alzheimer's disease (AD)-like pathology in HIV-1 infected individual. Here we determined underlying mechanisms whereby HIV-1 Tat induces de-acidification of endolysosomes and its consequences. It is known that HIV-1 Tat enters neuronal endolysosomes via receptor-mediated endocytosis, but little is known about how HIV-1 Tat escapes from endolysosomes other than the suggestion that high luminal H+ gradients are required. Our observation that HIV-1 Tat de-acidifies endolysosomes indicates that HIV-1 Tat escaping from endolysosomes appears to be linked to proton leakage out of endolysosomes and a proton-dependent peptide transporter might be involved. Of all known proton-dependent peptide transporters, we demonstrated that proton-coupled oligopeptide transporter 2 (Pept2) is present on neuronal endolysosomes and that siRNA knockdown of Pept2 blocked HIV-1 Tat-induced enlargement of endolysosomes. Thus, Pept2 might be a proton-dependent peptide transporter through which HIV-1 Tat de-acidifies endolysosomes and escapes endolysosomes. Elevation of endolysosome pH could affect a variety of endolysosome functions and one such function is endolysosome calcium homeostasis. We have demonstrated that elevation of endolysosome pH not only induces calcium release from endolysosomes but also activates a novel endolysosome-dependent calcium influx across plasma membranes, a phenomenon we have termed "acidic store-operated calcium entry." Thus, de-acidification of endolysosomes may play a central and early role in the pathogenesis of HAND and the development of AD-like pathology, and intervention against HIV-1 protein-induced de-acidification of endolysosomes and pathological changes to endolysosomes may become a new therapeutic strategy against HAND and AD.

P26
Microglial Regulation of Amyloid Homeostasis During HAND

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Abnormal Amyloid protein processing and consequent accumulation have been shown to be a component of HIV Associated Neurocognitive Deficits (HAND) in the post cART era. Resident macrophage/microglia of the central nervous system (CNS) maintain amyloid equilibrium between the extracellular and intracellular compartments through endocytosis. The role of microglia in regulating amyloid homeostasis during HAND remains to be clarified. Immunofluorescence staining of human brain tissues from patients with HAND demonstrates Iba+microglia cells in close proximity to neurons with intracellular accumulation of Amyloid protein as well as surrounding diffuse extracellular Aβ deposits. An in vitro model was then established to examine how exposure to HIV-1 viral particles affect the ability of microglial cells to clear Aβ1-42 monomers. Preliminary experiments suggest reduced microglial uptake of Aβ1-42 post infection by VSV-G pseudotyped HIV. The use of HIV virions with GFP inserted in the nef region further demonstrates significant reduction of Aβ1-42 internalization in GFP- cell population, which suggests that HIV exposure induced loss of microglial Aβ1-42 clearance is mediated by secondary factors. Furthermore, increased level of latent TGF-β1 was detected in the supernatant of primary human fetal microglia post infection suggesting reduced TGF-β1 activation. Since active TGF-β1 has been shown to promote microglial phagocytosis of Aβ in other studies, we propose to determine whether reduced microglial clearance of Aβ is dependent on suppressed TGF-β1 activation post infection.

P27
Defining the origins and functional properties of HIV-1 Vpr variants associated with neurocognitive impairment

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HIV-1-positive patients continue to develop neurological deficits, which are referred to as HIV-associated neurocognitive disorders (HAND). Relatively recent studies of HAND have identified viral protein R (Vpr) as a neuropathogenic factor. Extracellular Vpr, which has been detected in the sera and cerebrospinal fluids of HIV-1-infected patients, causes adverse changes in brain resident cell types, including macrophages, astrocytes, and neurons. Our recent efforts have

turned to investigating protein sequence variations in Vpr that may impact the role of Vpr in HAND. Analyses of peripheral blood-derived viral sequences from 150 HIV-1-infected patients have revealed amino acid changes in Vpr associated with neurocognitive impairment, as indicated by patient Total Hopkins Modified Dementia Scores (THMDS). These amino acid changes - E2K, P14T, V31I, T53P, R62S, V83Q, and A89T - are located in regions that have been previously associated with known functions of Vpr, including cell cycle arrest, virion packaging, nuclear localization, and DNA binding. Current efforts are focused on amplification of additional PBMC-derived Vpr variants from HIV-1-infected patients who have undergone a Comprehensive NeuroPsychological Assessment (CNPA) or who have been categorized as never impaired or always impaired with respect to longitudinally recorded THMDS. Further studies will associate Vpr variants with specific immune cell sub-populations and determine the impact of each amino acid variation on the functions of Vpr in the context of infection and as an extracellular protein potentially involved in neuropathogenesis. These studies will form the foundation for investigations designed to establish functional links between Vpr variation in the peripheral blood and neurocognitive deficits in HIV-1-infected patients. These studies were supported by a developmental grant from the NIMH-supported Comprehensive NeuroAIDS Center (CNAC) and CNAC core facilities awarded to Temple University. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, and T32 MH079785.

P28

Human immunodeficiency virus type 1 genetic variation is a result of a combination of reactivation and replication involving the introduction of new mutations

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The widespread use of combination antiretroviral therapy has resulted in effective long-term maintenance with undetectable viral loads for prolonged periods of time measured in years. However, despite long-term therapy-induced viral latency in the peripheral blood based on detectable viral replication, we have now clearly demonstrated the continued generation of new mutations during long-term cART. These results suggest the presence of a low level viral replication in some patients, even in the presence of cART. To examine this, a set of patients from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort, whom have been sampled longitudinally for more than 7 years have been used. In this regard, we modeled shifts in the predominant integrated proviral quasi-species and de novo variation due to mutations combined with selection pressures such as therapeutic interventions, AIDS-defining illnesses, and other factors. Using phylogenetic methods, LTR and Tat exon 1 sequences isolated from the peripheral blood mononuclear cells of these patients estimated HIV-1 to have an average mutation rate of 5.71/Kb/year, which was reduced by an average of 1.02/Kb/year upon introduction of cART. These studies represent the initial steps in quantifying rates of genetic variation across longitudinally sampled sequences from patients at multiple stages of disease progression. Notably, while long-term therapy reduced estimated mutation rates, they were still non-zero, even in the absence of detectable viral load. The sequence variation observed may be due in part to differential activation of latent proviral DNA quasispecies and/or low-level viral replication in various reservoirs that occur even in well-controlled patient populations over prolonged time and ultimately detected in the peripheral blood compartment. Understanding what affects mutation rates may lead us to a new understanding of HIV-1 disease progression and the mechanisms that drive it. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785.

P29

Glutamate biosynthesis in HIV-1 infected macrophages: Role of HIV-1 Vpr.

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HIV-1 infected macrophages play a significant role in the neuropathogenesis of AIDS. HIV-1 viral protein R (Vpr) not only facilitates HIV-1 infection but also promotes long-lived persistence in macrophages. Our previous studies using SILAC-based proteomic analysis showed that the expression of metabolic enzymes in the glycolytic pathway and tricarboxylic acid (TCA) cycle were altered in response to Vpr expression in macrophages. We hypothesized that Vpr induced

modulation of glycolysis and TCA cycle could regulate glutamate biosynthesis and secretion in HIV-1 infected macrophages. Stable isotope-labeled glucose and a multiple reaction monitoring (MRM) targeted metabolomics assay were used to evaluate the de novo synthesis and secretion of glutamate throughout the metabolic flux of glycolytic pathway and TCA cycle activation in HIV-1 infected primary macrophages and in Vpr overexpressing macrophages. In addition, a confirmation of the specific metabolite changes induced by Vpr in macrophages was performed at the intracellular and extracellular level in a time-dependent manner. The metabolic flux studies demonstrated an increase in glucose uptake, glutamate secretion and accumulation of α-ketoglutarate and glutamine in the extracellular milieu of HIV-1 infected primary macrophages and in Vpr expressing macrophages. Interestingly, glutamate pools and other intracellular intermediates (glucose-6-phosphate, fructose-6-phosphate, citrate, malate, α-ketoglutarate, and glutamine) showed a decreased trend except for fumarate; in contrast, glutamine accumulation was observed in the extracellular space. Our studies demonstrate that dysregulation of mitochondrial glutamate metabolism in macrophages by Vpr can contribute to neurodegeneration via neuroexcitotoxic mechanisms in the context of NeuroAIDS. (Supported by the Comprehensive NeuroAIDS Center grant (P30MH0921777), Developmental Core and Basic Science Core I to CB, and in part by R01 DA033213 and 1P01DA037830-01A1 to PKD)

P30
Polyamines: Predictive Biomarker for HIV-Associated Neurocognitive Disorders

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Spermidine/spermine-N1-acetytransferase (SSAT) is the key enzyme in the catabolism of polyamines that are involved in regulating NMDA functioning. Over expression of SSAT leads to abnormal metabolic cycling and may disrupt NMDA receptor signaling. In fact, the HIV protein Tat induces neurotoxicity involving polyamine/NMDA receptor interactions. Thus, we investigated abnormal polyamine cycling in HIV+ participants with varying degrees of HIV-associated neurocognitive disorders. Acetyl-polyamine (SSAT products) levels were assessed by HPLC in CSF from 99 HIV-infected participants (no cognitive impairment (NCI, n=25), asymptomatic neurocognitive impairment (ANI, n=25), mild cognitive and motor disorders (MCMD, n=24), and HIV-associated dementia (HAD, n=25)). Polyamine levels in brain tissues from a subset of participants [uninfected (n=3), NCI (n=3), and MNCD (n=3)] were also assessed. Human primary astrocytes expressing HIV Tat were assessed for levels of the SSAT activity. Activation of the polyamine catabolic enzyme, SSAT increases polyamine flux in brain and CSF of HIV infected individuals with HIV-associated neurocognitive disorders. CSF levels of acetylated polyamine increase with the degree of HAND severity as indicated by significantly increased acetylpolyamine levels in HAD participants compared to NCI and ANI (p<0.0001) and between MCMD and NCI and ANI (p<0.0001). In vitro studies suggest that the HIV protein Tat may be responsible in part for astrocyte-derived acetyl polyamine release. Our data suggest that polyamine metabolism may play a pivotal role in the neurodegeneration process among HAND patients. Changes in polyamine flux may serve as a potential predictive diagnostic biomarker for different severities of HAND. Funded by CNAC NIMH Grant Number P30MH092177 to KK and in part by 5R01DA033213 to PD.

P31
Methamphetamine decreases voltage-gated K+ channel activity and alters resting membrane potential in primary human fetal astrocytes

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Methamphetamine (Meth) is a potent and commonly-abused psychostimulant. Chronic exposure to Meth induces decreased neuronal activity in the medial prefrontal cortex and nucleus accumbens (two key regulators of cognition and addiction in the reward pathway), which may contribute to mechanisms underlying Meth addiction. It is not fully understood whether such decrease results from alterations in synaptic/intrinsic excitability of neurons, and/or dysregulation of the extracellular environment (e.g., glutamate and K+ levels) mediated by surrounding astrocytes. To fill this knowledge gap, we assessed the effects of Meth on functional activity of certain voltage-gated ion channels in the cell membrane of primary human fetal astrocytes (HFA) using whole-cell

voltage-clamp recording. We found that HFA displayed a large out-flowing voltage-gated K+ current (VGKC, a characteristic of immature or reactive astrocytes), while voltage-gated Ca2+ currents were not seen. Further, exposure of HFA to Meth (20 μM, 100 μM, or 300 μM for 3–6 h) induced significant depolarization of the resting membrane potential (RMP), and decreased VGKC (100 or 300 μM Meth) at 60–100 mV membrane potential levels, as compared to vehicle-treated HFAs (all $p<0.05$). Preliminary data suggest that blockade of VGKC decreases outflowing current in control and Meth-treated HFAs. Whether other K+ channel(s) are involved in Meth-induced RMP depolarization is currently under investigation. These novel findings reveal that Meth disturbs HFA activity by altering RMP and VGKC. Given that Meth-induced decrease of VGKC from astrocytes can consequentially reduce local extracellular K+ levels, such a reduction could ultimately contribute to decreased excitability in neurons surrounded by these astrocytes.

P32

Blood brain barrier disruption and inflammatory biomarkers analysis in CSF with pleocytosis compared with serum in HIV+ participants

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Background: Blood–brain barrier (BBB) disruption is associated with markers of neuronal injury including hippocampal atrophy and loss of synapses and dendrites. This study evaluated BBB disruption and inflammatory biomarkers in relation to pleocytosis in HIV infected patients. Methodology: We studied CSF and serum albumin and inflammatory biomarkers in 52 HIV infected individuals from Curitiba, Brazil and 19 demographically matched HIV negative individuals from San Diego, CA. BBB integrity was evaluated by the CSF/serum albumin quotient (QAlb). Interleukins (TNF-alpha, IFN-gamma, IL-1beta, IL-2, IL-4, IL-6, IL-7, IL-10) and chemokines (MCP-1/CCL2, MIP-1alpha, MIP-1beta, RANTES, IP-10) were measured using the Luminex multiplex immunoassay platform or ELISA HD (RANTES/CCL5). The groups with CSF WBC >5 cells/mm3 and <5 cells/mm3 were compared using independent group *t* test (unadjusted analysis) and linear regression (adjusted analysis), controlling for nadir CD4, and CSF and plasma HIV RNA suppression. Results: CSF WBC >5 cells/mm^3 was seen in 14 (27 %) of HIV+ subjects, in this group 45 % had higher CSF HIV RNA than in peripheral blood, indicating isolated intra-tecal HIV replication. The CSF/serum albumin ratio in the group with CSF WBC>5, mean+SD 16X10-3+22X10-3 and in the group with WBC <5, 7X10-3+ 3X10-3 ($p=0.018$). CSF levels of TNFalpha, IFNgamma, IL-2, IL-6, IL-7, IL-10, IP-10, MIP1alpha, MIP1beta and RANTES were significantly higher in the group with increased CSF WBC ($p<0.0001$; 0.026; 0.018; 0.022; 0.036; <0.00001; <0.0001; 0.0027; 0.0087; 0.008 respectively); although in serum these biomarkers but IP-10 ($p=0.052$) were not different between the two groups. Conclusions: BBB disruption is frequent in HIV-infected individuals, and was related with the increase of CSF WBC. In the group with CSF pleocytosis, the immunological response is almost restrict to CSF despite of the presence of BBB disruption. This finding supports the concept of the CNS as an isolated and specific compartment.

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IFN-gamma Inhibits JC Virus Replication in Glial cells by Suppressing T-Antigen Expression

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Patients undergoing immune modulatory therapies for the treatment of autoimmune diseases such as multiple sclerosis, and individuals with an impaired-immune system, most notably AIDS patients, are in the high risk group of developing progressive multifocal leukoencephalopathy (PML), an often lethal disease of the brain characterized by lytic infection of oligodendrocytes in the central nervous system (CNS) with JC virus (JCV). The immune system plays an important regulatory role in controlling JCV reactivation from latent sites by limiting viral gene expression and replication. However, little is known regarding the molecular mechanisms responsible for this regulation. Here, we investigated the impact of soluble immune mediators secreted by activated PBMCs on viral replication and gene expression by cell culture models and molecular virology techniques. Our data revealed that viral gene expression and viral replication were suppressed by soluble

immune mediators. Further studies demonstrated that soluble immune mediators secreted by activated PBMCs inhibit viral replication induced by T-antigen, the major viral regulatory protein, by suppressing its expression in glial cells. This unexpected suppression of T-antigen was mainly associated with the suppression of translational initiation. Cytokine/chemokine array studies using conditioned media from activated PBMCs revealed several candidate cytokines with possible roles in this regulation. Among them, only IFN-γ showed a robust inhibition of T-antigen expression. Further analysis of IFN-γ signaling pathway revealed a novel role of Jak1 signaling in control of viral T-antigen expression. Furthermore, IFN-γ suppressed JCV replication and viral propagation in primary human fetal glial cells, and showed a strong anti-JCV activity. These observations demonstrate a novel role for IFN-γ in regulation of JCV gene expression, provide a new avenue of research to understand molecular mechanisms of viral reactivation and may lead to development of novel strategies for the treatment of PML.

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The agnoprotein of Polyomavirus JC is Released by Infected Cells: Evidence for Its Cellular Uptake by Uninfected Neighboring Cells

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Polyomavirus JC replicates in glial cells in the brain, and causes the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). PML is usually seen in patients with underlying immunocompromised conditions, notably among AIDS patients and those on chronic immunosuppressive regimens. The late leader sequence of JC virus contains an open reading frame encoding a small regulatory protein called agnoprotein. Agnoprotein contributes to progressive viral infection by playing significant roles in viral replication cycle. Here, we demonstrate that agnoprotein can be detected in cell-free fractions of glial cultures infected with JCV, transfected with expression plasmids or transduced with adenovirus expression system. We also provide evidence that extracellular agnoprotein can be taken up by uninfected neighboring cells. These studies have revealed a novel phenomenon of agnoprotein during the viral life cycle with a potential of developing diagnostic and therapeutic interventions.

P35
Human Polyomavirus 6 DNA in the cerebrospinal fluid of an HIV-positive patient with leukoencephalopathy

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BACKGROUND: Leukoencephalopathies in HAART-treated, HIV-positive patients include progressive multifocal Leukoencephalopathy (PML), a result of lytic infection oligodendrocytes by JC polyomavirus (JCV), and another form characterized by the absence of JCV genome in cerebrospinal fluid (CSF) which we call here leukoencephalopathy of unknown origin (LEUO). OBJECTIVES: To test the potential viral etiology of LEUO. STUDY DESIGN: CSF was collected from 43 HIV-positive patients with MRI suggestive of leukoencephalopathies. DNA was isolated and real-time PCR assays for neurotropic viruses (Herpes Simplex Viruses 1/2, Varicella Zoster Virus, Epstein Barr Virus, Human Cytomegalovirus, Human Herpesvirus 6, JCV and HIV) were conducted. CSF from 14 non-reactive cases were subjected to random nucleic acid amplification, deep sequencing, and in silico search for viral sequences. RESULTS: JCV genome was detected in the CSF of 19 PML patients, HIV genome in the CSF of 5 PML patients including 2 JCV negative patients, and no viruses were detected in 22 LEUO patients. Human Polyomavirus 6 (HPyV6) DNA was detected by deep sequencing in one CSF sample. CONCLUSIONS: HPyV6 has not been previously reported in CSF or associated with any disease. HPyV6 DNA was detected in CSF of a case of demyelinating disease. Demonstrating a causative role will require further studies.

P36
Role of HDACs in the regulation of glutamate transporter, EAAT2 expression

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Glial glutamate transporter EAAT2 plays an essential role in cognitive functions and decreased expression of EAAT2 protein is observed in NeuroAIDS. In the current study, we investigated whether the pro-inflammatory cytokine IL-1beta

represses EAAT2 expression and whether histone deacetylation a landmark modification associated with transcriptional repression of gene transcription at the promoter level plays a role in astrocytic EAAT2 expression. We assessed the effects of HDAC inhibitor SAHA (suberoylanilide hydroxamic acid; Vorinostat) and overexpression of HDAC 1, 2, and 3 on EAAT2 expression in astrocytic cells, U251 and U86, and primary human fetal brain astrocytes in the context of IL-1 beta. Findings from both approaches demonstrated that SAHA (5 μM) induces EAAT2 expression and overexpression of HDACs 1, 2, and 3 inhibits EAAT2 expression. Furthermore, HDAC 1, 2, and 3 mediated repression can be relieved by SAHA by 50 %. This observations demonstrate that HDAC activity regulates EAAT2 expression and HDACi's such as SAHA represent a potential novel approach for upregulation of EAAT2 expression.

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Characterization of extracellular vesicles (exosomes) from HIV-1 infected macrophages treated with HIV-1 protease inhibitor, Ritonavir.

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Antiretroviral therapy (ART) prevents HIV-associated neurocognitive disorders (HAND). However, milder forms of HAND are still prevalent despite widespread use of ART. Ritonavir-boosted protease inhibitor monotherapy is a maintenance strategy that prevents nucleoside reverse transcriptase inhibitor toxicity and has been effective in maintaining long-term viral suppression in the majority of patients. In this study, we assessed whether extracellular vesicles (EVs) released from HIV-1 infected monocyte/macrophages cultured in the presence of HIV-1 protease inhibitor, Ritonavir, play a role in neurodegeneration. EVs were derived from conditioned media of U937 cells and U1 cells that are latently infected with HIV-1 and cultured in the presence and absence of HIV-1 protease inhibitor, Ritonavir (2.5 and 5 micro molar) by ultracentrifugation and Iodixanol (optiprep) gradient centrifugation. The EVs were characterized for markers of exosomes such as Tsg101 and Alix, and Acetylcholinesterase enzyme (AchE) activity, viral proteins Nef and Gag, and scanning electron microscopy. We have observed significant increase in Nef and Gag proteins in exosomes derived from ritonavir treated cells. Neuronal cultures treated with U1 and U1-ritonavir exosomes were found to be severely compromised in their ability to maintain existing neuronal network as well as their ability to form neurites in a scratch-wound assay. We observed a significant down regulation of cAMP-response-element-binding protein (CREB) phosphorylation in neurons treated with U1 exosomes and U1 exosomes derived from Ritonavir treated cells in comparison to exosomes derived from U937 cells. Furthermore, we observed significant down regulation of CREB regulated gene expression in these neurons. Collectively, these observations demonstrate that exosomes derived from HIV-1 infected cell and cells treated with Ritonavir can cause neuronal dysfunction and degeneration by targeting CREB signaling pathway. The studies were funded by a pilot grant to PD (CNAC NIMH Grant Number P30MH092177) and ultilized services offered by core facilities of CNAC.

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Chronic low level expression of the HIV viral protein TAT causes neurodegeneration in older mice

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Despite the introduction of anti-retroviral therapies (ART) the incidence of HIV-associated neurological disorders is estimated at ~50 %. The causes of these complications remain unclear. MRI studies have revealed that over time there is a loss of brain volume in HIV-infected subjects despite ART. It has long been postulated that low-level production of non-structural proteins from HIV-infected glia may contribute to neurological damage. To test this hypothesis, we utilised a leaky Tetracycline-inducible promoter in the rtTA-Tat transgenic mouse to determine if very low-level Tat production is associated with brain volume changes over time. Brain volume measures performed by T2-weighted MRI on 11–12 month old rtTA-Tat(+) mice treated with (n=4), or without (n=4) doxycycline inducing Tat expression (28 days). Age matched rtTA-Tat(−) animals were used as controls (n=4). Volumetric measurements of multiple brain regions, including ventricles, motor-cortex, striatum, hippocampus, anterior-cingulate, and somatosensory-cortex were performed. Tat mRNA expression was apparent in non-induced rtTA-Tat(+) mice, consistent with constitutive low-level rtTA promoter activity and also was increased three-fold following a 28-day induction of the rtTA promoter with doxycycline. Ventricle size was increased in non-induced rtTA-TAT(+) mice (19.55±0.92 mm^3) compared to controls (11.43±1.70 mm^3), and primary motor cortex was reduced (1.36±0.06 mm^3) compared

to controls (1.46±0.11 mm^3). No changes in size were observed in other brain regions measured. Protein levels of PSD95, synaptophysin, and beta(III) Tubilin were all reduced in the cortex of non-induced rtTA-Tat(+) mice compared to controls. No further reductions in ventricle (18.04±3.1 mm^3), motor cortex (1.24±0.10 mm^3), or neuronal protein expression were observed doxycycline-induced Tat expression. Very low-level expression of Tat was associated with reduced brain volume and neuronal damage in older mice. As ART is unlikely to affect expression of non-structural HIV-proteins in infected glia, these findings suggest that very low-level Tat expression over prolonged periods of time may contribute to ongoing neurological damage in ART treated HIV-infected individuals.

P39
HSV-1 replication kinetics and immune response in the lip scarification model of infection

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Herpes simplex virus type 1 (HSV-1) is a human pathogen which replicates in epithelial cells of mucosal surfaces before establishing a lifelong latent infection within the trigeminal ganglion. The immune system is critical with respect to the establishment and maintainence of latency. HSV-1 disease occurs with a spectrum of severity and can include corneal scarring and blindness to recurrent mild lesions following infection of the eye and lip, respectively. There is an established ocular infection model in the laboratory mouse which reproduces primary infection and latency observed in humans. However, the majority of primary human infections occur within the lip and latency is established within a different branch of the trigeminal ganglion from ocular tissue. In this regard, we set out to define the kinetics of HSV-1 replication and CD8+ T-cell response in the lip scarification model. The lower lip of 3 month old mice were scarified and inoculated with HSV-1 and tissue was collected at 7 time points up to day 60 post infection for detection of infectious virus and responding CD8+ T cells. We found high virus titers in the lip at early time points that resolved after 8 days of exposure. The virus infiltrated the trigeminal ganglion during primary infection in the lip and latency was established by 30 days post infection. CD8+ T cells were observed infiltrating the trigeminal ganglion 8 days post infection. CD8+ T-cells could be found in the trigeminal ganglion 60 days after infection. These results demonstrate that the lip scarification model can be used to study viral kinetics and the role of immune cells in the trigeminal ganglion.

P40
Latent toxoplasmosis is associated with neurocognitive impairment in a cohort of young adults with chronic HIV infection from Romania

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Introduction: Latent infection with T. gondii has been linked to psychiatric disorders and behavioral changes. We evaluated its impact on neurocognitive (NC) and neurobehavioral functioning in a group of young adults with chronic HIV infection since childhood. Methods: NC performance was evaluated using a standardised test battery, and self-reported apathy, disinhibition, and executive dysfunction was assessed using the Frontal System Behaviour Scale (FrSBe). Results: 194 young adults (median age 24 years, 48.2 % males) with parenterally-acquired HIV infection (HIV+) (median duration 21 years) and 51 HIV seronegative (HIV-) participants with similar demographic characteristics were included. All HIV+ individuals had good current immunological status (median CD4: 479 cells/μL), despite a low CD4 nadir (median: 93 cells/μL). Latent toxoplasmosis (positive anti-Toxoplasma IgG antibodies) was present in 32.4 % of the patients (Toxo+). Impairment rates increased with infection status: HIV-/Toxo- (6.1 %); HIV-/Toxo+(22 %), HIV+Toxo- (31 %), HIV+/Toxo+(49 %), which translated into an increased risk of NCI in the HIV+/Toxo+compared to the HIV+Toxo- group (RR=1.6, P=0.001). Within the HIV+ group, latent toxoplasma infection was associated with worse performance globally (t=2.8, p=0.006), as well as in memory (t=−2.6, p=0.009), speed of information processing (t=−2.7, p=0.01, verbal (t=−2.7, p=0.02) and learning (t=−2.4, p=0.02) domain, even after controlling for differences in HIV characteristic. In the subgroup limited to participants with undetectable plasma HIV load (118), those with positive anti-Toxoplasma antibodies (33.9 %) had worse NC performance (median GDS: 0.34 vs. 0.58, p=0.03). Compared to the HIV+/Toxo-, controlling for relevant demographic characteristics the HIV+/Toxo+ group had greater symptoms of disinhibition (p=.03). Conclusions: Latent Toxoplasmosis might be a cause of NC

impairment in young patients with and without chronic HIV infection. Longitudinal follow-up is warranted in order to evaluate the long-term impact of latent toxoplasmosis on cognitive and behavioural changes and psychiatric conditions in HIV-infected patients and possible interventions.

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CMV impact on neurocognitive impairment in a cohort of Romanian young adults with chronic HIV-infection

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Introduction: Cytomegalovirus (CMV) infection can contribute to accelerated cognitive decline in the elderly. We explored the potential role of CMV reactivation on neurocognitive impairment in a cohort of young adults with chronic HIV-infection. Methods: We evaluated in 41 Romanian young adults chronically infected with HIV-1 subtype F since childhood the association between: (1) CMV-DNA levels in PBMCs (digital droplet PCR), (2) T-cell responses to CMV antigen (QuantiFERON-CMV®), (3) B-cell responses to CMV (IgG levels), (4) plasma IFN-y levels (Meso Scale Discovery™), and (5) neurocognitive functioning measured by standard neurocognitive testing with appropriate norms. Results The participants in our study (46.3 % males) had a median age of 23.3 years and received antiretroviral therapy for a median of 10.8 years. Half had HIV-RNA levels less than 50 copies/ml at the time of sampling. Their median CD4 nadir count was 105 cells/ml and current median CD4 count was 488 cells/ml. While all participants had positive CMV-IgG antibodies, only 32 (78 %) had reactive QuantiFERON-CMV and 7 (18.9 %) had detectable CMV-DNA in PBMCs. Fourteen participants (34.1 %) had neurocognitive impairment. There was no correlation between neurocognitive scores and CMV antibody titers, CMV-DNA levels or CMV specific T-cell responses. Neurocognitive impairment did correlate with plasma concentrations of IFN-gamma, with unimpaired participants having the lowest concentrations of IFN-gamma (rho=0.27, p=0.07). This correlation was not maintained if we excluded the 21 participants with detectable HIV-RNA (rho=0.20, p=0.39). Interestingly, CMV-DNA was absent in all participants with normal neurocognitive functioning who also had undetectable HIV-RNA in plasma. Conclusions In this cohort of young adults with life-long chronic HIV-infection, CMV-specific T-cell and B-cell immune responses did not seem to impact neurocognitive function

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Comparative analysis of HIV DNA latent reservoir in CSF and PBMC during suppressive ART

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Background: Little is known about the HIV DNA reservoir in the central nervous system (CNS) and its impact on neurocognitive functioning during suppressive antiretroviral therapy (ART). Methods: Paired blood and cerebrospinal fluid (CSF) were prospectively collected from 16 HIV-infected individuals who started ART during early infection and with suppressed HIV RNA in blood and CSF (<50 copies/ml). HIV DNA levels were measured in peripheral blood mononuclear cells (PBMC) and CSF cells by ddPCR; soluble inflammatory markers (sCD163, IL-8, MCP-1) and marker of neuronal damage (neurofilament chain [NFL]) were measured in blood plasma or CSF supernatant by immunoassay. Viral diversity and compartmentalization analyses were performed using HIV-env sequences obtained by next generation sequencing from 6 paired PBMC and CSF cellular pellets. Neurocognitive functioning was measured by Global Deficit Score (GDS). Associations between GDS, virologic and inflammatory markers were performed using Pearson correlation. Results: In our cohort of suppressed HIV-infected individuals, HIV DNA was detected in 50 % of CSF cellular pellets and 86 % of PBMC. No association was found between GDS and HIV DNA levels in blood or CSF. Evidence of viral compartmentalization was observed in 4 out of 6 cases with available HIV-env sequences. Interestingly, the one subject with the highest HIV-env diversity in CSF presented the highest levels of NFL. Overall, worse GDS was associated with higher levels of sCD163 (r=0.65, p=0.02) and IL-8 (r=0.88, p=0.004) in blood plasma and higher levels of NFL (r=0.80, p=0.003). Conclusions: HIV DNA in CSF was detectable in half of HIV-infected individuals despite long-term suppressive ART started during early HIV-infection. In our limited dataset, HIV DNA populations were

mostly compartmentalized in CSF when compared to PBMC. A confirmation of these results on larger and diverse cohorts is necessary to increase our knowledge about the relationship between HIV molecular evolution/characteristics and neuropathogenesis during suppressive ART.

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Highly precise measurements of HIV DNA in CSF and blood by droplet digital PCR

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Background: Developing new tools to measure HIV DNA reservoirs will help to understand viral dynamics in the central nervous system (CNS). For the first time, we used droplet digital PCR (ddPCR) to investigate associations between HIV DNA reservoir in CSF and blood in subjects with or without antiretroviral therapy (ART) and to gauge how well the HIV DNA reservoir in CNS can be predicted by measurements in periphery. Methods: We analyzed paired peripheral blood mononuclear cells (PBMC) and CSF cell pellets from 20 subjects with undetectable HIV RNA (<50 copies/mL) and 9 with detectable HIV RNA in CSF and blood. Genomic DNA from PBMC was extracted using silica-based columns; CSF pellets were directly lysed. HIV DNA levels were measured by ddPCR. Non-parametric tests for differences between groups and associations between HIV DNA and RNA levels in CSF and blood were performed. Results: HIV DNA was detected in 19 (66 %) CSF pellets, including 10 (52 %) samples with undetectable HIV RNA in CSF. HIV DNA levels in CSF pellets positively correlated with HIV DNA levels in PBMC (P=0.03) and HIV RNA in CSF (P=0.05) but not with the number of CSF leukocytes. HIV DNA levels in PBMC positively correlated with HIV RNA levels in blood (p=0.001). Similarly, HIV RNA levels significantly correlated between both compartments (P<0.0001). While HIV DNA levels in blood were significantly lower in HIV RNA suppressed compared to non-suppressed subjects (P=0.01), HIV DNA levels in CSF did not differ between these groups. Conclusions: HIV DNA levels in CSF pellets can be measured by ddPCR. HIV DNA and RNA levels correlate within and across compartments. The association between suppressive ART and lower HIV DNA levels in blood, but not in CSF, is consistent with poorer ART penetration into the CNS, or slower decay of CNS HIV DNA compared to blood.

P44

Tripartite Containing Motif 32 Mediates HIV-1 Tat Induced Quiescence of Human Neural Precursor Cells

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HIV-1 infection of the brain results in altered stemness of multipotent neural precursor cells (NPCs). In pediatric neuroAIDS cases, HIV-1 infection of neural stem cell can lead to delayed developmental milestones, cognitive impairment as well as deranged brain development, brain atrophy and cerebrovascular abnormalities. Wherein, in adult NeuroAIDS, infection of NPCs leads to perturbed endogenous neurorestoration of the CNS following brain damage by HIV-1, compounding the severity of dementia in these cases. We recently demonstrated that exposure with HIV-1 Tat protein interferes with cell cycle kinetics of human neural precursor cells (hNPCs). Using primary cultures of human fetal brain derived multipotent neural precursor cells, we gained insights into role of a neural stem cell determinant, Tripartite containing motif 32 (TRIM32), in HIV-1 Tat induced quiescence of NPCs. Cellular localization and levels of TRIM32 have been attributed to be critical regulators of stemness of NPCs. Our data exhibits altered cellular localization of TRIM32 in hNPCs upon HIV-1 Tat exposure. The in vitro data was validated by studying TRIM32 localization in frontal cortex of HIV-1 seropositive adult patients collected at postmortem. Further, we observed HIV-1 Tat perturbs TRIM32 levels via dysregulation of a miRNA which controls the translation of TRIM32 mRNA transcripts. Hereby, we report a novel molecular cascade involving a microRNA and TRIM32 leading to HIV-1 Tat induced attenuated proliferation of NPCs.

P45

Evidence for a role of the inflammatory cytokine IL-1 beta and the iron storage protein ferritin heavy chain in HIV-induced synaptodendritic injury and cognitive deficit

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HIV-associated neurocognitive disorders (HAND) are characterized by a number of pathological changes, including synaptodendritic injury. While the molecular mechanisms of HAND are only partially understood, both host (e.g. inflammatory cytokines) and viral (e.g. HIV gp120) factors are thought to contribute to neuronal injury. We recently discovered that HIV gp120 regulates neuronal levels of the iron storage protein ferritin heavy chain (FHC), an effect mediated by gp120-evoked release of IL-1 beta from glial cells. Of note, our previous work also shows that FHC induction impairs the homeostatic function of the CXCL12/CXCR4 chemokine receptor axis, including CXCL12's ability to regulate spine density. To further investigate the consequences of neuronal FHC alterations by viral proteins in vivo, we utilized two rodent models of HAND (gp120-treated and HIV-Tg rats). We observed reductions in both dendritic spine density and basal dendrite branching in layer II/III pyramidal neurons of the medial prefrontal cortex (PFC), an area that shares strong anatomical homology with the dorsolateral PFC in primates. Changes in these measures suggest deficits in neuronal connectivity and complexity; this is further highlighted by the observation that in gp120-treated rats, dendritic spine density negatively correlated with reversal learning (Pearson $r = -0.8036$), a measure of cognitive flexibility shown to be impaired in these animals. Interestingly, elevated levels of FHC were observed in whole brain lysates obtained from the same HIV-Tg rats used for spine analysis. Ongoing studies focused on spine morphology suggest that certain spine types (e.g. mushroom) may be preferentially affected by gp120, and pilot studies in other brain regions (motor/somatosensory cortices) revealed different susceptibilities to synaptodendritic injury in gp120-treated animals. Taken together with our previous findings in HIV patients and SIV-infected non-human primates, these data validate FHC's role in cognitive impairment and points to IL-1 beta as a potential therapeutic target for HAND.

P46
Alterations in mitochondrial biogenesis during HAND: Implications for mechanisms of neurodegeneration

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Approximately 50 % of HIV+ persons are afflicted by some variant of HIV-associated neurocognitive disorders (HAND). HIV enters the brain causing infection of resident CD4+ cells, viral replication, viral protein/toxin production and neuroinflammation. Some, or all, of these mechanisms culminate in neurodegeneration and HAND. We hypothesized that HIV proteins released from infected CNS cells enter bystander neurons and affect mitochondrial biogenesis via fission/fusion processes. To test this we assayed brain tissues from a well-characterized cohort of HIV+ donors for expression of key mitochondrial fission/fusion proteins, mitofusin (MFN) 1/2 and dynamin-related protein (DRP1), and complimented these studies with in vivo experiments using gp120tg mice and in vitro assays using HIV proteins and SH-SY5Y neuronal cells. MFN1 was significantly increased and DRP1 decreased in HIVE frontal lobe lysate membrane fraction from HIVE donors, compared to HIV+ donors without encephalitis. DRP1 and etc. complex I protein levels were decreased with increased neurocognitive impairment in frontal lobe specimens from HAND patients. Immunostaining revealed increased neuronal MFN1 expression and enlarged mitochondria in HIVE brains; similar changes were seen in gp120 tg mouse brains. Mitochondrial ultrastructure showed increased mitochondria size, presence of electrodense regions and altered morphology in HIVE and gp120 tg brains. Recombinant gp120 induced similar changes in MFN1 and DRP1 levels in neuronal cells, and also increased extracellular acidification rate; these changes were reversed with MFN1 knockdown or DRP1 overexpression. These data suggest that HIV infection of the brain may lead to abnormal mitochondrial function and biogenesis leading to neurodegeneration and HAND. Interfering with this neurodegenerative mechanism may provide relief for HAND patients.

P47
Mitochondrial DNA copy number and common deletion quantity alterations in peripheral nerve tissues from donors with HIV sensory neuropathy

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Background: Neuropathy and neuropathic pain are common, under-treated conditions in the rapidly growing population of older individuals with HIV. Both HIV itself and certain antiretroviral drugs (ARVs) have been shown to impair mitochondrial function and affect mitochondrial DNA (mtDNA) replication via the mitochondrial γ-polymerase. A previous study linked mtDNA damage, as indexed by increased frequency of

the "common deletion," to neuropathy in ARV-treated HIV+ individuals. Methods: Here we investigated dorsal root ganglion (DRG) and sural nerve (SN) specimens from a well-characterized cohort of HIV+ patients; 6 with no signs of HIV sensory neuropathy (HSN), and 5 with 2 or more signs of HSN. All patients were currently on cART or had previous exposure. After isolating total DNA from DRG and SN specimens, the amount mtDNA per cell and the proportion of mtDNA carrying the "common deletion" were quantified via digital droplet polymerase-chain reaction, a method well suited to measure rare events. Results: We found that mtDNA copy number per cell was lower in HSN+ donors in the DRG and SN than those without HSN. Further, the mtDNA "common deletion" was detected in a higher proportion of the mtDNA in the SN of HSN+ donors, than those without HSN. Conclusions: These data suggest that mtDNA copy number and integrity may contribute to HSN in HIV+ patients. Future studies to protect mitochondrial integrity and function might help treat or prevent HIV-associated peripheral nerve damage.

P48

Proliferation of perivascular macrophages in macaque models of lentiviral encephalitis: a potential mechanism for HIV/SIV persistence in the brain?

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In lentiviral encephalitis, the number of brain perivascular macrophages (PVM) is increased. This may be due to recruitment of precursors from peripheral blood and/or in situ proliferation in the central nervous system (CNS). We therefore investigated if macrophage proliferation occurs in the CNS during simian immunodeficiency virus (SIV) infection of adult macaques. Using immunohistochemistry and fluorescence microscopy, we examined the expression of proliferation markers including Ki-67 and incorporation of thymidine analogs by PVM in the brain of uninfected macaques and SIV-infected macaques with or without encephalitis. Double-label immunohistochemistry using antibodies against the pan-macrophage marker CD68 and Ki-67 showed that there was a significant increase in Ki-67+CD68+ cells in macaques with SIV encephalitis (SIVE) compared to uninfected controls and SIV-infected animals without encephalitis. Triple-label immunofluorescence against CD163 (PVM marker), Ki-67, and the nuclear stain DAPI, confirmed that the vast majority of Ki-67-positive nuclei were localized to CD163+ PVM in perivascular cuffs and lesions that characterize SIVE. The proliferative capacity of Ki-67+ PVM was further suggested by their nuclear incorporation of bromodeoxyuridine and 5-ethynyl-2′-deoxyuridine. Examining the SIVE lesions further, using double-label immunofluorescence with antibodies against Ki-67 and SIV gag p28, revealed that not only were the Ki-67+ cells productively infected but that there was a significant positive correlation, in animals with SIVE, between the size of lesions and the percent of Ki-67+ cells in these lesions. Ki-67 expression by most p28+ cells suggests either that PVM begin proliferation after SIV infection or that SIV preferentially infects proliferating PVM in the brain. Altogether this study suggests that local proliferation of PVM may contribute to the persistence of HIV and accumulation of infected cells in the CNS.

P49

Mechanisms of Antiretroviral Drug-induced Changes in Amyloid Precursor Protein Processing: Implications for HAND

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HIV-associated neurocognitive disorders (HAND) persist in 30–50 % of HIV positive patients despite viral control by antiretroviral therapy (ART). Several studies indicate a potential role for antiretrovirals in the persistence of HAND and evolution from a subacute, subcortical dementia to a cortical, neurodegenerative disease. Based on their ability to induce ER stress in a wide variety of cell types, we hypothesized that HIV protease inhibitors (PI) induce ER stress in the CNS, resulting in chronic dysregulation of the unfolded protein response (UPR), which in turn alters amyloid precursor protein (APP) processing by inducing the β-site APP cleaving enzyme-1 (BACE1). Utilizing in vitro and in vivo models, we demonstrate that PIs induce neuronal ER stress leading to PKR-like ER kinase (PERK)-dependent phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2-alpha), and enhanced translation of BACE1. Additionally, we demonstrate enhanced Aβ production, by the PI, ritonavir, in primary rodent neuroglial cultures as well as in Chinese Hamster Ovary (CHO) cells expressing human APP. Genetic excision of PERK in primary neurons abrogated the ability of PIs to induce the ER stress, phosphorylation of eIF2-alpha and translational upregulation of BACE1. Consistent with these findings, ARVs administered to SIV-infected macaques resulted in elevated levels of BACE1 in the CNS, coinciding with markers of neuronal damage. Altogether, these findings implicate PIs as potential contributory mediators of neurodegeneration in HAND.

P50

cFMS inhibition as a potential therapeutic strategy for reducing neuroinflammation and long-lived macrophage/microglial reservoirs of HIV infection in brain

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The neuropathogenesis of HIV-associated neurocognitive disorders (HAND) is poorly understood, particularly the less severe forms of HAND, mild neurocognitive disorder and asymptomatic neurocognitive impairment. Here, we report evidence of neuroinflammation in autopsy tissues from patients with HIV and varying degrees of neurocognitive impairment but without HIV encephalitis (HIVE), one of the neuropathologies underlying HIV-associated dementia, or productive virus in brain. Macrophages and microglia appear activated, as determined by CD163, CD16, and HLA-DR expression, many having a rounded or ramified morphology with thickened processes. Astrocytes also show morphological alterations consistent with an activated state and have increased expression of GFAP and vimentin, as compared to seronegative controls. In some areas, astrocyte activation appears limited to perivascular locations, suggesting events at the blood–brain barrier may influence astrocyte activity. These data demonstrate neuroinflammation, not virus production, is a common feature of the lesser and more severe forms of HAND and supports the notion that HAND is a disease continuum. To begin to explore factors involved in promoting and/or advancing neuroinflammation in the setting of HIV, we investigated macrophage-colony stimulating factor (M-CSF) production in SIV encephalitis (SIVE). In SIVE brain, Mϕs comprising perivascular cuffs and nodular lesions are the principle source of M-CSF and also serve as the primary reservoir of productive SIV infection. We also show that M-CSF and IL-34, which share the same receptor, cFMS, promote expansion of CD163+CD16+ monocytes and enhance HIV production by microglia in vitro. This is attenuated by the addition of GW2580, a receptor tyrosine kinase inhibitor with high specificity for cFMS. These data suggest that M-CSF and IL-34 may influence monocyte/Mϕ activation and receptor expression. Further, M-CSF may contribute to the development and maintenance of a long-lived viral Mϕ/microglial reservoir and, as such, cFMS signaling may be an attractive target for eliminating long-lived Mϕ reservoirs of HIV.

P51

Heme oxygenase-1 deficiency in HIV-infected macrophages: target for adjunctive therapy for prevention of HIV-mediated neurodegeneration

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Expression of the essential cytoprotective enzyme heme oxygenase-1 (HO-1) is significantly reduced in the prefrontal cortex of HIV+ individuals with HIV-associated neurocognitive disorders (HAND). Furthermore, this HO-1 deficiency correlates with CNS viral load and brain markers of macrophage activation and type I interferon responses. In vitro, HIV infection of monocyte-derived macrophages (MDM) selectively reduces HO-1 expression and induces production of neurotoxic levels of glutamate; correction of this HO-1 deficiency reduces neurotoxic glutamate production. We now demonstrate that MDM HO-1 deficiency and associated neurotoxin production is a common feature of prototypic

and primary macrophage-tropic HIV-1 strains, and this is also a feature of HIV-2 macrophage infection. We provide evidence that this HO-1 deficiency in HIV-MDM does not depend on expression of HIV accessory gene proteins Nef, Vpr, or Vpu, but rather on HIV replication itself, however limited. Despite altered replication kinetics of mutated accessory gene HIV strains, infected MDM show decreased HO-1 protein expression ($p<0.05$) and increased supernatant neurotoxicity ($p<0.05$) as measured by neuron-based MAP2 ELISA and extracellular glutamate. Infection of MDM with 11 of 13 macrophage-tropic HIV-1 strains significantly reduced HO-1. HO-1 protein deficiency across all 13 HIV-1 strains correlated significantly with increased viral replication, supernatant neurotoxicity, and extracellular glutamate ($p<0.001$). This HO-1 deficiency was observed even with HIV-1 strains that showed limited viral replication. We further demonstrate that treatment of HIV-MDM with antiretroviral therapy (ART) post-infection did not prevent HIV-mediated HO-1 loss or associated neurotoxin production. Thus, approaches that correct this HO-1 deficiency in HIV-MDM can provide rationale adjunctive therapy for neuroprotection in addition to ART, which is ineffective at limiting neurotoxin production and HO-1 loss once HIV-infection is established in macrophages. In conclusion, modulation of HO-1 expression in HIV-infected MDM is one feasible approach for adjunctive therapy to ART in preventing HIV-induced neurodegeneration.

P52
HTLV-1 infection and neuropathogenesis in the context of Rag1−/−γc−/− (RAG1) mice.

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HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a disabling chronic inflammatory disease of the central nervous system (CNS) with similarities to multiple sclerosis (MS). To date, the lack of a suitable small animal model has hindered our quest to understand the immuno- and neuropathogenesis of HTLV-1 in an in vivo system. Previous work from others and have established that host immune response plays a critical role in the outcome of HTLV-1 infection, which could be better tested in the context of humanized (hu) mice. Thus, we employ here the neonatal and adult Balb/c-Rag1−/−γc−/− or Rag1 as well as Bone marrow-Liver-Thymic (BLT) mouse models for engraftment of human CD34+ hematopoietic stem cells. Flow cytometry and histological analyses revealed reconstitution of Rag1 mice with human immune cells, including macrophages, dendritic cells, T cells and B cells. Proviral load (PVL) was determined in the peripheral blood, spleen, and other organs of Rag1 and BLT mice by droplet digital PCR. Within blood, PVL and viral protein Tax was detected as early as 2 weeks post-infection (wpi). Tax showed peak expression at 14 wpi in Rag1 mice with continued expression until 16 weeks. Both PVL and Tax expression was considerably higher in the adult Rag1 mice as compared to the neonates with the latter showing less than 20 % PVL in the peripheral blood, brain, and liver. Successful infection followed by immune activation and Tax expression within lymphoid organs. Moreover, signs of lymphocytic infiltration with concomitant Tax expression and resulting myelin disruption were observed in the spinal cord and brain of the infected mice. This data represents the first attempt to establish HTLV-1 neuropathogenesis in the context of RAG1 and BLT mice suggesting possibility of developing a small animal model of HAM/TSP in humanized mice.

P53
Apigenin, a natural flavonoid, attenuates EAE severity through modulation of dendritic and other immune cell functions

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Apigenin, a natural flavonoid, found in parsley, chamomile and other plants, fruits, vegetables, herbs, and spices is known to have anti-oxidant and anti-inflammatory properties. The use of Apigenin containing plants for centuries as medicinal approaches to treat asthma, intransigent insomnia, Parkinson's disease, neuralgia, and shingles have been indicative of its role in the regulation of inflammation. However, there is a considerable dearth of information regarding its effect on immune cells, especially dendritic cells (DC) that maintain the critical

balance between an immunogenic and tolerogenic immune response, in an immunospecialized location like the central nervous system (CNS). Thus we looked at the anti-inflammatory properties of Apigenin in restoration of immune function and the resultant decrease in neuroinflammation. In vitro, Apigenin inhibited IL-6 and TNF-alpha secretion in mouse monocyte-derived MDDCs and splenic DCs stimulated with LPS and the cell surface expression of alpha4 integrin (adhesion), CD86 (co-stimulation), CLEC12A (antigen uptake) and MHC II (antigen presentation) molecules on DCs. To test if Apigenin treatment ameliorates disease after onset of experimental autoimmune encephalomyelitis (EAE) and its relapse, C57BL/6 and SJL mouse models of multiple sclerosis were immunized with MOG35-55 and PLP139-151 respectively, followed by treatment with Apigenin. A significant reduction in severity of EAE progression and relapse was observed in the treated mice. Apigenin treated EAE mice show decreased expression of alpha4 integrin and CLEC12A on splenic DCs and an increased retention of DCs and macrophages in the periphery compared to untreated EAE mice. This correlated with immunohistochemistry findings of decreased immune cell infiltration and reduced demyelination in the CNS. These results indicate a protective role of Apigenin against the neurodegenerative effects resulting from the entry of DC stimulated pathogenic T cells into the CNS. Apigenin can thus serve as a potential therapy for neuroinflammatory disease through its regulation of immunogenic T cell response.

P54

Whole-brain MR Spectroscopic Imaging and Diffusion Kurtosis Imaging in HIV-1 Clade C Infection

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Though HIV is spread throughout the brain during chronic infection, its distribution varies across the brain anatomy. Consequently, the degree of alterations to tissue microstructure and metabolite concentration is expected to go along with the viral distributions and/or their infection status within the brain. However, due to the non-availability of appropriate MR technologies, previous brain MR studies have evaluated the metabolite and microstructural alterations of HIV infection in limited anatomical or mostly in white matter regions. Furthermore, the brain changes in individuals with HIV-1 clade B infection are extensively studied albeit incompletely to identify imaging correlates for the neurocognitive deficits found in them. However, there is scarcity of similar studies in clade C, in particular from India where >95 % of infected individuals are with clade C. In this work, we used advanced whole-brain MR methods, proton MR spectroscopic imaging (MRSI) and diffusion kurtosis imaging (DKI), to fully characterize the brain tissue metabolite concentration and microstructural integrity changes, including the cortical gray matter regions, in individuals with HIV-1 clade C infection. Eight individuals with HIV-1 infection and seven age-matched healthy subjects were scanned at 3Tesla using whole-brain MRSI and DKI techniques. Data were analyzed by lobar, anatomical regional and tissue type levels to make between-group comparisons. Metrics compared include N-acetyl aspartate, total-creatine, total-choline, myo-inositol, glutamate+glutamine, their ratios (NAA/Cre, Cho/NAA and m-Ins/NAA), mean diffusivity, axial diffusivity, radial diffusivity, fractional anisotropy, mean kurtosis, axial kurtosis, and radial kurtosis. All lobar and 19 brain regions showed substantial changes for m-Ins/NAA and few other metabolites in the HIV group. Significant between-group differences were found for several DKI/DTI metrics in several gray matter and white matter regions; these regions corresponded with the known viral distributions within the brain. Our neuroimaging methodology will be useful to evaluate the impact of HIV and its therapeutics throughout the brain.

P55

Chimeric HIV Infected Mice Carry Latent-inducible HIV in T cells, Active HIV in Macrophages and Develop Neurocognitive Disease

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Antiretroviral therapies (ART) largely eliminated AIDS and HIV-associated dementia but not milder neurocognitive impairments (HIV-NCI) which afflict about 50 % of HIV infected individuals on treatment. HIV persistence in viral reservoirs likely contributes to NCI but the mechanisms involved are difficult to study in patients. Here we show that infection of conventional mice with chimeric HIV, EcoHIV, recapitulates conditions for development of NCI in HIV infected humans on ART without immunodeficiency. EcoHIV infected mice display transiently high virus burdens followed by virus decline and establishment of chronic infection. EcoHIV control in mice arises, at least in part, from antiviral innate and adaptive immune responses. Chronically infected mice remain aviremic and immunocompetent with normal CD4+ cell levels but carry integrated provirus in CD4+ cells, macrophages, bone marrow, and CNS. Spleen cells from chronically infected mice and PBMC from patients on effective ART had similar burdens of minimally expressed integrated virus. As in such patients, latent provirus in murine CD4+ lymphocytes was stable and ART-insensitive but could be induced ex vivo by prostratin and chromatin modifying agent SAHA. In contrast to T cells, macrophages persistently express EcoHIV in vivo and can be further induced by TNF-alpha. Infectious virus could be rescued from either cellular reservoir by culture or transfer to uninfected mice. Murine leukemia virus (MLV), the source of mouse-tropic envelope in EcoHIV, failed to replicate in macrophages; although both EcoHIV and MLV entered the brain of infected mice only EcoHIV induced NCI as determined by impaired spatial learning and working memory in radial arm water maze and poor memory in contextual fear conditioning. These results establish a model of HIV infection of conventional mice for investigation of HIV reservoirs in T cells, macrophages, and CNS in vivo, their contribution to HIV neuropathogenesis, and testing interventions to purge HIV reservoirs and prevent NCI.

P56

EBV nuclear antigen-1 epitope reactive to intrathecal antibodies in the cerebrospinal fluid of patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of unknown etiology. The most common laboratory abnormality associated with MS is increased intrathecal IgG synthesis and the presence of oligoclonal bands (OCBs) in the brain and cerebrospinal fluid (CSF). However, the major antigenic targets of the antibody response are unknown. The risk of MS is increased after infectious mononucleosis and MS patients have higher serum titers of EBV antibodies than control populations. Objectives: To identify disease-relevant epitopes of IgG antibodies in MS. Methods: We screened phage-displayed random peptide libraries (12-mer) with total IgG purified from an acute MS brain. We characterized phage peptide binding specificity to intrathecal IgG from patients with MS and controls by ELISA, phage-mediated Immuno-PCR, and isoelectric focusing. Results: Two phage-displayed peptides were identified that share linear sequence homologies with EBV nuclear antigens 1 and 2 (EBNA-1 and EBNA-2), respectively. The specificity of the EBV epitopes to panning MS brain IgG was confirmed by ELISA and competitive inhibition assays. Using a highly sensitive phage mediated immuno-PCR assay, we determined specific bindings of the two EBV epitopes to CSF from 50 MS and 5 inflammatory control (IC) patients. Antibody binding to EBNA-1 epitope, but not to EBNA-2 epitope, was found in 25 of the 50 MS patients and 1 of the 5 IC patients. Furthermore, EBNA-1 epitope was recognized by OCBs in multiple MS CSF by isoelectric focusing. Conclusions: EBNA-1 epitope is reactive to MS intrathecal antibodies corresponding to oligoclonal bands.

P57

Molecular Mechanisms involved in regulation of Peli1 activity by HIV-TAT & Cocaine: Implications for HAND

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Intravenous drug use (IVDU) and HIV infections are two linked global health crises. Cocaine, abused by infected patients, worsens HIV-associated neurocognitive disorders (HAND) via unknown mechanisms. While it is well recognized that both HIV-TAT and cocaine can activate microglia, mechanism(s) underlying neuroinflammation remain poorly understand. Here we sought to investigate the role of Pellino1 (Peli1) in neuroinflammation in the context of HIV-TAT & cocaine. Peli1 is an E3 ubiquitin ligase that positively regulates Toll-like receptor (TLR) mediated-pathways in

microglia. In this study, we first demonstrated that in both BV2 microglia as well as rat primary microglial cells, TAT was capable of increasing Peli1 levels both temporarily and dose-dependently. Next we sought to examine the effect of cocaine on Peli1 activity. In our effort to identity the protein kinase that was critical for modulating Peli1 activity, we focused our study on the Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) since this kinase is highly expressed in the mammalian brain and is known to be regulated by cytosolic Ca^{2+}, which in turn, can be modulated by cocaine. Our data demonstrated that exposure of BV2 cells to cocaine resulted in up-regulation of CaMKII activity via increased phosphorylation. Activated CaMKII was shown to bind with Peli1 as evidenced by multiple experiments. There are six candidate motifs in Peli1 that are compatible with the CaMKII minimum recognition indicating thereby that Peli1 is a direct substrate of CaMKII. In summary, our data suggests HIV-TAT and cocaine modulate Peli1 activity through transcriptional and post-translational regulation, respectively. Peli1, play a critical role in the development of experimental autoimmune encephalitis, could also have ramifications for microglial activation in the pathogenesis of HAND and could, potentially be harnessed as a therapeutic target for dampening neuroinflammation in HAND (This work is support by grants DA036157 and DA027729).

P58
Novel Tools for Programming Extracellular Vesicle Cargo for Delivery to Target Cells

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Extracellular vesicles (EVs) are nano-sized organelles that are shed by a variety of cell types in the body. They function as messengers of intercellular communication by functionally transferring active biomolecules such as proteins and RNA species including miRNA. Their potential to serve as biomarkers for several neurological pathologies as well as vehicles for delivery of therapeutic agents has only begun to be explored. Development of tools that allow manipulation of the exosomal cargo and tracking exosome cargo delivery are key to optimizing EVs as therapeutic shuttles. Here, we show that EVs can be effectively transfected with siRNAs, mRNAs as well as plasmid DNA using a simple two-step protocol developed for Exo-Fect. Importantly, we show that the transfected nucleic acids are translated into protein when delivered to recipient cells suggesting that they retain their functionality within the vesicles. This technology can be exploited to deliver pharmacological agents to the brain as EVs have been shown to cross the blood brain barrier. Additionally, delivery of EV cargo to the recipient cells can be monitored by labeling of internal EV RNAs and proteins using unique fluorescent reagents. Lastly, host producer cells can be engineered to generate engineered EVs that contain specified RNA, protein cargo and are also programmed to target the brain by presentation of neural-specific ligands on the EV surface. Taken together, the tools presented offer a comprehensive system for cargo labeling and manipulation to enhance applications in using EVs for therapeutic delivery to the CNS.

P59
TAR DNA binding protein 43 (TDP-43) enhances HIV replication in macrophages

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Macrophages are an important reservoir of HIV infection in the brain. The life cycle of the virus in these cells is different compared to lymphocytes and astrocytes where it can form a persistent infection. But the mechanism by which the virus is regulated in these cells is not clear. TAR DNA binding protein 43 (TDP-43) is an important regulator of RNA metabolism including miRNA processing, RNA splicing and mRNA stability. TDP-43 can associate with both DNA and RNA elements to control gene expression. Given that TDP-43 has previously been shown to bind directly to the HIV long terminal repeat (LTR) to repress HIV gene expression in vitro, we examined the role of TDP-43 in regulating HIV replication in various HIV-susceptible cell types including macrophages, T cells and astrocytes. Surprisingly, we show that over expression of TDP-43 enhanced HIV transcription in primary monocyte-derived macrophages as measured by qRT-PCR and enhanced viral release as measured by presence of reverse transcriptase (RT) in culture supernatant. These effects were independent of TDP-43 binding to HIV LTR, as mutation of putative TDP-43 binding sites in a TAR-spanning region of the HIV promoter abrogated binding but had no significant effect on TDP-43-mediated enhancement of HIV LTR activity. These findings suggest that TDP-43 plays a positive regulatory role in HIV infection of macrophages via a mechanism that is independent of association with the HIV promoter.

P60

HIV-1 viremia accelerates age of human cortex and blood cells

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Background: Infection with the Human Immunodeficiency Virus-1 (HIV) is associated with clinical symptoms of accelerated aging, as evidenced by increased incidence and diversity of age-related illnesses at relatively young ages that is supported by findings of organ and cellular pathology. However, without an accurate method for measuring biological (as opposed to chronological) age, further delineation of the pathogenesis of age-related pathologies in HIV is problematic. Here, we applied an epigenetic biomarker of aging based on host DNA methylation levels to detect and measure accelerated aging effects due to HIV infection in peripheral blood mononuclear cells (PBMC) and brain tissue. Methods: DNA from PBMC and brain tissue was obtained from several independent samples and assayed via the Illumina Infinium Methylation 450 K platform. Age acceleration was calculated based on residual values from the difference of chronological age and biological age, as determined via the Epigenetic Clock. Results: Using three novel DNA methylation data sets, we show that HIV leads to an increase in epigenetic age both in brain (7.4 years) and PBMC (5.2 years). While the observed accelerated aging effects in PBMC may reflect changes in blood cell composition (notably exhausted cytotoxic T cells), it is less clear what explains the observed accelerated aging effects in brain tissue. Conclusions: Overall, our results demonstrate that the Epigenetic Clock is a useful biomarker for detecting accelerated aging effects due to HIV viremia. This tool can be used to accurately determine the extent of age acceleration in individual tissue and cells, and as demonstrated here particularly in the context of NeuroHIV.

P61

Binding of exosomal miR-138 to TLR7 promotes microglial activation and contributes to morphine-associated neuroinflammation

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Opiate abuse and HIV-1 have been described as two linked global health crises, and despite the advent of anti-retroviral therapy, abuse of opiates like morphine has been shown to result in increased neuroinflammation in HAND, which was further evidenced by the morphine-dependent model of rhesus macaques (RMs) infected with CCR5-utilizing SIVR71/17E model. However, the detailed molecular mechanism of how morphine contribute to the increased neuroinflammatory response is unclear. In recent years, microRNA (miR) that participate in translational regulation have emerged as paracrine signaling mediators to control the disease pathogenesis. Specifically, crosstalk between CNS cells via extracellular vesicles (EV)-mediated miRNAs has been shown to play an important role in regulating the progress of CNS disease. In addition, our recent study demonstrated that EV-mediated shuttling of miR-29 regulates HIV Tat and morphine-mediated neuronal dysfunction. In this study, using microarray analysis from the basal ganglia region of the RM brains we demonstrated increased expression of miR-138 among other miRs in morphine-dependent SIV-infected RMs compared with virus-infected animals. Interestingly, we detected that morphine-mediated induction of miR-138 showing a cell-type specificity with the highest expression level in astrocytes by realtime PCR. We further found that morphine exposed astrocytes upregulate expression & release of miR-138 in the EVs, which, following uptake by the microglia, results in their activation via the TLR7-dependent pathway. Moreover, miR-138, containing GUUGUGU motif, can bind endosomal TLR7 and contributes to microglial activation. Finally, by using TLR7−/− mice, we confirmed the unique pathway of EV-mediated miR-138 in triggering microglia activation in vivo. These studies are likely to reveal the therapeutic potential of EV-loaded miRNA in regulating neuroinflammation in HIV-infected opiates abusers. (Grant support: DA035203, DA036157)

P62

Glial activation, recruitment, and survival of B-lineage cells during MCMV brain infection

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Proinflammatory cytokines, chemokines, and other mediators produced by activated glial cells drive immune cell infiltration of the brain in response to viral infection. In the present study, in vivo and in vitro experiments were undertaken to investigate the role of reactive glia in recruitment and survival B-lineage

cells within the brain. Flow cytometric analysis of brain-infiltrating CD19+ B-cells was performed at 7, 14, 30, and 60 days post-infection (dpi) to assess their chemokine receptor phenotype. Infiltrating B-cells, which were found to be present within the CNS during acute through chronic phases of infection, expressed CXCR3, CXCR5, CCR5, and CCR7 chemokine receptors. Correspondingly, mRNA for the chemokines CXCL9, CXCL10, CXCL13, CCL3, and CCL19 was detected within infected brain homogenates, with peak expression at 7 dpi. Dual immunohistochemical staining indicated that both CXCL10 and CXCL13 were predominantly localized to astrocytes and microglia. CXCL9, CXCL10, and CXCL13 were produced in vitro by primary astrocytes, but were produced at much higher levels by cultured microglial cells. In mixed glial cultures, expression of CXCL13 protein was induced by TNFα and IL-1β, but was interferon (IFN)-independent; while CXCL9 and CXCL10 were induced following TNFα, IL-1β, and IFNs. In addition, expression of mRNA for B cell-activating factor belonging to the TNF family (BAFF) was upregulated within the brain from acute through chronic infection; and dual immunohistochemical staining localized BAFF expression to GFAP-positive astrocytes. Finally, in vitro analysis suggested that these glial cell-derived chemokines drive CD19+ B-cell chemotaxis, as antibodies to CXCL9, CXCL10 and CXCL13 reduced (approx. 63 %) the migration of murine splenic B-cells toward supernatants obtained from TNFα-activated mixed glial cell cultures. Taken together, these data indicate that reactive glia drive infiltration of B-lineage cells in response to MCMV infection and promote their survival within the brain.

P63

Rabies Virus Infection Causes Neuronal Injury Mediated by Mitochondrial Dysfunction and Oxidative Stress

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Our previous studies in a mouse model of experimental rabies showed neuronal process (dendrites and axons) degeneration in association with severe clinical disease. Cultured adult rodent (mouse and rat) dorsal root ganglion neurons infected with the challenge virus standard-11 (CVS) strain of rabies virus (RABV) showed axonal swellings and reduced axonal growth with evidence of oxidative stress. We have shown that CVS infection alters a variety of mitochondrial parameters and increases reactive oxygen species (ROS) production and mitochondrial Complex I activity. We have hypothesized that a RABV protein targets mitochondria and triggers dysfunction. Mitochondrial extracts of mouse neuroblastoma (MNA) cells analyzed with a proteomics approach showed that extracts were highly enriched with the RABV phosphoprotein (P). P was also detected by immunoblotting in RABV-infected purified mitochondrial extracts and in Complex I immunoprecipitates from the extracts. A plasmid expressing P in cells increased Complex I activity and increased ROS generation, whereas expression of other RABV proteins did not. Expression of a peptide from amino acid 139–172 of the P increased Complex I activity and ROS generation similar to expression of the entire P protein, whereas peptides that did not contain this region did not. These results indicate that a region of the RABV P interacts with Complex I in mitochondria causing mitochondrial dysfunction, increased generation of ROS, and oxidative stress. Hence, the RABV P plays a key role in the induction of mitochondrial dysfunction and generation of ROS resulting in oxidative stress in rabies virus infection through a direct interaction with mitochondrial Complex I. The resulting mitochondrial dysfunction produces oxidative stress in neurons that causes acute degenerative changes affecting neuronal processes and plays an important role in producing severe clinical disease with a fatal outcome. This information will be important for the future development of novel therapies for rabies.

P64

HIV-related cognitive impairment shows association with polymorphisms within the dopaminergic system in substance dependent and independent populations

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It has been postulated that drugs of abuse act synergistically with HIV, leading to increased neurotoxicity and neurocognitive impairment. The CNS impacts of HIV and drug use converge on the mesocorticolimbic dopamine (DA) system. Using an advanced-stage HIV+ population, previous studies in our laboratory have implicated a role of polymorphisms of two receptors within this system (DRD1 and DRD2) and neuropsychological performance as well as opiate and cocaine dependence. To replicate and expand these studies, we have increased our sample size and added additional polymorphisms within the dopaminergic system. In Caucasian subjects, we observe significant associations for opiate and cocaine dependence with polymorphisms within DRD2 and COMT genes, while no significant associations were found in African-American individuals. Using linear regression analysis, we next examined these polymorphisms for associations with neuropsychological performance in global and cognitive domain T-scores (Motor, Processing Speed, Verbal Fluency, Learning, Memory, Executive Functioning, Working Memory) while controlling for opiate and cocaine dependency. While significant associations were observed in nearly every domain across both populations for multiple polymorphisms, the most significant effects in Caucasian subjects were observed in the motor domain with several DRD2 polymorphisms while African-American subjects had its most significant associations in working memory (COMT and DRD3) and memory (SLC6A3) domains. For all of these associations, the effects differed for substance dependence groups as the direction of the correlations were opposite to what was seen in subjects without dependency. Future studies will focus on increasing the sample size of the population as well as increasing the number of genes within the dopaminergic circuitry. We conclude that studies to examine genetic risk for HAND must carefully account for substance dependence patterns when assaying dopaminergic systems, as the neurobiological substrates of cognition in HIV populations may vary with tonic alterations secondary to chronic substance exposures.

P65
Antiretroviral Compounds Disrupt Oligodendrocyte Maturation and Myelin Maintenance

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HIV-Associated Neurocognitive Disorders (HAND) afflict approximately half of HIV(+) individuals despite effective viral suppression through combined antiretroviral therapy (cART). A prominent category of persistent pathologies are those affecting white matter, with evidence of diffuse myelin pallor, loss of structural integrity of corpus collosum, and decreased myelin protein mRNAs. Loss of myelin can contribute to neurocognitive dysfunctions, as the myelin membrane generated by CNS oligodendrocytes is essential for rapid signal transduction and axonal maintenance. We hypothesized that myelin changes in HAND may be attributed, in part, to effects of antiretrovirals on oligodendrocyte survival and maturation. Using our primary mouse oligodendrocyte culture model, we have shown that therapeutic concentrations of HIV protease inhibitors (PIs), Ritonavir and Lopinavir lead to dose-dependent decreases in oligodendrocyte maturation. Drug removal led to a complete and rapid reversal of deficits, suggesting proper differentiation pathway activation, and a post-translational effect of PIs which prevented proper cell surface localization of synthesized myelin proteins. In contrast, Zidovudine, a nucleoside reverse transcriptase inhibitor, did not produce any alterations in maturation. To examine the effects of antiretrovirals on the maintenance of formed myelin, we employed an in vivo jugular vein administration model in adult mice. Following twice-daily treatment with 20 mg/kg Ritonavir, we demonstrated a reduction of myelin proteins in only 14 days. Furthermore, examination of post-mortem prefrontal cortex specimens from the National NeuroAIDS Tissue Consortium demonstrated a significant decrease in myelin basic protein in HIV(+) individuals with HAND who had been cART-medicated for >12 months, when compared to both HIV(+) cART-naïve HAND patients and uninfected, age-matched controls. Overall, our in vitro and in vivo findings are the first to demonstrate the potential negative consequences of antiretrovirals in myelin production and maintenance, and implicate potential long-term negative consequences of cART in both juvenile and adult patients who will be on lifelong therapy.

P66
Immune Markers of Cognitive Impairment Among HIV-infected Individuals in Nigeria

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Introduction: Mononuclear cells play key roles in the pathogenesis of HIV associated neurocognitive disorders (HAND). Viral trafficking into the central nervous system occurs mainly within these cells. Activation of monocytes/macrophages has been shown to correlate with severity of HAND. Limited studies have looked at markers of immune activation vis-à-vis HAND classification in Africa. We examined the association between levels of markers of monocyte activation and HAND among HIV/AIDS patients in Nigeria. Methods: A total of 149 antiretroviral-naïve HIV infected participants in a prospective cohort study underway in Nigeria were administered a 7-domain comprehensive neuropsychological test battery and assessed for function using activities of daily living questionnaire. Demographically adjusted T scores based on HIV negative control normative values were generated. Mean scores were calculated for tests within each domain. Using baseline data, participants were classified as either unimpaired, having asymptomatic neurocognitive impairment (ANI), minor neurocognitive disorder (MND), or HIV associated dementia (HAD) in line with the "Frascati" criteria. Plasma levels of soluble CD14, soluble CD163, monocyte chemo-attractant protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), and Neopterin were measured. Results: About 76.5 % were unimpaired, 14.1 % had ANI, 9.4 % had MND, and none had HAD. In a multivariable linear regression adjusting for age and gender, mean levels of soluble CD14 were higher among MND and ANI compared to the unimpaired (p-values: 0.004 and 0.03 respectively; Cohen's d: 0.82 and 0.51 respectively). Mean levels of Neopterin and, for women, soluble CD163 and TNF-α were greater for MND than for unimpaired individuals (p-values: 0.05, 0.05, and 0.07, respectively; Cohen's d: 0.57, 0.63, and 0.59, respectively). Conclusion: Levels of monocyte activation markers correlate with the severity of impairment among individuals with HAND. The mechanisms that underlie these effects and the potential role of gender require further study.

P67
CSF Biomarkers of Iron Transport and Angiogenesis are Associated with HIV-Associated Neurocognitive Disorder (HAND)

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Background: Dysregulated iron transport may promote glial-cell activation and angiogenesis, which are implicated in blood–brain barrier compromise and some neurodegenerative disorders. We hypothesized that cerebrospinal fluid (CSF) ceruloplasmin (CP), a ferroxidase involved in iron transport, haptoglobin (HP), an iron-binding protein and ligand for the macrophage-monocyte scavenger receptor CD163, and vascular endothelial growth factor (VEGF) are associated with HAND. Methods: CP, HP, and VEGF levels were quantified by immunoassay in CSF from 405 participants enrolled in CHARTER, an observational neuro-HIV study. CSF biomarker associations with HAND, defined by global deficit score (GDS)$\geq$0.5 or Frascati criteria, were evaluated for tertile (T)3 vs. T1, adjusting for antiretroviral therapy, CD4 nadir, genetic ancestry by principal components, and comorbidity; GDS was also analyzed as a continuous measure. Analyses stratified by comorbidity (contributing vs. incidental to neurocognitive impairment), and excluding subjects with multiple biomarker values >2 SD above the mean, were also performed. Results: Higher CP and HP levels were associated with GDS-defined HAND in participants without contributing comorbid conditions (N=270), both before [adjusted odds ratio (OR, T3 vs. T1) 2.37 for CP, p=0.02; OR 2.13 for HP, p=0.04] and after [OR 2.52 for both CP and HP, both $p<0.05$] excluding outliers. CP levels were also associated with HAND by Frascati

criteria before and after excluding 18 participants with outlier values, but HAND remained associated with HP only after excluding outliers (OR 2.08, $p=0.03$). In analyses of all participants, higher VEGF was associated with HAND (OR 2.00, $p=0.02$). CP and HP were also positively associated with continuous GDS ($p<0.01$ and $p<0.05$, respectively) in participants lacking contributing comorbidities. Conclusions: CSF HP, CP, and possibly VEGF, show strong associations with HAND, depending on comorbidity status, suggesting the potential benefit of interventions aimed at disordered iron transport and angiogenesis in preventing or treating HAND.

P68

Eradication of integrated HIV-1 genome from latently infected T-cells by targeting LTR sequences using CRISPR/Cas9 genome editing system.

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Longevity, homeostatic proliferation and resistance to antiviral therapy of latently infected cells represents the principal challenges toward curing AIDS. The main latent HIV-1 reservoir resides in the subset of CD4+ T-cells called resting, memory T-cells. Using CRISPR/Cas9 genome editing technology to target two unique sequences in U3 region of HIV-1 LTR (called target A and B), we were able to completely eliminate proviral sequences from the genome of latently infected T-cell lines. We tested and validated two delivery approaches: plasmid DNA transfection/single cell clones selection and inducible lentiviral delivery system. Both strategies resulted in abrogation of HIV-1 reactivation as a result of removal of the proviral sequences from the host cell genome by long range PCR genotyping. This result was further confirmed by sequencing of cleavage lariat from integration locus in chromosome 16. Surveyor assay and sequencing of potential off target sites in the host genome showed no detectable off target effects. Furthermore, removal of the proviral sequence had no significant impact on the expression in neighboring genes. Finally, stable expression of Cas9/gRNA complexes targeting LTR was able to protect cells against new infections. Our results indicate that CRISPR/Cas9 system can be used to specifically remove integrated viral sequences from the genome of latently infected cells. This proof of concept study provides a new avenue to cure AIDS.

P69

Interplay of Rad51 with NF-κB pathway stimulates expression of HIV-1

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Transcription of the HIV-1 promoter is controlled by a series of ubiquitous and inducible cellular proteins, some with the ability to enter the nucleus and interact with the specific DNA sequences spanning the 5′ long terminal repeat, LTR. Here we show that Rad51 overexpression activates LTR promoter. This activation requires stimulation caused by NF-κB p65 and is abrogated by the expression of dominant negative IκBa mutant. Our results also indicate that activation of NF-κB pathway by PMA treatment or overexpression of its subunit p65, promotes the association of Rad51 with the LTR sequence. Accordingly, stimulation of the viral promoter by Rad51 relies, in part, on its interplay with p65 and the NF-κB pathway. Treatment of the cells with PMA that promotes nuclear entry of Rad51 or inactivation of the NF-κB pathway by a dominant negative mutant of IκBa modulates the ability of Rad51 to stimulate LTR transcription. Infection of primary peripheral blood mononuclear cells with HIV-1 induces Rad51 expression and treatment of the infected cells with Rad51 inhibitor suppressed lentiviral replication in this cells, suggesting the operation of positive feedback pathway between HIV-1 and Rad51. These observations ascribe a new role for Rad51 in transcription of the HIV-1 genome and offer a new avenue for the development of anti-HIV-1 therapeutics.

P70

Exosomes from HIV-1 Infected Cells Stimulate Production of Pro-inflammatory Cytokines through TAR RNA

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HIV-1 infection results in a chronic illness since long-term HAART can lower viral titers to an undetectable level. However, discontinuation of therapy rapidly increases virus burden. Moreover, patients under HAART frequently develop various metabolic disorders and HIV-associated neuronal disease (HAND). We have previously shown that exosomes containing TAR RNA enhance susceptibility of undifferentiated naïve cells to HIV-1 infection. The current study indicates that exosomes from HIV-1 infected primary cells as well as CSF samples are highly abundant with TAR RNA as detected by RT-real-time PCR. Interestingly, up to a million copies of TAR RNA per microliter were also detected from HIV-1 infected humanized mice suggesting that HIV-1 TAR RNA may be stable in vivo. Incubation of exosomes with primary macrophages resulted in a dramatic increase of proinflammatory cytokines, IL-6 and TNF-α indicating that exosomes containing TAR RNA could play a direct role in control of cytokine gene expression. The intact TAR molecule was able to bind to PKR, TLR7, and 8 effectively, whereas 5′ and 3′ stems bound best to TLR3 and none to PKR. Binding of TAR to PKR did not result in its phosphorylation and therefore TAR may be a dominant negative decoy molecule. The single stranded 5′ or 3′ stem RNA binding to TLRs activates the NF-κB pathway and regulate cytokine expression. Collectively, these results imply that exosomes containing TAR RNA could directly affect the proinflammatory cytokine gene expression and may explain a possible mechanism of inflammation observed in HIV-1 infected patients.

P71

Role of Bruton's Tyrosine Kinase inhibitors in HIV-1 infected cells

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Many cellular cofactors have been documented to be critical for various stages of viral replication. Using high throughput proteomic assays, we have previously identified Bruton's tyrosine kinase (BTK) as a host protein that was uniquely upregulated in the plasma membrane of HIV-1 infected T-cells. Here, we have further characterized the BTK expression in HIV-1 infection and show that this cellular factor is specifically expressed in infected myeloid cells. Significant up-regulation of the phosphorylated form of BTK was observed in infected cells. Using size exclusion chromatography, we found BTK to be virtually absent in the uninfected U937 cells, however new BTK protein complexes were identified and distributed in both high molecular weight (~600 kDa) and a small molecular weight complex (~60–120 kDa) in the infected U1 cells. BTK levels were highest in cells either chronically expressing virus or induced/infected myeloid cells and that BTK translocated to the membrane following induction of the infected cells. BTK knockdown in HIV-1 infected cells using siRNA resulted in selective death of infected, but not uninfected, cells. Using BTK specific antibody and small molecule inhibitors including LFM-A13 and a FDA approved compound, Ibrutinib (PCI—32765), we have found that HIV-1 infected cells are sensitive to apoptotic cell death and result in a decrease in virus production. Overall, our data suggests that HIV-1 infected cells are sensitive to treatments targeting BTK expressed in infected cells.

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Therapeutic doses of irradiation activate viral transcription and induce apoptosis in HIV-1 infected cells.

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The highly active antiretroviral therapy reduces HIV-1 RNA in plasma to undetectable levels. However, the virus continues to persist in the long-lived resting CD4+ T cells, macrophages and astrocytes which form a viral reservoir in infected individuals. Selective activation of viral transcription is critical since the host immune response in combination with antiretroviral therapy may eradicate the virus following reactivation ("Shock and Kill"). X-ray irradiation (IR), a well-defined stress signal that is widely used for many therapeutic purposes, has been shown to be capable of activating HIV-1 transcription, progeny virion formation and eventual apoptosis of infected cells. Using chronically HIV-1 infected T lymphoblastoid and monocytic cell lines, primary resting CD4+ T cells and humanized mice infected with dual-tropic HIV-1 89.6, we examined the effect of IR-induced cellular stress on HIV-1 transcription and viability of infected cells. Treatment of both T cells and monocytes with therapeutic IR doses led to dramatic increase of HIV-1 transcription, as

evidenced by the presence of Pol II and reduction of HDAC1 and methyl transferase SUV39H1 on the HIV-1 promoter using ChIP assay. Increased HIV-1 replication after IR correlated with higher cell death as compared with uninfected cells. Importantly, the level of phosphorylated Ser46 in p53 responsible for apoptosis induction was markedly higher in HIV-1 infected cells following IR treatment. Exposure of HIV-1 infected humanized mice treated with antiretrovirals, which did not display viral RNA in the plasma and PBMCs, to IR resulted in a significant increase of HIV-1 RNA in plasma, lung and brain tissues of these animals. IR-induced cellular stress facilitates enhanced apoptotic death of infected cells possibly via p53. Collectively, these data point to the use of combination of low to moderate dose of IR with HIV-1 transcription activators as a potential application for the "Shock and Kill" strategy for latently infected cells.

P73

Neurocognitive Impairment in EcoHIV-infected Mice Correlates with Diffuse Dendritic Damage and Defective Neuronal Function in the Hippocampus without Neuronal Apoptosis: A Model for Study of Mild HAND

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Antiretroviral therapy has significantly reduced the prevalence of HIV-associated dementia but not of milder neurocognitive impairments (NCI) which are currently diagnosed in about 50 % of HIV-infected individuals on treatment. We have shown that intracerebral inoculation of chimeric HIV (EcoHIV) in conventional mice permits efficient HIV expression in mouse brain tissue with accompanying pathologies resembling human HIV brain disease. Here we show that 2 weeks after this infection, mice exhibited significant impairments in spatial learning and memory in water maze tests and in associative learning and recall in auditory-cued fear conditioning. NCI persisted for 2 months despite subsequent seroconversion of infected animals. Murine leukemia virus used as a control mouse pathogen that shares the envelope gene with EcoHIV caused neither brain pathology nor behavioral impairment despite robust infection. No cellular apoptosis was seen in TUNEL assays with either virus despite macrophage/microglia activation and significant increases in the production of neuroimmune-associated cytokines and chemokines. EcoHIV-infected mice demonstrated significant decreases in MAP2 immunofluorescence in the CA1, CA2, and CA3 regions of the hippocampus and impaired theta-burst induced LTP in CA1 region, indicative of hippocampal dysfunction. The HIV-induced cognitive disease in mice was prevented by systemic treatment of animals with antiretroviral drugs used clinically in humans, showing HIV specificity and replication-dependence of the disease. Our results suggest that brain infection of an immunologically competent host with HIV is sufficient to induce persistent infection and enduring cognitive deficits, which at least in part can be attributed to hippocampal dysfunction. Because no neuronal apoptosis was observed, this model system may help in experimental characterization of pathophysiology of mild HAND. Supported by DA037611, MH104145, and DA017618.

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HIV Distal Neuropathic Pain is associated with Smaller Ventral Posterior Cingulate

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Despite modern antiretroviral therapy, HIV neuropathy affects approximately 50 % of HIV subjects. The clinical expression of HIV neuropathy is highly variable: many individuals report few symptoms, but about half report distal neuropathic pain (DNP), making it one of the most prevalent, disabling and treatment-resistant complications of HIV disease. The presence and intensity of DNP is not fully explained by the degree of peripheral nerve damage. A better understanding of brain mechanisms for HIV DNP may help explain why some patients with HIV neuropathy report pain while the majority does not. Previously, we reported that more severe DNP was associated with smaller total cerebral cortical gray matter volumes. Here we present a voxel-based morphometric analysis that investigates which regional cortical volumes are smaller for subjects with DNP compared to subjects without DNP. We report that the left ventral posterior cingulate cortex is the only regional brain volume which is smaller for subjects with DNP compared to subjects without DNP (peak $p=0.017$; t-score= 5.15; MNI coordinates $x=-6$, $y=-54$, $z=20$, cluster average t-

score=0.97). We discuss that smaller posterior cingulate cortex volume for subjects with DNP may indicate that DNP disrupts brain activity in the default mode network.

P75

HIV-1 regulatory protein, TAT, decreases brain reward function and enhances methamphetamine reward

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Psychostimulant abuse and depression are common comorbidities among humans with immunodeficiency virus (HIV) disease. The HIV regulatory protein TAT is one of multiple HIV-related proteins associated with HIV-induced neurotoxicity. TAT-induced neuropathology in corticolimbic brain areas may result in impaired reward function and, thus, contribute to depressive symptoms and psychostimulant abuse. Transgenic mice with doxycycline-induced TAT protein expression in the brain (TAT+) show neuropathology resembling brain abnormalities in humans with HIV disease. We evaluated brain reward function in response to TAT expression, nicotine and methamphetamine administration in TAT+ and TAT- (control) mice. Brain reward function was assessed using the intracranial self-stimulation procedure. Elevated current-intensity brain reward thresholds served as a measure of reward deficits, a core symptom of depression. Threshold lowering served as a measure of reward enhancement. After establishing stable baseline thresholds, TAT expression was induced by doxycycline administration for 7 days. Assessment of nicotine and methamphetamine dose–response functions in the intracranial self-stimulation procedure was initiated 2 weeks after the final doxycycline treatment. During doxycycline administration, thresholds were elevated by 20 % in TAT+ mice compared with TAT- mice. After the termination of doxycycline treatment, thresholds decreased in all mice, regardless of TAT expression. However, thresholds of TAT+ mice remained significantly higher than those of TAT- mice for 2 weeks. Methamphetamine induced dose-dependent threshold lowering in all mice, with TAT+ mice showing greater methamphetamine-induced threshold lowering compared with TAT- mice. Nicotine tended to elevate reward thresholds with no significant differences between TAT+ and TAT-mice. These results indicate that TAT expression in mice leads to reward deficits and a greater sensitivity to methamphetamine-induced reward enhancement. Our findings suggest that the TAT protein may contribute to increased depressive-like symptoms and continued methamphetamine use in HIV-positive individuals.

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Hyper-activation of L-type Ca^{2+} channels, independent of NMDA receptor, mediates increased Ca^{2+} influx in pyramidal neurons from the rat medial prefrontal cortex in the context of HIV infection.

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HIV-1 infection induces neurological and neuropsychiatric impairments. The medial prefrontal cortex (mPFC) regulates cognition, emotion and motivation-driven behavior, which are disrupted by HIV. We previously demonstrated that HIV infection induces an abnormal increase in the excitability of mPFC pyramidal neurons as modeled in HIV-1 transgenic (Tg) rats, which express seven of the nine HIV proteins driven by the HIV-1 promoter. In the current study, we assessed the contribution of voltage-gated Ca^{2+} channels (VGCCs) to HIV-induced hyper-excitability of mPFC pyramidal neurons in HIV-1 Tg rats. Five-six week old male adolescent non-Tg and HIV-1 Tg rats were used for electrophysiological assessment. To dissect Ca^{2+} influx (reflected by Ca^{2+} plateau potentials) specifically through VGCCs, whole-cell patch-clamp recordings were performed with blockade of glutamate- and GABA-mediated excitatory and inhibitory inputs, respectively, as well as voltage-gated Na^{+} and K^{+} channels. We report that HIV-induced neuronal hyper-excitation was associated with abnormally-enhanced Ca^{2+} influx, evidenced by prolonged Ca^{2+} potential duration and area ($p \leq 0.05$) in neurons from HIV-1 Tg rats compared to those from non-Tg rats. L-type Ca^{2+} channel (L-channel) activity significantly contributed to this increased Ca^{2+} influx because acute perfusion of nifedipine (5 μM, an L-channel blocker) reduced ($p \leq 0.001$) Ca^{2+} influx in HIV-1 Tg mPFC neurons to levels similar to those of untreated, control neurons from non-Tg rats. However, L-channel blockade in neurons from non-Tg rats showed significantly lower Ca^{2+} influx ($p \leq 0.05$) than in neurons from HIV-1 Tg rats, suggesting that non-L-type VGCC over-activation also contributes to the enhanced Ca^{2+} influx in HIV-infected mPFC. Our findings show that HIV infection alters mPFC neuronal function by dysregulating membrane excitability and voltage-sensitive Ca^{2+} influx, specifically at the L-channel, and most importantly independent of NMDA receptor. Overall, these findings demonstrate that HIV renders mPFC pyramidal neurons more susceptible and vulnerable to excitatory stimuli, which could contribute to HIV-associated neuropathogenesis.

P77

Treatment of EcoHIV-infected Mice with Intranasal Insulin Abrogates Cognitive Impairment and Normalizes Energy Metabolite Alterations Associated with HAND

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HIV-associated neurocognitive disorder (HAND) remains among the most prominent features of chronic infection with HIV, despite almost universal use and success of antiretroviral therapy (ART). Growing evidence supports insulin-mediated signaling pathways as key components in normal cognitive processes. Dysregulation of insulin signaling is implicated in inflammatory processes and energy metabolism and has been associated with the pathogenesis of several neurodegenerative diseases, including HAND. Recent clinical studies have shown that intranasal delivery of insulin to the CNS enhances cognitive processes in normal, Alzheimer's and diabetic patients. We hypothesized that intranasal insulin administration may also be effective in improving cognitive deficits in HAND. In the present work we evaluated this hypothesis using EcoHIV-infected immunocompetent mice, which were shown to reproduce the mild course of HIV infection such as seen in patients on effective ART and manifest neurocognitive impairment (NCI) when tested for learning and memory acuity in water maze. We report that daily intranasal administration of insulin (2.4 IU) increases brain insulin levels in mice approximately 50-fold from 10–20 pM at baseline to 700 pM at 1 h after administration with no effect on plasma insulin. Although insulin treatment (2.4 IU for 9 days) had no effect on virus burden in the brains of EcoHIV-infected mice, it completely reversed their NCI. Similar to human HIV, EcoHIV-infected mice exhibited altered brain metabolite levels including citrate, creatine, myoinositol, and glutamate which were partially normalized by insulin. Common with brain tissues from patients with HAND, brain from infected mice also showed dysregulation of gene families involved in energy metabolism and insulin signaling. The results highlight the relevance of the EcoHIV model to mild HAND and support the use of intranasal insulin for the treatment of HAND. *Supported by DA017618 and DA037611 to DJV and P30 MH075673-06 to BSS.

P78

Involvement of BAG3 in heart failure, HIV-1 associated comorbidity

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Survival rates of patients infected with HIV-1 have increased dramatically due to the introduction of combined antiretroviral therapy (cART) and the suppression of active viral replication. However, the incidence of HIV-1 comorbidities has also steadily risen. One of the commonest HIV-1-associated sequelae is heart failure (HF) secondary to left ventricular dysfunction. The exact mechanisms detailing the contribution of HIV-1 to the rapid development of HF in HIV-1-infected vs. non-HIV-1-infected individuals have not yet been elucidated. It is known that many HIV-1 proteins are constitutively expressed, and consequently, may be released by a latent viral reservoir into the systemic circulation. One HIV-1 protein of interest, Tat, is constantly produced in vivo, and it is well documented that previously uninfected cells that take up this protein experience significant toxic effects. Tat directly binds to cellular stress-induced/autophagy-related protein Bag3, which is essential for normal cardiac function. In response to mechanical stress to metabolically active cardiomyocytes, Bag3 protects myofibril structures at the Z-disk. While targeted knockdown of Bag3 in neonatal cardiomyocytes leads to cardiac dysfunction, Bag3 knockout mice develop a fulminant myopathy characterized by non-inflammatory myofibrillar degeneration - where the architecture of the Z-disc is disrupted—with apoptotic features and early death. Bag3 knockout mice die 4 weeks postnatally. Further, recent data suggest that Bag3 protein levels are decreased in heart tissue isolated during heart transplants from patients with HF compared to controls with normal heart function. Thus, it may be suggested that the interaction between

Bag3 and HIV-1 Tat proteins may lead to the inability of Bag3 to perform its normal cellular function in cardiomyocytes. Here we also show that exogenous expression of Bag3 can improve cardiac function in a mouse model of heart failure.

P79

IFN gamma drives Heme Oxygenase-1 deficiency in astrocytes: Role in HIV neuropathogenesis

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Expression of the cytoprotective enzyme heme oxygenase-1 (HO-1) is significantly reduced in the prefrontal cortex of HIV+ individuals and negatively correlates with CNS viral replication and neuroinflammation. In vitro, HIV infection of monocyte-derived macrophages (MDM) reduces HO-1 protein expression and induction of HO-1 reduces neurotoxic glutamate production. We now present data that IFN gamma, which is elevated in the CNS of HIV+ individuals, drives HO-1 protein deficiency within astrocytes. Chronic exposure to IFN gamma significantly reduces HO-1 protein, but not RNA, in primary human astrocytes. Similarly, HO-1 RNA is not reduced in HIV+ brain, despite reduced HO-1 protein, suggesting that HO-1 protein deficiency occurs through a post-transcriptional mechanism. We hypothesized that IFN gamma may drive accelerated degradation of HO-1 protein by induction of the immunoproteasome, a variant of the constitutive proteasome that exhibits distinct proteolytic properties and is elevated in HIV+ brain. Chronic exposure to IFN gamma dramatically altered proteasome composition in primary human astrocytes, as evidenced by induction of the catalytic immunoproteasome subunits LMP2 and LMP7 and concurrent loss of the corresponding constitutive subunits beta-1 and beta-5. This exchange of constitutive subunits for immunoproteasome subunits appears to be necessary for IFN gamma-driven HO-1 protein deficiency. Notably, HO-1 protein deficiency was only observed at higher doses of IFN gamma that promote near complete replacement of constitutive subunits by immunoproteasome subunits. Furthermore, HO-1 protein was reduced only after long exposures (12, 15 days) to IFN gamma, coinciding with an enhanced loss of constitutive subunits. Finally, pulse chase experiments demonstrate that IFN gamma decreases HO-1 protein half-life in a proteasome-dependent manner. Our results suggest that persistent elevation of IFN gamma within the CNS of HIV infected individuals promotes a switch to the immunoproteasome and enhances degradation of HO-1 protein. Therapeutic interventions targeting the immunoproteasome may provide neuroprotective benefit in HIV+ individuals.

P80

Neural Stem/Progenitor Cells: Impact of anti-viral cytokines on cell fate choice and proliferation during a CNS infection

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Viral infections in the central nervous system (CNS) are characterized by lymphocyte infiltration into the brain parenchyma and release of pro-inflammatory cytokines. Interferon-gamma (IFNγ) is a key anti-viral cytokine that is required for non-cytolytic clearance of many viruses from the CNS. Our goal is to define the role of IFNγ and the underlying signaling mechanisms in proliferation and differentiation of NSPCs. We hypothesize that IFNγ decreases NSPC proliferation and induces astrocytic differentiation in NSPCs via the Jak/STAT signaling pathway. Our data demonstrates that IFNγ reduced neurosphere diameter and blocked cell cycle progression at the G1/S checkpoint in vitro. IFNγ also increased astrocytic differentiation and reduced neuronal differentiation in NSPC cultures. IFNγ-mediated cell cycle blockade was STAT1-dependent, and was associated with reduced expression and phosphorylation of the Retinoblastoma (Rb) protein. During an in vivo CNS infection with measles virus, IFNγ was required to maintain the NSPC pool, suggesting that IFNγ is protective overall in the context of a CNS infection. Through these studies, we will define the role of the inflammatory response in modulating NSPC activity during viral infections. These studies will also identify new targets for therapy that could salvage the CNS from harmful effects of an anti-viral immune response.

P81

HIV-1 in the CNS: Serotonergic Deficits and Depression

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Background: In the cART era, HIV-1 infection has become a chronic disease and co-morbidities, including depression continue to remain a challenge for treatment. It is reported that up to 50 % of HIV-1 infected individuals experience depression which is twice as high compared to the non-infected people. Such high incidence of depression has led to the concept of "different type" of HIV-1 associated depression, particularly, since majority of individuals do not respond to treatment with antidepressants, such as SSRIs. Since serotonin (5-hydroytryptamione, 5-HT) is implicated in regulation of mood and behavior, we propose that HIV-associated depression may be the result of neurodegenerative changes in different brain regions leading to overall dysfunctional serotonergic system as a consequence of decreased availability of 5-HT in HIV-1+ depressed individuals compared to that in the non-depressed HIV-1+ and non-infected individuals. Objectives: We investigated changes in the availability of 5-HT in different regions of postmortem brains of HIV-1+ individuals (n=11) who were diagnosed with depression during life, and in the same brain regions of those who were diagnosed not to have depression (n=11). The well characterized brain regions (frontal cortex, basal ganglia, hippocampus, hypothalamus, raphe nuclei, amygdala, and substantia nigra) were procured from the NIH-supported NNTC centers. Results: We found that the availability of 5-HT (log_{10} pg/g tissue) was significantly decreased in some brain regions of HIV-1+ individuals who were diagnosed with depression compared to those who did not have depression (BG; 5-HT=3.598±0.69, vs 5.084± 1.138, p<0.005); (Amygd; 5-HT=3.43±0.45, vs 4.18±0.32; p<0.001). Similar differences were observed in the metabolite, 5-HIAA levels in some brain regions. No significant difference was found in 5-HT availability between non-depressed HIV-1+ and HIV-individuals. Conclusion: Our data suggest that in some of the brain regions of HIV-1+ depressed individuals, availability of 5-HT is significantly decreased leading to an overall serotonergic system dysfunction.

P82

Elevated sCD137 in plasma and DRG and its potential role in monocyte recruitment to DRG during SIV peripheral neuropathy

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SIV-infected CD8 T-lymphocyte-depleted rhesus macaques are used as a rapid-progression model of neuroAIDS with a high incidence of SIV encephalitis and mild-severe dorsal root ganglia (DRG) pathology. DRG histopathology, including satellitosis, Nageotte nodules, and neuronophagia and loss of intraepidermal nerve fiber (IENF) density, in SIV infected macaques are similar to the changes found in patients diagnosed with HIV-peripheral neuropathy. Previously, we have demonstrated that monocyte traffic into DRG (as measured by BrdU+ monocytes) correlated with the severity of DRG pathology and IENF density loss. Here, we sought to identify potential mechanisms of monocyte recruitment into the DRG. In this study, we used 12 SIV-infected CD8-depleted rhesus macaques that were given serial BrdU pulses to measure monocyte turnover and traffic and were sacrificed with AIDS. We measured chemokine and cytokine levels in DRG and plasma by MILLIPLEX assay (Luminex). This revealed elevated soluble CD137 (sCD137). CD137, a member of the TNFR family, enhances monocyte migration in vitro and in vivo and can be expressed by blood vessels at sites of inflammation, including atherosclerotic lesions where macrophages are CD137L+. CD137-CD137L bidirectional signaling mediates recruitment of monocytes to sites of inflammation and skews hematopoiesis toward myelopoiesis. Here, sCD137 was increased in plasma during late SIV infection and the percent change from pre-infection to necropsy was greatest in animals with severe DRG pathology. The number of circulating BrdU+ monocytes correlated with sCD137 in the plasma. Additionally, sCD137 was only detected in DRG tissue lysates with severe pathology, where BrdU+ monocytes were most abundant. These data suggest that sCD137 is a potential novel biomarker for the development SIV-PN and that CD137 signaling may play a role in recruiting monocytes to the DRG where they incite inflammation and tissue damage.

P83

Gene expression changes in monocytes predict neurocognitive dysfunction 2 years later

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BACKGROUND: Early pathogenic events leading to HIV-associated neurocognitive disorders (HAND) may be mediated by peripheral monocytes. We previously identified dysregulated genes and gene expression networks in peripheral blood monocytes that were correlated with neurocognitive functioning in HIV-1 infected (HIV+) adults. Here, we examine the same patients 2 years later, and probe for early gene expression biomarkers that predict change in neurocognitive functioning and HAND status. METHODS: mRNA was isolated from the monocytes of 93 HIV+ and 76 HIV-seronegative adults and analyzed with the Illumina HT-12 v4 Expression BeadChip. Sixty-five of the HIV+ participants were follow-ups. Neurocognitive functioning, HAND diagnosis, clinical and virologic variables were determined via standardized protocols. Data were analyzed using standard expression analysis and weighted gene co-expression network analysis (WGCNA). Bonferroni correction was applied. RESULTS: Standard expression analysis revealed one probe (GPR97) whose expression at baseline was significantly associated with change in neurocognitive functioning and HAND severity 2 years later. Another probe, reflecting expression of small nucleolar RNA, was also significantly associated with change in neurocognitive functioning. WGCNA revealed that baseline gene networks involved in mitotic cell cycle and immunoglobulin predicted change in neurocognitive functioning. CONCLUSIONS: These data implicate specific cellular alterations in monocytes that predict neurocognitive changes 2 years later.

P84
Restriction of HIV entry into astrocytes is overcome by cell-to-cell contact

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Studies show that astrocytes are a critical reservoir for HIV in the brain. However, HIV infection in astrocytes is extremely inefficient in vitro as astrocytes do not express detectable CD4 even though they express high level of CXCR4. We found that there was no significant intrinsic barrier for restricting HIV replication in astrocytes because the viruses could be constantly produced following either infection with VSV-G pseudotyped HIV-1 or transfection with HIV-1 proviral DNA. As expected, HIV was able to efficiently infect the cells pre-transfected with CD4 plasmid. In fact, we could consistently detect low levels of CD4 mRNA in primary fetal astrocytes and neuroprogenitor-derived astrocytes and small amounts of CD4 protein by immunoprecipitation. Interestingly, CD4 was able to be significantly up-regulated by pro-inflammatory cytokines at level of mRNA, but not at level of protein. Therefore, increased infection of HIV was not consistently seen in the cells pre-treated with pro-inflammatory cytokines. However, the efficient, productive infection was consistently observed in the cells co-cultivated with HIV-infected T lymphocytes. This cell-to-cell contact infection was further confirmed via a CD4-independent, CXCR4-dependent mechanism. A novel model of HIV infection in astrocytes is proposed, by which immature HIV particles are able to directly bind to CXCR4 before occurrence of their maturation. These observations indicate that HIV may infect astrocytes using alternative mechanisms in vivo rather than a classical mechanism.

P85
Suicidal gene mediated latent HIV-1 elimination

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HIV-1 eradication has become a challenge in the era of combined anti-retroviral therapy (cART). Even if HIV-1 replication is fully controlled with under cART, dormant HIV-1 still resides in the host genome, and the regulatory HIV-1 proteins like Tat and Rev are constantly produced. HIV-1 infection may re-emerge from this latent reservoir at any time. New approaches and therapies must be developed to eliminate latent HIV-1. One strategy is to use Tat- induced expression of suicidal genes. However, HIV-1 LTR has relatively high intrinsic activity even without Tat activation, which may cause off-target killing. Here we report an improved LTR construct (LTRd2) that has reduced basal activity, while it still maintains the inducibility by Tat. In addition, a re-arranged capase-3 gene (revCasp3) is used as suicidal gene. This gene is constitutively active, thus no protease or other cellular factors are

needed to activate it. By using this gene together with the improved HIV LTR (LTRd2-revCasp3), we are able to specifically target cells with Tat expression and eliminate them. When co-transfected with HIV-1 Tat plasmid, LTRd2-revCasp3 caused apoptotic cell death in HeLa cells and other tested cell lines, while it alone was not toxic. When cells with HIV-1 infection were transfected with LTRd2-revCasp3, they showed significant amount of apoptosis. In summary, our new construct could be an efficient suicidal gene for gene- therapy based HIV-1 eradication.

P86
Microglial activation and cocaine addiction

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Recent research shows that glia are intimately involved in synaptic plasticity, and that drugs of abuse in turn, affect glial activity. Specifically, microglia contribute to synaptic plasticity via direct interactions with dendritic spines, synaptic pruning, and regulation of hippocampal neurogenesis. We have combined a series of in vivo, ex vivo, and in vitro experiments to test the hypothesis that cocaine exposure can lead to significant alterations in microglial activation, enhancing cocaine-induced neuroplasticity and contributing to peripheral immune cell invasion, priming the neuroimmune axis for a cycle of neuroinflammation in the context of cocaine addiction. We found that cocaine self-administration increases microglial activation within reward regions of the brain, alongside increases in expression of the transcription factor and synaptic plasticity marker MeCP2. We then determined in vitro that microglia express MeCP2 and that this expression increases significantly following cocaine exposure, suggesting that cocaine's effects on MeCP2 expression may participate in cocaine-induced neuroplasticity. Moreover, we observed that chronic cocaine administration increases cerebrovascular leukocyte rolling and adhesion and subsequent BBB weakening that persists during withdrawal, setting up the likelihood for a persistent dysregulation of neuroimmune signaling that may mirror the cycle of cocaine addiction. This developing narrative identifies novel neuroimmune targets such as activated microglia for treating psychostimulant abuse, for which not a single approved medication exists. Our findings also illustrate mechanisms in the cocaine abuse paradigm that are likely important in CNS disease progression in HIV-infected patient who uses cocaine.

P87
Interplay of Polyomavirus JCV oncoprotein large T-antigen, Bag3-induced autophagy, and brain-derived neoplasms

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Replication of the human neurotropic JC Polyomavirus (JCV) in glial cells causes the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML). JCV possesses oncogenic activity, and expression of its transforming protein, large T-antigen (T-Ag), has been shown to induce tumors of neural origin in several experimental animal models. The presence of JCV DNA and T-Ag has been repeatedly observed in tissues of several human malignancies, including primitive neuro-ectodermal tumors and glioblastoma. Early studies from our laboratory have shown that Bag3, a member of the Bcl-2-associated athanogene (Bag) family of proteins implicated in protein quality control (PQC), autophagy and apoptosis, is down-regulated upon JCV infection of glial cells, and JCV T-Ag is responsible for suppressing the activity of the BAG3 gene promoter. Here, we examined the putative interaction of Bag3 and T-Ag, and the possible impact of Bag3 on T-Ag expression levels in JCV-infected human primary glial cells as well as cells derived from T-Ag-induced medulloblastoma in transgenic animals. Our results reveal that endogenous over-expression of Bag3 drastically decreases T-Ag expression by inducing autophagic degradation of the viral protein. Of note, this event leads to inhibition of JCV infection of glial cells, suggesting that Bag3-induced reduction of T-Ag has a biological impact on the viral lytic cycle. Further, protein-protein interaction studies show that there is a direct, physical association between T-Ag and Bag3, mediated through the zing-finger of T-Ag and the proline-rich-repeat (PXXP) domain of Bag3; this interaction, consequently, is necessary for the autophagic degradation of T-Ag. Current research is focusing on the role of Bag3 in glioblastoma maintenance and survivability, potential interacting partners of Bag3 in glioblastoma in vivo, and autophagy in glioblastoma tumor initiating/tumor stem cells (TICs/TSCs).
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P88

Hepatitis C Virus (HCV) Interaction with Astrocytes: Non-productive Infection and Induction of IL-18

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Hepatitis C virus (HCV) infection causes the central nervous system (CNS) abnormalities in more than 50 % of chronically infected subjects. However, the underlying mechanisms are largely unknown. In this study, we characterized the HCV interactions with astrocytes, one of the putative HCV target cells in the brain. We demonstrated that primary human astrocytes (PHA) were very inefficiently infected by HCV, either in the cell-free form or through cell-cell contact. We then determined the potential restriction steps of HCV infection and replication in these cells. PHA expressed all known HCV receptors but failed to support HCV entry. HCV IRES mediated RNA translation was functional in PHA and further enhanced by miR122 expression. Nevertheless, PHA did not support HCV replication regardless of miR122 expression. To our great surprise, we found that HCV exposure induced robust IL-18 expression in PHA and exhibited direct neurotoxicity. Taken together, these results showed that astrocytes did not support productive HCV infection and replication, but HCV interactions with astrocytes and neurons alone might be sufficient to cause CNS dysfunction.

P89

Application of a novel cell ablation for dissecting the role of immune cells in the pathogenesis of experimental allergic encephalomyelitis

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The roles of various immune cells in the pathogenesis of experimental allergic encephalomyelitis (EAE), a rodent model widely used for multiple sclerosis study have been previously investigated with different methodologies. However, the relative roles of different immune cells have not been studied with the same model system. Here, we utilized intermedilysin (ILY)-mediated cell ablation model to compare the pathogenic roles of immune cells in EAE. ILY, secreted by Streptococcus intermedius (SI), binds exclusively to human membrane protein CD59 (hCD59) but not to CD59 of any other species. Once bound, ILY rapidly and potently lyses the targeted cells. Using this unique feature, we previously developed a novel cell ablation method. To streamline this approach, we recently generated the floxedSTOP-hCD59 or inducible hCD59 knock-in mice (ihCD59) where hCD59 expression only occurs following Cre-mediated recombination. To express the hCD59 in each type of the T cells, monocytes, and B cells, we crossed ihCD59 with Lck-Cre, Lyz-Cre or CD19-Cre mice respectively to generate three different strains of the mice: ihCD59/Lck-Cre, ihCD59/CD19-Cre or ihCD59/Lyz-Cre. As expected, the specific Cre expression in T cells, monocytes, or B-cells mediated the hCD59's expression in each strain of these mice. Systemic ILY administration to each strain specifically ablated the corresponding cells. ILY administration was initiated 3 days after immunization with myelin oligodendrocyte glycoprotein peptide and continued for 14 days (daily i.p.) to ablate T cells, monocytes and B cells, respectively. By monitoring the clinical EAE scores in these mice, we demonstrated that ablation of T cells completely prevented EAE development and that ablation of monocytes largely attenuated the EAE scores, in contrast to moderate reduction of EAE scores in B-cell ablated mice. Therefore, for the first time, we have successfully dissected the roles of different immune cells with the same approach and demonstrated their distinct functions in EAE's pathogenesis.

P90

Epileptic seizures are induced by intracerebral ablation of astrocytes in the brain, a novel model for dissecting the interaction of neurons with glial cells

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Astrocytes play a number of active and crucial roles in the central nervous system (CNS), including vulnterability and neuroprotective responses to viral infections, as well as close

interaction with neurons for maintenance of proper neuronal activity. However, how astrocytes interact with the neuronal cells in the brains and maintain the regional neuronal activity still requires the further investigation. To this end, we utilized a novel cell ablation approach, namely, intermedilysin (ILY)-mediated human CD59 (hCD59) expressing cell damage model to locally eliminate the astrocytes in the CNS. We generated a new hCD59 transgenic mouse line, in which hCD59 is expressed under the control of human glial fibrillary acidic protein (GFAP) promoter, a widely used promoter for transgenically expressing the gene in astrocytes in mice (hCD59/GFAP). We demonstrated that ILY injection caused a dramatic reduction in the number of GFAP+ (GFAP positive) reactive astrocytes in the vicinity of CNS injury models in the hCD59/GFAP, but not WT mice without any off-target effects. By directly intracerebral injection ILY to the brains, we observed that the specific damage of the astrocytes induced fatally epileptic seizures in the hCD59/GFAP, but not WT mice. Further, micro electrode array (MEA) recordings of acute brain slices derived from the above-mentioned mice revealed that ILY treatment immediately induced the epileptiform electrophysiological changes in the hippocampus area of the hCD59/GFAP, but not WT. These results provide direct evidence to support the notion that the astrocytes plays a crucial role in maintaining normal neuronal activities and indicates that we successfully generated a novel mouse model for dissecting the interaction of neurons with astrocytes in response to CNS viral infections.

P91
Cell-cell contact viral transfer contributes to HIV infection and persistence in astrocytes

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Astrocytes are the most abundant cells in the central nervous system and play important roles in HIV/neuroAIDS. Detection of HIV proviral DNA, RNA and early gene products but not late structural gene products in astrocytes in vivo and in vitro indicates that astrocytes are susceptible to HIV infection albeit in a restricted manner. We as well as others have shown that cell-free HIV is capable of entering CD4- astrocytes through human mannose receptor-mediated endocytosis. In this study, we took advantage of several newly developed fluorescence protein-based HIV reporter viruses and further characterized HIV interaction with astrocytes. First, we found that HIV was successfully transferred to astrocytes from HIV-infected CD4+ T cells in a cell-cell contact- and gp120-dependent manner. In addition, we demonstrated that compared to endocytosis-mediated cell-free HIV entry and subsequent degradation of endocytosed virions, the cell-cell contact between astrocytes and HIV-infected CD4+ T cells led to robust HIV infection of astrocytes but retained the restricted nature of viral gene expression. Furthermore, we showed that HIV latency was established in astrocytes. Lastly, we demonstrated that infectious progeny HIV was readily recovered from HIV latent astrocytes in a cell-cell contact-mediated manner. Taken together, our studies point to the importance of the cell-cell contact-mediated HIV interaction with astrocytes and provide direct evidence to support the notion that astrocytes are HIV latent reservoirs in the central nervous system.

P92
HIV-1 mediated disruption of Wnt/β-catenin signaling in astrocytes lead to neuronal injury

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Neuronal injury characterized by synaptodendritic dysregulation and/or apoptosis is a prominent feature of HIV-Associated Neurocognitive Disorders (HAND). Neuronal injury may arise, in part, from impaired function of surrounding non-neuronal supporting cells. Abnormal astrocyte activity will result in decreased neuroprotection due to increased release of inflammatory mediators, abnormal glutamate cycling and dysregulation of secretory factors critical for astrocyte-neuronal communication. Wnt/β-catenin signaling in astrocytes regulates a number of genes responsible for cell survival, structure and function. We have previously shown that inflammatory mediators, HIV infection, and HIV Tat down regulate Wnt/β-catenin signaling in astrocytes. We evaluated here the impact of down regulation of astrocytic β-catenin signaling on neuronal injury. Towards this end, primary human fetal astrocytes (HFAs) were infected with HIVBaL or knocked down (KD) for β-catenin using siRNA and the astrocyte conditioned media (ACM) was added to Lund human mesencephalic (LUHMES) cells, which are differentiated into dopaminergic neurons. In some experiments, treated HFAs were co-cultured with LUHMED neurons. Microtubule-associated protein 2 (MAP2) expression was assessed at 72 h post-treatment by western blot or immunofluorescence. ACM from HIV infected or β-catenin KD HFAs resulted in ~80 % decrease in neuronal β-catenin expression with no effect on the housekeeping protein GAPDH. ACM from HIV infected or β-catenin KD HFAs also resulted in reduction of MAP2 expression by ~50 and 80 %, respectively, as measured by WB. Co-culturing β-catenin KD astrocytes with neurons also led to

a significant reduction in neuronal MAP2 expression by immunofluorescence. Taken together, HIV-mediated disruption of Wnt/β-catenin signaling in astrocytes lead to significant neuronal injury, highlighting the importance of Wnt/β-catenin signaling in astrocytes for astrocyte-neuronal communication and neuroprotection. This work is supported by F32 NS0889442-01 (VL) and 5R01 NS060632-06 (LA).

P93
Monocyte/macrophages that traffic from bone marrow are a source of CNS virus in Rhesus Macaques that develop SIVE

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Previously we demonstrated CNS macrophages are replenished from bone marrow (BM) hematopoietic stem cells, and there are increased numbers of BM-derived monocytes in SIV encephalitic rhesus macaques. We hypothesized that BM-derived monocytes are the source of SIV-infected macrophages in SIV encephalitic (SIVE) lesions. Twenty-two rhesus macaques were i.v.-infected with SIVmac251; 11 were CD8+ lymphocyte-depleted for rapid AIDS and SIVE. Two depleted and 2 naturally progressing animals were sacrificed at 21 days post-infection (dpi). Eighteen animals progressed to AIDS; 5 depleted and 1 naturally progressing animal developed SIVE. Numbers of monocyte/macrophages (CD68+) and T-lymphocytes (CD3+) were counted in BM, monocytes were sorted, and monocyte numbers were determined by flow cytometry. SIV RNA sequences were obtained from laser-capture microdissected macrophages in BM, sorted monocytes, plasma, and CNS tissues. Bayesian intra-host phylogeography of sequences were used to investigate viral gene flow (migration) from BM to the CNS. Depleted and naturally progressing animals with SIVE had a 2-fold increase in CD68+ BM monocyte/macrophages compared to 21 dpi controls ($p<0.05$) and AIDS animals without encephalitis (SIVnoE) ($p=0.001$). Increased numbers of CD68+ BM macrophages at necropsy correlated with a higher percentage of CD14+CD16+ monocytes and soluble CD163 in plasma of SIVE animals. Viral sequences obtained from CD68+ BM macrophages of SIVE animals shared >99 % identity with sequences from CD14+ monocytes and CNS tissues. Gene flow analysis revealed that SIV sequences from brain tissues shared a most recent common ancestor with sequences from BM and demonstrated that, in both depleted and naturally progressing animals, the last viral migration to the brain originated from BM just before terminal illness and development of SIVE. These data demonstrate an expanded monocyte/macrophage population in BM and blood with SIVE and provide evidence of BM viral sequences seeding the CNS late in infection.

P94
HIV-1 Vpr induces NLRP3 inflammasome activation: Regulation by a caspase-1 inhibitor

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Human immunodeficiency virus type 1 (HIV-1) infects the brain during seroconversion and eventually results in neuroinflammation and neurodegeneration in susceptible individuals. Inflammasomes are cytosolic protein complexes that serve as platforms for caspase-1 activation and ensuing cleavage and release of interleukin (IL)-1beta and IL-18. Our group recently showed that HIV-1 infection of human microglia induces the NLRP3 inflammasome. The viral protein R (Vpr) is an accessory protein encoded by HIV-1, which is essential for macrophage infection. We detected Vpr (~10 nM) in cerebrospinal fluid of HIV-1 infected patients and in supernatants (ranging from 50 to 100 nM) of HIV-1 infected microglia. We investigated the actions of Vpr on inflammasome activation in cells of monocytic lineage. Extracellular Vpr exposure to differentiated human monocytic cells (THP-1) and primary human microglia caused caspase-1 activation and reduced cell viability ($p<0.01$). Infection of THP-1 s and microglia with Vpr-deficient HIV-1 showed significantly reduced caspase-1 activation and IL-1beta production ($p<0.01$), compared to cells infected with Vpr-encoding HIV-1. Intracellular Vpr expression in THP-1 cells, transfected with vpr, led to enhanced caspase-1 activation and reduced cellular viability ($p<0.001$) but without significant IL-1beta release. We extended these observations in vivo by showing increased NLRP3, caspase-1 and IL-1beta in vpr transgenic mice, following Complete Freud's Adjuvant (CFA) subcutaneous stimulation. Treatment with the caspase-1 inhibitor, VX-765, suppressed inflammasome activation in CFA-stimulated vpr transgenic mice. Open field behavioral testing of CFA-stimulated vpr transgenic mice showed reduced anxiety levels in VX-765 treated animals. Thus, Vpr-induced NLRP3 inflammasome

activation likely contributes to HIV-1 associated neuroinflammation and can be abrogated by caspase-1 inhibition. Our findings provide insights into role of Vpr in immunopathogenesis and evidence that caspase-1 inhibition might provide therapeutic interventions in controlling HIV-1 associated neuroinflammation.

P95

PD-L1:PD-1 pathway directly correlate with heightened proviral load, dysregulated dendritic cells' functions, less polyfunctional T cells, and reduced MIP-1α expression during HTLV-1 associated oncogenesis and neuroinflammation

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiologic agent of two immunologically distinct diseases: adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the HTLV-1-specific CD8+ cytotoxic T cell response is seen in both pathogenic states, its actual significance in preventing viral load and controlling disease progression remains questionable. Utilizing a newly standardized dendritic cell and a T cell polychromatic antibody cocktail, we investigated the immune activation of these cells in a patients' cohort from Jamaica including HTLV-1 seronegative controls, asymptomatic carriers (ACs), ATL, and HAM/TSP. Extensive immune profiling revealed that DCs from HTLV-1-diseased individuals exhibited an altered maturation and adhesion phenotype as compared to ACs. Similarly, CD8+ T cells from both HAM/TSP and ATL patients demonstrated less polyfunctionality and poor recall response to Tax antigen but not to control antigen, SEB or CEF samples demonstrated some functional responses, albeit to a much lesser extent than those responses seen in ACs. As an underlying mechanism(s), the expression of an inhibitory molecule PD-1 and its ligand, PD-L1 was upregulated in CTLs and DCs, respectively in both diseased groups. PD-1 expression showed a direct whereas MIP-1alpha expression showed an indirect correlation with the proviral load providing new insights about the immunopathogenesis of HTLV-associated diseases. Collectively, these results suggest that modulation of both DC and CD8 T cell functions by blockade of the PD-L1/PD-1 pathway alone or in combination with other inhibitory molecules may provide future immunotherapies for ATL and/or HAM/TSP patients, for whom there is no definite treatment, cure, or vaccine.

P96

Selective vulnerability to HIV-1 Tat in a subset of CA1 hippocampal interneurons

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One of the areas of the brain that is known to be strongly affected in neuro-acquired immunodeficiency syndrome (neuroAIDS) is the hippocampus, which has a large variety of interneuron subtypes with varying protein expression profiles, functions, and metabolic demands, which give rise to a complex processing network. Attenuation of spatial learning and memory, a common deficit observed in HIV-associated neurocognitive disorders (HAND), is linked to hippocampal pathology. HIV-1 Tat is known to be a strong contributor to the pathological processes observed in neuroAIDS. We hypothesize that some of these diverse interneuron subtypes may respond differentially to HIV-1 Tat. To test this hypothesis, neuronal vulnerability was assessed in GFAP-driven, doxycycline-inducible Tat transgenic mice, with control mice lacking only the tat transgene. The layers of the hippocampus CA1 region were probed in brain slices using antibodies for neuronal markers, including parvalbumin (PV), neuronal nitric oxide synthase (nNOS), neuropeptide Y (NPY), and neuronal nuclear marker (NeuN), combined with fluorescent tagged secondary antibodies. Cell nuclei were counterstained with Hoechst. Probes for PV revealed no difference in PV positive cells in stratum oriens or the pyramidal layer of CA1 between groups. The percentage of nNOS-immunoreactive interneurons without NPY immunoreactivity was significantly decreased in the pyramidal layer of the CA1 region of the hippocampus, as was the percentage of nNOS-immunoreactive interneurons in the stratum radiatum of CA1 in Tat expressing animals compared to controls. The findings indicate a subset of CA1 nNOS-expressing interneurons is selectively vulnerable to HIV-1 Tat and suggest that a subset of hippocampal interconnections is preferentially susceptible to neuroAIDS. This finding suggests novel structural and functional deficits in hippocampal circuitry that underlie

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P97

Inducible expression of CXCL1 from astrocytes amplifies demyelination that is associated with increased neutrophil infiltration into the CNS following CNS viral infection

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The functional role of the ELR+ chemokine CXCL1 in host defense and following infection of the central nervous system (CNS) with the neurotropic JHM strain of mouse hepatitis virus (JHMV) was examined. Mice in which expression of CXCL1 is under the control of a tetracycline-inducible promoter active within GFAP-positive cells were generated and this allowed for selectively increasing CNS expression of CXCL1 in response to JHMV infection and evaluating the effects on neuroinflammation, control of viral replication, and demyelination. Inducible expression of CNS-derived CXCL1 resulted in increased levels of CXCL1 protein within the serum, brain and spinal cord that correlated with increased frequency of Ly6G+CD11b+ neutrophils present within the CNS. Elevated levels of CXCL1 did not affect generation of virus-specific T cells and there was no difference in control of JHMV replication compared to control mice indicating T cell infiltration into the CNS is CXCL1-independent. Sustained CXCL1 expression resulted in increased mortality that correlated with increased neutrophil infiltration, diminished numbers of mature oligodendrocytes, and increased demyelination. Neutrophil ablation in CXCL1-transgenic mice reduced the severity of demyelination, but did not completely abrogate the increase in disease severity in mice that overproduced CXCL1. These findings demonstrate that sustained neutrophil infiltration into the CNS is associated with increased demyelination in a model of viral-induced neurologic disease.

P98

Effects of Sex and HIV Serostatus on Spatial Learning Performance of Drug Users

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Rationale: Recent studies have reported verbal memory impairment and decreased hippocampal activity on fMRI among HIV+ compared with HIV- women. The current study compared spatial memory performance of 173 HIV+ and HIV- substance dependent men and women, using a virtual spatial learning task dependent on the integrity of hippocampal systems. Our goals were to build on previous same-sex studies by comparing nonverbal learning performance of HIV+ and HIV- men and women directly and investigating potential interactive effects of sex and HIV serostatus. Methods: We tested 108 HIV- and 65 HIV+ substance dependent individuals (SDIs), primarily users of cocaine and alcohol. The sample was approximately 50 % women and 85 % African American. All subjects performed the Memory Island Task, a computerized measure of spatial learning and memory, requiring the subject to traverse a virtual island and locate a series of target objects. On four trials visual cues direct the subject toward the target location, but no cues are provided for a second block of trials. Subjects were abstinent at testing and well matched on demographic, substance use, and comorbid variables. Results: Male SDIs located the targets significantly more rapidly compared to female SDIs, $p<.0001$. HIV+ SDIs were significantly less efficient compared with HIV- SDIs in finding the exact location of the targets, $p<.05$. Task performance was not significantly associated with current or nadir CD4 or with HIV RNA levels. Recent cocaine use predicted significantly slower performance among women. Conclusions: A positive HIV serostatus was associated with less efficient spatial learning and memory but not with psychomotor slowing. This impairment was not sex-specific, although recent cocaine use predicted greater slowing among female SDIs. These results extend previous reports that HIV serostatus affects neurocognitive processing dependent on hippocampal integrity. Supported by the National Institute on Drug Abuse.

P99

Reward, Attention, and HIV-Related Risk within the Context of HIV+ Status and Drug Addiction

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Background: HIV is primarily contracted through reward-motivated, risky behaviors (e.g., IV drug use, unprotected sex). Avoiding risk requires goal-directed control over one's actions. Understanding the relation between reward and behavioral control could help identify those at greatest risk for HIV acquisition or transmission. Methods: We examined the relation between reward-based visual attention, visual working memory, motor control, and impulsivity in three groups: HIV+, HIV- and opioid-dependent, and healthy controls. Visual attention: Subjects searched for color-defined targets which were probabilistically associated with high- and low-value monetary rewards. In a follow-up test, subjects searched for shape-defined targets; color was irrelevant. Yet, on some trials, non-target shapes were presented in the color of formerly rewarding high- or low-value targets. Our measure of interest, "attentional capture," was the additional time taken to locate a target with a distractor in the background. To assess the robustness of attentional capture, the HIV+ group was retested 6 months later. Visual working memory: Colored squares were briefly presented on a computer screen. After a short delay, a single square returned from the initial presentation. Subjects indicated whether the returning square was the same or different color than when first presented. Motor control: Halstead Finger Tapping Test Impulsivity: Barratt Impulsiveness Scale Results: The HIV+ and opioid-dependent groups showed abnormally high attentional capture (i.e., longer time to locate a target). High attentional capture correlated with poor visual working memory. Uniquely in the HIV+ group, high attentional capture was associated with high impulsivity and slow finger tapping. Attentional capture persisted in HIV+ subjects at follow-up, which was strongly predicted by impulsivity and finger tapping measures obtained 6 months earlier. Conclusions: Individuals with predisposing factors (low working memory, low motor control, high impulsivity) may be particularly vulnerable to reward-motivated, risky behavior and are, therefore, at increased risk for acquiring or transmitting HIV.

P100
Measles Virus Trafficking at the Neuronal Synapse

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Despite an effective vaccine, measles virus (MV) remains a formidable worldwide pathogen, resulting in >100,000 deaths annually. In addition to the acute peripheral infection, MV can infect the brain, causing rare, but often fatal diseases, such as subacute sclerosing panencephalitis. Previous work from our lab and others has shown that MV inter-neuronal spread is vastly different than the classical mechanism of spread described in non-neuronal cells: MV spreads trans-synaptically between neurons in the absence of extracellular virus and syncytium formation. Moreover, unlike the lytic infection in non-neuronal cells, MV infection does not result in neuronal death. Using permissive hippocampal mouse neurons, we continue to define mechanisms of MV trafficking in neurons, specifically exploring the roles of molecular motors, such as myosin, dynein, and kinesin. Immunoaffinity experiments have indicated an interaction between the actin-based molecular motor, myosin Va (myoVa), and a measles envelope protein, fusion (MV-F). Complementary confocal studies confirm this interaction, and show localization of MV-F to dendritic spines. Both the actin-destabilizing agent cytochalasin D and a dominant-negative myosinVa block this localization to dendritic spines, as well as subsequent inter-neuronal viral spread. While this interaction would provide transport within the immediate vicinity of the synapse, other virus-molecular motor protein interactions would be needed for transport along the axon, possibly with a member of the kinesin family since myoVa has been shown to form a heteromotor with the ubiquitous kinesin heavy chain (Kif5B). Interestingly, our lab has also shown that MV-F plays a key role in trans-synaptic spread, interacting with the neurotransmitter receptor neurokinin-1 to form microchannels, which may contribute to the unique CNS disease observed in infected transgenic mice. Together, this work will help to connect the different aspects of the virus's journey through a neuronal network with viral neuropathogenesis.

P101
Tight junction protein expression in a blood–brain barrier model of HIV-1 Tat and/or morphine exposure

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Injection drug use is linked to the acquisition of approximately one-third of human immunodeficiency virus type 1 (HIV-1) cases resulting in acquired immunodeficiency syndrome (AIDS) in the United States. Moreover, opioid abuse within this population is a confounding factor in disease progression in multiple ways, including increased viral replication and peripheral viral load, as well as incidence and severity of neurocognitive impairment and the development of dementia, as compared to non-users. Compromise of blood–brain barrier (BBB) integrity is undoubtedly involved in the pathological progression to HIV-1-associated neurocognitive disorders (HAND). HIV-1 proteins, as well as various drugs of abuse, have been implicated in the observed breaching of the BBB. Previous studies suggest that exposure to both HIV-1 Tat protein, as well as mu-opioids, alters BBB permeability, resulting in increased cellular transmigration, as well as overall barrier leakiness. In this study, a human brain microvascular endothelial cell line, hCMEC/D3, was utilized to establish an in vitro model of the BBB to investigate the effects of chronic Tat and/or morphine exposure on tight junction protein (TJP) expression of the BBB. Changes in mRNA transcripts of TJPs were observed throughout the course of chronic exposure. Differences in TJP expression and localization was also observed on the protein level following cellular fractionation and western immunoblot analysis. Overall, these studies demonstrate that exposure to Tat and/or morphine induces changes in TJP expression patterns at both the mRNA and protein levels. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785, and R01 NS089435.

P102
Uncovering Early Neurologic Deficits in the HIV-1 Transgenic Rat

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Despite the effectiveness of combination antiretroviral therapy (cART), pediatric AIDS, caused by human immunodeficiency virus type 1 (HIV-1) remains a leading cause of worldwide childhood mortality. HIV-1 Tg rats, which express 7 of the 9 HIV-1 genes constitutively throughout development, provide a useful model for the development of neurologic impairments in pediatric AIDS. Specifically, male and female Fischer HIV-1 Tg and F344 control rats, sampled from 11 litters, were repeatedly assessed during early development, for locomotor activity - an index of motor function (Postnatal Days (PD) 12, 16 and 20), and prepulse inhibition (PPI) of the auditory startle response (ASR) - an index of sensorimotor gating (PD 14, 17 and 21). Preliminary analyses suggested no significant difference between HIV-1 and F344 control animals as inferred from ambulation distance (cm), although significant alterations as a function of the HIV-1 transgene were apparent for within-session habituation at the earlier test ages. PPI was tested with both auditory and visual modalities, an auditory startle stimulus, and interstimulus intervals (ISI) of 0, 30, 50, 100, 200, and 4000 msec. The 0 and 4000 msec ISI provided the baseline from which to evaluate response inhibition. With visual prepulses, HIV-1 Tg animals exhibited a temporal insensitivity to ISI on PD 17 (<20 % inhibition), whereas control animals displayed robust PPI with a peak inhibition (73 %) at the 100 msec ISI. By PD 21, HIV-1 Tg rats displayed robust PPI at the 100 msec ISI, albeit with residual impairment relative to controls (52 vs. 98 % PPI, respectively). With auditory prepulses, both HIV-1 Tg and control animals showed similar temporal shifts in their inhibition curves from 30 to 100 msec across PD 14 to PD 21. Alterations in temporal processes may provide an important early diagnostic marker for the neurocognitive deficits reported in adulthood. Funded by NIH grants DA013137 and HD043680.

P103
PrPc: Friend or Foe in HIV CNS Pathogenesis?

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HIV-1 enters the CNS soon after peripheral infection and causes chronic neuroinflammation and CNS damage that leads to cognitive impairment in greater than 50 % of HIV infected people. Protease resistant prion protein, PrPc, is the non-pathogenic cellular isoform of human prion protein that is constitutively expressed in the CNS and is involved in several physiological processes that are disrupted during HIV neuropathogenesis. PrPc is expressed on monocytes and brain microvascular endothelial cells (BMVEC) and is essential for transmigration of monocytes across the blood brain barrier (BBB). HIV infection of monocytes results in increased surface PrPc as well as increased shedding. To determine the effect of shed PrPc on transmigration of monocyte across the BBB, we used our in vitro BBB model of astrocytes and BMVEC co-cultured on opposite sides of 0.3 μm pore insert. Soluble PrPc blocked the transmigration of both HIV+ and HIV- monocytes across the BBB, suggesting that it could be

an initial mechanism of protection against the influx of monocytes across the barrier during early stages of HIV infection that could otherwise result in viral seeding of the CNS and neuroinflammation. Treatment of astrocytes and BMVEC with CCL2, a chemokine that is highly elevated in the brain of HIV infected people, causes increased PrPc shedding. This shed PrPc induces the production of inflammatory mediators, including CCL2, IL-6, and IL-8, from astrocytes suggesting that sPrPc contributes to neuroinflammation. Additionally, treatment of astrocytes and BMVEC with TNF-alpha and VEGF results in loss of cell associated PrPc as demonstrated by Western blot and flow cytometry. Since PrPc is an adhesion molecule important for forming a tight BMVEC monolayer, we hypothesize that its loss will result in reduced BBB integrity. Thus, our data indicate that PrPc may be both protective and damaging depending upon its temporal and spatial expression during HIV neuropathogenesis.

P104
Leptin is associated with obesity measures in HIV-seropositive women

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Background: The increased life span of HIV positive individuals is accompanied by the side effects of long-term cART treatment and aging co-morbidities including obesity and metabolic syndrome (dyslipidemias and glucose intolerance) (Falutz 2011; Reust 2011). The lateral hypothalamus (LH), which is altered with HIV infection, is associated with feeding behavior and body weight due to the secretion of hypocretin (hcrt), also known as orexin, and the actions of leptin. The objective of our study was to investigate whether hcrt-1 levels and leptin levels correlated with obesity measures in HIV seropositive women. Methods: In a retrospective study, we measured serum and CSF hcrt-1, and serum leptin levels of 39 HIV-seropositive women without a history of drug abuse evaluated for metabolic syndrome. CSF and serum hcrt-1 levels were determined using the fluorescent immunoassay kit (Phoenix Pharmaceuticals), with an intra- and inter-assay validity of 10 and 15 % respectively. Serum leptin levels were determined using a Sandwich ELISA (Millipore, MA), with an intra- and inter-assay validity of 2.6 - 4.6 and 2.6 - 6.2 % respectively. The associations between hcrt-1 and leptin levels with obesity measures were tested using non-parametric statistics (IBM SPSS Statistics version 20). Statistical significance was determined at p less than 0.05. Results: In our sample of HIV-seropositive women, serum leptin levels were positively associated with obesity measures such as body mass index (BMI), waist circumference (WC), total fat percentage and negatively correlated with a new shape index (ABSI). There was no correlation between hcrt-1 levels in neither serum nor CSF with any of the obesity measures tested. No correlation was found between serum leptin and hcrt-1 levels. Conclusions: Our findings show that leptin, but not the hypocretin system, is associated with obesity measures in HIV-seropositive women. GRANT SUPPORT: R21MH095524, R25MH080661, R25MD007607, U54MD008149, U54NS043011.

P105
Discrepancy between Years of Education and Reading Level Affects Neuropsychological Performance in an Urban HIV Infected Cohort

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The use of educational attainment as an appropriate demographic correction for neuropsychological performance among diverse populations has recently been challenged. This may be of particular concern when assessing minority groups, due to disparities in quality of education between racial/ethnic factions. Instead, acquired reading level may serve as a better representation of educational achievement. In HIV infection, reading level is maintained, irrespective of the development of neurocognitive impairment. Here, we evaluated the relationship of reading level, years of education, and CD4+ T-cell nadir with global neuropsychological performance in an urban HIV-infected cohort. For this study, 62 HIV infected subjects from an urban population were recruited through the Comprehensive NeuroAIDS Center at Temple University School of Medicine. Our cohort comprised 36 males and 26 females, 23–71 years of age (mean=42.9± 11.27). Pearson correlations, Student's *T*-test, and multiple regression analyses assessed the predictive abilities of reading level, as measured by the Word Reading subtest of the Wide Range Achievement Test-4 grade equivalency (WRAT-4ge), years of education, and CD4+ T-cell nadirs on overall neuropsychological performance [Global Cognitive Index (GCI)]. These studies reveal a significant correlation ($p<0.0001$)

between WRAT-4ge and GCI, and a non-significant relationship (p=0.453) between years of education and GCI. Paired-samples t-test, t(57)=7.780, p<0.0001, demonstrate the average years of education (mean=11.98±2.10) significantly differ from average WRAT-4ge (mean=8.47±3.62). A multiple regression analysis with education discrepancy and CD4+ T-cell nadir as predictor variables accounted for 60 % of the variance in GCI, with reading discrepancy identified as the only significant predictor (p<0.0001). In conclusion, our data show the discrepancy in educational attainment and acquired reading level is a better predictor of neurocognitive performance than severity of disease burden in HIV-infected, urban subjects. As such, using years of education as a demographic corrector on neuropsychological tests for this population may overpathologize these groups.

P106
Interleukin-7 Treatment of Progressive Multifocal Leukoencephalopathy in a Patient with Idiopathic Lymphocytopenia

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Background: Progressive multifocal leukoencephalopathy (PML), caused by the polyomavirus JC (JCV), is a demyelinating disease of the central nervous system for which there is no cure. Interleukin-7 (IL-7) is a multifunctional cytokine which induces expansion of both CD4+ and CD8+ T cells and restores T cell functionality. Case study: A 66-year-old man presented with moderate expressive aphasia. MRI showed multiple confluent T2/FLAIR hyperintense lesions in the left hemispheric white matter with mild enhancement post-contrast. Cerebrospinal fluid (CSF) was unremarkable and MR spectroscopy showed an elevated choline peak, suggestive of brain tumor. A brain biopsy performed 4 months after symptom onset, showed scattered "bizarre" astrocytes with enlarged nuclei and numerous SV-40-positive nuclear inclusions establishing the diagnosis of PML. Polymerase chain reaction (PCR) testing for JCV DNA in CSF was negative, but JCV DNA was detected by PCR in brain parenchyma. Antibodies against HIV-1 and HIV-1 RNA were undetectable in blood. Absolute lymphocyte counts (ALC) revealed profound lymphocytopenia at 168/ul and T cell subsets showed CD4+ count=87/uL, CD8+ count=7/uL. Absolute B cells were low (4-8/uL), but quantitative immunoglobulin (IgG, IgA, and IgM) levels in blood were within normal limits. There was no evidence of hematological malignancy. We obtained eIND approval for investigational use of IL-7 for treatment of the lymphocytopenia. Results: The patient received 3 weekly intramuscular injections of IL-7 at a dose of 10 ug/kg with no adverse effects. His neurologic exam showed stable moderate expressive aphasia. There was an increase in ALC from 168 to 595/ul, CD4+ T-cells from 87 to 301/ul, and CD8+ T-cells from 7 to 34/ul. JCV-specific T cell responses measured by intracellular cytokine staining did not appear altered after treatment with IL-7. Conclusion: This case further supports the investigational use of IL-7 in patients developing PML in the setting of idiopathic lymphocytopenia.

P107
Alterations of Noradrenergic Fibers in the Prefrontal Cortex of HIV-1 Transgenic Rats Reduced by the Phytoestrogen Metabolite S-Equol

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HIV-1-associated neurocognitive disorders (HAND) affect approximately 50 % of HIV-1-positive individuals despite the effectiveness of combination antiretroviral therapy (cART) in reducing the prevalence of more severe neurocognitive impairment (i.e., dementia). Deficits in executive function are a distinguishing feature of HAND in the cART era, decaying more rapidly during HIV disease progression than other cognitive domains. We have previously demonstrated deficits in attention and core components of executive function in the HIV-1 transgenic (Tg) rat, as well as the effectiveness of the phytoestrogen metabolite S-equol in both improving cognitive performance and protecting against these deficits when administered at an early age. In order to determine anatomical correlates of the deficits and the effects of the S-equol treatment, we used unbiased stereology to quantify dopamine beta-hydroxylase (DBH)-immunostained varicosities in the prefrontal cortex (PFC). The PFC, where the estrogen beta receptor is highly expressed, supports executive function and attention. Preliminary estimates, obtained with

a coefficient of error less than 0.1, suggest that the HIV-1 Tg animals have a greater overall number of DBH-immunostained varicosities in the PFC than the control animals, and further, that treatment with S-equol reduced the number of varicosities in the HIV-1 Tg group. Alterations in norepinephrine release in the PFC may underlie the deficits in attention and executive function observed in the HIV-1 Tg rats. The noradrenergic system in the PFC may be an important target for the therapeutic effects of S-equol on HAND. Funded by NIH grants DA013137 and HD043680.

P108

Frequency and distribution of varicella zoster virus and pathological changes in temporal arteries from patients with clinically-suspect, biopsy-negative giant cell arteritis

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Recent clinical, virological and pathological analyses provide compelling evidence that varicella zoster virus (VZV) infection triggers the inflammatory cascade that characterizes giant cell arteritis (GCA). Because temporal artery (TA) biopsies of patients with clinical and laboratory abnormalities characteristically seen in GCA are frequently negative and may represent early VZV vasculopathy, we determined the frequency and distribution of VZV antigen in GCA-negative TAs compared to normal TAs and previously reported GCA-positive TAs. Immunohistochemical analysis of 3400 sections from 68 GCA-negative TAs (50 slides/TA) detected VZV antigen in 41 (60 %) TAs compared to 11/49 (22 %) normal TAs and 61/82 (74 %) GCA-positive TAs. In GCA-negative TAs, VZV antigen was seen in the adventitia, media and intima 37 %, 24 % and 38 % of the time compared to 15 %, 50 % and 35 % in normal TAs and 49 %, 32 % and 19 % in GCA-positive TAs. In the adventitia of GCA-negative TAs, viral antigen was seen in the nerve bundle, vaso vasorum and adventitial cells 56 %, 4 % and 40 % of the time, respectively, compared to 9 %, 6 % and 85 % in normal TAs and 26 %, 4 % and 70 % in GCA-positive TAs. VZV antigen was significantly more likely to be present in nerve bundles in GCA-negative and GCA-positive TAs than in normal TAs ($p<0.0001$). Importantly, histopathological analysis of sections adjacent to those containing viral antigen revealed adventitial inflammation in 23/41 (58 %) of GCA-negative subjects; in contrast, inflammation was not seen in any sections adjacent to viral antigen from normal TAs. Overall, the frequency of VZV in GCA-negative TAs (60 %) was only slightly less than the frequency of VZV in GCA-positive TAs (74 %). Combined with the presence of adventitial inflammation adjacent to VZV in more than half of GCA-negative subjects, our findings suggest that before GCA pathology develops, VZV infects the arterial adventitia via nerve fibers followed by an inflammatory response.

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Investigating the role of Purα in functional activity of primary neuronal cultures using micro electrode (MEA) array technology

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Culturing dissociated cortical neurons on micro electrode array (MEA) dishes is a powerful experimental tool for investigating functional and structural characteristics of in vitro neuronal networks. Over the past few decades, MEAs have been used to investigate the mechanisms that take place at the network level among cultured neuronal preparations and to answer fundamental questions regarding the cellular basis of brain function and synaptic connectivity. The present work for the first time aims to utilize MEA technology to investigate and quantify changes in neuronal activity and development induced by the knock-down of the cellular protein, Purα. Purα is a multifunctional protein that plays a major role in the regulation of the cell cycle and oncogenic transformation. The creation and use of transgenic mice with inactivation of the PURA gene has unraveled a critical role for Purα in postnatal brain development and in dendrite-specific transport of mRNAs and the establishment of the postsynaptic compartment in developing neurons. Furthermore, Purα has been shown to interact with the HIV-1 regulatory Tat protein, thus acting as an important mediator of HIV-1 gene expression and Tat-associated neurotoxicity. In order to further investigate Purα's contribution to neuronal development and its role in the neurodegenerative effects of HIV-1 infection, in this work we designed and used the RNA-guided genome editing tool, CRISPR/Cas9, combined with a lentiviral delivery system to knock down the PURA gene in both human and mouse primary neurons. Mouse primary neurons were then cultured on micro electrode array dishes that allowed us to run in vitro neuronal functional activity experiments. Such experiments allowed us to quantify changes in neuronal activity during in vitro development and to investigate the effects induced by the absence of Purα, both on neuronal development and in the context of HIV-1 degenerative disorders.

P110

Differential responses of inhibitory neuronal subpopulations to morphine mediated Ferritin Heavy Chain upregulation

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Many HIV+ patients also abuse drugs, with opioid use being particularly common as sharing needles represents an important avenue of infection. These patients can show accelerated progression of HIV-associated neurocognitive disorders (HAND), the mechanism of which is not clear. Our lab has demonstrated that morphine and other mu-opioids can antagonize the homeostatic chemokine receptor CXCR4 via upregulation of the iron binding protein subunit Ferritin Heavy Chain (FHC). Neuronal CXCR4 blockade and upregulated FHC are associated with dendritic spine reduction, and correlated with enhanced cognitive decline in humans and animal models of HAND. Our experiments aim to gain insights into this novel mechanism of mu-opioid mediated CXCR4 regulation, as these systems have been previously implicated in HAND progression. Morphine upregulates FHC in cortical neurons, while astrocytes seem to be unaffected in vitro, suggesting exclusivity for particular CNS cell types. FHC upregulation specifically occurs in the cytoplasm as demonstrated by fractionation and confocal imaging studies, resulting in a distribution that is more consistent to that of CXCR4. Preliminary imaging studies show that certain neurons are more susceptible to FHC upregulation than others, and that GABA transporter-1 expressing neurons represent one of these susceptible populations. Current work is focused on exploring FHC expression after morphine treatment in inhibitory neuronal subpopulations. Pilot studies suggest that calretenin expressing interneurons do not upregulate FHC after morphine, but interestingly have higher basal levels of FHC compared to calretenin negative cortical neurons. Additionally, FHC upregulation is likely dependent on G-protein signals, as pertussis toxin pretreatment blocks this effect. These experiments suggest that mu-opioid usage may cause specific deficits in inhibitory neuronal circuits via FHC upregulation and subsequent CXCR4 blockade, which may induce or sustain particular features of HAND such as excitotoxicity, or other neurochemical adaptations leading to cognitive impairment.

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Loss of CCR2 Expressing Non-Classical Monocytes are Associated with Cognitive Impairment in Antiretroviral Therapy-Naïve HIV-Infected Thais

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HIV-associated neurocognitive disorder (HAND) in the era of combination antiretroviral therapy (cART) still remains a substantial problem. Our previous work identified the HIV DNA content of CD14+ enriched cell populations as a correlate to HAND. A more in-depth functional characterization and insight of monocyte subsets based on surface biomarkers expression patterns in association to HIV neuropathogenesis remains incompletely understood. We carried out a prospective study of 29 cART-naÌøve, HIV-infected Thai individuals, with varying degrees of cognitive impairment compared to 44 demographically matched HIV-uninfected controls. Monocyte subsets were profiled, evaluating chemokine receptor (CCR)-2, CCR5 and CD163 expression using a comprehensive multiparametric flow cytometric panel design. Markers of inflammation (neopterin, tumor necrosis factor (TNF)-alpha, interleukin-6, monocyte chemoattractant protein-1, interferon-alpha and -gamma) in plasma and cerebrospinal fluid (CSF) were measured by Luminex multiplex assay and ELISA, whereas HIV DNA content was determined by real time-PCR. Lower numbers of CD16 expressing non-classical monocytes, as well as the CCR2 expressing subset of non-classical monocytes, were associated with impaired neuropsychological testing performance (r=0.43, p=0.024 and r=0.43, p=0.024, respectively). Direct associations were noted specifically with the psychomotor testing cognitive domain. The number of CCR2 non-classical monocytes also inversely correlated with CSF levels of the macrophage scavenger, neopterin (r=−0.43, p=0.035), and plasma levels of the inflammatory cytokine, TNF-alpha (r=−0.40, p=0.041). These data benchmark the loss of CCR2 expressing subset of circulating non-classical monocyte mobilization as independent an index of cognitive impairment and central nervous system (CNS) inflammation that can be tracked after cART or therapeutically targeted to limit CNS impairment.

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Trafficking of conventional dendritic cells (DCs) into the central nervous system in response to SIV and retrograde transport to germinal centers to within cerebral lymph nodes

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Neuroinvasion by HIV leads to neurocognitive diseases and alters the permeability of the BBB. Conventional dendritic cells (cDCs) infiltrating the CNS can potentially encounter HIV from infected perivascular macrophages lining the BBB. They can thereafter carry HIV to cervical lymph nodes (CxLNs), where HIV particles will be transmitted to CD4 T cells and eventually get trapped in CD35+ follicular DCs (fDCs) to create a viral reservoir within the germinal center. HIV patients using drugs of abuse such as morphine, can further compromise the BBB and enhance infectivity of the CNS. Research demonstrating the presence and role of DCs in the CNS during HIV infection has not been developed yet. In this respect, we explored the presence of DCs in the brain parenchyma and CxLNs of rhesus macaques infected with SIV and administered with morphine. Cells positive for cDC markers were consistently found in the brain parenchyma of SIV-infected macaques and enhanced within infected macaques given morphine. Examination of CxLNs in SIV-infected macaques confirmed presence of SIV p27+ cDCs entering CD20+ germinal centers further trapping of virus within CD35+ fDCs. We are currently researching the effects of chronic infection on fDC viral entrapment. These results provide first evidence of DC trafficking and establishment of viral reservoir in NeuroAIDS vis-à-vis drugs of abuse.

P113

Peripheral T Cell Activity and Basal Ganglia Functional Connectivity in HIV Infection

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HIV infection can adversely affect performance in cognitive and motor domains. While cerebral function often improves after initiation of combination antiretroviral therapy (cART), the neural mechanisms associated with infection related behavioral deficits, and their improvement with treatment, are poorly understood. To investigate whether measures of inter-regional functional connectivity might serve as central markers of disease activity, we used resting state functional MRI (rs-fMRI) to examine longitudinal treatment effects in functional connectivity. In nine cART-naive HIV infected participants, we collected structural and functional MRI data before and after 3 and 6 months of therapy, to determine: (1) if inter-regional connectivity was influenced by commencement of cART, (2) whether such changes were most evident in brain regions known to be either affected by HIV infection and (3) where such changes were related to peripheral markers of disease activity such as plasma viral load, CD4 and CD8 cell counts. We recorded longitudinal changes in rs-fMRI connectivity, plasma viral load, CD4/CD8 T cell counts. Over 24 weeks of therapy, our cohort averaged increases in CD4 cell counts of 6.8 cells/week ($p<.001$), decreases in viral load ($p<.001$) and no change in CD8 cell counts ($p=.64$), results that are typically seen with in HIV subjects after initiation of cART. Longitudinal seed-based connectivity analysis of the rs-fMRI data revealed a positive relationship between increasing CD4 cell counts and connectivity among caudate, pallidum and prefrontal cortex. The average connectivity across all basal ganglia nuclei with medial prefrontal cortex, orbital prefrontal cortex and the posterior cerebellum was positively associated with CD4 cell count ($p<.05$ FDR-corrected). Inter-regional brain connectivity is a promising and practical potential biomarker for efficient and sensitive monitoring of HIV treatment effects. Our results suggest that rs-fMRI may be useful for monitoring treatment response in small groups, or even individual patients with further optimization of imaging techniques.

P114
HIV viral load in the cerebrospinal fluid (CSF) from AIDS-related opportunistic meningitis is a mixture of viral particles originating in T-cell and monocyte/macrophage cell lineages

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Opportunistic infections (OI) of the CNS in AIDS patients produce a substantial increase in the trafficking of HIV and CSF HIV-load. HIV-1 directly contributes to the high morbidity and mortality associated with CNS-OI and triggers chronic astroglial and microglial responses that may persist even in successfully OI-treated patients. To gain insights on the biological significance of the burden of HIV-1 during CNS-OI, we aimed to characterize at molecular level the origin of CSF HIV-load in patients with AIDS-associated meningitis. Furthermore, we investigated the HIV-1 genetic clades associated with the most frequent CNS-OI in Colombia. To determine the cellular sources within the CNS contributing to CSF HIV-1-viral load, we evaluated paired CSF/plasma samples from 12 newly diagnosed AIDS patients who presented with tuberculous or cryptococcal meningitis. The viral particles were purified into two fractions based on the differential expression of CD26 (lymphocyte-origin) or CD36 (monocyte/macrophage-origin) molecules on the viral envelope, using an immune-magnetic capture assay. Genomic RNA within the purified viral envelopes was examined by reverse-transcription and polymerase chain reaction to determine virion integrity. CSF samples contained a mix of T-cell-derived and macrophage-derived viral particles. In contrast, the viral load in plasma was comprised mostly by viruses of T-cell origin. Analysis of the coreceptor usage by virions in CSF is being performed. Heteroduplex mobility assays performed in all AIDS cases, 25 with CNS-OI and 5 without CNS disease, correspond to the pattern of HIV-1 subtype B. In conclusion, opportunistic meningeal infection triggers HIV-1 replication in monocyte/macrophage cell lineage contributing to the cerebral viral burden, which may increase the brain viral genetic heterogeneity associated with long-term microglial and astroglial activation and infection. These results also confirm clade B is the prevalent HIV subtype circulating in Colombia and contributes additional evidence to the global HIV-1 distribution and their association with neurological disease.

P115
A non-replicative senescence in HIV-1 infected and uninfected macrophages

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Combination anti-retroviral therapy (cART) has significantly increased the lifespan of HIV-1 infected patients. By 2015,

half of the population living with HIV-1 in the US will be above the age of 50. However, non-infectious HIV-related comorbidities are emerging. Chronic infection with HIV-1 and long-term usage of cART can cause cellular senescence and premature aging, making patients more at risk for old age cancers and multiple organ disorders. Cellular senescence is an anti-tumor mechanism activated in response to a variety of stresses. It is primarily a beneficial cellular program to prevent tumor growth; however, it has been shown to have antagonistically pleiotropic functions such as promotion of cancers and age-related disorders later in life. Accumulation of certain senescent markers has been shown in chronically infected HIV-1 patients in both the CNS and peripheral tissues. However, senescence or a senescence-like phenotype has not been thoroughly examined in macrophages, and not at all during HIV-1 infection. We have found that human monocyte-derived macrophages (MDM) display markers of a non-replicative senescence phenotype after induction of senescence via continuous culture or oxidative damage. To confirm the presence of a senescent phenotype, we used ELISAs to determine the presence of a senescence associated secretory phenotype, senescence-associated beta-galactosidase assay, and western blot and immunofluorescence analyses of the levels of p16, p21, and p53, which have been shown to increase in senescent cells. The phenotype consists of increased soluble IL-6 levels, p53, p21 expression, and an enlarged flattened morphology. Interestingly, we see a similar phenotype when we infect human primary macrophages with HIV-1 pseudotyped virus. Therefore, we propose that macrophages develop a non-replicative senescence phenotype in response to senescence-inducing stimuli and to HIV-1 infection.

P116
Role of pericytes in the regulation of the neurovascular unit in HIV-1 infection

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Brain pericytes are uniquely positioned within the neurovascular unit to provide crucial support to blood brain barrier (BBB) formation, maintenance, and stability. Neurologic conditions, such as HIV-1-associated neurocognitive disorder (HAND), are associated with BBB compromise due to chronic inflammation. Little is known about pericyte function during HIV-1 infection and the subsequent contribution to HAND. We found decreased expression of pericyte markers in human brains from HIV-1 infected patients as compared to controls. To examine functional changes of primary human brain vascular pericytes we assessed expression of pericyte markers [alpha1 integrin, alpha-smooth muscle actin (alpha-SMA), and platelet derived growth factor-B receptor, PDGF-R beta] and CX-43 (a gap junction marker) after exposure to TNFalpha and IL-1beta by western blot and FACS. We found down regulation of alpha1 integrin, alpha-SMA, PDGF-Rβ and CX-43 in cytokine-treated pericytes. Factors promoting BBB formation (angiopoietin-1, transforming growth factor-beta1) showed a decrease after exposure of pericytes to virus or cytokines. mRNA for basement membrane components assessed by qPCR was reduced in pericytes treated with cytokines or HIV-1. TNF alpha and IL-1 beta enhanced expression of adhesion molecules in pericytes paralleling increased monocyte adhesion to pericytes. Monocyte migration across BBB models composed of human brain endothelial cells and pericytes demonstrated a diminished rate in dual models (60 % of models composed only of brain endothelial cells). However, exposure to the relevant chemokine, CCL2, enhanced the magnitude of monocyte migration when compared to BBB models composed of brain endothelial cells only. These data suggest an important role of pericytes in BBB regulation in neuroinflammation

P117
Blood brain barrier shielding and anti-inflammatory properties of cannabinoid type 2 receptor (CB2) agonists: Novel approach to treatment of HAND

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Previous studies have shown that modulation of the receptor-mediated cannabinoid system during neuroinflammation can produce potent neuroprotective and anti-inflammatory effects. However, in this context, little is known about how selective activation of CB2 affects the activated state of the brain endothelium and blood–brain barrier (BBB) function. Using human brain tissues and primary human brain microvascular endothelial cells (BMVEC), we demonstrate that the CB2 is highly upregulated during HIV infection and inflammatory insults. Next, we examined whether the CB2 agonists could attenuate inflammatory responses at the BBB using a mouse model of LPS-induced neuroinflammation. Visualization by intravital microscopy (IVM) revealed that administration of CB2 agonists attenuated leukocyte adhesion in cortical

venules and prevented barrier leakiness after LPS administration. The CB2 agonist increased barrier tightness and increased the amount of tight junction proteins in BMVEC in vitro. CB2 agonists decreased the induction of adhesion molecule surface expression in BMVEC exposed to various proinflammatory mediators. Next, we tested the idea that selective CB2 activation in human monocytes regulates their ability to engage the brain endothelium and migrate across BBB preventing its injury. IVM was used to quantify adhesion of leukocytes to cortical vessels in LPS-induced neuroinflammation, following injection of ex vivo CB2-activated leukocytes into mice. CB2 agonists decreased adhesion (91-96 %) of ex vivo labeled cells in vivo. In an in vitro BBB model, CB2 activation in monocytes reduced adhesion (100 %) to and migration (60 %) across monolayers of BMVEC and diminished BBB damage. CB2 stimulation in monocytes downregulated active forms of integrins and led to increased expression of inhibitory sites of the actin binding proteins upstream regulators of conformational integrin changes. CB2 stimulation decreased formation of lamellipodia. These results suggest that pharmacological CB2 ligands offer a new strategy for BBB protection during neuroinflammation.

P118
Modulation of the gut microbiota to promote health

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Obesity and metabolic disorders are significant co-morbidities associated with HIV infection. The link between the gut microbiota, obesity and metabolic syndrome have been established. We have found that C57BL/6 J mice weaned on high fat diet (HFD) supplemented with 2 % mannose (HFD+M), display reduced weight gain compared to mice on a HFD alone. Mannose treated mice display improved insulin sensitivity and glucose tolerance, increased endurance and reduction in fat in adipose and liver compared to mice reared on HFD. The feces of HFD+M mice have higher energy content and elevated short chain fatty acids than mice on HFD, indicating that the observed lean phenotype is due, at least in part, to reduced caloric absorption by the host. Mannose treatment results in alterations in the gut microbiota of mice and tracks longitudinally with weight and fat mass alterations. Functional analysis of cecal gut microbiota by RNA-Seq shows that mice treated with mannose undergo profound and coherent changes in metabolism and expression of inflammatory flagellins. This combination of studies has provided important clues toward our goal of understanding the diet/microbiome/host interactions of the mannose-induced lean phenotype. Our data support the view that mannose supplementation in human diets may represent a safe and effective way to combat the growing obesity epidemic.

P119
The human gut microbiome is associated with HIV-associated neurocognitive impairment

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Background: The gut microbiome has been implicated in the development and function of brain circuits that wire cognitive and emotional functions. HIV infection often results in neurocognitive impairment (NCI) even with successful antiretroviral therapy (ART). We previously demonstrated that a specific gut bacterial profile, driven by higher proportion of Lactobacillales, was associated with higher CD4 and less microbial translocation, markers previously associated with better neurocognitive function. Here, we compared the neurocognitive status of the individuals in our study in relation to their gut microbiome. Methods: We evaluated the gut microbiota of recently HIV-infected men (n=8) who were participating in a randomized, double-blind controlled trial of combination ART. Bacterial populations were pyrosequenced from anal swabs collected before and after the initiation of ART. We classified bacterial sequences at the order level and evaluated the relationship of the microbiome with neuropsychological performance using the Global Deficit Score (GDS). NCI was defined as having a GDS of≥0.5. Results: This study classified proportions of distal gut bacterial flora at the order level (13 orders) using the V6 region of the bacterial 16 s rDNA. Using principal component analysis, individuals with NCI could be segregated by their gut microbial profiles as assessed prior to the initiation of ART. Individuals with and without NCI could still be distinguished from each other based on their gut microbial profile after 24 weeks of ART. Conclusions: In the current study, we showed that the specific gut bacterial profiles were associated with NCI independently of ART. Further studies are needed to determine whether the gut microbiome is a marker or a mediator of NCI during HIV infection.

P120
Cerebrospinal Fluid Cell-Free Mitochondrial DNA Levels are Elevated in HIV-Associated Neurocognitive Disorder

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Background: HIV-associated neurocognitive disorder (HAND) involves persistent neuro-inflammation. Mitochondrial DNA (mtDNA) carries CpG motifs and has pro-inflammatory properties. We previously observed that cell-free mtDNA in cerebrospinal fluid (CSF) was associated with systemic and CNS inflammation in HIV-infected persons, and with severity of neurocognitive impairment among individuals with HAND. Here, we investigated relationships between demographics, HIV disease characteristics, HAND and CSF cell-free mtDNA levels in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) cohort. Methodology: In this cross-sectional study of 335 HIV-infected subjects, we quantified levels of cell-free mtDNA (ND2) in CSF using droplet digital PCR. CSF samples from the earliest available time-point with concurrent, comprehensive neurocognitive assessments were analyzed. We evaluated mtDNA levels in relation to demographics, HIV disease characteristics, and HAND, defined by Global Deficit Score≥0.5, and by Frascati criteria at the time of CSF sampling, adjusting for potential confounders. Results: Individuals with mild neurocognitive disorder had higher median CSF cell-free mtDNA than either neurocognitively normal individuals (13,000 vs. 7940 copies/ml, $p=0.04$), or those with asymptomatic neurocognitive impairment (13,000 vs. 7680 copies/ml, $p<0.001$). These results remained significant after controlling for HIV RNA detectability in CSF. Individuals on combination antiretroviral therapy (CART) had lower CSF mtDNA than CART-naïve individuals ($p=0.05$), and individuals on CART with undetectable CSF HIV RNA had lower mtDNA than those with detectable HIV RNA (6620 vs. 10,580 copies/ml, $p=0.02$). After additional adjustment for CSF HIV RNA and CD4 nadir, women had significantly lower CSF mtDNA levels than men ($p=0.01$). Conclusions: CSF cell-free mtDNA levels are significantly elevated in HIV-infected individuals with mild neurocognitive disorder and with higher CSF HIV RNA. Future in vitro studies are needed to clarify whether CSF mtDNA is a biomarker or a mediator of neuro-inflammation; if the latter, targeted strategies to reduce its impact may ameliorate HAND.

P121
MicroRNA regulation of SAMHD1 mediated HIV restriction

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Although, highly active antiretroviral therapies (HAART) have significantly reduced the morbidity and mortality in HIV patients, virus continues to reside in central nervous system (CNS) reservoir. Hence a complete eradication of virus remains as a challenge. HIV productively infects microglia / macrophages, but astrocytes are generally restricted and neurons are highly restricted to HIV infection. The relative importance of the possible replication blocks in astrocytes, however, is yet to be delineated. Recently identified restriction factor, sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1), has been shown to restrict HIV infection in resting CD4 T cells and in monocyte-derived dendritic cells. However SAMHD1 expression and HIV-1 restriction activity regulation in CNS cells is unknown. Though, certain miRNAs have been implicated in HIV restriction in resting CD4+T cells, their role in CNS HIV restriction and their mode of action are not established. We hypothesized that varying SAMHD1 expression leads to restricted HIV infection and host miRNAs regulate SAMHD1 expression in CNS cells. Our data show that astrocytes have higher level of SAMHD1 and low level of miR155 compared to microglia. Here we provide a proof of concept that SAMHD1 in astrocytes is responsible for the HIV restriction, silencing of which relieves this restriction. We report for the first time that miR-155 and -181a regulate SAMHD1 expression and they mediate HIV regulation by optimizing RT step through SAMHD1 regulation. Reactivation of HIV replication is accompanied by decrease in SAMHD1 expression. In conclusion, our data demonstrate that if optimal combinations of both of these could be effectively delivered to astrocytes along with HAART in in-vivo condition, eradication of CNS viral reservoirs may be possible, opening novel avenues to improve

current strategies to trigger reactivation of the latent CNS reservoir in patients.

P122

Cocaine use in HIV-1-infected African Americans in the Drexel Medicine CARES Cohort results in altered immunomodulatory profiles and disease progression

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This study evaluated the relationship between illicit drug use and HIV-1 disease severity in HIV-1-infected patients enrolled in the Drexel Medicine CNS AIDS Research Eradication Study (CARES) Cohort. Since cocaine is known to have immunomodulatory effects, the cytokine profiles of preferential non-users (PN), cocaine users (PCo) or multi-drug users (MDU) were analyzed to understand the effects of cocaine on cytokine modulation and HIV-1 disease severity. Patients within the cohort were assessed longitudinally for HIV-1 clinical parameters and history of illicit drug, alcohol, and tobacco use. The Luminex human cytokine 30-plex panel was used for cytokine quantification. Analysis was performed using a newly developed biostatistical model. Substance abuse was found to be common within the cohort. Utilizing the drug screens at the time of each visit, it was determined that the cohort could be categorized as PN, PCo, and MDU. The overall health of the PN population was better than that of the PCo population, with peak and current viral loads in PN substantially lower than those in PCo and MDU patients. Among the 30 cytokines investigated, differential cytokine profiles were established within the three populations. The Th2 cytokines, IL-4 and IL-10, known to play a critical role during HIV-1 infection, were positively associated with increasing cocaine use. Clinical parameters such as latest viral load, CD4+ and CD8+ T-cell counts, and CD4:CD8 ratio were also significantly associated with cocaine use. Based on these assessments, cocaine use appears to associate with more severe HIV-1 disease. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785.

P123

The Contribution of IgG Glycosylation to Oligoclonal Bands in Multiple Sclerosis

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Phage-displayed random peptide libraries were previously used in our lab to identify peptides specific to IgG antibodies in MS patients (Yu et al., 2011). In the present study, we applied phage-mediated real-time immuno-PCR (IPCR) and isoelectric focusing electrophoresis (IEF) techniques to characterize the interactions of phage peptides to MS IgG. We demonstrated that the peptides had a higher binding affinity to the corresponding patient's CSF than serum, indicating that they bound to intrathecally synthesized IgG in MS. Although no single peptide shared binding specificity among multiple MS CSF, cross reactivity between 2 pairs of patients was confirmed by IPCR and IEF. We further performed an IgG deglycosylation study on CSF from six MS patients with the hypothesis that the oligoclonal bands (OCBs) in MS are due to IgG glycosylation. After complete deglycosylation with the Enzymatic DeGlycoMx Kit (QA bio) which removes all N-linked oligosaccharides and many O-linked oligosaccharides from glycoproteins, phage peptide specific OCBs shifting to a more acidic region was observed in both CSF and sera of four patients. A slight reduction of bands in addition to shifting was observed in one patient, and band shifting to a more basic region was observed in an additional patient. Complete band collapsing did not occur in any of the patients studied. Our results suggest that glycosylation is a contributing factor to the characteristic oligoclonal IgG bands in MS, and may play a role in disease pathogenesis.

P124

Insulin signalling regulates lentivirus replication in brain: Reduced viral burden and improved neurological outcomes

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HIV-1 infection of the brain is a challenging viral reservoir that contributes to the development of HIV-associated neurocognitive disorder (HAND). Insulin resistance is major complication of contemporary HIV/AIDS care. Experimental studies report insulin treatment suppresses HIV-1 replication in leukocytes. Clinical studies show increased soluble insulin receptor levels are present in HAND patients. Herein, we investigated the expression of the insulin receptor (IR) and the insulin-like factor-1 (IGF-1) receptor as well as the consequences of the insulin receptor activation on lentivirus infections in vitro and in vivo. IR and IGF-1R transcript levels were similar in brains from HIV-infected versus uninfected patients and IR immunoreactivity was localized chiefly on neurons and macrophage lineage cells. Human fetal microglia (HFM) and PMA-differentiated human monocytic cells (d-THP-1) were infected with HIV-1 and treated with insulin; p24 levels in supernatants from HIV-infected HFM were reduced in a dose-dependent manner ($p<0.05$) but not in supernatants from infected d-THP-1 cells. Human fetal neurons (HFNs) were exposed to the HIV-1 protein, Vpr, with or without prior insulin treatment. Insulin treatment reduced neurotoxicity mediated by Vpr exposure in HFN ($p<0.05$). Domestic cats infected with a neurovirulent feline immunodeficiency virus (FIV) strain, or mock- infected, were treated daily for 6 weeks with intranasal insulin or PBS; all animals underwent neurobehavioral testing followed by neuropathological and molecular studies. Intranasal insulin treatment improved neurological status in FIV-infected cats including both memory and motor functions ($p<0.05$) although blood CD4 T cell levels were unaffected. FIV pol levels were reduced in brains of insulin-treated FIV-infected animals compared to PBS-treated FIV-infected animals, which was accompanied by reduced gliosis in cerebral cortex and white matter ($p<0.05$). Insulin receptor signalling exerts antiviral in vitro and in vivo actions and might represent a potential therapeutic strategy for reducing lentivirus expression in the brain and improving clinical outcomes in HAND.

P125
TNF-alpha/TNFR2 regulatory axis stimulates EphB2-mediated neuroregeneration

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HIV-1 infection of the central nervous system (CNS) causes neuronal injury and synaptic loss, which correlate with cognitive decline in HIV-1/AIDS patients. Neuronal damage results from CNS viral replication and neurotoxic effects of inflammatory chemokines and cytokines, including TNF-alpha. To determine the transcriptional effects of TNF-alpha-mediated signaling on synaptic plasticity, the expression of genes central to synaptic alterations during learning and memory were analyzed in human neurosphere cultures using quantitative RT-PCR. TNF-alpha treatment affected the expression of many genes, but particularly induced expression of Ephrin receptor B2, which is involved in NMDA signaling. We utilized a scratch assay to demonstrate the response of primary human neurons to injury upon treatment with TNF-alpha, and our results show that TNF-alpha induced more neurite outgrowth and increased EphB2 expression in the cell body and neurites. Studies have shown neuroprotective effects of TNF-alpha through the NF-kappaB pathway, therefore, we propose that upon TNF-alpha treatment following induced injury, neurite outgrowth occurs primarily through EphB2 signaling via stimulation of NF-kappaB1. EphB2 promoter activity was analyzed with TNF-alpha treatment and increased with NF-kappaB overexpression, which was abolished with IkappaB overexpression. We also determined that NF-kappaB p65 directly binds to the promoter via multiple binding sites, but primarily through the second nearest binding site to the transcriptional start site. Neuroprotective effects of TNF-alpha have been suggested to occur through TNF-alpha receptor 2 (TNFR2) activation, thus we blocked both TNFR1 and TNFR2 in human primary fetal neurons and showed that EphB2 is transcriptionally upregulated upon blocking TNFR1 compared to blocking TNFR2. This suggests that TNF-alpha neuroregenerative effects via EphB2 induction occurs through TNFR2. Our observations provide a new avenue for the investigation on the impact of HIV-1 in neuronal cell damage and the involvement of TNF-alpha, as well as providing a potential therapeutic target in TNFR2 activation of EphB2.

P126
Tregs modulate proliferation of T-cells and microglia following viral brain infection

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Regulatory T-cells (Tregs) are well known to play crucial roles in suppression of immune responses during infection and autoimmunity. Accumulation and retention of Treg cells has been reported within post viral-encephalitic brains. However, the extent to which these Tregs modulate neuroinflammation is yet to be elucidated. Here, we used Foxp3-DTR-GFP knock-in transgenic mice, which upon administration of low dose of diphtheria toxin (DTx) results in specific deletion of

Tregs. We investigated the proliferation status of various immune cell subtypes within inflamed central nervous system (CNS) tissue. We observed that depletion of Tregs resulted in increased proliferation frequencies of CD8+ and CD4+ T-cells, as well as brain-resident microglial cells during the acute phase of viral infection (i.e., 7 days post-infection, dpi). In contrast, immune cell proliferation rate was controlled in untreated animals by 14 dpi when compared to Dtx-treated mice. Previous studies by us and others have demonstrated that Treg numbers within the brain rebound by 20 dpi following Dtx treatment to higher numbers than in untreated animals. Despite this rebound, microglia and CD4+ T-cells proliferated at a higher rate when compared to that of Treg-sufficient mice, thus maintaining sustained neuroinflammation. Furthermore, at 30 dpi we found the majority of CD8+T cells were CD127hi KLRG1- indicating that the cells were long lived memory precursor cells. These memory cells showed marked elevation of CD103 expression (a marker of tissue resident-memory T-cells, Trm), from DTx-treated animals. In contrast, a small percentage of CD4+ T-cells expressed CD103, which was also found at negligible levels in cervical lymph nodes. In summary, our findings demonstrate that Tregs limit neuroinflammatory responses to viral brain infection by controlling proliferation of immune cell types within the brain and may direct a larger proportion of brain-infiltrating CD8+ T-cells to be maintained as Trm cells.

P127
Adult Neurogenic Defects in HIV-1 Tg26 Transgenic Mice

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While combination antiretroviral therapy has significantly diminished the incidence and intensity of HIV-associated neurocognitive disorders (HAND), a more moderate form of HAND has become prevalent. Unfortunately, the underlying mechanisms remain poorly understood. HIV-1 infection has been previously observed in neural stem/progenitor cells (NSCs/NPCs) both in vitro and in vivo. HIV-1 viral proteins Tat and gp120 not only damage differentiated neurons, but some studies have shown that they also inhibit the proliferation and differentiation of NSCs/NPCs. To explore the potential role of HIV-1 viral proteins in regulating early neurogenesis and lineage differentiation, we performed in vitro and in vivo studies using HIV Tg26 transgenic mice harboring the entire pNL4-3 HIV-1 genome with a gag/pol deletion. RT-PCR analysis confirmed viral mRNA expression in brain tissue (2 copies per 200 ng RNA). Neurosphere assays performed with subventricular zone (SVZ) NSCs showed a statistically significant decrease in the numbers of total primary neurospheres and neurospheres smaller than 300 μm from Tg26 mice, when compared to their wild-type littermates. However, a slight increase in the numbers of neurospheres larger than 300 μm was also observed in NSCs dissected out from the SVZs of Tg26 mice, when compared to wild-type littermates. These results suggest that HIV-1 viral proteins inhibit NSC differentiation into NPCs. In addition, the proliferative capability of NSCs/NPCs was reduced by 50-70 % in Tg26 mice as compared to littermates. These results were confirmed in vivo by multi-labeled immunohistochemical staining and confocal image analysis of serial brain sections. Neural lineage differentiation analysis by multi-labeled immunocytochemistry revealed that Tg26 NSCs exhibited a 25 % diminished capacity to differentiate into neurons and an increase in glial lineage differentiation. In conclusion, these results demonstrate that adult neurogenesis is impaired in HIV-1 Tg26 mice, and these impairments may contribute to the development of HAND.

P128
NIBP/TRAPPC9 restricts HIV-1 reactivation in human CHME5 microglial cells

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Even though cART has significantly increased the life expectancy and quality of life for patients affected with HIV-1, viral latency remains a significant barrier to finding an HIV-1 cure. Several HIV-1 latent reservoirs including CD4+ memory T cells, monocytes/macrophages, and microglial cells have been well described. NIBP/TRAPPC9 is a novel protein that enhances cytokine-induced NF-κB activation and regulates trans-Golgi networking. We demonstrated previously that NIBP/TRAPPC9 suppresses HIV-1 LTR promoter activation in neural stem cells, fibroblasts and TZM-bI cell line, which is well-known model containing HIV-1

luciferase reporter for studying HIV-1 latency/reactivation. Since microglial cells do not express NIBP, we hypothesized that NIBP/TRAPPC9 delivery may suppress HIV-1 replication/reactivation in these HIV-1 permissive cells. To test this hypothesis, we infected CHME5 microglial cell line with an adenovirus overexpressing N-terminal mCherry tagged full length NIBP (1139 aa), a C-terminal mutant NIBP (N506), or an empty mCherry negative control. At 24 h after adenovirus infection (MOI=20), we induced HIV-1 LTR promoter activation using the NF-κB activator TNFα (50 ng/ml), HDAC inhibitor TSA (100 ng/mL), or PKC activator PMA (1 ng/mL). At 24 h after treatment, the level of HIV-1 reactivation was determined by flow cytometric analysis of EGFP-expressing cells. The results showed that adenoviral delivery of NIBP/TRAPPC9 to these cells significantly inhibited constitutive and TNFα/TSA/PMA-induced activation of the HIV-1 EGFP reporter, but the NIBP(N506) C-terminal deletion mutant had no effect as compared to empty adenoviral-mCherry control. These data present evidence that functional NIBP/TRAPPC9 effectively suppresses HIV-1 transcription and reactivation. Delivery of NIBP/TRAPPC9 in HIV-1-permissive cells may provide a novel approach for HIV-1/AIDS therapy. (This work was partially supported by a Ruth L. Kirchstein National Research Service Award (NIH 1 T32 MH079785); Core facility services supported by CNAC at Temple, NIMH P30 MH092177).

P129

Intrinsic pro-inflammatory cytokine production and microglial activation during West Nile virus infection of ex vivo CNS slice cultures

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West Nile virus (WNV) neuroinvasive disease causes significant morbidity and mortality in the United States. Mouse models for WNV infections have elucidated the importance of pro-inflammatory peripheral immune cell invasion in viral clearance from the central nervous system (CNS), but how viral infection invokes this response is not completely understood. We used ex vivo slice cultures of mouse brain (BSC) and spinal cord (SCSC) to investigate intrinsic responses of the CNS to WNV infection. Consistent with in vivo infection, WNV robustly infected neurons and some astrocytes in ex vivo slice cultures, inducing tissue injury that was associated with astrogliosis and activation of caspase-3 dependent apoptosis. WNV infection resulted in the significant up-regulation of pro-inflammatory cytokines over time, including CXCL10, CCL5, CCL3, CCL2, IL-6, TNF, and TRAIL. Increased expression of pro-inflammatory genes was associated with activation of microglia, which demonstrated amoeboid-like morphological changes and phagocytosis of WNV-infected cells and antigenic debris. These results demonstrate that CNS cells can respond to WNV infection in the absence of a peripheral immune response. Minocycline is a broad-spectrum tetracycline antibiotic that is commonly used to inhibit microglial function in vitro. In minocycline-treated slice cultures there was a delay in the activation of microglia and a significant reduction in the WNV-induced expression of CCL5, CCL3, CCL2, IL-6, TNF, and TRAIL over the course of infection, suggesting that these cytokines are produced by microglia during WNV-infection of the CNS. In contrast, minocycline did not reduce expression of CXCL10, which is known to be up-regulated in WNV-infected neurons. Minocycline treatment also decreased viral growth and reduced neuronal death in WNV-infected SCSC suggesting that pro-inflammatory cytokines expressed by microglia contribute to neurotoxicity following WNV infection of the CNS. These studies were supported by a Veterans Administration merit award and NIH grants RO1-NS076512 and R33-AI101064.

P130

A subset of CD8+ T cells (CD4DIMCD8Bright Cells) control HIV infection in the brain

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CD8+ T cells are prominent in brains of humans and macaques with HIV-E and SIV-E, respectively, yet their role in HIV control in the CNS is not entirely clear. A subset of CD8+ T cells, CD4dimCD8bright T cells (referred to as DP cells), is enriched in anti-HIV responses and in blood of HIV+ Long Term Non-Progressors. We evaluated the ability of DP T cells to control HIV infection in the brain in comparison to CD8 single positive (SP) T cells as modeled in HIV+ NOD/SCID/IL-2rcγ−/− mice reconstituted with human PBMCs (NSG-huPBMCs). We demonstrate that CD8SP (≈40 % of CD3+ T cells) and DP T cells (≈15 % of CD3+ T cells) are found in the brains of HIV+NSG-huPBMCs mice. DP cells harbor HIV, but interestingly, at 3 weeks post-infection, greater than 90 % of CD4SP cells were depleted, while 25 % of DP cells survived. Intracranial injection of CD8SP cells into the brain

of NSG-huPBMCs mice induced DP expression by 10-fold indicating that the microenvironment of the brain is conducive to the DP phenotype. Both CD8SP and DP cells continue to proliferate in the CNS. One week after injection≈90 % of DP cells and 80 % of CD8SP cells were Terminal Effector Memory (TEMRA) cells (CCR7-CD27-CD28-CD45RA+). Importantly, increased percentage of DP cells inversely correlated with expression of HIV-gag mRNA ($R=-0.61$, $p\leq 0.001$) but no such correlation existed between CD8SP and HIV-gag mRNA ($R=-0.23$, $p\leq 0.3$). Lastly, we show the presence of DP T cells in brains of HIV-infected individuals. Collectively, our studies point to DP cells as a subset of CD8+ T cells responsible for HIV control in the CNS.

P131

The role of antioxidants abrogate meth-induce HIV-1 replication and oxidative stress in human glial cells

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PURPOSE: It has been reported that methamphetamine (METH) contributes to the increase of human immunodeficiency virus (HIV-1) transmission leading to neuronal damage. METH abuse induces oxidative stress by depleting key antioxidants, including glutathione, and thereby enhances the progression of HIV-1 pathogenesis. The accumulation of reactive oxygen species (ROS) affects the function of the glial cells in the brain leading to neuronal damage and cytotoxicity. There is an immediate need for the development of new treatment options to counteract the effect of methamphetamine abuse by HIV-1 patients. A potential treatment option to address oxidative stress/inflammation induced damage, will be the use of antioxidants that may elicit an anti-viral activity by suppressing the oxidative stress and HIV replication and could modulate the effect of pro-inflammatory molecules caused by methamphetamine that affects glial cells. Therefore, we evaluated the effect of antioxidants on glial cells following exposure to METH and HIV. DESIGN METHODS: Using glial cells we have determined the effects of antioxidants on METH and HIV-1 induced inflammation/oxidative stress. Various oxidative stress parameters, such as malondialdehyde (MDA) and ROS were measured. In addition we measured inflammatory cytokine production, and HIV replication. RESULTS: Our results indicate that antioxidants modulate inflammatory cytokines such as IL-1b, IL-6, and that an antioxidant treatment abrogates HIV-1 replication and oxidative stress induced by METH in human glial cells. CONCLUSION: These data suggest that antioxidants have a novel anti-viral activity. This project provides, for the first time, the molecular mechanism of antioxidants in human glial cells and its new natural therapeutic potential for HIV-1 infection and AIDS.

P132

Spatial Memory Test (MI) as an Early Predictor of Progression in HAND

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Background: The prevalence of HIV-associated neurocognitive disorders (HAND) is increasing as HIV infected persons live longer. Memory Island (MI) is a computer-based test useful in identifying susceptibility to cognitive impairments. Performance on MI strongly correlates with neuropsychological tests (NP) performance. MI has the advantage of being a non-invasive and easy to administer test. Our objective is to use MI as reliable tool in screening and predicting HAND progression. We hypothesize that MI will predict the occurrence of HAND in HIV-seropositive women. Methods: 35 women were evaluated (20 HIV-seropositive and 15 matched controls) with neurological and NP tests at baseline and at 6 months follow-up. Cognitive performance was determined using the HAND criteria. Non-parametric statistical analyses and correlations were performed. Results: Three HIV-seropositive women presented worse HAND diagnosis after follow-up: one with normal cognition and 2 with asymptomatic neurocognitive impairment (ANI). No differences were observed between HIV-seropositive and control groups in speed of navigation to locate the target during the trials containing a visible or hidden target. During the visible target trials, no differences among groups were observed in the ability to locate the target location. However, during the trials with the hidden target, NP-impaired HIV-seropositive women required more time and longer distance than NP-normal women to locate the object, especially at follow-up ($p<0.05$). Thus, HIV-seropositive women presented less efficient acquisition (learning) and worse performance at follow-up ($p<0.05$). When spatial memory retention was assessed (probe trial, no target present), HIV-seropositive women

spent less time in the quadrant of the island that contained the target when compared with controls at baseline or follow-up ($p<0.01$). Conclusions: The MI test is useful in detecting spatial learning and memory deficits in HIV-seropositive women and is a valuable instrument for the screening and predicting HAND progression in HIV-seropositive women.

P133

Cambinol, a novel inhibitor of neutral sphingomyelinase 2 shows neuroprotective properties

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Neutral sphigomyelinase2 (nSMase2) catalyzes the hydrolysis of sphinghomyelin (SM) to phosphorylcholine and ceramide. Ceramide production through nSMase2 upregulation has been associated with cellular processes ranging from apoptosis to modulation of synaptic plasticity to manufacturing of ceramide-rich exosomes. While transient nSMase2 upregulation is part of normal brain functioning, chronic upregulation of the enzyme has been implicated in the pathogenesis of HIV-Associated Neurocognitive Disorders (HAND), Alzheimer's disease, Multiple Sclerosis and Amyotrophic Lateral Sclerosis. Inhibition of nSMase2 using antisensene knockdown or pharmacological agents has been shown to reduce apoptosis and offer neuroprotection in neurodegenerative disease models. However, while nSmase2 is emerging as an important player in neurodegeneration, the current armamentarium of nSMase2 inhibitors is inadequate to explore the role of the enzyme. The objective of this work is to develop chemical probes of nSMase2 to evaluate its role in HAND. In order to identify new inhibitors of nSMase2, two screening assays were characterized using recombinant human nSMase2. The primary assay (1536-well format) is a fluorescence-based high throughput compatible assay while the confirmatory assay (96-well format) uses [^{14}C]-sphingomyelin. The assays were then used to carry out two pilot screens that included 2300 pharmacologically active compounds and approved drugs. The screens identified cambinol as a novel uncompetitive nSMase2 inhibitor (Ki= 7 åμM). Cambinol's inhibitory activity for nSMase2 was approximately 10-fold more potent than for its previously known target, silence information regulator 1 and 2 (SIRT1/2). Cambinol decreased tumor necrosis factor-alpha or interleukin-1 beta-induced increases of ceramide and cell death in primary neurons. Even though cambinol exhibits better solubility than currently available nSMAse2 inhibitors and could be used in vitro, it exhibits poor metabolic stability making it of limited use in animal models. We are currently carrying out an HTS campaign using the Molecular Libraries Small Molecule Repository (>400,000 compounds) to identify additional nSMase2 inhibitors.

P134

Poly(ADP-ribose) polymerase-1 inhibition in protects the blood–brain barrier in HIV-1 associated neuroinflammation

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Blood–brain barrier (BBB) dysfunction seen in neuroinflammation contributes to mortality and morbidity in multiple sclerosis, encephalitis, traumatic brain injury, and stroke. Identification of molecular targets maintaining barrier function is of clinical relevance. We used a novel in vivo model of localized aseptic meningitis where tumor necrosis factor alpha (TNFα) was introduced intracerebrally and surveyed cerebral vascular changes and leukocyte-endothelium interactions by intravital videomicroscopy. Poly (ADP-ribose) polymerase-1 (PARP) inhibition significantly reduced leukocyte adhesion to and migration across brain endothelium in cortical microvessels. PARP inactivation diminished BBB permeability in an in vivo model of systemic inflammation. PARP suppression in primary human brain microvascular endothelial cells (BMVEC), an in vitro model of BBB, enhanced

barrier integrity and augmented expression of tight junction proteins. PARP inhibition in BMVEC diminished human monocyte adhesion to TNFα-activated BMVEC (up to 65 %) and migration (80-100 %) across BBB models. PARP suppression decreased expression of adhesion molecules and decreased activity of GTPases (controlling BBB integrity and monocyte migration across the BBB). PARP inhibitors down-regulated expression of inflammatory genes and dampened secretion of pro-inflammatory factors increased by TNFα in BMVEC. In monocytes, PARP inhibitors down regulated the active form of β-integrin that paralleled RhoA/Rac1 suppression. PARP inhibitors decreased expression of pro-inflammatory molecules (known neurotoxins associated with HIV infection) and diminished HIV replication in human macrophages. In vivo treatment with a PARP inhibitor decreased enhanced BBB permeability in mice with systemic inflammation. These results point to PARP suppression as a novel approach to BBB protection in the setting of endothelial dysfunction caused by inflammation.

P135
MiR-29a: a potential marker of neurocognitive impairment in HIV-infected patients

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Background and Objectives: Micro RNAs (miR), non-coding RNA molecules that regulate the post-translational gene expression, have been implicated previously in experience-dependent adaptive changes of neural circuits. We aimed to explore the relationship between altered miR expression, in particular miR-29a which was associated with the progression of HIV infection. Methods: Expression levels of miR-29a were measured by quantitative real-time PCR (Life Technologies - TaqMan® MicroRNA Assays) in 90 HIV infected patients and normalized against an age- matched control group. Neurocognitive functioning was assessed by a comprehensive testing battery on 7 cognitive domains; a global deficit score (GDS) or domain specific dysfunction scores above 0.5 predicted neurocognitive impairment (NCI). Results: All patients (median age 24 years, 48.8 % males), parenterally infected during childhood, were followed for a median period of 14.6 years, 90 % were antiretroviral (ARV) experienced (median length on ARV 142 months). The median HIV viral load was 2.7 $\log_{10}$ copies/mL, while the median CD4 count was 464.1 cells/mm^3 (20 % of the patients with severe immunosuppression). Neurocognitive impairment was diagnosed in 47.8 % of the patients, correlated with current CD4 count (rho=0.20, p=0.05). In all patients, a weak but statistical significant correlation was observed between miR-29a levels and dysfunction scores for memory (rho=0.22, p=0.03) and executive functions (rho=0.20, p=0.04). In addition, for the neurocognitive impaired patients, miR-29a expression was directly correlated with the GDS score (rho=0.39, p=0.01), and the dysfunction scores for speed information processing (rho=0.30, p=0.04) and executive function (rho=0.33, p=0.02). Conclusion: Our study suggests the potential role of miR-29a as a marker for neurocognitive impairment in HIV infected patients.

P136
Activation of CD38, AMPK, MAPK and NADPH Oxidases is Increased in Brains of HIV-1 Transgenic Rats

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Neurocognitive impairment in HIV-1 infection has been associated with the development of oxidative stress in brain and neurodegenerative responses. Factors that underlie the development of oxidative stress include the generation of reactive oxygen species by NADPH oxidases and activation of the ectoenzyme CD38. CD38 is a bifunctional molecule that can generate second messengers from the co-enzyme nicotinamide adenine dinucleotide (NAD) that result in release of calcium from intracellular stores. Persistent activation of CD38 results in significant energy expenditure and a decrease in intracellular levels of ATP. In these studies, levels of the CD38 and activation of the mitogen activated protein kinases (MAPK) Erk1/2, Erk5, p38 and SAPK/JNK, the brain-specific NADPH oxidase DUOX1 and of the master energy sensor AMPK were examined in brains from HIV-1 transgenic (HIV1Tg) and wild-type Fisher 344/NHsd (F344) rats. Levels of p38 and JNK/SAPK activation and of CD38 and DOUX1 expression were higher in the brains of the HIV1Tg than in the F344 animals. This increase was associated with increased activation of AMPK and of Erk1/2 and ERK5, which trigger neuroprotective responses. These studies, therefore, suggest that mechanisms which are linked to nervous system dysfunction in HIV infection can trigger responses that

are capable of at least partially compensating for such effects to modulate the risk of the development of neurotoxicity in HIV-1 infection.

P137

Prefrontal cortical volume loss is associated with stress-related deficits in verbal learning and memory in HIV-infected women

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Deficits in verbal learning and memory are a prominent feature of neurocognitive function in HIV-infected women, and are associated with high levels of perceived stress. To understand the neurobiological factors contributing to this stress-related memory impairment, we examined the association between stress, verbal memory, and brain volumes in HIV-infected women. Participants included 38 HIV-infected women (Mean age=43.9 years) from the Chicago Consortium of the Women's Interagency HIV Study (WIHS). Participants underwent structural magnetic resonance imaging (MRI) and completed standardized measures of verbal learning and memory, and stress (Perceived Stress Scale-10; PSS-10). Brain volumes were evaluated in a priori regions of interest, including the medial temporal lobe (MTL) and prefrontal cortex (PFC). Compared to HIV-infected women with lower stress (PSS-10 scores in lower two tertiles), HIV-infected women with higher stress, (scores in the top tertile) performed worse on measures of verbal learning and memory and showed smaller volumes bilaterally in the parahippocampal gyrus, superior frontal gyrus, middle frontal gyrus, and inferior frontal gyrus (p's<0.05). Reduced volumes in the inferior frontal gyrus (right hemisphere) and bilateral superior frontal gyri were negatively associated with verbal learning and memory performance. Prefrontal cortical atrophy is associated with stress-related deficits in verbal learning and memory in HIV-infected women. The time course of these volume losses in relation to memory deficits has yet to be elucidated, but the magnitude of the volumetric differences between women with higher versus lower stress suggests a prolonged vulnerability due to chronic stress and/or early life trauma.

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A novel viral determinant of HIV neuropathogenesis in Tat protein basic domain

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HIV-1 infection of CNS can cause HIV Associated Neurocognitive Disorders (HAND) via chronic neuro-inflammation. Delineating the viral determinants of HAND is key to developing neuroprotective agents. It has been reported that HIV-1 in some parts of the world - such as clade C HIV-1 (HIV-1C) in India - leads to lower incidence of the severe form of HAND, HAD. A C30C31 motif in HIV-1 Tat, absent in most HIV-1C isolates, mediates monocyte recruitment to CNS, induces inflammatory cytokines and causes direct neurotoxicity. We now describe a novel Tat determinant of HIV neuropathogenesis and an associated polymorphism that may be responsible for the reduced neuropathogenesis of HIV-1C. Basic domain of Tat, which is responsible for cellular uptake of extracellular Tat includes an Arg57 in subtype B while the lower-HAD risk HIV-1C has a Ser57. We examined the consequence of R57S substitution for cellular uptake using fluorescently labeled decapeptides corresponding to the Tat CPP of HIV-1B and HIV-1C. Flow cytometry and confocal microscopy of peptide-treated cells revealed a greater uptake of R57-containing peptides. We tested the biological relevance of this difference using a transcellular transactivation assay, in which we transfected HeLa cells with Tat expression constructs, and used them as "Tat-producer" cells in a co-cultivation system with "Target" cells containing LTR-reporters (TZMbl or HLM-1 cells). TatArg57 led to a higher degree of transactivation compared to TatSer57, even though

transactivation upon direct transfection into reporter cells was similar for both. Finally, using PBMCs ("Target" cells) co-cultivated with "Tat-producer" cells, we observed a higher induction of proinflammatory cytokines with TatArg57 compared with TatSer57. Thus, the R57S polymorphism may contribute to subtype-specific differences in HAND pathogenesis by impacting the efficiency by which the extracellular Tat protein is taken up into uninfected bystander cells contributing to both maintaining and propagating an inflammatory environment in the brain.

P139

The highly enriched c-type lectins on dendritic cells provide potential therapeutic strategy to ameliorate neuroinflammatory diseases

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The potential of dendritic cells (DCs) as sentinels for immune therapy is beginning to emerge against various neuroinflammatory diseases. However, there has been no attempt to establish a clinically viable target to impede the migration of DCs and other myeloid cells across the blood brain barrier (BBB). The need for studying specific molecular mechanisms of DCs trafficking into the CNS directed us to investigate the role of C-type lectins (CLRs) in chemoattraction to brain microvescular endothelial. We showed that CCL2 driven process involves Src homology region 2 domain-containing phosphatase (SHP)1/2-mediated signaling for coordination of actin polymerization in podosomes that express the WASP Interacting Protein (WIP). Further, antibody blockade of CLEC12A in mice with progressive and relapsing-remitting EAE (experimental autoimmune encephalomyelitis), significantly ameliorated the disease through inhibition of myeloid cell infiltration into the brain and spinal cord. Anti-CLEC12A antibody also restored DC numbers in the spleen along with a decreased TH17 phenotype within CD4 T cells. Our studies indicate that DC-specific therapeutics especially those that are CLR-targeted serve as promising candidates to curb the propagation of inflammation within the CNS. Thus, the prospect of selectively regulating DC entry into the CNS will substantiate the promise of DC-based immunotherapies to battle diseases that overpower the body's immune capabilities, and can be directed against inflammatory lesions or tumors.

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Cocaine activated MSK1 promotes both the initiation and the elongation phases of HIV transcription via NF-κB and P-TEFb

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Cocaine is one of the most widely abused drugs in the United States, which both impairs the normal functioning of brain cells and also activate human immunodeficiency virus (HIV) expression in central nervous system (CNS). As a result, HIV-infected individuals who abuse cocaine experience more severe and rapid onset of NeuroAIDS than non-abusing individuals. It has been known that cocaine affects the expression of numerous cellular genes by modulating various signaling and epigenetic pathways. Some of those pathways also influence the expression of integrated HIV proviruses and eventually end up enhancing HIV replication and transmission. Here, we report that cocaine promotes the initiation phase of HIV transcription by activating selective protein kinases; they in turn activate NF-κB by phosphorylating RelA/p65 subunit at Ser536 residue. Cocaine also activates Mitogen- and stress-activated kinase 1 (MSK1). MSK1 subsequently phosphorylates the P65 subunit of NF-κB at residue 276 that enhances the interaction of NF-κB with histone acetyl transferases (HATs). HATs consequently facilitate the establishment of transcriptionally active chromatin structures at HIV LTR and promote the initiation phase of HIV transcription. Besides NF-κB phosphorylation, MSK1 catalyzes the phosphorylation of histone H3 at ser10. This phosphorylation event promotes the recruitment of positive transcription elongation factor b (P-TEFb) at HIV LTR, which eventually enhances the elongation phase of HIV transcription. Thus by activating MSK1 cocaine promotes both initiation and elongation phase

of HIV transcription and facilitates the generation of complete HIV transcripts, which eventually enhances the rate of HIV replication.

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HIV-1 gp120, methamphetamine and antiretrovirals all compromise neuronal energy homeostasis in association with varying degrees of synaptic and neuritic damage

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Combination antiretroviral therapy (cART) prolongs survival of HIV patients; however the prevalence of HIV-associated neurocognitive disorders (HAND) continues to grow. Numerous HIV patients also use recreational drugs such as methamphetamine (METH) which may compromise the efficacy of cART. The combined effect of recreational and therapeutic drugs and virus on the brain is poorly understood. Therefore we exposed mixed neuronal-glial cerebrocortical cells to different antiretroviral (ARV) compounds and METH in the presence or absence of gp120 for 24 h and 7 days. First, we assessed neuronal injury and death in fixed cells using specific markers for neuronal dendrites (MAP-2) and pre-synaptic terminal (synaptophysin) in combination with nuclear DNA staining and fluorescence microscopy. When we analyzed the effect of cART compounds representing four different pharmacological categories (AZT, NVP, SQV and 118-D-24) in the presence or absence of gp120, none caused a significant neuronal injury in 24 h. However, after 7 days in the presence of gp120, only neuronal synapses were compromised, while MAP-2 positive dendrites were spared. We also analyzed potential cellular and neuronal toxicity using an ATPlite assay, a well established method used in large-scale drug screening for evaluation of cytotoxicity. The ATP assay similarly indicated that the occurrence of cytotoxicity was context-specific for each of the drugs or combinations thereof. To recover from energetic stress, autophagy is generally activated in order to preserve the vital cellular functions. To address this hypothesis activation of AMPK, and processing of LC3 and p62 were assessed. Our results indicated that autophagy is induced during exposure of cerebrocortical cells to METH, ARVs and gp120 but fails to restore normal levels of cellular ATP. Altogether, our findings indicate that the overall positive effect of cART in HIV infection is accompanied by some neurotoxicity which is possibly aggravated in the presence of abused drugs.

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Human polyoma JC virus late transcripts generate an unpredicted open reading frame, capable of encoding a 58 amino acid long protein

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JC virus (JCV) causes a fatal disease in the brain known as progressive multifocal leukoencephalopathy (PML) in a subset of immunocompromised individuals. The early and late coding regions of JCV genome is known to transcribe one primary transcript respectively and produce several predicted and alternatively spliced gene products, capable of encoding regulatory (large and small T antigens, T'-proteins and agnoprotein) and structural (VP1, VP2 and VP3) proteins. Our recent nuclear magnetic resonance (NMR) structure-based mutational analysis of the dimerization domain of agnoprotein transcripts by RT-PCR has led us discover an unpredicted open reading frame (ORF) for JCV late gene transcripts. This new ORF results from both (i) a trans-insertional process of the 5′-short coding region of VP1 between the coding regions of agnoprotein and VP2 after replacing the intron 1 and (ii) occurrence of a frame-shift within the 5′-short coding sequences of VP2. The new ORF has the capacity to encode a 58 aa long protein. Initial characterization of the new ORF by RT-PCR utilizing total RNA isolated both from the infected primary glial tissue culture cells and the PML brain tissue samples has confirmed that the new ORF indeed is expressed not only in vitro but also in vivo. Subsequent analysis of the possible protein coding capacity of the new ORF by immunoblotting studies using newly raised polyclonal rabbit antibodies against its predicted amino acid coding sequence has demonstrated that it can indeed encode a predicted size protein. Immunocytochemistry studies further revealed that it localizes mostly to the nucleus of the infected cells, implicating regulatory roles in the several aspects of the viral life cycle including, but not limited to, replication, transcription or virion biogenesis. We are currently in the process of investigating these implicated roles by employing various genetical, biochemical and virological approaches.

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HIV-1 Nef Blocks Autophagy and is Released in Exosomes Derived from Astrocytes; Evidence for Exosomal Nef mediated Neurotoxicity

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HIV-associated neurological disorders (HAND) affect the majority of AIDS patients and are a significant problem among HIV-1-infected individuals who live longer due to combined anti-retroviral therapies (cART). The virus utilizes viral proteins and subsequent cytokine inductions to unleash its toxicity on neurons. Among these viral proteins, Nef is a small HIV-1 protein expressed abundantly in astrocytes of HIV-1-infected brains and has been suggested to have a role in the pathogenesis of HIV-Associated Neurocognitive Disorders (HAND). In order to explore its effect in the CNS, Nef was expressed in primary human fetal astrocytes (PHFA) using an adenovirus vector. We observed that Nef expression triggered the accumulation of autophagy markers. Similar results were obtained with Bafilomycin A1, an autophagy inhibitor which blocks the fusion of autophagosome to lysosome. Furthermore co-expression of tandem LC3 vector (mRFP-EGFP-LC3) and Ad-Nef in these cells produced mainly yellow puncta strongly suggesting that autophagosome fusion to lysosome is blocked in PHFA cells in the presence of Nef. HIV-1 Nef expressed in infected human astrocytes and macrophages has been shown to be released in extracellular vesicles called exosomes. We purified the Nef-carrying exosomes and studied its neurotoxic effects on neurons. We observed that exosomal Nef was readily taken up by these neurons as evidenced by immunoblotting and immunocytochemistry. Furthermore, treatment with the Nef carrying exosomes induced oxidative stress as evidenced by a decrease in glutathione levels in neuronal cells indicating neurotoxicity. To further investigate its neurotoxic effects, we expressed Nef in primary neurons by transduction. Intracellular expression of Nef led to significant axonal and neurite degeneration. Interestingly, expression of Nef down-regulated phospho-tau protein levels while enhancing total tau in primary neurons. Collectively, these data show that HIV-1 Nef can be a formidable contributor to neurotoxicity along with other factors which leads to HAND in HIV-1 infected AIDS patients.

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Immune mediated regulation of JCV reactivation mediated by interplay between SF2 and Pur-alpha

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PML is a rare and fatal demyelinating disease of the CNS caused by the human polyomavirus, JC virus (JCV) which occurs in AIDS patients and those on immunosuppressive monoclonal antibody therapies (mAbs). We sought to identify mechanisms by which mAb therapy could stimulate reactivation of JCV in a cell culture model system and targeted mechanisms which could effect early gene transcription and JCV T-antigen production, which are key potential steps for blocking reactivation of JCV. Two important regulatory partners we have previously identified for T-antigen include Pur-alpha and SF2. SF2/ASF, an alternative splicing factor, is a potential regulator of JCV whose overexpression in glial cells strongly suppresses viral gene expression and replication. Pur-alpha has been most extensively characterized as a sequence-specific DNA- and RNA-binding protein which directs both DNA replication and gene transcription, as well as mRNA translation, and is a potent inducer of the JCV early promoter and the HIV-1 LTR, through binding to the key viral regulatory proteins, T-antigen and Tat, respectively. We observed downregulation of SF2 in response to co-culture with conditioned media from PBMCs treated with mAbs and a concomitant up-regulation of Pur-alpha. Pur-alpha and SF2 both act directly as transcriptional regulators of the JCV promoter and we now observed they also impact JCV T-antigen production at the post-transcriptional level. In addition, we also observed that SF2 can negatively regulate Pur-alpha levels. These cell culture studies suggest potential mechanisms by which local production of cytokines in brain tissues and the direct impact of mAbs on brain cells in proximity to active PML lesions can lead to JCV reactivation. Further elucidation of the mechanism behind the observed SF2/Pur-alpha regulation of JCV reactivation will point toward future therapeutic strategies to block JCV reactivation in at risk patients. Core facility services supported by CNAC at Temple (NIMH P30 MH092177).

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Selective vulnerability of D1- and D2-expressing medium spiny neurons after Tat±morphine treatments

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HIV and drug abuse are co-morbid conditions that significantly burden society. Combined antiretroviral therapy has improved and extended the life-spans of HIV-1 patients, yet, HIV-1 associated neurocognitive disorders (HAND) persist, and can be exacerbated by drug abuse. HIV-1 patients show selective vulnerability in the basal ganglia and the HIV-1 protein transactivator of transcription (Tat) has been shown to contribute to neuron cell death and increase the neurotoxicity and dendritic pathology of medium spiny striatal neurons (MSNs). Yet, how Tat and morphine affect subgroups of MSNs, which are responsible for many cognitive and motor functions, is unclear. The main categorization of MSNs consists of dopamine 1 receptor-expressing MSNs (D1-MSN), and dopamine 2 receptor-expressing MSNs (D2-MSN). To investigate possible D1- or D2-MSN selectively vulnerability to Tat±morphine, reporter mice (Drd1a-tdTomato or Drd2-EGFP) were crossed with Tat-transgenic mice to allow for selective examination D1- or D2-MSNs. Striatal-associated motor deficits were evaluated via rotarod coordination, open-field locomotion and Kondziela's inverted screen test, and electrophysiological and morphological abnormalities via patch-clamp recording, biocytin injection, confocal imaging, and 3D reconstruction. Preliminary data suggest that Tat and morphine did not affect the intrinsic electrophysiological properties of D1-MSNs, such as resting potential, threshold, and rheobase, although additional D1-MSNs remain to be sampled; nevertheless, there was a drug-induced dendritic spiny density reduction. Tat and morphine in combination initially decreased open field spontaneous locomotion compared to all other groups (first 5 min), while later, morphine increased locomotion compared to saline and Tat treated animals (15–30 min). However, morphine also increased anxiety-like behaviors compared to Tat and saline animals, though motor coordination and muscle stamina were similar between all groups. Therefore, we speculate that locomotor differences are related to affective changes rather than motor deficits, and that this effect is related to morphine-induced reductions in D1-MSNs dendritic spine density.

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Recruitment of HIV and CMV specific cells into the CSF and correlations to HIV Neurocognitive Disorders (HAND)

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Background: Trafficking of CD8+T-cells through the CSF and CNS may be either protective or pathogenic. Analysis of T-cell responses of 31 HIV infected individuals demonstrated that frequency of CD8+IFNgamma+T-cells in CSF correlated with neurocognitive impairment. Both HIV and CMV specific T-cells were identified in CSF, despite the absence of CMV in CSF. Methods: To investigate the roles of HIV antigen and CSF chemokine levels on frequency of virus specific T-cells in CSF, HIV and CMV specific CD8 and CD4 T-cell cytokine responses and CXCL10 levels were compared under 3 conditions: 1) HIV RNA detectable >40 copies/ml (LOD) in both CSF and plasma, 2) HIV RNA>40 in plasma, but <40 in CSF, and 3) HIV RNA<40 in both plasma and CSF. Results: Although absolute chemokine levels in CSF were lower when HIV RNA was suppressed, CXCL10 levels remained higher in CSF than in plasma. The frequency of CMV specific IFNgamma expressing cells did not vary with presence/absence of HIV. However, the frequency of HIV specific CD4 and CD8 IFNgamma expressing T-cells was significantly lower in CSF when HIV was detectable in plasma but not CSF. Surprisingly, when HIV was suppressed in both CSF and plasma, the frequency of HIV specific cells in CSF was comparable to the levels when HIV was detectable in both CSF and plasma. Conclusions/implications: 1) CD4 and CD8 T-cells primarily distribute to compartment(s) containing cognate antigen. 2) In the absence of cognate antigen, effector T-cells distribute according to levels of chemokines. 3) The increased frequency of CD8 effector cells in CSF when HIV RNA is suppressed in both compartments may be the basis of "immune reconstitution syndrome" 4) CMV specific CD8 T-cells could contribute to CNS inflammation if activated by CMV antigen in a remote compartment followed by recruitment to CNS by the CXCL10 gradient.

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Host genetic factors and miRNA-linked dendritic cell responses associated with the outcome of treatment response in HIV-1/HCV co-infected individuals

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HIV-1/HCV co-infection is a significant burden on global economy and public health. PEGylated interferon (PEG-IFN) and ribavirin (RBV) remain the essential components of anti-HCV treatment despite recently developed direct acting antiviral drugs. Herein, we investigated the host genetic and immunological correlates (in particular myeloid and plasmacytoid DCs) of successful treatment response in conjunction with mechanisms by which PEG-IFN is able to clear the virus in a cohort of HIV-1/HCV co-infected individuals undergoing IFN/RBV treatment. First we demonstrate that functional state of DCs before/during therapy influences the treatment outcome. Further, upon genotyping IFNL3 polymorphisms rs12979860, rs4803217 and ss469415590, we found rs12979860 to be a better predictor of treatment outcome. Next, we compared the expression of forty-six ISGs (IFN-stimulated genes) prior to and after the treatment and observed that pre-treatment levels of several ISGs were higher in SVRs compared to NRs. In continuation with molecular mechanisms, we identified miRNAs whose expression can be regulated by IFN in periphery. Specifically, we examined miRNA expression patterns in mDCs and pDCs in response to IFN-α and observed miR-221 downregulation via IFN induced STAT3 inhibition in both. Using in silico approaches followed by experimental validation, CDKN1C, CD54 and SOCS1 were identified as miR-221 targets. Moreover, miR-221 overexpression in mDCs enhanced their secretion of pro-inflammatory cytokines IL-6 and TNF-alpha but reduced the secretion of anti-inflammatory cytokine IL-10. These observations were extended and correlated with those obtained with patients' PBMCs as well as total liver cells and kupffer cells (antigen presenting cells in liver) from HCV infected individuals as well as individuals with alcoholic cirrhosis. In summary, these studies demonstrated the role of IFN-alpha/miR-221 axis in HCV pathogenesis and response to IFN-based treatments.

P148
The role of Rig-I-like RNA helicases during viral infection of the central nervous system

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Retinoic acid-inducible gene 1 (Rig-I)-like RNA helicases (RLRs), including Rig-I and Melanoma Differentiation-Associated protein 5 (Mda5) recognize pathogen-associated molecular pathogens (PAMPs) and play an important role in host anti-viral defense by signaling through mitochondrial antiviral signaling protein (IPS-1) to up-regulate type I interferon (IFN). Laboratory of Genetics and physiology 2 (Lgp2) and Ddx60 have more recently been identified as novel RLRs. Lgp2 and Ddx60 do not contain a specific signaling domain but may cooperate with Rig-I and Mda5 to regulate IFN signaling. The role of RLRs in viral infection of the central nervous system (CNS) has not been well characterized. We used microarray and quantitative PCR (qPCR) analysis to demonstrate that Rig-I, Mda5, Lgp2 and Ddx60 are up-regulated in the brain following in vivo infection of mice with West Nile virus (WNV), Japanese encephalitis virus (JEV) or reovirus. Rig-I, Mda5, Lgp2 and Ddx60 were also up-regulated following WNV and reovirus infection of ex vivo brain and spinal cord slice cultures demonstrating their increased expression in the CNS did not require peripheral immune responses. Additionally, we observed the formation of a large novel protein complex (~300 kDa) at early times following in vitro reovirus infection which may indicate a signaling cascade facilitated by Ddx60. We hypothesize that this complex contributes to type I interferon production.

P149
Impact of HIV-1 gp120 on mitochondrial bioenergetics and neuronal function

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Combinatory antiretroviral therapy (cART) has increased the lifespan of HIV-1 infected individuals but the prevalence of mild form of HIV-associated neurocognitive disorders (HAND) has increased. HIV-1 viral proteins released by the infected cells have been suggested to be responsible for the neuronal damage. Here, we hypothesize that HIV1- gp120 is able to cause deleterious effect to the neurons by affecting the mitochondria. Using differentiated SH-SY5Y cell and primary culture treated with HIV-1gp120IIIB we see altered expression of several genes and proteins involved in mitochondrial functions: PGC-1α, COXI, COXIII, Drp1, Fis1, MIRO, TRAK; and neuronal factors: CREB, BDNF, synaptophysin and miRNAs (miR499-5p, miR34a, miR132). Gp120 also caused decreased ATP production, increased depolarization of mitochondrial membrane potential and decreased mtDNA:nuDNA. Overexpression of CREB was able to rescue some of the effects. Therefore, HIV-1 gp120 affects mitochondria which lead to neuronal damage.

P150
Polyomavirus JC longitudinal seroprevalence in a cohort of Italian newborns

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BACKGROUND: JC virus (JCV) is a widespread member of the Polyomaviridae family. Serological studies determined that JCV primary infection occurs asymptomatically in 60-100 % of the population during childhood, but the age of JCV exposure has not been yet characterized. This study was conducted to defining the kinetic of maternal antibodies against JCV in infants and to dating the viral primary infection. MATERIAL AND METHODS: an home made indirect Enzyme Linked Immunosorbent Assay (ELISA) was employed, using JCV Viral Protein 1-GST fusion protein to test for the presence of IgG to JCV in serum samples taken from 167 newborns and followed up to 3 years (time points: 1, 2, 3, 4, 6, 9, 12, 24, 36 months). The cut-off point was determined in each assay by the mean OD reading of the negative controls which were added to standard deviation and multiplied three times. RESULTS: JCV IgG were detected in 92.8 %, and in 77.9 % of the newborns within the first and the second month of life, respectively (chi quadro test, $p<0.05$). The prevalence increased during the time, up to 91.4 % and 96.3 % after 12 and 24 months, respectively. Antibody amount also significantly increased ($p<0,001$) after 3 months of life (OD:2.3 times the value of the cut-off), up to 36 months (OD: 4.1 times the value of the cut-off). CONCLUSION: Maternal JCV antibodies wane after about 2 months from the birth, as it happens for other viruses such as rubella and varicella. Infants are thus susceptible at very young age for JCV infection, and almost all the children are subjected to the primary infection within the first year of life.

P151
Temporal lobe and olfactory pathway injury in subjects with autopsy-proven human herpesvirus-6 infection

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Previous studies of human herpesvirus-6 (HHV-6) have suggested its neurotropic nature and lifelong persistence. Controversial results on HHV-6 affected brain regions and neural cell types suggest that this issue is far from definitive. Regulatory S100 proteins, predominantly located in astrocytes, exhibit varying effects on neural homeostasis. In this study we evaluated possible associations between immunoexpression of HHV-6 and S100 in glial cells across different brain regions. Brain autopsy samples from 19 unspecified encephalopathy cases were compared to 19 age-matched and 12 younger cases without neuropathology. Brain tissue samples were assayed for HHV-6 DNA using nested and real-time PCR. HHV-6 and S100 antigens were detected immunohistochemically, expression assessed semi-quantitatively with an additional quantitative estimation of immunopositive cells. SPSS 22.0 and non-parametric tests were used for statistical analysis. The HHV-6 DNA was detected in 41.6 % of the encephalopathy group tissue samples. There were more HHV-6 positive astrocytes in the gray matter of the temporal lobe than in the olfactory pathways ($p<0.001$). The same applied to the oligodendrocytes both in the gray and white matter ($p<0.05$). There were significantly more HHV-6 positive glial cells in the white matter of the encephalopathic subjects when compared to both controls ($p<0.05$). S100 expression was significantly ($p<0.05$) higher in astrocytes of both gray and white matter in the encephalopathy group when compared to young controls. Also in this group the S100 expression in astrocytes was more pronounced in the temporal cortex and subcortical white matter when compared to the olfactory pathways ($p<0.001$). Heterogeneity, both architectural and in cellular response, appears in brain tissue injury in case of HHV-6 infection. S100 estimations suggest that regulation of calcium ion homeostasis by astrocytes may be altered and further aggravated by HHV-6, leading to brain tissue damage.

P152
Local Interferon Responses in Axons Limit Retrograde Transport of an Alpha Herpesvirus in Neurons

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Natural infection by the neuroinvasive alpha herpesvirus, pseudorabies virus (PRV), begins at an epithelial surface where a local inflammatory response is induced. The axonal termini of the highly polarized peripheral nervous system (PNS) neurons are in direct contact with this infected peripheral site while their cell bodies reside far away in the ganglia. This means that in many cases only axons experience the events (i.e. infection, damage, inflammation) happening at peripheral tissues. How neurons respond to such remote events remains a fundamental question in neuroscience and virology. Recent findings suggest that new protein synthesis is induced locally in the axons upon environmental stimulation, and these new proteins regulate many biological processes in neurons. The overarching aim of this study is to understand how PNS neurons respond to the cytokine milieu produced during the inflammatory response following infection of epithelial tissues. Using in vitro compartmented cultures of rodent sympathetic neurons, we found that pre-exposure of axons to type I interferon (IFN alpha and IFN beta) significantly diminished the number of PRV particles moving in the retrograde direction. In further characterizing this response, we found that antibodies blocking type I IFN receptor restored PRV particle transport in IFN beta treated samples, while blocking transcription with actinomycin D in cell bodies did not. Interestingly, exposure of axons to type I IFN induced local phosphorylation of STAT1 only in axons. These results suggested that IFN beta signaling is responsible for the local antiviral response in axons. However, this signaling differs from the canonical pathway in that transcription of interferon-stimulated genes in the neuronal nucleus is not required. Using a label-free proteomic approach, we discovered changes in the abundance of various proteins in axons upon IFN treatment. The activation of local events in axons represents a new paradigm for cytokine control of neuroinvasion.

P153

Methamphetamine dependence is associated with cerebral microgliosis in HIV-1 infected individuals

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Objective: Methamphetamine (Meth) use is a common comorbidity in HIV-infected individuals. To explore the potential combined brain insults of Meth and HIV, we studied cerebral parenchymal and microvascular changes in relation to Meth dependence in the context of HIV infection. Design: Clinicopathological cross-sectional study of 78 HIV-infected adults with or without Meth dependence enrolled in the California NeuroAIDS Tissue Network (CNTN). Methods: Using multivariable ordinal logistic regression models we investigated associations between lifetime Meth dependence and cerebral gliosis (quantitative immunohistochemistry for ionized calcium-binding adapter molecule 1 [Iba1] and glial fibrillary acidic protein [GFAP] in the frontal, temporo-parietal, and putamen-internal capsule regions), synaptodendritic loss (confocal microscopy for synaptophysin [SYP] and microtubule-associated protein 2 [MAP2] in the frontal cortex), beta-amyloid plaque deposition (immunohistochemistry in the frontal, temporo-parietal, and putamen-internal capsule regions), or arteriolosclerosis (standard histopathology in the forebrain). Results: We found that Meth dependence was associated with marked Iba1 microgliosis in the temporo-parietal region (odds ratio [OR] 4.42 [95 % confidence interval, CI 1.36-14.39], $p=0.014$, $n=62$), which remained statistically significant after adjusting for HIV encephalitis, white matter lesions, and opportunistic diseases (adjusted OR 5.76 [95 % CI 1.67-19.82], $p=0.006$, $n=61$). No significant association was found between Meth dependence and GFAP astrogliosis, SYP or MAP2 loss, beta-amyloid plaque deposition, or arteriolosclerosis. The presence of HIV encephalitis or white matter lesions was associated with moderate or severe SYP loss after adjusting for Meth dependence and opportunistic diseases (adjusted OR 11.77 [95 % CI 1.35-102.46], $p=0.025$, $n=51$); a similar trend was observed with moderate or severe MAP2 loss (adjusted OR 4.72 [95 % CI 0.88-25.32], $p=0.07$, $n=51$). Conclusions: Meth dependence might increase the risk of cerebral microgliotic neuroinflammation in HIV-infected individuals. The association of Meth dependence with

astrogliosis, synaptodendritic loss, beta-amyloid plaque deposition, or arteriolosclerosis was not evident in the present study.

P154
Morphine induces cell adhesion and cellular migration in an in vitro model of the blood–brain barrier

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Opioid abuse by human immunodeficiency virus type 1 (HIV-1)-infected individuals leads to more rapid disease progression, increased viral replication and peripheral blood viral load, and increased incidence and severity of neurocognitive abnormalities compared to non-drug abusers. The blood–brain barrier (BBB) is an obstacle that must be overcome during neuroinvasion with subsequent development of HIV-associated neurocognitive disorders (HAND). Previous studies of mu-opioids and alteration of BBB permeability have suggested that exposure increases cellular transmigration through an uncharacterized mechanism. In this study, a human brain microvascular endothelial cell (hBMEC) line, hCMEC/D3, was used to establish an in vitro transwell model of the BBB to investigate the effects of chronic (24, 48, 72 h) morphine treatment on barrier structure and function. We observed that hCMEC/D3 cells formed a confluent monolayer with a basal rate of passage of a tracer molecule comparable to primary hBMECs. It has also been shown that these cells express mu opioid receptor, and that prolonged morphine treatment induces changes in mRNA levels of cellular adhesion molecules. Functionally, an increase in PBMC transmigration and firm adhesion was observed following prolonged morphine exposure, in the absence of an increase in overall barrier leakiness. These results have suggested that morphine activates hCMEC/D3 cells leading to a cell environment permissive to transmigration. These studies may uncover a mechanism by which morphine disrupts periphery-CNS homeostasis leading to accelerated HAND. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785, and R01 NS089435.

P155
Single nucleotide polymorphisms (SNPs) in the HIV-1 LTR correlate with clinical disease parameters

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The HIV-1 genome including the long terminal repeat (LTR) is continuously under selective pressure, even in well-controlled individuals on HAART. Single nucleotide polymorphisms (SNPs) in the LTR are of interest due to their role in regulating viral transcription and overall viral fitness. Previous work has demonstrated SNPs in particular transcription factor binding sites (TFBSs) can alter viral transcription and virus production in a cell type-specific manner. In addition, disease severity was correlated with an increased frequency of SNPs in C/EBP site I and Sp site III from LTRs derived from the peripheral blood compartment. To further understand patient LTR signatures and SNPs that correlate with advanced stage and/or neurologic disease, the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort in Philadelphia, PA was used to conduct a prospective, longitudinal study on 489 HIV-1-seropositive patients. Numerous SNPs were strongly correlated with clinical disease parameters, such as CD4+ T-cell count and viral load. One of these SNPs located at position 108, a confirmed COUP/AP1 binding site, increased in frequency in patients with high viral loads and low CD4+ T-cell counts. The in silico transcription factor binding prediction algorithm JASPER, demonstrated that nucleotide changes away from the consensus nucleotide adenine to either thymine, cytosine or guanine resulted in a reduction in COUP-2 binding. JASPER also indicated position 108 as a novel TFBSs for other transcription factors. Current studies using electrophoretic mobility shift assay (EMSA) are being performed to confirm the in silico prediction analysis and determine if there is differentially utilization in selected cellular phenotypes. These results have demonstrated an increase in the prevalence of a specific LTR SNP during disease progression, with the impact of the SNP on transcriptional regulation and pathogenesis

remaining to be explored. This work is supported by R01 NS32092, R01 DA19807, P30 MH092177, T32 MH079785.

P156
IFN-beta protects neurons in a transgenic model of HIV-1 associated brain injury

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HIV-1 appears to invade the CNS soon after peripheral infection. However, severe neurologic symptoms may be delayed until a later stage of disease progression. The delay has been explained by the ability of the innate immune system to effectively suppress viral replication during the initial stages of CNS infection. Type I interferons (IFNs) are critical mediators of this response in the brain, and are a major first line of host defense against viral infections. We hypothesized, that IFN-beta production and signaling in the CNS might prevent the development of neurological symptoms. Therefore we investigated the potential neuroprotective effects of IFN-beta in the brain against toxicity of HIV/gp120 using in vivo and in vitro models. We used mixed rodent cerebrocortical cultures (RCC), containing neurons, astrocytes, and microglia, to show that treatment with IFN-beta can provide concentration-dependent neuroprotection against gp120-induced toxicity. Additionally, treatment with IFN-beta of RCC increased levels of natural ligands of the HIV co-receptor CCR5 and up-regulated expression of anti-viral IFN-stimulated genes. These ligands, MIP-1-beta/CCL4 and RANTES/CCL5, are known to suppress HIV-1 infection; disease progression and all can provide significant in vitro protection against gp120-induced injury. To investigate the neuroprotective effects of IFN-beta against HIV/gp120-induced neurotoxicity in vivo, we performed intranasal IFN-beta administration to transgenic mice expressing the viral envelope protein in the brain. HIV/gp120-tg animals manifest several neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia, and pronounced astrocytosis. After one month of once a week treatment, brains were analyzed by quantitative RT-PCR for specific changes in gene expression and by immunohistology for structural neuronal injury. We found that intranasal IFN-beta application triggered a biological response that was detectable as IFN-induced gene expression and ameliorated neuronal damage in HIV/gp120-tg mice.

P157
Identification and characterization of two novel alternatively spliced E2F1 transcripts in the brain

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E2F1 is a transcription factor classically known to regulate G0/G1 to S phase progression during the cell cycle. In addition, E2F1 also regulates a wide range of apoptotic genes and thus has been well studied in the context of neuronal death and neurodegenerative diseases. However, its function and regulation in the mature central nervous system are not well understood. Alternative splicing is a well conserved post-transcriptional mechanism that is common in cells of the CNS and is necessary to generate diverse functional modifications to RNA or protein products from the same gene. Despite this, physiologically significant alternatively spliced E2F1 transcripts have not been reported. In the present study, we report the identification of two novel alternatively spliced E2F1 transcripts: an E2F1 transcript retaining intron 5 and an E2F1 transcript excluding exon 6. These alternatively spliced transcripts are specific to the brain and neural cell types including the neurons, astrocytes, and oligodendrocytes. Additionally, the expression of the E2F1 transcripts are distinct during the maturation of primary hippocampal neuroglial cells. Pharmacologically induced global translation inhibition with cycloheximide or thapsigargin lead to significantly reduced expression of classic E2F1 and E2F1 transcript excluding exon 6. On the other hand, although anisomycin induced translation inhibition lead to reduced expression of these two transcripts, it had no effect on the expression of E2F1 transcript retaining intron 5. Increasing neuronal activity by elevating concentration of potassium chloride selectively increased the expression of E2F1 transcript retaining intron 5 but not the other transcripts. Taken together, our data suggest that the alternatively spliced E2F1 transcripts behave differently than the classic E2F1 transcript and provide a foundation for future investigation to study their functions in the brain.

P158
Regulation of HIV replication in human blood–brain barrier pericytes by cellular occludin levels

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Entry of HIV into the brain and damage to the blood–brain barrier (BBB) occur during the early stages of infection. Here we show that BBB pericytes have a biphasic response to HIV infection, initially loosing occludin in a p38 MAPK-dependent manner. Lower occludin levels are associated with enhanced nuclear translocation of the repressor CtBP1 and activation of NF-κB, and reduced expression and activation of the class-II histone-deacetylase SIRT-1. Afterwards, pericytes transition to a second stage recovering occludin by a ROCK-mediated mechanism, restoring SIRT-1 levels and activation, decreasing NF-κB activation, and reducing HIV-replication. The influence of occludin on HIV replication was confirmed in HEK-293 cells, monocytic U937 cells, and human macrophages. Mechanistically, we characterized the C-terminal domain of occludin as an NADH oxidase. Our results indicate that occludin, typically considered to be exclusive of epithelial/endothelial cells, has previously unsuspected metabolic properties that confer antiviral properties and explain its non-canonical expression in non-barrier-forming cells. Importantly, our work portrays occludin as a potential target for pharmacological intervention. Supported by the NIH, grants DA039576, HL126559, MH098891, and MH072567. We also acknowledge support from the Miami Center for AIDS Research (CFAR) grant P30AI073961.

P159

gp120 Induced Neurocognitive Deficits in the Rat Correlate with Increased Senescence in the Brain

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HIV-1 associated neurocognitive disorder (HAND) is a growing problem as the HIV-1+ population ages. This disease affects the learning, memory and executive functions of the subjects. The HIV-1 surface protein gp120 is neurotoxic and is implicated in reduced neuron survival during HAND. Cellular senescence is an irreversible growth arrest that can be triggered by replicative shortening of telomeres or other stresses. Astrocytes, the most numerous cell type in the brain, protect and maintain neurons. Astrocytes senesce and the number of senescent astrocytes increases in aging and Alzheimer's disease. Moreover, a senescence-like phenotype has been reported in neurons. We therefore asked whether gp120 induces senescence and cognitive deficits. We had 3 aims: (1) to determine if gp120 induces senescence in cultured human astrocytes, (2) to determine if gp120 administration results in behavioral deficits in the rat, and (3) whether this correlates with an increase in senescence in the brain. Percentage of human astrocytes that senesced was higher by beta-galactosidase (β-Gal) assay upon treatment with gp120 compared to control. We observed an increase in senescence by β-Gal assay in the brain sections comprising the motor cortex, striatum and basal ganglia of adult rats treated with intra-cerebroventricular infusions of gp120, which correlates with previously observed deficits in performance of the Morris Water Maze task. In other behavioral studies using the attention set shifting protocol (adapted from Birrell and Brown, 2002), gp120 rats were significantly impaired in their performance of the task relative to controls. Thus, induction of senescence by gp120 parallels presence of cognitive deficits. It remains to be seen if other brain cells are susceptible to this damage, whether our findings in attention set shifting correlate with increased senescence in the pre-frontal cortex, which is responsible for executive functions, and whether co-treatment with anti-senescence agents such as rapamycin can reverse these symptoms.

P160

HIV-Tat regulation via Toll-like receptor4 of endogenous retroviruses of the W family: inference for neuroAIDS

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Working hypothesis: to verify whether activation of the neuropathogenic and immunopathogenic MSRV and Syncytin-1 elements of the HERV-W family of endogenous retroviruses could contribute to pathogenic phenomena leading to HIV-related neurodegeneration. To this end peripheral blood mononuclear cells, monocyte-macrophages and astrocytes were either infected by HIV or exposed to HIV-Tat, and/or other treatments, and the expression of transcripts and proteins of interest was evaluated by real-time RT-PCR and Western Blotting. The results indicate that HIV and Tat increase the levels of MSRVenv mRNAs and HERV-Wenv proteins in astrocytes and in blood cells. In monocyte-macrophages, Tat induces also high levels of CCR2, CD16 and Toll-like receptor 4 (TLR4) molecules. Syncytin-1 response to Tat depends on the cell context: in monocytes Tat stimulates MSRVenv and inhibits Syncytin-1, while in differentiated macrophages, it stimulates both elements. In primary

astrocytes, Tat stimulates the HERV-Ws indirectly, through interaction with TLR4 and induction of TNFα, without internalization. The in vivo consequence would be that, through increase of CD16 and CCR2, Tat promotes neuroinvasion by HIV-infected monocytes/macrophages, but also by the potentially neuropathogenic HERV-Ws. Also the novel finding that Tat stimulates TLR4 may be relevant, since TLR4 is critical in neuroinflammation. Within CNS, Tat-induced TNFα could induce high levels of the HERV-Ws in both macrophages and astrocytes, also without HIV replication. It is known that TNFα is the most abundant proinflammatory cytokine in the brain of neuro-AIDS patients: in this context of neurodegeneration, MSRV and Syncytin-1 stimulation by the induced TNFα seems a concrete possibility. The indirect mechanism by which Tat activates the HERV-Ws through induction of TNFα could add a new piece to the puzzle of CNS pathogenesis, since in the brain of patients, the HERV-Wenv protein could cause neuroinflammation, neurodegeneration, alterations of the immune system and stress responses, thus contributing to contribute to HIV-related neurodegeneration.

P161

Increased Transmigration across the BBB of HIV-infected HIV+CD14+CD16+ monocytes as compared to HIV-exposed HIVexpCD14+CD16+ monocytes: HIV viral seeding of the CNS and HIV associated neurocognitive disorders

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HIV associated neurocognitive disorders (HAND) affect 40-70 % of HIV infected individuals despite successful combined antiretroviral therapy (cART), and manifests even in people with undetectable viral loads. Monocytes bring virus into the brain early after peripheral infection and continue to mediate ongoing viral seeding and low levels of neuroinflammation. Mature CD14+CD16+ monocytes are highly susceptible to HIV infection and preferentially transmigrate across the blood–brain barrier (BBB) in response to the chemokine CCL2. The CD14+CD16+ monocyte population in HIV infected individuals, even when on cART, is heterogeneous consisting of cells that are productively or latently infected with HIV, HIV+CD14+CD16+ monocytes (HIV+), and cells that have been exposed to viral proteins and inflammatory mediators but are not infected, HIVexpCD14+CD16+ monocytes (HIVexp). It is not known whether HIV infection or just merely exposure to HIV is necessary to increase CCR2 or junctional proteins including JAM-A and ALCAM essential for monocyte transmigration across the BBB, and how this affects BBB transmigration of HIV+ and HIVexp monocytes. Our laboratory previously determined that transmigration of CD14+CD16+ monocytes across the BBB is significantly greater in HIV infected individuals with HAND compared to those without HAND, in part due to increased expression of CCR2, the CCL2 receptor. We now show, using uninfected and latently HIV infected human monocytic cell lines, that active viral replication increases CCR2 and junctional proteins in comparison to expression on latently infected and uninfected monocytes. Moreover, we demonstrate that CCR2 and junctional proteins are expressed differently on HIVexp and HIV+ monocytes. Using FACS and viral DNA quantification we found that there is preferential selection for primary human HIV+CD14+CD16+ monocytes during BBB transmigration. These data suggest that in HIV infected individuals, even while on cART, small numbers of HIV+ mature monocytes express increased surface makers facilitating their entry into the CNS and mediating HAND pathogenesis.

P162

MicroRNAs as biomarkers for HIV-1 associated neurocognitive disorder and dementia: Development of sensitive diagnostic tool

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HIV-1 virus replication and disease progression including neuropathogenesis and HIV-1 associated neurocognitive disorders (HAND) wide varies in HIV-1 infected subjects. Among the infected population, 40-60 % of the individuals are likely to develop HIV-1 associated neurocognitive impairment. Recent studies support the notion that host cellular gene expression profile as a response to virus infection is directly correlated with disease patterns. Host cellular gene expression is regulated by a combination of transcriptional and post-transcriptional regulators such as microRNAs (miRNAs)

resulting in loss of immune control, cognitive disorders and disease progression. We hypothesize that changes in expression profile of these miRNAs occur over the course of disease development and could serve as a valuable biomarker diagnostic tool. To test our hypothesis, we performed a comprehensive high throughput miRNA profiling using a "discovery cohort" of HIV-1 positive subjects with and without HIV-1 associated dementia (HAD) to identify novel biomarkers. Using the cross sectional samples, differentially regulated miRNAs in HIV-1 positive subjects with and without HIV-1 associated dementia (HAD) have identified 94 miRNAs that are highly significant between the two groups. A custom array was generated with these miRNA primer pairs and longitudinal samples from subjects (N=12) who developed HIV-1 associated dementia (HAD). Among the 94 miRNA tested 40 miRNAs showed difference at 6 years and 31 miRNAs at 3 years prior to diagnosis. Finally, we have identified interesting biomarker candidates that are highly correlated with disease progression and additional biomarkers that are highly associated with clinical diagnosis of HAD in HIV-1 positive patients.

P163
Macrophage Infiltration and Chronic Immune Activation Correlates with Encephalitis and Cardiac Fibrosis

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HIV+ individuals are at increased risk for cardiac inflammation and neurocognitive disorders compared to seronegative individuals. Increased cardiac risk factors are associated with lower baseline cognitive performance and elevated numbers of activated CD14+CD16+ monocytes. We hypothesized that CNS pathology and cardiac inflammation and fibrosis are correlated and linked by increased macrophage accumulation in the brain and heart. We examined cardiac tissues from SIV-infected, CD8-lymphocyte depleted rhesus macaques with encephalitis (SIVE, n=8) and SIV-infected, non-depleted animals without encephalitis (SIVnoE, N=7). Cardiac tissues from HIV+ individuals with encephalitis (HIVE, n=10) and HIV+ individuals without encephalitis (HIVnoE, n=10) were examined. Cardiac tissues were graded on the degree; a) inflammation, b) fibrosis, and c) cardiomyocyte degeneration, and scored as no significant findings, mild, moderate, or severe pathology. Macrophage numbers in cardiac tissues were quantified after immunohistochemistry. In SIVE animals, there were significant increases in CD163+, CD68+, and MAC387+ macrophages in cardiac tissues correlating with increased fibrosis compared to SIVnoE (t-test, $p<0.05$). SIVE animals had a significant 2.5-fold increase in the percentage of collagen per total tissue area (fibrosis) in cardiac tissues compared to SIVnoE animals (t-test, $p<0.05$), and increased graded severity of inflammation and fibrosis. SIVE animals had significant early changes in CD14+CD16+ monocytes compared to SIVnoE animals (1.5-fold increase, t-test, $p<0.05$), which also correlated with cardiac fibrosis ($P<0.05$). In parallel, HIVE individuals had elevated CD163+, CD68+, and MAC387+ macrophages in cardiac tissues correlating with enhanced fibrosis. The inflammation and fibrosis in cardiac tissues was more severe in HIVE compared HIVnoE individuals. A significant 2-fold increase in the percentage of collage per total cardiac tissue area was seen in HIVE compared to HIVnoE individuals (t-test, $p<0.05$). These data suggest that monocyte/macrophage activation and infiltration into CNS and cardiac tissues occurs and the degree of cardiac fibrosis correlates with development of HIVE.

P164
Wnts suppress CD14+CD16+ phenotype of monocytes

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Monocytes infiltrate the central nervous system for a variety of pathological and/or homeostatic purposes where they differentiate into either pro-inflammatory M-1-or alternative M-2-like macrophages (MDMs). Furthermore, M-1 macrophages promote neurodegeneration by inhibiting axonal growth and neuronal repair. We have demonstrated that monocytes express robust levels of Wnt/beta-catenin signaling which is down-regulated as monocytes differentiate into MDMs. We evaluated here the impact of Wnts; small secreted glycoproteins which trigger beta-catenin dependent or independent signaling, on monocyte/MDMs phenotype. Human monocytes were cultured with conditioned media from primary human fetal astrocytes (HFA). HFAs are a rich source of Wnts 1, 2b, 3, 5b and 10. We used DKK-1 to antagonize Wnts for lack of commercially available Wnt neutralizing

antibodies. Astrocyte conditioned media (ACM) added to monocytes at 50 % concentration had no effect on CD14+ CD16+ expression in comparison to cDMEM cultures. However, antagonizing Wnts in the ACM through addition of DKK-1 resulted in CD14+CD16+ induction by 6.4 folds compared to ACM treatment alone. ACM was also much more potent in inducing CD14+CD163+ expression compared to cDMEM, cDMEM with M-CSF or GM-CSF treated monocytes (89.41 vs. 61.8, 42.9 and 0.46 % respectively). Finally, DKK-1 treated ACM inhibited MDM adherence in comparison to untreated ACM, cDMEM, and cDMEM with M-CSF or GM-CSF culture conditions. Although preliminary, these data suggest that Wnts inhibit CD14+CD16+ phenotype of monocytes, which are a critical cell type for neuroinvasion of HIV. Ongoing studies are assessing the impact of Wnts on differentiation and functionality of the MDMs from each experimental group. Ultimately, our studies will provide a better understanding of how Wnts within the CNS skew differentiation and function of infiltrating monocytes.

P165
HIV-1 Vpr disrupts axonal transport through induction of alpha-Synuclein

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Disruption of mitochondrial axonal transport, essential for the maintenance of synaptic and neuronal integrity and function, has long been known as a probable marker of neurodegenerative diseases. The effect of HIV-1 proteins on mitochondrial disruptions remains unclear therefore we sought to examine the impact of HIV-1 viral protein R (Vpr) on mitochondrial functions. Using primary neuronal cultures, we demonstrated that Vpr slowed the motility of mitochondria, reduced ATP production and caused loss of mitochondrial bioenergy. Reduction in mitochondria motility was associated with fragmented distribution of acetylated alpha tubulin, decreased in HDAC6 expression and alteration of microtubule stability leading to impaired function of neurons. Addition of recombinant Vpr also led to deregulation of Miro and pink1, proteins involved in mitochondrial trafficking. Loss of microtubule stability was accompanied with increased tau aggregates and elevated expression level of alpha synuclein protein. Using immunohistochemistry, similar results were obtained when using hippocampal tissues isolated from 16-month-old (aged) mice but not with 5-month-old (young) mice. This latest in particular and our data in general led to the conclusion that HIV-1 Vpr protein altered neuronal functions through inhibition of mitochondria transport and may accelerate neuronal ageing.

P166
Crisper/Cas9 system as an agent for eradication of polyomavirus JC infection

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease of the CNS caused by lytic infection of oligodendrocytes, which produce myelin in the brain, with human neurotropic JC virus. PML lesions are areas of demyelination containing oligodendrocytes with viral nuclear inclusion bodies and bizarre astrocytes, which are also productively infected by JCV. PML was first reported as an obscure disorder afflicting leukemia patients but with the onset of the AIDS pandemic, the number of PML patients with HIV/AIDS overwhelmed other predisposing conditions and it is becoming an important disease with high public health relevance in that it impacts many different groups of people including HIV/AIDs patients and patients with MS and Crohn's disease. Although JCV was isolated over 40 years ago and has been extensively studied since, there is still no effective therapy for PML. Recently, novel genome-editing method was developed based on clustered regularly interspaced short palindromic repeat (CRISPR) systems. Crisper system was originally discovered in the bacterium streptococcus pyogenes that work as a mechanism to defend against viruses and foreign DNA. The system uses a nuclease, CRISPR-associated (cas9), that complexes with small RNAs as guides (gRNAs) to cleave DNA in a sequence-specific manner upstream of the protospacer adaptor motif (PAM) in any genomic location. Here, we use CRIS PER/Cas9 system as a potential tool for JCV elimination by

designing guide RNAs (gRNAs) targeting regions within the early gene encoding viral protein, T-antigen. Our results indicated that JCV T-antigen Cas9gRNAs effectively delete target DNA sequence, reduce T-antigen expression and its late promoter activity and inhibit JCV DNA replication.

P167
Rad51 activates Polyomavirus JC early transcription

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The human neurotropic polyomavirus JC (JCV) causes the CNS demyelinating disease progressive multifocal leukoencephalopathy (PML). JCV infection is very common and after primary infection, the virus is able to persist in a latent state. Rarely, and usually only under conditions of immune impairment, JCV re-emerges to productively infect the astrocytes and oligodendrocytes of the brain causing PML. The regulation of initiation of replication in PML (reactivation) is not well understood but previous studies have implicated the importance of the transcription factor NF-kappaB acting at a well characterized site in the JCV noncoding control region (NCCR). NF-kappaB in turn is regulated in a number of ways including activation by proinflammatory cytokines such as TNF-alpha, interactions with other transcription factors such as C/EBPbeta and NFAT4 and epigenetic events involving protein acetylation - all of which regulate JCV transcription. We previously showed that active JCV infection is marked by the occurrence of DNA damage and the induction of the expression of cellular DNA repair proteins including Rad51, a component of the homologous recombination-directed double-strand break DNA repair machinery. Here we show that increased Rad51 expression activates the JCV early promoter. This activation is co-operative with the stimulation caused by NF-kappaB p65, abrogated by mutation of the NF-kappaB binding site, inhibited by the LIP isoform or siRNA to NF-kappaB p65 and enhanced by the histone deacetylase inhibitor sodium butyrate. These data indicate that the Rad51 induction by JCV infection acts through the NF-kappaB via its binding site to stimulate JCV early transcription. We suggest that this provides a positive feedback mechanism to enhance viral gene expression during the early stage of JCV infection.

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Effects of DNA recombination between JC virus and Epstein-Barr virus upon neurovirulence of JC virus in immunosuppression and AIDS

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HIV-1 infection of the brain influences infection of two different DNA viruses which are asymptomatic in most individuals but which are activated in the CNS in AIDS. JC virus (JCV) inhabits uroepithelial cells, but in AIDS or in response to certain immunosuppressive agents, it causes a frequently fatal brain demyelinating disease, PML. Epstein-Barr virus (EBV) is latent in B lymphocytes but causes lymphomas and certain epithelial cancers in AIDS. The Tat protein of HIV-1 stimulates JCV DNA replication, whereas HIV-1 regulates the cell cycle of B cells containing EBV. We have found a novel sequence interaction between JCV and EBV that can help adapt JCV to infect the brain. Our results begin to address questions of how JCV can make the transition from the urinary tract to the brain and of how it can adapt to infect oligodendroglia. Sequence changes in the archetype JCV control region (NCCR) are necessary for JCV neurovirulence, although additional steps are also necessary for activation of JCV in glial cells. We have found that the three most critical NCCR rearrangements, two deletions and a duplication, occur at sequences of homology with sites in EBV. No herpesvirus other than EBV has homology to one critical site in JCV. Most NCCR rearrangements result from homologous recombination initiated by double-strand breaks at stalled replication forks. We demonstrate recombination between JCV and EBV, at a JCV deletion site essential for JCV neurovirulence, in living cells harboring both viruses and in cerebrospinal fluid of multiple sclerosis patients undergoing immunosuppression, a population susceptible to PML. Insertion of JCV sequences into EBV is demonstrated by interviral PCR and by Southern blotting of EBV DNA segments. This insertion confers advantages on JCV, not only by altering a critical NCCR sequence,

but also by exploiting EBV DNA abilities to transfer among different cell types.

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A TRPML1 agonist facilitates lysosomal clearance of proteins and lipids in a mouse model of gp120 facilitated amyloid deposition

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HIV-infection may be associated with alterations in endolysosomal functions as manifest by disturbances in transluminal ion gradients, intracellular deposition of proteins and lipids. Previously, we reported that expression of gp120 in an animal model of abnormal amyloid processing (APP/PS1/gp120) modified the pattern of β-amyloid deposition to an intraneuronal phenotype with prominent deposition in lysosome compartments that were also engorged with sphingomyelin and calcium. Activation of TRPML1 in cultured neurons released intraluminal calcium from lysosomes, reduced endolysosomal pH, and cleared lysosomes of sphingomyelin and Aβ. Here, we evaluated in vivo if the TRPML1 agonist ML-SA1 reduced lysosomal dysfunction in APP/PS1/gp120 mice. Vehicle (15 % DMSO) or ML-SA1 (127.5 μg/kg/d) was administered by infusion pumps into the lateral ventricles of 5 month-old WT, gp120, APP/PS1, and APP/PS1/gp120 mice for 28 days. Sphingomyelin, Aβ, and lysosomal structural content were analyzed in hippocampus and cortical structures. Concentrations of human Aβ1-40, and Aβ1-42 were highest in APP/PS1/gp120 compared to APP/PS1 mice, and were undetectable in WT or gp120 mice. Sphingomyelin content and expression of the lysosomal protein LAMP-1 were increased in APP/PS1/gp120 mice compared with all other stains, and were similar in APP/PS1, gp120 and WT mice. Intraventricular treatment with ML-SA1 did not alter Aβ-peptide concentrations in APP/PS1 mice, but reduced Aβ-peptide accumulations in APP/PS1/gp120 mice. ML-SA1 reduced sphingolipid content and LAMP-1 expression in APP/PS1/gp120 mice compared with APP/PS1 mice. These preliminary in vivo findings suggest that therapeutic approaches targeting lysosomal TRP channels could protect neurons in HIV-infected individuals by enhancing lysosomal function. Therapeutic development of TRPML1 agonists is ongoing.

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Dickkopf-related Protein 1 is inversely correlated with HIV-Associated Neurocognitive Impairment and positively correlated with Methamphetamine density

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Dickkopf-related protein 1 (DKK1) is a soluble antagonist of the Wnt/beta-catenin pathway. Because diminished Wnt/beta-catenin signaling perturbs neuroprotection and methamphetamine (Meth) inhibits Wnt/beta-catenin signaling, we hypothesized that Meth users will have higher levels of plasma DKK1 and that increased DKK1 would be associated with an increased risk for neurocognitive impairment (NCI) in HIV+ subjects. To test this hypothesis, we obtained plasma samples from 41 HIV+ and 43 HIV- adults from the UCSD TMARC cohort. Plasma DKK1 and MCP-1 (a comparison marker) were measured by immunoassay. All subjects were assessed using a standardized comprehensive NC battery that adhered to Frascati guidelines. NC performance was summarized using the global deficit score method. Levels of MCP-1 (p=0.02) but not DKK1 (p=0.65) were higher in HIV+ subjects than in HIV- subjects. Among HIV+ subjects, higher levels of DKK1 (d=0.63, p=0.05) but not MCP-1 (p=0.59) were associated with NCI. The association between NCI and DKK1 but not MCP-1 strengthened using recursive partitioning analysis: among the 41 HIV+ subjects, those who had DKK1 levels of at least 1,129 pg/ml had a 6.0-fold increased odds of having NCI (75 % vs 33 %, 2-tail FET p=0.04). The effect size was large and the association between DKK1 and NCI was highly specific (92 %), although the sensitivity was poor (35 %). Multivariable analysis among HIV+ subjects identified that the association between higher DKK1 levels and NCI remained statistically significant after accounting for the effects of nadir CD4+ T-cell counts, drugs of abuse, and ART use. Higher DKK1 levels (p=0.02) were associated with frequency of meth use (meth density) in all subjects. These findings underscore the potential specificity of

DKK1 as a biomarker for NCI in HIV+ adults and among Meth users. Supported by 2 R01 NS060632 (LA), R01 DA 033966 (LA), K24 MH097673 (SL), and P50 DA26306 (TMARC).

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Wnt Signaling in the Pathogenesis of HIV-1/AIDS-Associated Sensory Neuropathy

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Peripheral sensory neuropathy (SN) is a common neurological pathology developed in HIV-1/AIDS patients. SN is causally associated with the clinical symptom of neuropathic pain, which drastically deteriorates the patients' life quality. Our goal is to understand the pathogenic mechanism, and ultimately develop rationale-based therapeutic approaches. Using an interdisciplinary approach, we recently identified HIV-1 gp120 as a critical pathogen factor and Wnt5a as an important host factor in the development of HIV-associated pain (Yuan et al., 2014, Annals Neurol.; Li et al., 2013, J Biol. Chem.; Shi et al., 2012, J Neurosci., Shi et al., 2013, JNIP). Extended from these studies, we will report here our findings of the development of HIV-associated "dying-back" neuropathy of sensory neurons innervating glabrous skins in the gp120 mouse models that extensively phenotype the pathology of "pain/neuropathy-positive" HIV patients. Using conditional knockout mice, we are determining the role of Wnt5a in the pathogenic process, and the mechanism by which Wnt5a contributes to the development of gp120-induced SN.

P172
CRISPR/Cas9 synergistic activation mediator induces target-specific reactivation of HIV-1 latent reservoir for "shock and kill" therapy

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Due to insufficient reactivation, non-specific cell targeting and drug toxicity, the current strategy, called "shock and kill" that is used for first reactivating the latent reservoir of HIV-1 and then for elimination of this reservoir, has yet to generate any promising outcome. Here we report the feasibility of employing novel CRISPR/Cas9 synergistic activation mediator (SAM) technology to reactivate the HIV latent reservoir in a cell- and target-specific manner. Our initial attempt using a single guided RNA (sgRNA) targeting various sites of HIV-1 long term repeats (LTR) and a transcription activation domain (VP64) fused with a nuclease-deactivated Cas9 (dCas9) did not detect any noticeable impact on the reactivation of HIV-1 in reporter cell model. By employing a MS2-mediated Cas9-SAM system that includes lentivirus-mediated dCas9-VP64 and additional p65 and HSF1 activators fused with MS2 bacteriophage coat protein along with various HIV-1 LTR targeting MS2-binding sgRNAs, we identified several specific sgRNAs that dramatically and continuously stimulate the activation of HIV-1 latent reporter in various cell models. This latency-reversing dCas9-SAM system offers a highly specific, highly efficient and lowly toxic new biological approach for employment in the "shock and kill" strategy to eliminate HIV-1 latent reservoir in patients.

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HIV-1 gRNA screening and functional characterization for HIV-1 eradication in vitro

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There is an urgent need for the development of HIV-1 genome eradication strategies that will lead to a permanent or "sterile" cure of HIV-1/AIDS. The RNA-guided endonuclease Cas9 technology has emerged as a simpler and more versatile technology to edit any genes/genomes in mammalian cells. We previously reported that stable transfection of human cell cultures with plasmids expressing Cas9 and HIV LTR-A-D successfully eradicated part and/or the entire HIV-1 genome without compromising host cell function (Hu et al. PNAS 2014, 111:11461–6). In this study, we employed lentivirus-mediated Cas9/gRNA delivery system and screened 23 gRNAs targeting HIV-1 LTR and viral structural region. The target seed sequences were selected according to the best

bioinformatics score for both editing efficiency and off-target prediction. Most of them can be also paired for Cas9 nickase and RNA-guided FokI nuclease (RFN), which can reduce up to 1500-fold potential off-target effects. Using inducible or constitutive Cas9 stable expression system in HEK293T cells, we cotransfected a pEcoHIV-NL4-3-eLuc reporter vector with single gRNA or different combinations of gRNAs and examined DNA cleaving efficiency by PCR genotyping and HIV-1 replication activity by a luciferase reporter assay. The results indicate that most of the designed gRNAs are effective to eradicate the predicted HIV-1 genome sequence between selected two targeting sites and affect luciferase reporter activities. In particular, a combination of viral structural gRNAs with one or two LTR gRNAs provided a higher efficiency of genome eradication and an easier approach for PCR genotyping. In conclusion, our data suggest that the carefully designed gRNAs targeting HIV-1 LTR and structural region caused effective cleavage of HIV-1 genome. Supported by grants awarded by NIH to KK/WH (R01NS087971) and KK (P30MH092177)

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Mitochondrial apoptotic activity of P53 contributes to neuronal apoptosis and pathogenesis during reovirus encephalitis

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The tumor suppressor p53 plays a critical part in the determination of cell fate after various insults. Prior studies have shown that p53 and subsequent mitochondria-mediated apoptosis signaling play an important role in avian-reovirus-induced apoptosis and reovirus oncolysis in vitro. However, the role of P53 and mitochondrial signaling in neuronal apoptosis and pathogenesis occurring in reovirus-infected brains during viral encephalitis is not known. We now demonstrate that p53 is up-regulated in the brains of neonatal mice infected with Type 3 (T3) strains of reovirus. We further show that specific inhibition of mitochondrial p53 translocation by Pifithrin μ reduces T3 reovirus-induced caspase 3 activity and tissue injury in ex vivo brain slice cultures (BSC). Direct interaction of p53 with the pro-apoptotic Bcl-2 family member, Bak, results in the activation and oligomerization of Bak and mediates permeabilization of the outer mitochondrial membrane, release of cytochrome c from the mitochondria and apoptosis. Bak is activated in infected neurons during reovirus encephalitis and Bak−/− mice show decreased injury and increased survival following reovirus infection compared to wild type controls. In addition, p53 and Bak form a complex with cyclophillin D (CypD) in reovirus-infected brain tissue suggesting that these molecules co-operate to open the mitochondria permeability transition pore (mPTP) during reovirus encephalitis. In support of this, administration of Cyclosporine A, which binds to CypD and functions as a potent and specific inhibitor of mPTP formation, significantly improves the survival time of mice with reovirus encephalitis. These studies imply that p53-induced mitochondrial apoptotic activity, including Bak activation and formation of P53-Bak-CypD complex, contributes to neuronal apoptosis and pathogenesis during reovirus encephalitis. These studies were supported by NIH grants NS076512 and AI101064 and by a Veterans Administration Merit award.

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HIV-1 Tat affects oligodendrocyte viability and function via iGluR-mediated Ca^{2+} dysregulation, and activation of GSK3β and CaMKIIβ

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Myelin pallor is frequently reported in HIV patients, and can occur in the CNS prior to other evidence of disease process. Previous work from the lab showed that oligodendrocytes (OLs) are direct targets of HIV-1 Tat (transactivator of transcription). Tat induces a dose-dependent increase of intracellular Ca^{2+} level ($[Ca^{2+}]i$) in cultured murine OLs, which can be attenuated by ionotropic glutamate receptor (iGluR) antagonists MK801 and CNQX. Interestingly, this Tat-induced $[Ca^{2+}]i$ increase leads to increased death in immature (O4+, MBP-), but not mature (O4+, MBP+) OLs, over 96 h. In addition, Tat-induced $[Ca^{2+}]i$ increase also reduced myelin-like membrane production by mature OLs. Calcium/Calmodulin dependent kinase IIβ (CaMKIIβ) and glycogen synthase kinase 3β (GSK3β) have been known to regulate differentiation, myelination and apoptosis in OLs. Since both CaMKIIβ and GSK3β are important downstream modulators of $[Ca^{2+}]i$ change, we hypothesized that the detrimental effects of Tat on immature/mature OL viability and function are mediated via CaMKIIβ and GSK3β activation. Our results showed that Tat activates both CaMKIIβ and GSK3β in immature OLs, but only activates CaMKIIβ in mature OLs. MK801 completely blocks Tat-induced CaMKIIβ and

GSK3β activation in both immature and mature OLs, while CNQX blocks GSK3β activation, but has only a partial effect on CaMKIIβ activity. iGluR blockade or GSK3β inhibition both rescue Tat-induced immature OL death, but only MK801 reverses the membrane injury in mature OLs. Together, these data strongly suggest that 1) activity of CaMKIIβ and GSK3β in OLs can be regulated by Tat-induced iGluRs activation and 2) OLs at different developmental stages show different responses to Tat, possibly due to activation of different signaling pathways. The support of grant 1F31NS084838-01A1 and NS069216 from NIH/NINDS is gratefully acknowledged.

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