Title

Cerebral amyloid-beta plaques link host genotypes to neurocognitive impairment among HIV-infected adults

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Publication Date

2018

Peer reviewed

Journal of Neuroimmune Pharmacology (2018) 13(Suppl 1):S1–S102
https://doi.org/10.1007/s11481-018-9786-5

ABSTRACTS

Abstracts from the Joint Meeting of the International Society for NeuroVirology (ISNV) and the Society on NeuroImmune Pharmacology (SNIP) April 10-14, 2018, Chicago, Illinois, USA

P1

TIMBD modulates neurotransmission and improves HIV-1 Tat-associated anxiety-like behavior and memory deficits in Tat-transgenic mice

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HIV-1 Tat has been implicated in Human Immunodeficiency Virus (HIV)-associated neurocognitive disorder (HAND). Patients with HAND suffer from both cognitive and motor dysfunction which is characterized by memory loss and changes in personality. A variety of natural products including resveratrol (RES) have been tried as interventional agents to improve neurocognition. Resveratrol is one of the known phytoestrogens with potential antioxidant and anti-inflammatory properties that has been tested for its inhibitory effects in neurodegeneration. However studies suggests RES's poor specificity and bioavailability as a reason for its poor efficacy. To overcome these problems associated with RES, our research group has developed novel RES analog (TIMBD). Our in vitro studies suggest that TIMBD has better anti-inflammatory and antioxidant properties than RES. TIMBD decreased the expression of Tat-induced inflammatory cytokines and increased the expression of antioxidant enzymes significantly compared to RES. In this study, we demonstrate that TIMBD improves the behavioral deficits associated with HIV-1 Tat and improves neuronal transmission in Tat-transgenic(tg) mice. Briefly, wild-type control and Tat-tg mice were treated with either vehicle or TIMBD pellets (10mg, s.c.) for 12 weeks. These mice were then tested for anxiety-like behavior and memory impairment using open field test, light/dark box and Morris water maze. Mice were sacrificed at the end of experiment and proteins collected from different brain regions, were used for western blot analysis. Our results demonstrate that TIMBD is able to improve the anxiety-like behavior and memory impairment associated with HIV-1 Tat. TIMBD is also able to restore the expression of neurotrophic proteins (BDNF), pre-synaptic proteins (synaptophysin) and post-synaptic proteins (PSD95) compared to Tat in both male and female mice ($p<0.05$). Therefore, we suggest that TIMBD is able to modify the behavior deficits associated with HIV-1 Tat and the mechanism of improvement of behavioral deficits may be through modulation of neurotransmission genes.

P2

AURKA activation acidifies endolysosomes in neurons and may inhibit Alzheimer's disease pathogenesis

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Alzheimer's disease (AD) is the leading cause of dementia worldwide, affecting approximately 46 million people. Currently, there are no effective preventative or therapeutic interventions against AD. Endolysosomes are the main organelles in which amyloidogenesis occurs and endolysosome dysfunction contributes to AD pathogenesis; de-acidifcation leads to amyloidogenesis and this increased production of amyloid beta is blocked by agents that acidify endolysosomes. Thus, endolysosome acidification may represent a new therapeutic strategy against AD pathogenesis. v-ATPase is the major proton pump on endolysosomes and increased v-ATPase activity acidifies endolysosomes. Because aurora kinase A (AURKA) has been shown to increase v-ATPase activity in kidney carcinoma cells and the activity of AURKA is reduced in postmortem brain tissues of AD patients, here we determined the extent to which and mechanisms by which activation of AURKA acidifies endolysosomes. Using immunoblotting and immunocytochemical techniques, we demonstrated that AURKA was expressed in primary cultured rat neurons and brains of C57BL/6J mature mice. Furthermore, endolysosome pH of neurons was measured ratiometrically using LysoSensor Dye DND-160, and we found that activation of AURKA with anacardic acid (5 μM and 50 μM) resulted in a concentration-dependent acidification of endolysosomes. Our findings that AURKA activation acidifies endolysosomes in neurons suggests that activators of AURKA might be useful in decreasing the pathogenesis of AD.
Supported by P30GM103329, R01MH100972, MH105329 and R21DA040519.

P3

Utilizing a gene therapy approach to purge latent HIV-infected cells

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HIV-1 persistence during antiretroviral therapy is a major hurdle to a cure. Genomic editing techniques, like the CRISPR/Cas9 system, hold promise to permanently excise or inactivate integrated virus from a host cell. However, due to the intrinsic mutation rate during HIV-1 replication and numerous intra- and extracellular selective pressures, the virus in patients exists as a collection of distinct ever changing genomic variants, termed quasispecies even during highly effective suppressive therapy. Presented here is a methodology for designing gRNA sequences to cleave a spectrum of HIV-1 quasispecies utilizing technologies to minimize off-target impact. PBMC genomic DNA was isolated from patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from brain and spleen tissue from the National NeuroAIDS Tissue Consortium (NNTC) and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using Next Generation Sequencing (NGS). A package of 4 or 10 gRNAs were selected based on a training data set, which in silico is predicted to cleave the quasispecies within the testing data set and a majority of the NNTC

patient samples. These gRNA packages have been cloned and have been transfected into HIV-1 reporter cell lines. The results of these experiments have shown that that the SMRT packages efficiently reduced reporter gene expression. In addition, the 4 SMRT gRNAs have now also been cloned into a single lentiviral vector. Preliminary studies have shown that delivery of the lentivirus was able to attenuate reporter gene expression. These studies represent a step towards understanding the complex task of using CRISPR/Cas9 for HIV-1 targeted excision therapy. Future studies will incorporate the evaluation of integration sites and their effect on CRISPR/Cas9 binding and cleaving. These studies will incorporate the use of latency reversal agents in conjunction with CRISPR to assess overall cleavage efficiency.

P4

HAM/TSP CSF B-cell receptor (BCR) derived antibodies demonstrated reactivity to HTLV-1 specific proteins

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Antibody secreting cells (ASC) are a subset of B cells responsible for antibody production. In humans, this encompasses short-lived plasmablasts and plasma cells. Although it has been well established that ASCs are vital to the regulation and elimination of other infections, their role in the control of HTLV-1 is gaining more recognition. HIV has demonstrated how important a robust B cell response is for control of viral loads but the role of HTLV-1 specific antibodies is unclear. Indeed, ASC are increased in HAM/TSP patient CSF (manuscript submitted). Considering the presence of exosomes in the CSF of HAM/TSP patients, and their demonstrated HTLV-1 antigen content, we asked whether B cell receptors (BCRs) present on ASC in the CSF are preferentially enriched for those BCRs that recognize HTLV-1 antigens. Stimulation of B cells by exosomes has been reported, demonstrating the specific enhancement of B cell populations that recognized autoantigens packaged in the exosomes. We therefore asked whether BCRs present in the CSF of HAM/TSP patients recognize HTLV-1 antigens. In collaboration with the Virus Research Center, BCR libraries were formed based on the top clones from high throughput sequencing of HAM/TSP CSF B cells. Heavy and light chains were transfected into cells and resulting HAM/TSP CSF specific antibodies (abs) were isolated. We found that Abs formed from HAM/TSP BCR libraries showed increased reactivity to proteins expressed by HTLV-1 infected cell lines and HAM/TSP PBMCs while no reactivity was observed to uninfected cell lines. While reactivity to HTLV-1 Tax, and Env could not be confirmed, HAM/TSP CSF BCR Ab clones appeared to show reactivity to HTLV-1 p19. These data demonstrate that antibodies derived from patient CSF B cells can be screened against known antigens to test for reactivity and may have applications in neuroinflammatory disorders of unknown etiology.

P5

The role of RAGE in brain endothelial extracellular vesicle secretion and intercellular amyloid transfer

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We have shown previously that HIV-1 facilitates shedding of endothelial extracellular vesicles (ECV) from human brain endothelial cells, increases ECV Aβ content and mean ECV size. In addition, ECV can transfer Aβ to other cells of the neurovascular unit. In this work, we investigated a possible involvement of the receptor for advanced glycation end products (RAGE) in these events. We demonstrated earlier that HIV-1 increased RAGE levels, and HIV-1 induced Aβ accumulation was RAGE-dependent in brain endothelial cells. It was also reported that RAGE might be involved in microvesicle secretion from macrophages. Therefore, we hypothesized that RAGE may be involved in the HIV-1 induced brain endothelial ECV-shedding, ECV-Aβ level increase, and ECV-Aβ transfer to neurovascular unit cells. Blocking RAGE with a high-affinity specific inhibitor (FPS-ZM1) resulted in decreased protein and Aβ levels in brain endothelial-derived ECV. Unexpectedly, treatment with FPS-ZM1 also increased ECV number and size. In addition, RAGE-inhibition significantly blocked ECV-Aβ transfer to human astrocytes and neuroprogenitor cells, while it had no effect on ECV-Aβ transfer to pericytes. Because astrocytes are the key players in HIV-1 associated neuroinflammation, and neuroprogenitor cells are critical in regeneration after neuronal loss, our data suggests that inhibition of RAGE may be beneficial in the ECV-mediated amyloid pathology of the HIV-infected brain.

Support by MH098891, MH072567, DA039576, DA027569, and HL126559.

P6

microRNA-455-3p predicts HIV-associated symptomatic distal sensory polyneuropathy and suppresses NGF expression in human neurons

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Symptomatic distal sensory polyneuropathy (sDSP) in people with HIV/AIDS is common, often leading to disabling neuropathic pain. Host-encoded microRNAs (miRNA) regulate both host and viral gene expression and can serve as disease biomarkers. We investigated plasma miRNA profiles in HIV/AIDS patients with sDSP. Discovery and Validation Cohorts were studied that included HIV/AIDS patients with or without sDSP (nonDSP) as well as uninfected healthy controls (HCs). Plasma miRNA expression levels were measured by array hybridization and verified by quantitative real-time reverse transcriptase PCR (qRT-PCR). Biostatistical and bioinformatic analyses were applied to the data. MicroRNA targets were validated in neural cell cultures.Expression analyses identified several miRNAs in the Discovery Cohort (sDSP, n,=29; nonDSP, n,=40; HC, n=9) with increased levels (≥2.0 fold and $p \leq 0.05$) in the sDSP group compared with the nonDSP group. miR-455-3p displayed a 12-fold median increase in the sDSP patients group. In the Validation Cohort (sDSP, n,=24; nonDSP, n=16; HC n=19) significant upregulation of miR-455-3p was also observed in the sDSP group. Receiver-operating characteristic curves showed that miR455-3p was predictive of the sDSP diagnosis in the Discovery (AUC 0.82) and Validation (AUC 0.73) Cohorts. Bioinformatics analyses revealed that miR-455 targeted multiple genes implicated in peripheral nerve maintenance including nerve growth factor (NGF) and related genes. Transfection of cultured human dorsal root ganglia with miR-455-3p showed a concentration-dependent reduction in beta-III tubulin expression relative to cells transfected with a noncoding (control) microRNA. Human neurons transfected with miR-455 disclosed a reduction of NGF expression and reduced cellular processes that were reversed by co-treatment with an anti-miR455-3p antagomir. Plasma miR-455-3p expression was increased in HIV/AIDS patients with sDSP in two patient cohorts and targeted NGF that resulted in nerve degeneration. Plasma-derived miR-455-3p represents a potential biomarker and disease determinant for sDSP.

P7

Measuring the efficiency of HIV-1-specific gRNAs for CRISPR/Cas9 excision of patient-derived sequences

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Although antiretroviral therapy (ART) has been shown to dramatically improve the prognosis for HIV-1-infected patients, latent/persistent infection due to integrated proviral DNA remains in some tissues including the peripheral blood, lymphoid tissue, brain and gut. There is currently no effective strategy for the complete removal of persistent proviral DNA from infected patients and the end result of ART interruption is a resurgence of systemic viral load. However, recent success has been achieved in the removal of integrated HIV-1 from individual cells within infected cell lines and primary cell populations derived from infected patients using the CRISPR/Cas9 system suggesting a new approach to targeted elimination of latent infection. However, one of the challenges of designing and implementing a CRISPR/Cas9-based treatment is the evaluation of the effectiveness of the therapy across a wide range of individuals or even within a single patient. To address this challenge, PCR-amplified LTRs from HIV-1-infected patients have been inserted into a plasmid vector. Using the resulting recombinant plasmids as substrate in an in vitro cutting assay, the relative efficiency of different gRNAs was assessed by measuring the proportion of substrate that underwent a conformational change during the enzymatic reaction. In future experiments, the newly developed Circle-Seq technique, which also capitalizes on conformational changes to circularized DNA during CRISPR/Cas9 cutting, will be utilized to prepare patient DNA for next-generation sequencing. This will provide a highly quantitative measure of efficiency of different gRNAs to target the range of quasispecies present in the latent reservoirs of HIV-1-infected patients; a major hurdle to achieve an HIV cure.

P8

The role of neuronal cytoskeleton in NeuroAIDS

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Although combined antiretroviral therapy is extremely successful in eliminating the human immunodeficiency virus (HIV) to undetectable levels in the periphery and prolonging the life in HIV positive subjects, there is a growing evidence of HIV-associated neurocognitive disorders. The lack of documented evidence of direct infection of neurons has prompted studies to discover mechanisms of HIV mediated neurotoxicity. As a result of these studies, viral proteins such as gp120, Tat, Nef, and others have emerged as the leading, potentially neurotoxic agents underlying HIV-mediated neuronal degeneration. We have recently established that gp120 is internalized and binds with high affinity to class III beta-tubulin, a component of neuronal microtubules, through a conserved α-helical motif. Gp120 causes deacetylation of tubulin, a post-translational modification which impairs the functionality of microtubules. We hypothesize that the deacetylation of tubulin caused by gp120 impairs the axonal transport of organelles. We demonstrated that HDAC-6 selective inhibitor, tubacin, prevents gp120-meditaed deacetylation of tubulin, as well as gp120-mediated neurite shortening and cell death of primary rat cortical neurons. We also hypothesize that gp120 could interfere with Tau alternative splicing. We established that gp120 increases 4R/3R-tau isoform ratio in cortical neurons and confirmed this finding in the HAND brain samples. Overall, our data suggest that gp120 interaction with neuronal cytoskeleton is a novel mechanism of its toxicity.

P9

Development of a Sustained Release Long-Acting Darunavir Prodrug Nanoparticle

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Antiretroviral therapy (ART) has improved the quality and longevity of those living with HIV/AIDS. Despite the advances limitations in drug bioavailability, resistance, clearance, toxicities and poor patient adherence have impaired regimen success. We posit that such pharmacological limitations may be overcome by long acting slow effective release ART (LASER ART). This approach improves cell and tissues drug delivery, antiretroviral responses and extends dosing intervals. To this end, we synthesized a hydrophobic and lipophilic bioreversible prodrug of darunavir and transformed it into a LASER ART. Darunavir (DRV), a protease inhibitor was modified by covalent linkage of a 14-carbon chain hydrophobic fatty acid moiety to the parent drug by a cleavable hemiaminal bond. Stable poloxamer 407 coated prodrug nanoformulations (NMDRV) were then produced by high-pressure homogenization. Physicochemical properties of NMDRV and resultant particle cell uptake, cytotoxcitiy, antiretroviral efficacy, pharmacokinetic and biodistribution studies were performed using the pro and native drug formulations. These were tested in monocyte derived macrophages (MDM) and BALB/c mice. NMDRV was taken up in MDM at levels of 86 ug/106 cells over 24 hours and retained for up to 2 weeks. Comparative studies performed with native DRV formulation showed neither uptake or drug retention at equivalent time points. These results paralleled antiretroviral efficacy in MDM measuring protection against HIV-1 infection for two weeks. Plasma DRV concentration, at or above, the ED90 was detected through day 7 following a single dose of 40 mg/kg. The results highlight a new LASER ART formulation designed to positively affect ART distribution with limited toxicity for long-term treatment of HIV patients.

P10

Optical electrophysiology of human primary neurons: role of KCC2 in hyperexcitability induced by HIV ± morphine exposure

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Despite the introduction of combined antiretroviral therapy, the CNS remains highly susceptible to insult from HIV-1 and inflammatory factors which cause sublethal damage to bystander neurons, providing the neural basis of HIV-associated neurocognitive disorders (HAND). Opiate use is often comorbid with HIV infection and these patients show exacerbated HAND symptomology. Little is known about electrophysiological changes associated with HIV ± morphine co-exposure. We addressed this question by developing a dissociated primary human model derived from differentiating human neural progenitor cells (hNPC) into mixed neuron-astrocyte cultures containing glutamatergic and gamma-aminobutyric acid-(GABA)ergic neurons. Optical techniques were used for electrophysiological experiments, thus circumventing the biohazard of sharp electrodes in the presence of HIV. With the genetically encoded voltage indicator (GEVI), FlicR1, and genetically encoded calcium indicator (GECI), GCaMP6f, we measured human neuron electrophysiological and calcium activity to elucidate changes in excitatory-inhibitory

balance due to HIV ± morphine exposure. Additionally, we determined that HIV and morphine dysregulate neuronal [Cl-]i resulting in hyperexcitability. K-Cl cotransporter 2 (KCC2) maintains low [Cl-]i necessary for GABAAR mediated hyperpolarization. Thus, we hypothesized that HIV ± morphine decrease expression/activity of KCC2 leading to dysregulated [Cl-]i and loss of subsequent GABAAR hyperpolarization. This was confirmed by immunostaining experiments that showed significant loss of KCC2 in neurons exposed to supernatant from HIV-infected monocytes (250-500 pg/mL p24) and 500nM morphine in the absence of neuron death. We determined that the viral factors transactivator of transcription (Tat) and glycoprotein 120 (gp120; R5-tropic) contribute to KCC2 loss. These results correlate with significant defects of GABA-ergic signaling in primary human neurons exposed to HIV, or HIV proteins ± morphine. KCC2 expression and response to GABA were rescued by co-exposure with KCC2 enhancer, CLP257. Our data identify KCC2 and upstream activity as a promising, novel target for intervention to alleviate functional changes underlying HAND ± opiate use.

P11

Exosomes from Uninfected Cells Activate HIV-1 Transcription

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HIV-1 infection causes AIDS, infecting millions worldwide. It can persist in a state of chronic infection due to its ability to become latent. In this study, exosomes from uninfected cells were observed to increase viral transcripts from latent wild-type HIV-1-infected cells. A possible mechanism for this finding revealed that the exosomes cause an increase in RNA Polymerase II loading onto the DNA within the infected cells; this was possibly driven by the presence of specific kinases in the exosomes, some of which were unique to T-cell-derived and myeloid-derived exosomes. Subsequently transcribed viral transcripts, which include TAR and a novel RNA termed "TAR-gag", can then be packaged into exosomes and potentially be exported to neighboring uninfected cells. TAR-gag is a long, noncoding RNA that is 615 bases long, contains the TAR element, and terminates within the p17 region of HIV-1 gag (1). In the presence of transcription inhibitors F07#13 (a Tat peptide mimetic) and CR8#13 (an ATP-binding analog of Cdk9), TAR-gag is capable of binding to SWI/SNF components in the nucleus, including the mSin3A/HDAC-1 complex, and serves as a scaffolding RNA. Additionally, TAR-gag can recruit suppressive factors and RNA-binding proteins to the HIV-1 promoter, resulting in transcriptional gene silencing (TGS) (2). In recipient cells, exosomal-transported viral RNA products can elicit innate immune response, leading to activation of the TLR and NF-κB pathways and cytokine induction, including IL-1α, IL-6, IL-8, and TNF-β (3). Collectively, these results imply that exosomes from uninfected cells can activate HIV-1 from latency in infected cells and that a "true transcriptional latency" may not be possible in vivo, especially in the presence of cART.

P12

Virosomes: An interplay between Virus and Exosomes from infected cells

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Exosomes are small vesicles, 30-120 nm in length, released from all cell types in the body, can be found in various bodily fluids, such as semen and urine and are transported through the bloodstream and the lymphatic system. They are formed by inward folding of the endosomal membrane to form multivesicular bodies (MVBs), a process carried out by the endosomal sorting complex required for transport (ESCRT). The contents of exosomes depend on the originating cell, and we have found distinct markers (RNA and proteins) from T-cell vs. Myeloid infected cells that may control recipient cells in gene regulation. Finally, our current studies are aimed at addressing three fundamental points regarding exosomes and their potential significance in HIV-1 pathogens. First, we investigated the effect of exosomes derived from uninfected cells on latent HIV-1-infected cells and found that there is an abundant level of short noncoding RNA in infected cells, arguing that a true "transcriptional latency" may not exist in vivo since cells are constantly in contact with exosomes from uninfected cells. The infected cells (in presence of cART) still secret exosomes that contain viral products including TAR. Secondly, the effect of the exosomes and EVs are distinct for T-cells vs. CNS cells. Here the EVs may be pushing through control of cell cycle and show a classical "bell shape" curve over time when exposed to EVs. The net effect over time is exhaustion and death of the cells. Finally, when these exosomes are decorated around Nanoparticles, they can activate and mature Dendritic cells (DCs) suggesting they can be used in vaccine or adjuvant therapy.

P13

Cannabinoid receptor activation induces unique changes in the murine gut microbiome associated with the induction of anti-inflammatory myeloid-derived suppressor cells and T regulatory cells

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Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient found in the Cannabis plant, has been shown to activate cannabinoid receptors CB1 and CB2. Synthetic THC is currently being used to treat anorexia in people with HIV/AIDS, patients undergoing chemotherapy, multiple sclerosis, neuropathic pain, and spasticity. Moreover, use of marijuana for recreational and medicinal purposes is getting increased attention globally. The mammalian intestine harbors a diverse array of bacteria which are known to regulate and respond to many stimuli including that of the immune and nervous system. In the current study, therefore, we investigated the immune-modulatory capacity of THC, and its resulting effects on the gut microbiome. We tested the effect of acute or chronic exposure of C57BL/6 mice to THC on the murine immune system, and correlated these immunological changes to the flux of intestinal bacteria. Intraperitoneal exposure of mice to THC caused significant migration of CD11b+Gr-1+ myeloid-derived suppressor cells (MDSCs) from the bone marrow to the peritoneal cavity where they proliferated based on a dose dependent increase in Ki-67 expression. Mice administered THC displayed an increase in the number of colonic lamina propria RORγt+ FoxP3+ bacteria-associated T regulatory cells (Tregs). The acute induction of anti-inflammatory cells we observed was mirrored by an acute increase in the short chain fatty acid, butyrate, a bacterial metabolite known to have many beneficial effects in the colon. To determine the receptor mediating these changes, Cnr1-/-, Cnr2-/-, and Cnr1Cnr2-/- double knockout mice were administered THC, revealing that CB1 ligation is responsible for the alterations in butyrate levels; however, both CB1 and CB2 are needed to maintain normal levels of butyrate and acetic acid in the murine intestine. These data suggest a role for THC in reducing inflammation in the gut, as well as a role for cannabinoids in regulating the microbiome and healthy intestinal function.

P14

Ex vivo and in vivo CRISPR/Cas9 mediated excision of proviral DNA from the cells of HIV-1 positive patients using huPBMC-NOG mice model

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To further examine utilization of CRISPR/Cas9 gene editing to achieve curative virus sterilization in in vivo settings, we used huPBMC-NOG mice model and lentiviral delivery of Cas9/gRNAs targeting HIV-1 genome. In the first set of experiments, PBMCs from healthy donors were infected with HIV-1JR-fl and then treated with the lentiviral cocktail carrying Cas9 gene and two gRNA expressing cassettes targeting viral LTRs (LTR-A and LTR-B). In parallel, the same cells were transplanted into NOG mice followed by intravenous injection of the LV cocktail. Two weeks later animals were sacrificed and the spleens were collected and examined for viral sequences excision. In both: in vitro and in vivo LV-Cas9/gRNA treated samples we were able to detect CRISPR/Cas9 induced double cleaved/end-joined truncated viral LTR sequences. Next, similar experiment was performed using PBMCs isolated from HIV-1 positive patients from Temple University CNAC cohort. After PCR amplification and Sanger sequencing of proviral LTRs, cells from 3 patients were selected, based on the perfect match with our gRNAs pair. Next, the cells were treated with lentiviral Cas9/gRNAs in ex vivo culture and in vivo, after transplantation into NOG mice. Several tissues were examined for CRISPR/Cas9 induced excision of viral LTRs. Detection of the cleavage products varied between different tissues and patient donors with complete excision observed for all patients in blood and no any cleavage detectable in the brains of treated animals (blood 3/3, spleen and liver 2/3, lung 1/3, brain 0/3). The level of HIV-1 DNA in in vitro treated cells and spleens of humanized animals was quantified using digital droplet PCR and the expression Cas9 mRNA and gRNAs confirmed by RT-PCR. Our data provide for the first time evidence of successful CRISPR/Cas9 mediated excision of viral sequences in the cells derived from HIV-1 positive patients in in vivo settings.

P15

Eradication of HIV-1 from in vitro infected primary myeloid and brain cells using Tat-inducible CRISPR/Cas9 system

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The goal of the project was to examine the use of Tat inducible CRISPR/Cas9 gene editing platform to eradicate HIV-1 provirus or induce resistance to a new infection, using in vitro infected primary human macrophages, microglia and astrocytes. Cells were divided into two groups: HIV-1 infection first/CRISPR/Cas9 treatment second (eradication arm) and CRISPR/Cas9 first/HIV-1 infection second (resistance to a new infection arm). Monocyte derived macrophages were infected with HIV-1NL4-3-GFP-BAL and primary human fetal brain cells with VSV-g pseudotyped HIV-1NL4-3-GFP-P2A-Nef. In eradication arm experiments, at day 3 of HIV-1 infection antiretroviral drugs were added for 6 days to block productive infection and induce latency like state. CRISPR/Cas9 was delivered using lentiviral vectors. Cas9 was delivered using three different lentiviral constructs: RFP-Cas9 (stable expression from hEF1α promoter and fluorescent microscopy verification of transduction efficiency), CW-Cas9 (very robust doxycycline inducible expression) or LTR(-80/+66)-Cas9 (Tat inducible expression). Single guide RNAs (sgRNAs) targeting two unique sequences in U3 region of HIV-1 LTR (called target A and B) were delivered using lenti-KLV-BFP vector and were expressed from U6 promoter. After 5 or 7 days cells were harvested, genomic DNA and RNA was extracted. Viral expression was checked by GFP expression using flow cytometry and HIV-1 env mRNA expression in qRT-PCRs. Viral DNA was quantified using HIV-1 env gene specific Taqman qPCRs. Lentiviral delivery of Cas9 and sgRNAs targeting HIV-1 LTRs induced statistically significant reduction of virus expression in in vitro HIV-1 infected primary human fetal microglia and MDMs but not in astrocytes. The same treatment applied prior HIV-1 infection resulted in drastic suppression of viral replication in all primary cell types tested.

P16

Inhibition of HSV-1 replication by a gene editing strategy

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HSV-1 is a human neurotropic virus and its genome is present in trigeminal ganglia of 85% of human population worldwide, with a seroprevalence of 90% in normal asymptomatic individuals. Replication of HSV-1 in the central nervous system causes encephalitis, resulting from the infection and anti-viral inflammatory response within the frontal and the temporal lobes. Current treatments for primary HSV-1 infection and reactivation of diseases are non-selective, do not prevent establishment of latent infection and have adverse side effects, pointing to a strong need for improved and specific therapeutic strategies. In this study, we used RNA-guided CRISPR/Cas9 gene editing to specifically target three of the viral immediate-early proteins, infected cell protein 0 (ICPO), lCP4, and lCP27, that are implicated in controlling further viral gene expression and affecting normal host cell function. We found that CRISPR/Cas9 introduced the excision of all the three target genes, completely abrogated HSV-1 infectivity in permissive human cell culture models and protected permissive cells against HSV-1 infection. We conclude that RNA-guided CRISPR/Cas9 can be used to develop a novel, specific and efficacious therapeutic and prophylactic platform for targeted viral genomic ablation to treat HSV-1 diseases.

P17

CRISPR/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 with human polyomavirus JC (JCV). PML lesions are areas of demyelination containing oligodendrocytes with viral nuclear inclusion bodies and bizarre astrocytes, which are also productively infected by JCV. Although JCV was isolated over forty years ago and has been extensively studied, there is still no effective therapy for PML. Recently, a novel genome-editing method was developed based on clustered regularly interspaced short palindromic repeat (CRISPR) systems. The CRISPR 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 CRISPER/Cas9 system as a potential tool for JCV elimination by

designing guide RNAs (gRNAs) targeting a first site in the early gene encoding viral protein T-antigen and a second site located in non-coding control region (NCCR). Our results indicated that CRISPR Cas9/gRNAs system effectively delete target DNA sequence, reduce T-antigen expression and late promoter activity and inhibit JCV DNA replication. No off-target effects of the JCV-specific CRISPR/Cas9 editing were also detected. These data have shown that the CRISPR/Cas9 system could be a promising tool for the eradication of the JCV genome from cells and a valuable cure for PML.

P18

Targeting HIV-infected brain to improve stroke outcome

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In the era of highly active antiretroviral therapy, the HIV prognostic has changed from a deadly to a chronic disease. While the virus is repressed, several co-morbidities, including cardiovascular disease are still present in long term survivors. HIV positive individuals are more at risk of having strokes and also suffer from a less favorable recovery prognostic. Our hypothesis is that despite efficient HAART, residual HIV presence can contribute to stroke severity. In addition, we also hypothesize that viral reservoirs in the brain contribute to injury. Previous publications in our laboratory, based on the EcoHIV mouse model, demonstrated that infection affects the integrity of the functions of the blood-brain barrier. In the current study, we observed that brain infection by EcoHIV resulted in a significant increase in infract size both at early and late post-stroke when compared to mock infected animals. A recovery from stroke injury was seen in control animals, this reduction was not visible in EcoHIV infected mice. Upon further examination, we were able to demonstrate that the induction of stroke resulted in an increase in HIV presence in the affected hemisphere, with infected cells situated primarily near or at the border of the infract area. The majority of cells harboring the virus were from the macrophage/microglial lineage. We next employed several immune markers to examine if the immune reaction to the tissue injury and the more prominent viral presence could be responsible for the delay in infract recovery. We observed a trend for an increase in inflammatory markers in EcoHIV infected mice, especially those associated with the monocyte/macrophage/neutrophil response. We are currently investigating the potential therapeutic efficacy of targeting the HIV CNS reservoir using a high CNS penetrating efficacy therapy. The successful implementation of this regiment would be highly beneficial in HIV patients at risk of cerebrovascular disease.

P19

Zika Virus E protein alters properties of human fetal neural stem cells by disruption of microRNA circuitry

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Zika Virus (ZV) infection has gained worldwide attention following large outbreaks in Brazil during 2015-2016 epidemics. It has been linked to severe clinical condition called microcephaly where fetuses are born with abnormally small brain. ZV infection causes death and quiescence in neural stem cells hence reducing overall pool of cells leading to microcephaly. We have employed well characterized in-vitro model system of human fetal neural stem cells (fNSCs) for understanding the molecular mechanism of ZV induced microcephaly. We delineated that ectopic expression of ZV E protein induces quiescence in fNSCs. We found that mRNA level of pro-neuronal genes were upregulated in E protein expressing groups suggesting that E protein also induces immature neuronal differentiation. Differentiation of human fNSCs in the presence of E protein induces apoptosis at day-3 of differentiation. Migration of cells from differentiating neurospheres is also disrupted. To further probe into the mechanism we analyzed global miRNA expression (miRNA Seq) which shows disrupted miRNA circuitry in fNSCs. Among differentially expressed miRNAs (DEM), we validated miR-204-3p, miR-1306-5p, miR-6087, miR-23c and miR-676. Incidentally, these miRNAs are also reported in other viral infections. GO analysis for biological process of targets of differentially expressed miRNAs revealed particular enrichment of up regulated DEM targets in primary biological and metabolic processes. Whereas targets of downregulated genes were not only enriched in primary biological and metabolic processes but also very high enrichment of cell cycle and cell cycle processes which supports our findings that E protein alters cell cycle dynamics. PANTHER pathway analysis shows top most enrichment of developmental pathways including CCKR, PDGF, EGF, p53, FGF and notch signaling pathways. Our data not only provides novel insights into the mechanism of ZV induced complications but also provides valuable resource to the field for further understanding of mechanisms.

P20

HIV-1 Infection Upregulates Toll-Like Receptor-3 Expression In The Human Brain Endothelium

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Several advancements have been improved the strategies to treat the HIV/AIDS disease with the development of combination antiretroviral therapy (CART). However, exists the spectrum of neurocognitive dysfunction associated with HIV infection, described as HIV-associated neurocognitive disorder (HAND). HAND is associated with blood-brain-barrier (BBB) inflammation, involving toll-like receptors (TLRs) signaling. TLRs are pattern-recognition receptors that detect invading pathogens via their pathogen associated molecular patterns (PAMP). It is not known whether primary human brain microvascular endothelial cells (HBMEC) the major BBB component, express TLRs, or whether specific TLRs are involved in HIV-mediated BBB dysfunction and HAND. Using RT-PCR, we show that primary HBMEC express TRL3, 4, 5, 7, 9, 10 mRNA, and that TLR3 are the most abundant TLRs expressed on the human brain endothelium. We also showed that exposure of HBMEC to HIV-1 and TLR3 ligands significantly increased endothelial TLR3 transcription and expression. Ex-vivo studies using human brain tissues further showed significantly higher TLR3 transcription and expression in brain tissues of HIV-1-infected subjects, mostly on the brain blood vessels; with much higher TLR3 levels in brain tissues of subjects with HAND or HIV encephalitis (HIVE). Exposure of HBMEC to HIV-1 and TRL3 ligands activated c-jun (serine-63) and SAPK/JNK (Thr183/Tyr185), and this could be blocked by TLR3/dsRNA complex inhibitor (TRL3.CI). Functional studies showed that both HIV-1 and TLR3 ligands significantly increased monocytes adhesion and migration through in vitro BBB models, and both TLR3.CI and JNK inhibitors blocked HIV-1- and TLR3 ligands-induced increase in monocyte adhesion and trans-endothelial migration. These data suggests that viral recognition via endothelial TLR3 and JNK pathways are involved in HIV-1-induced BBB dysfunction and HAND.

P21

Toll-Like Receptor-3 Mediates HIV-1-Induced Interleukin-6 Expression In The Human Brain Endothelium Via TAK1 And JNK Pathways

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Our previous studies showed that HIV-associated neurocognitive disorders (HAND) are associated with blood-brain-barrier (BBB) inflammation, involving toll-like receptors (TLRs) signaling. We also showed that HIV-1 significantly increased interleukin-6 (IL6) transcription and

expression in human brain blood vessels and human brain microvascular endothelial cells (HBMEC), the major BBB component. Based on our data showing that TLR3 are the most abundant TLRs expressed in HBMECs, our present study investigate the relationship between TLR3 and HIV-1-induced IL6 expression on the brain endothelium. We demonstrate that exposure of HBMEC to HIV-1 or TLR3 ligands increased endothelial IL6 expression by 6-to-127-fold (P<0.001). HIV-1 upregulated IL6 through interleukin-1 receptor-associated-kinase (IRAK)-1/4/TAK1/JNK pathways, via ATP-dependent JNK activation. TLR3 activation upregulated IL6 through TAK1/JNK pathways, via ATP-dependent or -independent JNK activation. HIV-1 and TLR3 activation also upregulated transcription factors associated with IL6 and TAK1/JNK pathways (Jun, CEBPA, STAT1). Blocking TLR3 activation prevented HIV-1- and TLR3 ligands-induced upregulation of these transcription factors, and prevented IL6 transcription and expression. These data suggests that viral immune recognition via endothelial TLR3, TAK1 and JNK pathways, are involved in IL6 transcription and expression, and viral-induced endothelial inflammation.

P22

Rhesus Macaque Model of Zika and SIV/HIV Co-infection

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Interactions between HIV infection and neglected tropical infectious diseases have been suggested to affect pathogenecity and disease progression in co-infected individuals. These interactions are marked because of the potential for one pathogen to alter another pathogen's epidemiology, immunopathogenesis and response to therapy. The association between HIV infection and endemic diseases has been described in tropical regions with different levels of complications. As ZIKV infection expands its geographical range, exposed HIV immunosuppressed individuals may unveil new and more severe clinical manifestations. Therefore, due to the large geographical overlap of populations exposed to both ZIKV and HIV, close surveillance of HIV-positive individuals to mirror such co-infections are of particular importance. Therefore, we sought to determine if ZKIV infection with HIV positive individuals altered pathogenesis. We utilized rhesus macaques chronically infected with either SIVmac239 (n=4) or SHIV3618MTF (n=2). These macaques were then inoculated with 104 PFU of ZIKV (PRABVC59) subcutaneously. ZIKV plasma viral loads were found to peak up to 105 copies/ml with a mean value of 103 copies/ml. These values are similar to ZIKV infected animals in the literature as well as our own data. Plasma viral loads of SIV (106-107 copies/ml) and SHIV (102-103 copies/ml) did not change as compared prior to ZIKV inoculation. Next, necropsy was performed 6-7 months post ZIKV infection, and ZIKV/SIV/SHIV viral loads were measured in different tissues and organs. Using highly sensitive assays with detection limits as low as 3 copies/ml, we found no detectable ZIKV virus present suggesting that the virus had been cleared. Furthermore, levels of cytokines/chemokines in plasma measured using luminex assays revealed that minimal changes had occurred. While these data suggest that ZIKV infection in chronically infected HIV individuals may not significantly alter the pathogenesis and disease progression of HIV or ZIKV, this study warrants more epidemiological studies to validate these findings.

P23

Varicella zoster virus alters expression of cell adhesion proteins in human perineurial cells via IL-6

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Varicella zoster virus (VZV) vasculopathy occurs after virus reactivates from ganglia, spreads along nerve fibers to arteries and produces stroke. Temporal arteries from VZV vasculopathy patients contain virus in perineurial cells that form a protective barrier between peripheral nerves and surrounding tissue. We hypothesized that during VZV reactivation, VZV disrupts cell adhesion proteins in perineurial cells, potentiating infection of surrounding vascular cells. Thus, we mock- and VZV-infected primary human perineurial cells (HPNCs) and compared expression and distribution of cell adhesion proteins (claudin-1, E-cadherin and N-cadherin) at 3 days postinfection. No VZV-induced changes in claudin-1 transcripts were seen; however claudin-1 redistributed from the membrane/cytoplasm to the nucleus in VZV-infected cells compared to mock. Furthermore, while mock-infected cells expressed E-cadherin and not N-cadherin, VZV-infected cells did not express E-cadherin but expressed N-cadherin - supporting the novel possibility of a VZV-induced epithelial-to-mesenchymal-cell-like transition (EMT). These VZV-induced changes were confirmed in vivo in temporal arteries from VZV vasculopathy patients. Addition of conditioned media from VZV-infected cells to uninfected HPNCs revealed a soluble factor that was able to induce the same changes seen in VZV-infected cells, which was absent in conditioned media from mock-infected cells. Since IL-6 was induced in VZV-infected cells and was a candidate soluble factor for inducing EMT, an anti-IL6 receptor antibody was used to pretreat cells before addition of conditioned media. Indeed, the anti-IL6 receptor antibody prevented VZV-induced alterations in cell adhesion proteins in HPNCs. Overall, our findings indicate that VZV-induced redistribution of claudin-1, downregulation of E-cadherin and upregulation of N-cadherin is mediated by interleukin-6 and may lead to loss of perineurial cell barrier integrity, allowing viral spread from nerve fibers to surrounding vascular cells in VZV vasculopathy. Furthermore, the possibility is raised of a role for anti-IL6 receptor blockade in prevention of neurotropic virus entry and exit from peripheral nerves.

P24

Alcohol-Induced Inflammation Primes the Brain to Augment Meth-induced Dopaminergic Deficits

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A majority of methamphetamine (Meth) users are diagnosed with an alcohol use disorder. Despite the common comorbidity and the fact that each drug produces inflammation, little is known about the subsequent neurochemical effects. We hypothesized that co-exposure to ethanol (EtOH) and Meth produces greater inflammation-induced neurotoxicity compared to either drug alone. Male Sprague Dawley rats were allowed a 2-bottle choice of 10% EtOH or water every other day for a total of 28 days, followed by one day of binge Meth injections (10mg/kg x 4 inj.). Intake and preference increased over the 4 week period. 24h after the last day of EtOH drinking, LPS was elevated in serum and the striatum (*p<0.05 vs. Water). Cyclooxygenase-2 (COX-2) was also increased in the striatum after EtOH (*p<0.05 vs. Water). 7 days after Meth injections, supra-additive depletions of dopamine and 5HT were noted in the rats that received EtOH+Meth compared to Meth alone (*p<0.05 vs. Water+Meth). Importantly, EtOH alone did not affect dopamine or 5HT concentrations in the brain. These enhanced Meth-induced depletions occurred in a dose-dependent manner, in that the concentrations were negatively correlated with the amount of EtOH consumed over 28 days. The synergistic depletions of dopamine observed in the striatum after EtOH+Meth were paralleled by a decrease in tyrosine hydroxylase-positive immunoreactivity in the substantia nigra pars compacta as well as a decrease in motor function, measured via rotarod (*p<0.05 vs. Water+Meth). The enhanced dopamine and 5HT depletions produced by EtOH+Meth were blocked by administration of the COX inhibitor, ketoprofen injected during the intermittent EtOH withdrawal periods, indicating that EtOH-

induced inflammation is a key mediator in enhancing Meth-induced 5HT and dopamine depletions. Acute L-DOPA administration attenuated the decreases in motor function 7 days after Meth in EtOH-drinking rats. Studies are underway to investigate if administration of anti-inflammatory drugs blocks these deficits.

P25

The role of Pur-alpha in neuronal RNA transport and implications for the regulation of HIV-1 RNA

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In order to successfully replicate in the host cell, HIV must take advantage of the host transcriptional and translational machinery. Pur-alpha is a highly conserved protein that is essential for neural development and participates in the regulation of neurotropic viruses including HIV and JC virus. In addition to its well-established role as a single-stranded DNA and RNA binding protein, Pur-alpha has been identified as an important component of mRNA transport complexes in neurons. Dysfunctions of Pur-alpha have been linked to several CNS disorders and diseases, including Fragile X Tremor Ataxia Syndrome and C9orf72-mediated Amyotrophic Lateral Sclerosis. Recently, a neurological developmental disorder, PURA Syndrome, has been recognized. PURA Syndrome is characterized by de novo, heterozygous point mutations within the coding sequence of the PURA gene. Patients with this disorder display severe neurological developmental delays, encephalopathies, demyelination, hypotonia, and occasionally seizures. A similar phenotype is seen in Pur-alpha knockout mouse models, further implicating the importance of Pur-alpha in CNS function. In the past, Pur-alpha has been linked HIV-mediated neurodegeneration, particularly via its interaction with Tat to stimulate HIV transcription through the viral TAR RNA element. It has also been established that HIV is able to utilize host mRNA nuclear export components and pathways to aid in the trafficking and translation of viral RNA. However, the role Pur-alpha may play in this process has yet to be elucidated. Given the increasing evidence for the importance of Pur-alpha in neuronal mRNA transport and CNS function, as well as past studies showing the interaction between Pur-alpha and HIV TAR RNA, it provides an interesting pathway for further study. Elucidating the impact of Pur-alpha on RNA regulation in the brain may uncover mechanisms for blocking viral RNA transport as a therapeutic target for the treatment of HIV.

P26

Dysregulation of Sonic Hedgehog Pathway and Pericytes in the Brain after Lentiviral Infection

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Blood-brain barrier (BBB) dysfunction has been associated with cognitive decline in many CNS diseases, including HIV-associated neurocognitive disorder (HAND). Recent research suggests an important role for the Sonic hedgehog (Shh) signaling pathway in the maintenance of BBB integrity under both physiological and pathological conditions. In the present study, we sought to examine expression of Shh and its downstream effectors in relation to brain pericytes and BBB integrity in rhesus macaques infected with simian immunodeficiency virus (SIV), an animal model of HIV infection and CNS disease. Cortical brain tissues from uninfected (n = 4) and SIV-infected macaques with (SIVE, n = 6) or without (SIVnoE, n = 4) encephalitis were examined using multi-label, semi-quantitative immunofluorescence microscopy of Shh, Netrin-1, zona occludens 1 (ZO-1), glial fibrillary acidic protein, CD163, platelet-derived growth factor receptor b (PDGFRB), glucose transporter 1, fibrinogen, and SIV Gag p28. While no significant difference was found in the total amount of Shh in the brain between groups, both SIV-infected groups had significantly higher astrocyte coverage and more Shh appearing on the endothelium and in encephalitic lesions. The percentage of vessels with fibrinogen extravasation indicative of BBB leakiness was significantly higher in SIVE animals than in uninfected or SIVnoE groups. Both Netrin-1 immunofluorescence intensity and the size of PDGFRB+ pericytes, a cellular source of Netrin-1, increased significantly in SIVE animals when compared to uninfected or SIVnoE animals, but pericytes were completely absent from blood vessels in lesions. Hypertrophied pericytes were strongly localized in areas of fibrinogen extravasation and found in close proximity to SIVp28 positive staining in all 6 SIVE cases examined. The lack of pericytes and Netrin-1 in encephalitic lesions, in line with ZO-1 downregulation in the fenestrated endothelium, suggests that pericyte loss, despite the strong presence of Shh, may contribute to SIV-induced BBB disruption and neuropathogenesis in HAND.

P27

PERK Haplotype Function in HIV-Associated Neurocognitive Disorders

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The Unfolded Protein Response (UPR) is a signaling system which aims to re-establish protein homeostasis under conditions of ER stress. It does so by sensing misfolding events in the ER with three master regulators, IRE1, ATF6, and PERK, which then affect signaling to increase the cell's folding capacity and maintain survival. Several markers of UPR activation have been observed in the CNS of HIV infected individuals, including PERK and ATF6. Of these, the PERK protein has two major haplotypes, A and B, differentiated by three SNPs which encode amino acid changes in the resulting protein. Intriguingly, PERK Haplotype B is a risk factor for certain neurodegenerative diseases. Furthermore, PERK Haplotype B has been demonstrated to have increased kinase activity in lymphocytes compared with Haplotype A, when subjected to endoplasmic reticulum stress. We thus propose that the amino acid changes between PERK haplotypes cause PERK B to respond more severely than PERK A to the same ER stress in neurons, disrupting normal neuronal function. We seek to investigate the mechanisms behind the differences in PERK haplotype activities.

P28

New findings regarding the HIV-1 transgenic rat

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The HIV-1 transgenic (Tg) rat has been widely used to investigate the neurobiological basis of HIV-1 associated neurocognitive disorder (HAND). The HIV-1 Tg rat expresses 7 of 9 HIV-1 proteins under the control of the LTR. Despite wide experimental usage of the HIV-1 Tg rat, many genetic aspects of the HIV-1 Tg rat remain relatively unexplored. Specifically, this study addressed several critical issues regarding proviral

integration, HIV-1 expression patterns, and functional activity of the LTR in the HIV-1 Tg rat CNS. First, next-generation sequencing techniques and bioinformatics tools were used to detect insertion sites of the HIV-1 viral genome. Two independent insertion sites on rat chromosomes 10 and 13 were detected. There were multiple transgene concatemers, and the HIV-HIV junction patterns indicated there were 27 copies of HIV-1 DNA. Second, we used a highly sensitive mRNA in situ detection technology (RNAscope) to determine the HIV-1 mRNA distribution pattern in HIV-1 Tg rat CNS. We found that HIV-1 mRNA was differentially expressed in HIV-1 Tg rat brain regions. Specifically, the most abundant HIV-1 mRNA expression was located in the cerebral cortex. The mPFC, NAc and striatal brain regions also had high levels of HIV-1 mRNA expression; however, not all cells expressed the HIV-1 provirus. Finally, ex-vivo studies of HIV-1 Tg astrocytes confirmed activation of the LTR following vorinostat/SAHA stimulation and consequent increased HIV-1 viral protein production (tat, gp120). In summary, these findings suggest the HIV-1 Tg rat may be a unique genetic model for critically testing gene excision approaches as a strategy to remove the HIV-1 provirus from the brain and promote functional neurocognitive restoration.

P29

TAARgeting Astrogliosis During Meth And HIV-1 Exposure

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As a popular psychostimulant, methamphetamine (METH) use leads to long-lasting, strong euphoric effects. METH exacerbates the severity and onset of HIV-associated neurocognitive disorders (HAND), which affect 30-70% of the 37.6 million people globally infected with HIV. Most neurodegenerative diseases share neuroinflammation as a common pathogenic mechanism. Neuroinflammation, HIV and METH dysregulate a wide range of brain functions including neuronal signaling, glial activation, viral infection, oxidative stress and excitotoxicity. Since neuroglia often determine the outcomes of neurological disease, we investigate the mechanisms regulating astrocyte-mediated neurotoxicity in the context of METH and HIV comorbidity. To these ends, we examined the expression, localization and function of the novel METH astrocyte receptor, trace amine associated receptor 1 (TAAR1) in an in vitro model of HIV-associated activation wherein extended METH exposure is administered to mimic residual METH concentrations that occur in humans between binges of METH-taking. In this model, TAAR1 levels, and its localization to the endoplasmic reticulum (ER) and plasma membranes, increased with METH and HIV-induced astrogliosis. Calcium flux, which mediates ER, mitochondrial, and oxidative stress, also was increased, corroborating our prior studies on astrocyte mitochondrial dysregulation. The astrocyte responses to METH and HIV-relevant stimuli were blocked with the TAAR1-selective antagonist EPPTB. Extended METH and HIV activation impaired excitatory amino acid transporter 2 (EAAT2) expression and activity, which were recovered by following 24 hour exposure with EPPTB. Together, these data highlight several mechanisms regulating METH/HIV-induced, astroglia-mediated neurotoxicity and the potential for astrocyte targeted intervention via TAAR1 during chronic disease.

P30

Epigenetic Therapy Approach in HIV-mediated Neurocognitive Impairment in Mice

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Background: HIV infected individuals on antiretroviral therapy develop chronic mild neurocognitive impairment (NCI) that may affect everyday activities but do not progress to HIV dementia. The pathobiology of mild NCI is unclear and there is no treatment. We developed a chimeric HIV, EcoHIV, which can infect immunocompetent mice to investigate NCI biology and treatment. Infected animals develop chronic NCI that can be measured by behavioral tests and present transcriptional downregulation of pathways controlling neuronal signal transmission and memory similar to gene expression profiles from patients with HIV cognitive disease. Results: In order to analyze the epigenetic control of these functions we performed chromatin immunoprecipitation and next generation sequencing and found a high correlation between hypermethylation at histone 3 lysine 9 (H3K9) and transcriptional suppression of genes associated with synaptodendritic functions. To study the involvement of this epigenetic change in NCI we used valproic acid (VPA) a histone deacetylase inhibitor that also decreases histone methylation at H3K9. VPA was administered daily one day before EcoHIV infection or at different time points after infection on cognitive impaired mice. VPA treatment was able to both prevent and reverse NCI impairment, gene expression changes on key synaptic genes and histone hypermethylation on their promoters. The behavioral and molecular effects of VPA continued after VPA treatment discontinuation indicating a potential long term effect. Conclusion: The results suggest dysregulation of epigenetic control of memory in HIV induced mild cognitive dysfunction and indicate that the disease is prevented and treatable by epigenetic drugs targeting H3K9 methylation.

P31

The role of Sirtuin-1 in neuroHIV and in aging-associated inflammation

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Microglia and macrophages are the main non-neuronal subsets of myeloid origin in the brain, and are critical regulators in neurodegenerative disorders, where inflammation is a key factor. Since HIV infection results in neurological perturbations that are similar to those in aging, we examined microglial and infiltrating myeloid subsets in the search for changes that might resemble the ones in aging. For that, we used the SIV infection in rhesus macaques to model neuroAIDS. We found that Sirt-1, a molecule that impacts survival and health in many models, was decreased in cell isolates containing mostly microglia and myeloid cells from the brain of infected macaques. The role of Sirt-1 in neuroAIDS is unknown. We hypothesized that Sirt-1 silencing functions are affected by SIV. Mapping of Sirt-1 binding patterns to chromatin revealed that the number of Sirt-1-bound genes was 29.6% increased in myeloid cells from infected animals with mild or no detectable neuropathology, but 51% was decreased in severe neuropathology, compared to controls. Importantly, Sirt-1-bound genes in controls largely participate in neuroinflammation. Promoters of type I IFN pathway genes IRF7, IRF1, IFIT1, and AIF1, showed Sirt-1 binding in controls, which was consistently lost after infection, together with higher transcription. Loss of Sirt-1 binding was also found in brains from old uninfected animals, suggesting a common regulation. The role of Sirt-1 in regulating these inflammatory markers was confirmed in two different in vitro models, where Sirt-1 blockage modulated IRF7, IRF1 and AIF1 levels both in human macrophage cell lines and in blood-derived monocytes from normal donors, stimulated with a TLR9 agonist. Our data suggests that Sirt-1-inflammatory gene silencing is disturbed by SIV, resembling aging in brains. These findings may impact our knowledge on the contribution of innate immune subsets to neurological

consequences of HIV infection, aggravated by, and overlapping with, the aging process.

P32

HIV-1 Tat-induced matrix metalloproteinase remodeling of perineuronal nets

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The HIV-1 protein Tat is continually released from HIV-infected cells despite effective antiretroviral therapies. Although the mechanisms underlying HIV-associated neurocognitive disorders (HAND) are largely unknown, Tat-induced neuroinflammation is thought to play a role. Previous studies have linked both Tat and HIV infection to increased levels of specific neuronal- and glial-derived matrix metalloproteinases (MMPs). MMP activity is concentrated at the cell surface, and MMP substrates include components of the perineuronal net (PNN). PNNs are protective lattice-like structures that stabilize synapses and are predominantly localized to the soma and proximal dendrites of parvalbumin-positive (PV+) inhibitory interneurons, and PV+ basket cells in particular (PVBCs). The synaptic circuit between PVBCs and pyramidal cells is critical to rhythmic neuronal population events, including sharp wave ripples (SWRs) that underlie memory consolidation. Disruption of PNNs surrounding PVBCs leads to lateral diffusion of glutamatergic receptors and decreased PVBC excitability. Importantly, PNNs are reduced in the brains of HIV-infected individuals, and furthermore they are virtually absent in severely pathological HIV-encephalitis brains. Critical molecular effectors of PNN disruption in the HIV infected brain and the consequences of such on neuronal population dynamics have not, however, been well examined. In this study, we have sought to explore the role of Tat and specific MMPs in perineuronal net remodeling. We observe that Tat increased the expression and activity of MMP-3 and MMP-13. In vitro digest assays reveal that both MMP-3 and MMP-13 cleave the PNN proteins aggrecan, brevican, and neurocan. Furthermore, in HIV+ post-mortem brains of virally-suppressed individuals, active catalytic MMP-13 is abundantly detected. Future studies will evaluate MMP expression, PNN integrity, and MMP-dependent alterations in neuronal population dynamics in the Tat-injected brain. The identification of Tat-induced molecular effectors of PNN proteolysis and consequent effects on network activity may yield new therapeutic adjuncts for the treatment of HAND.

P33

Varicella zoster virus induces aberrant nuclear localization of neurokinin-1 receptor leading to cell process extension and viral spread that is blocked by aprepitant

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Latent varicella zoster virus (VZV) is present in 90% of the world population with >50% reactivating by 85 years of age leading to zoster, stroke, vision loss and other multisystem diseases. An under-recognized complication is VZV myelitis (paraparesis) that is recurrent and difficult to treat. Spinal astrocytes most likely play a significant role in the pathogenesis of VZV myelitis since these cells are preferentially infected by VZV in vivo and maintain a chronic inflammatory environment. Furthermore, neurokinin-1 receptor (NK-1R) signaling in astrocytes may be involved since it is critical in astrocyte network integrity and function. Thus, we hypothesize that NK-1R signaling is involved in cell-to-cell spread of VZV in primary human spinal astrocytes (HA-sp) contributing to VZV myelitis. Herein, HA-sp were mock- or VZV-infected and examined for localization of NK-1R, morphological changes and viral spread in the absence or presence of aprepitant, a commercially available pharmacological antagonist towards NK-1R. Results showed that VZV-infection of HA-sp induces aberrant nuclear localization of NK-1R in the absence of NK-1Rs normal substance P ligand that leads to extensive cellular processes and cell-to-cell spread of VZV. Aprepitant reduced process formation and subsequent viral spread. Overall, we: (1) showed a novel nuclear localization and proviral function of NK-1R in the absence of its ligand substance P in the primary human spinal astrocytes that differs from its cytoplasmic localization during substance P binding and subsequent role in pain signaling and immune cell activation; and (2) demonstrated that the commercially available antiemetic drug, aprepitant, has antiviral properties providing a much needed alternative to the currently available alphaherpesvirus nucleoside inhibitors.

P34

Plasma IP-10: a potential biomarker of HAND in HIV+ women

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Introduction: HIV associated neurocognitive disorders (HAND) is associated with persistent neuroimmune activation. We aimed to identify biomarkers associated with HAND and to investigate their association with cognitive function and sex, in a highly homogenous cohort of HIV+ young adults, parenterally infected during early childhood. **Methods:** A Romanian cohort of 145 HIV-infected subjects (51% females) without major confounders underwent standardized neurocognitive, psychological and medical evaluation in a cross sectional study. Levels of IL-1β, IL-6, IL-8, TNF-α, CXCL10/IP-10, MCP-1, and IL-8 were determined in plasma and cerebrospinal fluid (CSF) clinical specimens. Regression analysis was used to assess the effect of each log10 transformed biomarker level on neurocognitive outcomes, related to sex and clinical laboratory markers of HIV disease. **Results:** The median age was 23.7 years. In this cohort, 36.6% showed neurocognitive impairment (NCI). Median current CD4 was 481 cells/mm3 and 36.6% had detectable plasma viral load. Women had better previous and current neuromedical status as measured by HIV-associated clinical lab values. In plasma, controlling for sex, higher levels of IL-6 and TNF-alpha were significantly associated with increased odds of NCI ($p<0.05$). Plasma IP-10 showed a significant interaction with sex ($p=0.02$); higher values were associated with greater odds of NCI in females only ($p=0.02$). Individuals with undetectable viral load had significantly lower plasma IP-10 ($p<0.001$) and MCP-1 ($p=0.02$) levels, and CSF IP-10 ($p=0.01$), IL-6 ($p=0.03$), and TNF-alpha ($p=0.04$) levels. Current CD4 showed a negative correlation with IP-10 ($p<0.001$) and MCP-1 ($p=0.006$), and a positive correlation with IL-6 ($p=0.04$) and TNF-alpha ($p=0.006$). **Conclusions:** NCI was associated with elevated plasma IL-6 and TNF-alpha, but not CSF levels. Plasma IP-10 level is a closer correlate of NCI in women with chronic HIV infection, compared to men and other biomarkers.

P35

Mitochondrial dysfunction in methamphetamine-induced vulnerability for Parkinson's disease

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Methamphetamine (meth) abusers are at risk for developing Parkinson's disease (PD). We revealed that rats self-administering (SA) meth exhibit a forced abstinence (FA) time-dependent reduction in brain biomarkers for PD (Kousik et al. JPET 2014) indicating that meth initiates a pathological trajectory that may result in PD. Mitochondrial dysfunction underpins meth-mediated neuronal stress, and exposure to rotenone (a specific mitochondrial toxin) causes PD. Here we test the hypothesis that a meth-induced trajectory towards PD is associated with brain mitochondrial dysfunction. Male Sprague-Dawley rats self-administered meth (0.1mg/kg/0.1mL/infusion) daily for 2 weeks; controls were yoked to non-contingent (NC) infusions of saline or subcutaneous meth injections. To isolate the contribution of mitochondrial dysfunction in vivo, subclinical doses of rotenone were administered via subcutaneous osmotic minipumps during meth FA14-FA19. Assessments of mild PD-like motor deficits (forelimb bradykinesia) were performed weekly. Meth-SA rats exhibited progressive motor deficits on FA14 ($p<0.01$), while NC meth+rotenone rats exhibited deficits on FA21 ($p<0.01$). On FA56, meth+rotenone rats exhibited exacerbated deficits ($p<0.01$) compared to meth-SA+vehicle and NC meth+rotenone rats. These findings suggest that an interaction between meth and rotenone is present only with self-administered meth. Rats were sacrificed and PD-relevant brain regions were dissected out on FA62. Here, we report cytochrome c translocation, an early step in mitochondrial apoptosis, for the striatum. Meth-SA rats exhibited higher cytosolic levels ($p<0.01$) and lower mitochondrial levels ($p<0.01$) of cytochrome c compared to saline+vehicle and saline+rotenone, but no interaction between the two treatments was seen. Unexpectedly, mitochondrial cytochrome c was elevated in the NC meth+rotenone rats compared to all groups ($p<0.01$). These novel findings reveal that the mitochondrial toxin, rotenone exacerbates meth-induced PD-like motor deficits, but the behavioral effects are not associated with cytochrome c changes in the striatum. The findings also reveal dramatic differences in outcomes that depend on contingencies of meth administration.

P36

Osteopontin Counters HIV-Induced Neuronal Injury Through the Activation of mTOR and Beta-Integrin Signaling

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Collectively, we and other groups have shown that the multifunctional cytokine-like protein osteopontin (OPN) is elevated in the plasma, cerebrospinal fluid, and brain tissue of HIV-infected individuals with moderate to severe cognitive impairment. We have also shown that the source of OPN in the brain likely derives in part from its expression and release from cells of the myeloid, astrocytic and neuronal compartments. While it is known that OPN can signal through pro-survival pathways, as well as exacerbate pro-inflammatory ones, its impact on the integrity and function of neurons in the context of health and disease is largely unknown. In this study we used both differentiated human neuroblastoma cells and primary rat cortical neurons to ask whether OPN protects or enhances HIV IIIB envelope-induced neuronal injury. We found that OPN, in a dose-dependent fashion, significantly reduces HIV Env-induced neuronal cell death. The protective mechanism involves signalling through the mammalian target of rapamycin (mTOR), as the latter activity is blocked by rapamycin treatment. Western analyses showed that mTOR, as well as downstream targets including pS6 ribosomal protein, are activated in neurons exposed to HIV Env and OPN. Unexpectedly, we observed that OPN, in a dose-dependent manner in the presence or absence of HIV Env, increased neurite growth as measured by β3-tubulin expression. Further examination of primary neurons exposed to HIV Env and increasing doses of OPN using MAP2 staining revealed that dendritic processes are significantly increased, while axonal protein neurofilament levels were decreased. Interestingly, blocking integrin signaling differentially implicated these receptors in the neurite outgrowth phenotype. Our findings suggest a link between β-integrin and mTOR pathways in the protective and neurite outgrowth effects of OPN on neurons.

P37

Engineering Gesicles using the GPI-(MYC)linker-CCL2 construct to enhance CRISPR/Cas9 delivery to inactivate the HIV Provirus

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The CRISPR/Cas9 system has been used to target integrated HIV provirus, resulting in proviral mutation, excision, and decreased proviral activity. Therefore, strategies to increase the efficiency and the target cell specificity of CRISPR/Cas9 delivery would facilitate the translation of this method to human therapies. Utilizing a specific microvesicle termed a "gesicle", we can transiently deliver Cas9 in a ribonucleoprotein form. We further show that gesicle-mediated delivery of CRISPR/Cas9 can disrupt HIV proviral activity when coupled with guide RNA's targeted to the HIV long terminal repeat. To enhance specificity and efficacy of gesicles, we altered the membrane components of their producer cells (HEK293FT) to express a tethered chemokine CCL2, using the GPI-(Myc)linker-CCL2 construct. CCL2 is a well-established migratory factor for monocytes, macrophages, and microglia which express the cognate receptor CCR2. We show that transfection of HEK293FT producer cells with the GPI-(Myc)linker-CCL2 construct results in the expression of (Myc)linker and CCL2 based on immunocytochemistry and upregulated (Myc)linker expression confirmed by western blot. Gesicles were then prepared from either non-transfected producer cells (control gesicles) or producer cells transfected with the GPI-(Myc)linker-CCL2 construct (CCL2-GPI gesicles). Both gesicle populations containing Cas9 and LTR-targeted gRNAs were applied to HIV-NanoLuc CHME-5 E9 cells, a microglia-like cell line that harbors stably integrated HIV proviruses expressing NanoLuciferase. The CCL2-GPI-containing gesicles significantly decreased proviral activity both basally and after TNF-α stimulation to a greater effect when compared to control gesicles. These data suggest that membrane bound chemokines may serve as a strategy to enhance cellular specificity and efficiency of gesicle-mediated delivery of CRISPR/Cas9.

P38

HIV infected macrophages secrete cathepsin B/serum amyloid p complex in and outside exosomes contributing to neuronal dysfunction

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HIV-infected macrophages infiltrate the brain contributing to the development of HIV-associated neurocognitive disorders (HAND). Mild forms of HAND prevail in 20-50% of the patients. HIV-infected macrophages secrete the lysosomal protease cathepsin B (CATB) with serum amyloid p component (SAPC), as a neurotoxic protein complex. We hypothesized that CATB is released in exosomes and internalized by neurons, triggering dysfunction. We exposed neurons to histidine-tagged CATB and localized the histidine tag in neurons by western blot. His-CATB was internalized by neurons ($p=0.007$), triggered activation of caspase 3, and decreased synaptophysin. However, pre-treating the media with anti-CATB and SAPC antibodies reduced CATB internalization by 20% and 40%, respectively, and rescued the neurons from apoptosis and synapse loss. His-CATB slightly increased the amount of amyloid beta peptides, which was decreased by pre-treatment with SAPC antibodies. In addition, neurons exposed to His-CATB in HIV-infected macrophage-conditioned media (MCM) internalized more CATB than in uninfected media. We then examined the presence of CATB and SAPC in exosomes isolated

from MCM, by western blot and ELISA. Exosomes contain 34% of the total secreted CATB, as well as SAPC, CD63 and Hsp70. Exosomes from HIV-infected macrophages contain more cathepsin B (p=0.017), increased the activity of caspase-3/7 in neurons (1.5 fold), and triggered neuronal death (p=0.026; TUNEL assay), compared to exosomes from uninfected macrophages. Our results suggest a novel neuronal damage process in which CATB: (1) is internalized by neurons triggering apoptosis and decreasing synapses, (2) contributes to the amyloid beta load, and (3) is partially secreted in exosomes inducing HIV-neurotoxicity. Since CATB and amyloid beta are elevated in the brain of HAND and Alzheimer's patients compared to normal cognition, we conclude that CATB/SAPC complex plays an important role in neurodegeneration. Further studies will allow us to determine if CATB/SAPC inhibitors could be considered as therapy candidates against HAND.

P39

Effects of morphine on LP-BM5 murine retrovirus-induced CNS infection in gp120 transgenic mice

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HIV-associated neurocognitive disorders (HAND) affect nearly half of HIV-infected individuals. We recently demonstrated that LP-BM5 murine retrovirus (the agent that causes murine AIDS) infection in HIV gp120tg mice can be used as a model system to study the pathophysiology of HAND. As opioid drug abuse significantly increases the risk and severity of HAND, we investigated the effects of morphine, the prototypical opioid, on LP-BM5 induced central nervous system infection in gp120tg mice. As expected, LP-BM5 induced splenomegaly in gp120tg+ and gp120tg- mice (sign of immunodeficiency in MAIDS), which was slightly inhibited by morphine treatment in gp120tg+ mice. gp120tg+ but not gp120tg- mice that received LP-BM5+ morphine showed reduced performance in a spontaneous alteration T maze assay, a behavioral test that primarily measures hippocampus-related learning and memory function. To determine whether changes in CNS inflammation were involved in this behavioral change, a panel of proinflammatory factors was further assessed in the hippocampus. LP-BM5 alone significantly increased the transcript levels of TNFα, IL-12p40, CCL2, CCL3, CCL5, CXCL10, and iNOS. Although morphine alone also increased the RNA levels of these factors to varying degrees, morphine significantly reduced LP-BM5-induced upregulation of IL-12p40, CCL2, CCL3, CCL5, and CXCL10. Altogether, our results show a paradoxical relationship between morphine's inhibition of LP-BM5-induced hippocampal proinflammatory factors and morphine-facilitated learning/memory deficits in LP-BM5 infected gp120tg+ mice. Further investigation is necessary to identify factors that affect cognitive functions in gp120tg+ mice treated with morphine+LP-BM5.

P40

Pathogenic strains of ZIKV induce gene expression promoting inflammation and apoptosis in primary human placental trophoblasts

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Placental insufficiency is an uncommon complication of pregnancy in which the placenta does not deliver sufficient nutrients to the developing fetus resulting in miscarriages and/or small fetuses. Pregnant women infected with Zika virus (ZIKV) have recently been reported to have placental insufficiency which may contribute to ZIKV-associated fetal microcephaly. Infection with the pathogenic American/Asian strains of ZIKV is associated with fetal microcephaly, brain malformations, and placental insufficiency. However, the direct impact of ZIKV on the placenta is unclear. Here we examined functional effects and the gene expression profiles of placental human villous trophoblast (HVTs) infected with either the non-pathogenic ZIKV strain, MR766 (Ugandan) or the pathogenic ZIKV-FLR (Colombian). Using a quantitative polymerase chain reaction (qPCR) array we compared gene expression profiles of HVTs infected with either ZIKV-MR766 or ZIKV-FLR to uninfected HVTs. Genes integral to inflammatory, apoptotic, and immune response pathways, specifically CCL5, TNF, WISP1, BIRC3, and STAT1, were upregulated in HVTs infected with ZIKV-FLR. But these aforementioned genes did not present any significant change in gene expression in HVTs infected with ZIKV-MR766. Upregulation of GATA3 and STAT1, both of which promotes Th2 differentiation, was observed only in the HVTs infected with ZIKV-FLR. Downregulation of BCL2A1, another gene involved in an apoptosis modulating pathway, was observed only in HVTs infected with ZIKV-FLR. Interestingly, ZIKV-MR766 infection of HVTs resulted in the downregulation of WNT1, a gene integral to CNS development and the establishment of planar cell polarity, but is unaltered in ZIKV-FLR infection. Lastly, caspase-3 activation in HVTs was examined. Caspase-3 was found to be differentially activated in ZIKV-MR766 HVTs when compared to ZIKV-FLR HVTs. Together, these changes in HVT gene expression and caspase-3 activation demonstrate that the ZIKV-FLR strain may induce pathogenesis by modulating apoptosis, and facilitating a pro-inflammatory environment that may enable ZIKV entry through the placental barrier.

P41

Interferon alpha mediated suppression of JCV is PI3K/AKT and mTOR pathway dependent

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The severe demyelinating disease progressive multifocal leukoencephalopathy (PML) is caused by the neurotropic polyomavirus JC (JCV), which replicates in oligodendrocytes and astrocytes in the brain. Initiation of PML occurs occasionally under conditions of immune dysfunction and results from the reactivation of persistent virus from an inactivate state to replicate lytically. In this study, we have now examined the cytokine interferon-α (IFN-α), which, in contrast, has a negative effect on JCV gene expression and replication. IFN-α and IFN-β inhibited the replication of JCV in primary human fetal astrocytes and reduced transcription by JCV promoter reporter constructs in oligodendroglioma cells. We found that IFN-α treatment of glial cells induced expression of STAT1 and caused STAT1 phosphorylation and translocation to the nucleus. Other downstream signaling events were also examined including PI3K/Akt and mTOR and inhibition of PI3K with LY294002 was found to enhance JCV replication while rapamycin inhibition of mTOR affected STAT1 translocation to the nucleus. We conclude that pathways downstream of IFN-α negatively regulate JCV gene expression and replication and INF alpha induced PI3K/AKT and mTOR activity are important regulator factors for anti- JCV activity.

P42

Eradication of VZV Genome using Sacas9 gRNAs Based Gene Therapy

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Varicella zoster virus (VZV) belongs to the α-Herpesviridae family an is exclusively a human virus. VZV is the etiological agent of chickenpox

and herpes zoster (shingles). A primary exposure to VZV lead to Chickenpox, a relatively mild, self-limited childhood illness with a characteristic exanthem, but in immunocompromised children can be more severe. Reactivation of VZV causes dermatomal herpes zoster (HZ) and more rarely severe disseminated HZ including diffuse rash, encephalitis, hepatitis, and pneumonitis. Chickenpox is one of the most contagious infectious diseases, especially in the early stages of the eruption. Person-to-person transmission is due to aerosol, when an affected person coughs or sneezes, or by direct contact with varicella or zoster injury. Actually there is a vaccination against VZV, the Oka vaccine. Vaccination is usually carried out with a single dose to children between 12 months and 12 years, and with two doses in those over 12 years. In this study we examined the potential role of CRISPR/Cas9 gene editing on VZV. We designed 3 gRNAs based on SaCas9 targeting the ORF63, one of the most relevant gene expressed during latency. To test the ability of our construct to induce site specific cleavage and excision of VZV genome, we used a stable cell line for ORF63 gene. Our results indicate CRISPR/Cas9-mediated cleavage of ORF63 gene. To verify the specificity of our excision strategy in targeting the viral genome, we performed analysis of the predicted/possible off targets sites in the human genome. No cleavage were detected for our off-targets. Our experiment showed the efficiency and specificity of CRISPR/Cas9 in targeting the VZV genome.

P43

Murine leukemia virus expressing HIV Tat (MLV-Tat) transactivates HIV LTR in cells in culture and induces neurocognitive impairment in MLV-Tat infected mice

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HIV infects CNS early after primary infection and HIV infected individuals are at high risk of cognitive and motor diseases known in aggregate as HIV-associated neurocognitive disorders (HAND). Successful combination antiretroviral therapy (cART) prevents HIV dementia, the most severe HAND manifestation, but not the milder neurocognitive impairments known as NCI or mild HAND, which are currently observed in about 50% of HIV positive individuals on cART. cART suppresses HIV replication but HIV can persist at low-levels in cellular reservoirs of the virus, primarily latently infected but inducible T cells and chronically infected macrophages. These cells can produce pathogenic viral and cellular proteins that contribute to chronic diseases in these patients, including mild HAND. We have previously shown that EcoHIV, chimeric HIV that expresses MLV envelope protein in place of gp120 and can infect mice, causes cognitive impairment resembling mild HAND. EcoHIV carries all HIV genes except gp120, and it is not clear which of EcoHIV functions may be responsible for the cognitive disease observed in infected mice. Here we describe construction of modified infectious MLV that expresses HIV Tat, called MLV-Tat. We show that MLV-Tat can infect mouse cells in vitro and transactivate HIV LTR, demonstrating that MLV-expressed Tat is biologically active. MLV-Tat was also infectious in three different mouse strains we tested as indicated by expression of MLV envelope and tat message after virus inoculation. Most intriguingly, MLV-Tat but not MLV infected mice showed evidence of cognitive impairment when tested in radial arm water maze 30 days but not ten days after infection and we showed these mice developed peripheral infection tested in thymus. Our data show that HIV-Tat is a sole contributor to cognitive impairment observed in MLV- Tat infected mice and suggest critical role of this protein in pathogenesis of mild HAND in people.

P44

Cinnamic acid stimulates lysosomal biogenesis and enhances memory in an animal model of Alzheimer's Disease via PPARα

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Abnormal lysosomal function and dysregulation of autophagy are key drivers in the pathogenesis of many neurodegenerative and lysosomal storage disorders. In this study, we explored whether cinnamic acid (CA), a metabolite cinnamon, can induce lysosomal biogenesis in mouse primary brain cells and thus might have implications for lysosomal disorders. CA enhanced the total lysosome content in primary astrocytes indicated by the lysotracker staining which was further confirmed by the increase in autophagic vesicle demonstrated by electron microscopy. The mRNA expressions of different lysosomal markers including LAMP2, LIMP2, NPC1, CLN2 were increased following CA treatment. Immunocytochemistry studies showed increased LAMP2 expression in astrocytes as well as cortical neuronal cultures. In addition, the activity of lysosomal enzyme tripeptidyl peptidase 1 was augmented in CA-treated astrocytes, indicating that CA can enhance the functionality of lysosomal enzymes. Furthermore, TFEB, the master regulator of lysosomal biogenesis, was found to be upregulated by CA suggesting CA induces lysosomal biogenesis via TFEB. Interestingly, CA induced the activation of PPARα and upregulated TFEB and lysosomal biogenesis in astrocytes via PPARα. Accordingly, CA treatment increased the level of TFEB, reduced the cerebral load of amyloid plaques and improved memory and learning in 5XFAD, but not 5XFAD/PPARα-/-, mice. Our study identifies a novel role for CA in inducing lysosomal biogenesis via PPARα mediated transcriptional upregulation of TFEB and highlights the therapeutic potential of CA for Alzheimer's disease and other lysosomal storage disorders.

This study was supported by a merit award from the Department of Veteran Affairs (1I01BX003033) and a grant (AT006681) from National Institutes of Health.

P45

An anti-viral immune response is associated with increased neural stem/progenitor cell proliferation and neurogenesis in the adult brain

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Viral infections in the central nervous system (CNS) alter neural stem/progenitor cell (NSPC) activity and survival via direct infection by the virus or potentially through the effects of the anti-viral immune response. Our previous work demonstrated that neonatal NSPCs were protected by the anti-viral cytokine, interferon gamma (IFNγ), during a CNS infection, although the neonates ultimately succumbed to the infection. Here, we examined the response of adult NSPCs during CNS infection, where the anti-viral immune response controls the virus and the mice survive. In order to test the response of adult NSPCs in vivo, we utilized the NSE-CD46+ transgenic mouse model. This line expresses human CD46, a measles virus (MV) receptor, under the control of the neuron specific enolase promoter. Thus, only CNS neurons are infected by MV, sparing the NSPCs from viral infection. We infected adult mice at postnatal day 60 and analyzed the hippocampus by flow cytometry at 7, 21, and 60 days post-infection (dpi). In MV-infected adults, there was no change in nestin+ NSPCs at 7 and 21 dpi, but there was an increase in nestin+ cells at 60 dpi. A similar pattern was observed in immature neurons stained for doublecortin. In contrast, glial precursor cells (A2B5+) were unchanged by infection. In adult mice lacking IFNγ (CD46+/IFNγ-KO), there was an increase in NSPCs at 21 and 60 dpi, and an increase in the immature neuron population at 21 dpi only. This increase in NSPCs and immature neurons at 21 dpi might be attributed to a higher neuronal dropout in the CD46+/IFNγ-KO infected mice compared to the CD46+ infected mice. Current studies aim to understand age-dependent differences in IFNγ signaling in NSPCs. These studies highlight the importance of anti-viral immune responses on adult NSPCs, and may link the control of NSPC proliferation and neurogenesis to specific anti-viral cytokines.

P46

Serum levels of ATP is a biomarker of HIV cognitive disease: Role in Blood-Brain Barrier compromise and CNS function

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Despite widespread availability of antiretroviral medications in the developed world, HIV-associated neurocognitive disorders (HAND) remains prevalent in around half of the HIV infected population. After the initial infection, HIV infiltration into the CNS promptly occurs due to infected circulating monocyte/macrophages entering the brain, which result in the infection of CNS resident microglia, macrophages and a small population of astrocytes. In the current ART era, CNS damage is localized into small areas of the brain, and cognitive disease extremely complicates the predict. We recently found that all HIV infected individuals have constitutively open pannexin-1 channels resulting in the release of several intracellular factors including PGE2, and ATP. We identify that ATP, but not PGE2, is highly associated with cognitive disease and could be a biomarker of cognitive disease in the HIV infected population. Thus, we hypothesize that "ATP is a biomarker of cognitive impairment and a player in local HIV associated damage." In our study, we demonstrated that i.) ATP is a biomarker of cognitive, ii.) ATP help to disrupt the blood-brain barrier, iii.) ATP compromise TJP expression/localization, and iv.) ATP helps in transmigration of monocytes across the blood-brain barrier. All aspects observed in NeuroHIV. Our studies demonstrate the potential use of ATP and accompanying molecules including PGE2 as potential biomarkers for HIV related cognitive disease.

P47

Nitrosative damage during retrovirus infection-induced neuropathic pain

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Peripheral neuropathy is currently the most common neurological complication in HIV-infected individuals, occurring in 35-50% of patients undergoing combination anti-retroviral therapy. Data have shown that distal symmetric polyneuropathy develops in mice by 6 weeks following infection with the LP-BM5 retrovirus mixture. Previous work from our laboratory has demonstrated that glial cells modulate antiviral T-cell effector responses through the programmed death (PD)-1: PD-L1 pathway, thereby limiting the deleterious consequences of unrestrained neuroinflammation. Using the MouseMet electronic von Frey system, we observed hind-paw mechanical hypersensitivity in LP-BM5-infected animals and it was clearly associated with significantly increased lymphocyte infiltration into the spinal cord. We also observed elevated expression of IFN-γ in Lumbar spinal cord (LSC) and dorsal root ganglion (DRG) and MHC II (on resident microglia in LSC). Using Western blotting, we detected elevated levels of 3-nitrotyrosine within the LSC and DRG of LP-BM5 infected animals, an indicator of nitric oxide (NO)-induced protein damage. Additionally, infected PD-1 KO animals displayed significantly greater mechanical hypersensitivity than WT or uninfected mice at 4 weeks post-infection (p.i.). Accelerated onset of hind-paw hypersensitivity in PD-1 KO animals was associated with significantly increased infiltration of CD4+ and CD8+ T lymphocytes, macrophages and microglial activation at early time points. Importantly, we also observed elevated levels of 3-nitrotyrosine within the LSC and DRG in infected PD-1 KO animals when compared with WT animals. Results reported here connect peripheral immune cell infiltration and reactive gliosis with nitrosative damage. These data may help elucidate how retroviral infection-induced neuroinflammatory networks contribute to nerve damage and neuropathic pain.

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MicroRNA alteration in astrocytes caused by HIV-1 Tat protein and opiates elucidates potential mechanisms underlying HIV-associated neurocognitive disorders (HAND)

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HIV-associated neurocognitive disorder (HAND) impacts nearly one-half of HIV infected patients, including those on current therapy. Mechanisms underlying HAND remain unclear and targeted CNS therapy does not exist. Evidence for HIV-1 Tat protein and morphine have both been implicated in exacerbation of neuroinflammation. Tat protein can inhibit Dicer endoribonuclease cleavage necessary for microRNA maturation. We recently found that Tat binds and inhibits miRNAs to downregulate β-catenin signaling, a pathway shown to be protective against HIV-1 neuroinflammation. The direct mechanism behind Tat-miRNA on neuroinflammatory relevant pathways, such as β-catenin, is unknown. Our objective is to determine the role of miRNAs altered by Tat protein and morphine on HAND outcomes. We hypothesize worsening aberrations in miRNA and inflammation in combined treatment. To address this hypothesis, we cultured primary fetal astrocytes (PFA) and U-87MG astrocyte cell lines, transfected with Tat plasmid, and subsequently treated with morphine. Luciferase reporter assay was used to quantify β-catenin activity. We then profiled changes of 380 miRNAs by RT-qPCR on Tat and morphine treated cells. In PFA, morphine increased the suppressive ability of Tat on β-catenin activity compared to Tat alone in a dose-specific manner. Similarly, U-87MG cells treated with lower dosage of morphine showed increased ability of Tat β-catenin suppression. In U-87MG cells, 90 miRNAs were uniquely dysregulated by a 3-fold change or more in combined Tat and morphine treatment. We observed morphine ability to increase the suppressive ability of Tat on β-catenin activity in a dose-dependent manner. Moreover, there was greater miRNA dysregulation in combined treatments compared to individual treatments. Of these uniquely modified miRNAs in our combined group, we found relevant targets related to axonal guidance, neurotrophin signaling, cytokines/chemokine signaling, and glucocorticoid signaling. Our finding suggests worsening neuropathology in joined Tat and morphine interactions.

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Impaired Insulin Sensitivity Predicts Worsening Cognitive Function in HIV-infected Subjects

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To determine the association of insulin sensitivity and metabolic status with declining cognition in HIV-infected individuals, we conducted untargeted lipidomic analyses, targeted clinical, and metabolic measures in longitudinal plasma samples obtained from HIV-infected patients enrolled in the Central Nervous System HIV Anti-Retroviral Therapy Effects Research Study. Findings were validated using plasma samples

from the Multi Center AIDS Cohort Study (MACS). Subjects were grouped according to longitudinally and serially assessed cognitive performance as stably normal or declining cognition. Subjects with declining cognition exhibited baseline hyperinsulinemia (95.5±7.2 vs. 60.3±5.1 pmol/L; p<0.0002), elevated plasma triacylglycerides (TAG, n= 6 species) and monoalkyldiacylglycerides (MADAG, n= 6 species), with decreased phospholipids (PL, n= 28 species). C-peptide was elevated (2.8 ±0.3 vs. 1.6±0.2 ng/mL; p<0.002) with normal c-peptide/insulin ratios suggesting that insulin production was increased, but insulin clearance was normal. The association of a baseline insulin resistance with worsening cognition was further supported by low HDL (37.4±3.1 vs. 50.6 mg/dL; p<0.01), high LDL/HDL (2.7±0.3 vs. 1.9±0.2 mg/dL; p<0.03), and elevated cholesterol/HDL ratios (4.9±0.4 vs. 3.7±0.3; mg/dL: p<0.02) compared to subjects with stably normal cognition. The overall increase in circulating levels of neutral lipids and the association of impaired insulin sensitivity and cognitive decline was validated in the independent cohort of HIV-infected subjects (MACS). These findings suggest that insulin resistance and mobilization of fatty acid energy stores precedes cognitive decline in ART-treated HIV-infected subjects.

P50

Endolysosome de-acidification affects the structure and function of endolysosomes as well as their ability to interact with and signal between other intracellular organelles

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Endolysosomes are highly dynamic acidic organelles critical for a wide spectrum of biological functions including their classical roles in degrading various macromolecules, damaged organelles, and protein aggregates, and their new roles in signaling and plasma membrane repair. Endolysosomes are especially important for neural cells, because neurons are post-mitotic cells that require constant protein quality control and membrane turnover. The acidic environment ion the lumen of endolysosomes is maintained primarily by vacuolar H+ ATPase. Besides high concentrations of protons, endolysosomes contain a spectrum of highly active protease enzymes as well as high concentrations of a variety of cations including calcium and iron. Loss of the proton gradient in endolysosomes leads to de-acidification, impaired degradation capabilities, abnormal accumulation of various macromolecules, and a profound redistribution of endolysosomes inside of cells. Endolysosome de-acidification also leads to efflux of readily releasable stores of calcium and iron, and these released cations can affect endolysosome trafficking and the cross-talk between endolysosomes and other organelles. Here, we will present data from others and us showing that neuronal endolysosomes contain readily releasable stores of calcium, that calcium released from endolysosomes upon deacidification can induce the release of calcium from other organelles and increase influx through the plasma membrane, that deacidification-induced calcium release from lysosomes leads to lysosome exocytosis, and that deacidification-induced iron release can affect mitochondria function. Increased awareness of the complexities involved in inter-organellar signaling might help lead to a renewed interest in cell biology, classically defined.

This work was supported by P30GM103329, R01MH100972, R01MH105329, R21DA040519.

P51

Hyperactivity of medium spiny neurons in the dorsal striatum is associated with unique Ca2+ and K+ channel dysfunction in 12-month-old HIV-1 Tg rats

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Despite combination antiretroviral therapy (cART), HIV-associated neurocognitive disorders (HAND) occur in >50% of HIV+ patients. The striatum is a brain region in the basal ganglia, which is one of the key regulators of cognitive function, and susceptible and vulnerable to HIV-1. We have previously demonstrated a hyper-excitability of mPFC pyramidal neurons in HIV-1 transgenic (Tg) rats (that express 7 of 9 HIV-1 proteins), which is associated with altered voltage-gated Ca2+ channel (VGCC) activity and K+ influx, independent of NMDA receptor activation. mPFC neurons are glutamatergic, which provide excitatory drive to the striatum and certain other cortical/subcortical areas that are likely involved in HAND. Here we assessed the impact of HIV on medium spiny neurons (MSNs), a predominating neuron type in the dorsal striatum (the caudate-putamen) of 12-month-old (12m) HIV-1 Tg rats using whole-cell patch-clamp recording. We found that the majority of MSNs (~75%) in non-Tg rats responded to moderate excitatory stimuli (≤300pA depolarizing currents, which mimicked physiological excitatory inputs), though the others were less responsive. Evoked firing was abnormally increased in these MSNs from HIV-1 Tg rats compared to those from age-matched non-Tg rats. Intriguingly, this hyperactivity of striatal MSNs was associated with reduced Ca2+ influx through VGCCs; increased expression of less functional VGCCs, and enhanced K+ efflux through Kv channels in MSNs from 12m HIV-1 Tg rats; all could render MSNs less excitable. However, membrane hyperpolarization-induced K+ influx through inwardly-rectifying Kir channels was significantly increased in MSNs from HIV-1 Tg rats. Such increased K+ influx could elevate intracellular K+ during resting status, thereby facilitating membrane depolarization and firing in response to excitatory inputs. This Kir channel dysfunction is unmasked by blocking glutamate/GABA receptors, suggesting that abnormally-increased Kir activity in striatal MSNs of 12m HIV-1 Tg rats is also associated with dysregulation of glutamate/GABA transmission.

P52

HIV Nef and ART Disrupt Autophagy in Human Astrocytes and may Contribute to HIV-associated Neurocognitive Disorders

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HIV-associated neurocognitive disorders (HAND) affect 40-70% of HIV-infected people despite ART. The pathogenesis of HAND is multifactorial and not well-understood. Macroautophagy is a cellular self-digestion process with essential roles in defense against infection, aging, and neurodegeneration. Some studies demonstrate that HIV disrupts macroautophagy in CNS cells; however, this disruption is inadequately characterized. We treated primary human astrocytes with HIV Nef and/or Tenofovir+Emtricitabine+Raltegravir (ART), and cell lysates were collected for western blot analysis of LC3-II and p62, and RNA for qRT-PCR of p62, macroautophagy markers. We show that HIV Nef significantly increases LC3-II flux with minimal impact on p62 flux, suggesting Nef may increase in-bulk macroautophagy, i.e., it increases autophagy non-selectively. ART treatment results in a significant increase in LC3-II flux with a trend toward increased p62 flux, indicating selective macroautophagy is potentially increased in astrocytes relative to untreated cells. P62 transcription was not changed relative to control cells after treatment with either Nef or ART. When treated with Nef and ART, LC3-II and p62 flux in astrocytes are not significantly different from untreated cells, suggesting a "normalization" of the changes observed after treatment with either Nef or ART. Our initial study demonstrates that HIV Nef and commonly prescribed ART mediate changes in autophagy in astrocytes. To our knowledge, this is the first time changes in macroautophagy have been described due to these factors in astrocytes. Interestingly, the effect of Nef and ART on autophagy in astrocytes may be either beneficial for the cell (slow viral production if autophagy activation by Nef is dampened in the presence of ART), or, favor viral toxicity (if ART dampens cell-mediated autophagy activation due to the

presence of Nef). Ultimately, HIV Nef and ART may disrupt the homeostasis provided by autophagic flux, contributing to accelerated aging and HAND in HIV-infected people.

P53

Cocaine-mediated gut microbiome changes and peripheral inflammation: Implications on the gut-brain axis

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Cocaine is a psychostimulant that acts on brain cells to mediate its addictive effects. Emerging evidence indicates that the gut also contributes to the development of drug addiction. Cocaine-induced microbial dysbiosis and epithelial dysfunction could likely play a role in this process. We thus sought to examine the effect of cocaine on alterations in gut microbiota as well as inflammation and mucus production. Mice were administrated cocaine for 7 days (n = 10, i.p., 20 mg/kg) and sacrificed 24 h after the last injection. DNA was isolated from colonic digesta and sequenced to identify bacterial community profiles using the 16S rRNA gene. Cocaine exposure significantly decreased gut colonization of four genera i.e. Erysipelotrichaceae, Allobaculum, Parasutterella and Clostridium sensu stricto in the colon, and increased colonization of five other generas (Intestinimonas, Mycoplasma, Pseudonocardia, Chloroflexi and Clostridiales), suggesting alterations of gut homeostasis. Also, we found significantly increased levels of inflammatory mediator (IL-1β) and a concomitant decreasing trend in mucus production in the cocaine-treated mice compared with the controls. To elucidate the mechanism(s) of cocaine-induced gut alterations, we analyzed colon samples for endoplasmic reticulum stress (ER) induction and found upregulation of cleaved Activating Transcription Factor 6 (ATF6) that initiates the unfolded protein response during ER stress. In addition, colonic levels of Binding Protein (BIP), an ER chaperon protein, were also significantly increased in the cocaine-treated group compared with controls. Collectively, these findings suggest that cocaine induces ER stress, alters mucus production and microbiota in the gut, potentially contributing to decreased epithelial protection and increased peripheral inflammation. The future directions of this study will be to explore the contribution of cocaine-induced gut inflammation to neuroinflammation and its role in the development of drug addiction.

Supported by R01DA043138

P54

HIV-1 alters expression of connexin 43 in human neural progenitor cells

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Brain infection and its consequent neurocognitive impairment remain a prevalent comorbidity in HIV infected individuals, even when viral titers are well controlled by highly active antiretroviral therapy. Adult neural progenitor cells have recently drawn more attention given the vital and active nature of their participation in brain physiology, and generation of neuron and glial population in the central nervous system. We have recently demonstrated that a selective population of neural progenitor cells is prone to HIV infection, generating potential new cellular sites carrying latent HIV infection in the brain. In the present study, we hypothesize that injury signals are propagated from infected neural progenitor cells to neighboring cells. To address this notion, we investigated these bystander effects by focusing on the role of gap junctions (GJs) between neural progenitor cells. A dye coupling assay monitoring the dye transfer between adjacent cells demonstrated a significant increase after HIV infection. These results suggest that HIV infection can alter intercellular communication between non-infected and HIV-infected cells. To determine the impact of HIV infection on GJ protein expression in human neural progenitor cells, levels of connexin 43, a prominent GJ protein, were examined by immunoblotting and immunostaining. Our results demonstrated a marked up-regulation of connexin 43 in HIV-infected human neural progenitor cells. In conclusion, HIV infection markedly affects intercellular communication and connexin 43 levels in human neural progenitor cells.

Supported in part by NIH 1R01HL126559 and R01MH072567.

P55

Impact of human genetic variation and DNA accessibility on the off-target effects of anti-HIV-1 gRNA design

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Despite antiretroviral therapy, human immunodeficiency virus type 1 (HIV-1) infection remains a life-long clinical problem due to reservoirs harboring proviral DNA in a latent/persistent form in infected cells. Recently, gene-editing strategies utilizing the CRISPR/Cas9 system have been developed to excise the HIV-1 genome from infected cells by developing a set of guide RNAs (gRNAs) homologous to the integrated viral genome. However, the promiscuous nature of Cas9 system allows for the potential off-target cleavage of similar sequences across the human genome. This is further complicated by the genetic variability across the human population, as well as the chromatin structure at potential off-target sites. To predict the off-target effect accounting for genetic variation, we have developed a screening method employing a bloom filter constructed from a database of human genetic information from 2,504 individuals in the 1000 Genomes project. A bloom filter allows for an extremely compact representation of the database with a rapid ability to detect whether a sequence is present in the database. To test the results of this method, more than 140 anti-HIV-1 LTR gRNAs were collected from previously published studies. This analysis showed that various off-target sites identified among different demographic categories of the human population using multiple published models of CRISPR/Cas9 recognition. GUIDE-seq and DNase-seq data were used for DNA accessibility and tested on HEK293T and U2OS cells. Results from this analysis have shown that, in a given genomic region, the DNA accessibility is positively correlated with the frequency of CC9 activity. Compiling these results will greatly increase our ability to design gRNAs that are effective with respect to targeting integrated HIV-1 proviral DNA while avoiding off-target effects detrimental to host cell function and survival; an absolutely critical aspect of gRNA design for developing CRISPR-based gene therapies for HIV/AIDS and other infectious diseases as well as cancers.

P56

Regulation of ZIKV infection by SRSF1 and IFN-gamma in human astrocytes

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Zika virus (ZIKV) is an emerging human pathogen with a potential of causing significant health concerns in fetus, neonates, and adults. The major public health significance of ZIKV infection lies in the increasing body of evidence linking it to microcephaly in neonates and Guillain-Barré syndrome in adults. There is limited understanding of viral pathogenesis and consequences of neuro-invasion of the virus with no available anti-ZIKV therapy. Neurological complications associated with ZIKV infection suggest that virus can invade into the central nervous system (CNS). Recent studies of human neuronal progenitor cells (hNPCs) have been suggested that ZIKV can replicate in these cells and induce apoptosis resulting in depleted progenitor pool. On the other hand, intracranial

injection of ZIKV in to the wild-type adult mouse results in widespread infection and activation of glia suggesting that glia could also be targeted by ZIKV in the brain. There is no current report regarding the replication of ZIKV in human glial cells. Astrocytes are the main supporting glial cells in the brain and play crucial roles in neurogenesis and neuronal survival. Our data suggest that ZIKV can infect and replicate in human primary astrocytes. More interestingly, the replication rate of ZIKV in astrocytes is much higher than hNPCs and microglial cells suggesting that astrocytes could be the main target of ZIKV in the CNS. Our experiments have also suggested that ZIKV infection in astrocytes downregulates cellular splicing factor 2 / alternative splicing factor (SF2/ASF, SRSF1), a cellular protein which has critical roles in RNA processing as well as protein translation. Accordingly, overexpression of SRSF1 causes a dramatic reduction in ZIKV replication suggesting a novel role of SRSF1 in suppression of ZIKV. Moreover, treatment of primary human astrocytes with IFNγ increases SRSF1 expression and profoundly suppresses replication of ZIKV in astrocytes.

P57

HIV-1 Tat modulates cognitive performance in an assay-dependent manner

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Although antiretroviral therapy (cART) has decreased the severity of Human Immunodeficiency Virus (HIV)-associated dementia, cognitive impairment and the prevalence of HIV Associated Neurocognitive Disorders (HAND) persists. The means by which HAND persists in the presence of cART in HIV+ patients remains under investigation. In animal studies, exposure to the inflammatory HIV-1 regulatory protein transactivator of transcription (Tat) was found to produce cognitive learning and memory deficits. We hypothesized that brain exposure to HIV-1 Tat protein is sufficient to induce oxidative stress and neuroinflammation, ultimately impairing cognitive performance. Using the iTat bigenic mouse model, where brain-selective Tat expression is controlled by activation of a GFAP-linked doxycycline (Dox) promotor, we tested the effects of Tat protein on two non-hippocampal associated cognitive tasks, the Pre-Pulse Inhibition (PPI) model of sensorimotor gating and the prefrontal cortex (PFC)-dependent Attentional Set Shift task. Western blot analysis characterized the expression of Tat protein in iTat bigenic mouse brain that correlated with dose and duration of Dox treatment, findings confirmed with a novel aptamer-based fluorescent assay for Tat protein. Magnitude of exposure to Tat protein was shown to elevate total ROS/RNS levels in whole brain homogenate assessed by a fluorophore probe assay and activate microglia in the PFC as measured by Iba1 immunohistochemical labeling. Behaviorally, a 1, 7 or 14 day exposure to HIV-1 Tat protein attenuated pre-pulse inhibition. In contrast, a 14-day exposure to HIV-1 Tat protein improved performance in sessions of the Attentional Set Shift associated with discrimination learning. Overall, these data suggest that expression of HIV-1 Tat protein in mouse brain was sufficient to modulate cognitive performance in an assay-dependent manner. This suggests a direct biological means by which HIV infection may promote persistent HAND and cognitive impairment in many, but not all, modalities of cognition in HIV patients.

P58

Cocaine promotes astrocyte mitochondrial toxicity to regulate activation of antiviral innate immune responses to HIV-1 and Zika virus

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Cocaine abuse is correlated to heightened transmission of infectious diseases. This is partly achieved through signaling of innate immune pattern recognition receptors (PRRs) such as toll-like receptor (TLR)-2 and -4 to modulate neuroinflammatory cytokines. However, less studied is the role of cocaine in regulating central nervous system (CNS) antiviral responses, specifically type I interferon. We demonstrate that cocaine-mediated oxidative stress is sufficient enough to activate phosphorylation of serine/threonine-protein kinase (TBK) 1 and interferon regulatory transcription factor (IRF) 3 to stimulate type I interferon gene transcription, in the absence of viral infection. Interestingly, cocaine in combination with human immunodeficiency virus (HIV)-1, Zika virus (ZIKV) or polyinosinic-polycytidylic acid (poly I:C), reduces antiviral innate immune responses compared to virus alone. These results suggest cocaine influences activation and regulation of pattern recognition receptors involved in viral-induced innate immune signaling in astrocytes. Cytosolic mammalian PRRs, including cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), canonically activated by pathogen or self- genetic material, and mitochondrial antiviral signaling (MAVS) protein, regulated by changes in mitochondrial equilibrium, which ultimately result in interferon regulatory transcription factor 3/7 (IRF3/7) translocation to the nucleus for transcriptional activation of type I interferon. We show cocaine activates STING in a time dependent manner and results in mitochondrial alterations thereby regulating MAVS localization and distribution. We hypothesize that cocaine exposure triggers astrocyte innate immune signaling and regulates viral-mediated antiviral responses, via STING and MAVS. Thus, cocaine use activates CNS immune responses that compromise responses to subsequent viral pathogen exposure.

P59

β-adrenoceptors are expressed in non-small-cell lung cancer and affect cell proliferation

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Introduction: Several lines of evidence support β-adrenoceptor (AR) activation as a key mechanism affecting cancer growth, and β-AR antagonists (β-blockers) as potential novel anti-cancer drugs. Studying β-AR in different cancers is however necessary to identify tumor targets most likely to benefit from β-blockers (1). **Aim**: To characterize β1- and β2-AR expression in non-small-cell lung carcinoma (NSCLC), the most frequent of lung cancers, causing over 1 million deaths/year. β-AR function in NSCLC was also investigated in the A549 cell line, originating from adenocarcinoma human alveolar basal epithelial cells. **Methodology:** After approval by the local EC/IRB, β1- and β2-AR expression was evaluated by immunohistochemistry in surgical specimens from 80 NSCLC patients (age [mean±SD] 65.3±7.8 years; 15.2% females; 50% adenocarcinoma (ADC) and 50% squamous-cell carcinoma (SCC)). In A549 cells, β1- and β2-AR expression was studied by qRT-PCR, and the effect of noradrenaline, adrenaline, and of the β-AR agonist isoprenaline on cell proliferation was assayed by flow cytometry. **Results and Conclusions**: When compared with matched surrounding non-tumour tissues, β1-AR were less expressed in both ADC (H-score = 145.9±9.2 vs 82.1±10.0, P = < 0.0001) and SCC (134.4±8.5 vs 68.7 ±12.4, P = < 0.0001), while β2-AR were overexpressed in ADC (167.7 ±7.8 vs 200.1±10.4, P = 0.003) but not in SCC (159.4±8.8 vs 161.4±13.9, P>0.05). β1-AR were expressed to the same extent in ADC and SCC

(87.9±10.4 vs 66.4±11.0, P>0.05), whereas β2-AR were higher in ADC in comparison to SCC (204.0±10.2 vs 169.9±12.7, P = 0.040). In A549 cells, both β1- and β2-AR mRNA was detected and noradrenaline, adrenaline, and isoprenaline increased cell proliferation in a concentration-dependent manner. In conclusion, NSCLC express β-AR which may affect cell proliferation, however with remarkable differences depending on the histologic type. Studies are needed to establish the role of specific β-AR subtypes and their potential relevance as therapeutic targets.

P60

REGULATION OF EXOSOME-MEDIATED REACTIVE OXYGEN SPECIES SECRETION INDUCED BY HIV-1 TAT IN HUMAN NEURONAL CELLS

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It is well-known that oxidative stress in the brain is involved in the pathogenesis of HIV-associated neurocognitive disorders (HAND). However, the mechanisms responsible for the generation of these Reactive Oxygen Species (ROS) remain to be clarified. In this study we investigated if HIV-1 Tat can induce the secretion of ROS levels in exosomes from human neuronal cells and if these levels can be reduced using GW4869 and Allopurinol, inhibitors of exosome release and ROS generation, respectively. Human neuronal cells (SH-SY5Y;5x105 cells) were cultured in presence of Tat (100nM) for 24hrs and the percentage of exosomes with ROS and ROS levels per exosome were measured by flow cytometry. Exosomes with ROS were also analyzed in culture medium of cells exposed to Tat plus GW4869 (5 μM), Tat plus Allopurinol (10 μM), and Tat plus a combination of both drugs. Exosomes were isolated from medium by ultracentrifugation (100,000xg) and then incubated with aldehyde/sulfate beads. Exosome-coated beads were incubated with CD63-Alexa-647, Rab-5b-PE, and ROS detection reagent and then samples were analyzed using FACSAria flow cytometer. Our results indicated that: (1)The percentage of exosomes with ROS and ROS levels per exosome increased significantly ($p<0.05$) when the cells were exposed to Tat; (2)A significant decreased in the percentage of exosomes with ROS was observed when the cells were incubated with Tat plus GW4869; (3)A significant decreased in ROS levels per exosome was also observed when the cells were exposed to Tat plus Allopurinol; (4)Both the percentage of exosomes with ROS and ROS levels per exosome decreased significantly when Tat-treated cells were incubated with GW4869 plus Allopurinol. Our findings support that Tat has a role in exosomal ROS generation and secretion. The effects observed with both, GW4869 and Allopurinol, may help to identify targets that may lead to evaluate novel treatments and understand the mechanisms leading to HAND.

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VZV infection of corneal epithelial cells and corneal rims: a model for VZV keratitis

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The most common ocular complication of herpes zoster ophthalmicus (HZO) is keratitis, produced by varicella zoster virus (VZV) infection of human corneal epithelial cells (HCECs). VZV keratitis recurs frequently and can lead to vision loss. Corneal exam is remarkable for edema, pseudodendrites, poorly adherent epithelial cells and minimal inflammation. The mechanism(s) of recurrent VZV keratitis is not understood, but it has been proposed that chronic active infection may be contributory. This is supported by studies showing: (1) late epithelial cell dendriform keratitis lesions containing VZV DNA are improved following antiviral therapy, and (2) corneal buttons from HZO patients undergoing penetrating keratoplasty with stromal keratitis contain VZV DNA up to 51 years after HZO onset. Interestingly, it is unclear whether ocular cells that contain viral DNA or antigen harbor infectious virus particles. Herein, we developed a model to study VZV keratitis and test the hypothesis that VZV produces a persistent infection in primary HCECs. Infection of HCECs with VZV resulted in proliferation of HCECs and formation of pseudodendrites similar to that seen in patients with VZV keratitis. Importantly, VZV-infected HCECs were able to transmit infection to fibroblasts. When compared to VZV-infected fibroblasts, VZV-infected HCECs accumulated viral DNA and RNA at a slower rate and showed less cytopathology. Finally, human corneal rims were maintained ex vivo, scratched on their surface and infected with VZV. After 5 days, corneal clouding was observed, recapitulating the corneal edema seen in VZV keratitis. Overall, these early studies show VZV can infect HCECs and these virus-infected HCECs, which turn over every 3 days in vivo, can transmit VZV to uninfected cells. Furthermore, VZV-infection of HCECs and corneal rims recapitulate many of the findings seen in vivo, including cell proliferation, pseudodendrite formation, and corneal edema, providing a promising model to increase understanding of VZV infection of the eye.

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Effects of Cocaine and HIV on Astrocyte Cholesterol Synthesis: Implications for HIV-Associated Neurocognitive Disorders in Cocaine User Patients

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The human brain must maintain an intricate balance of cholesterol homeostasis to meet the high metabolic demands of neurons. Neurons cannot generate cholesterol and therefore, rely on cholesterol synthesized by astrocytes to maintain viability, synaptic connections and normal functioning. Liver X receptors (LXRs) are the master regulators of cholesterol homeostasis in the central nervous system (CNS). In astrocytes, LXR activation leads to the transcription of target genes involved in cholesterol trafficking and efflux. Disruption of LXR signaling in the brain can lead to significant dysfunctions in cholesterol homeostasis, and have been implicated in numerous neurological diseases including Alzheimer's disease. HIV+ cocaine users are associated with astrocyte and neuronal dysfunction. We propose that dysregulation in CNS cholesterol metabolism may be involved in the progression of HIV-associated neurocognitive disorders (HAND) and in cocaine-mediated neurocognitive impairments. Several studies report neurotoxic synergy between cocaine and HIV protein, Tat. Given the importance of astrocytes in LXR-mediated cholesterol regulation and providing metabolic support to neurons, we hypothesized that exposure of astrocytes to cocaine and Tat would lead to disruptions in LXR signaling. Alterations in these pathways affect the bioavailability of cholesterol provided by astrocytes leading to neuronal dysfunction and worsening HAND. Our data show that exposure of astrocytes to cocaine and Tat decreases the expression of LXRβ and its target genes, ApoE and ABCa1. Our findings also demonstrate that cocaine and Tat decrease overall bioavailability of cholesterol produced by astrocytes suggesting a deficiency in cholesterol supply and efflux to neurons. These findings were supported in our Tat transgenic mouse model of chronic cocaine exposure. Taken together, these data uncover novel alterations in a bioenergetic pathway in astrocytes exposed to cocaine and the HIV-1, Tat. Results from these studies point to a new pathway in the CNS that may contribute to HAND in HIV+ cocaine user patients.

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Neuroimmune Regulation of JC Virus by Intracellular and Extracellular Agnoprotein.

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JC virus (JCV) is a human polyomavirus and the etiologic agent of the demyelinating disease progressive multifocal leukoencephalopathy (PML). PML is observed in patients with underlying immunocompromising conditions, suggesting that neuro-immune interactions between peripheral immune cells and neuro-glia play an important role in controlling viral reactivation in the brain. There is little known about the immunobiology of JCV reactivation in glial cells and the role of immune, glial, and viral players in this regulation. We have previously showed that agnoprotein, a small JCV regulatory protein, is released from infected cells and internalized by neighboring bystander cells. Here we have investigated the possible role of extracellular and intracellular agnoprotein in the neuroimmune response to JC virus. Our findings suggest that glial cells exposed to agnoprotein secrete significantly less GM-CSF, which is mediated by agnoprotein induced suppression of GM-CSF transcription. Likewise, monocytes treated with agnoprotein showed altered differentiation and maturation. In addition, monocytes and microglial cells exposed to agnoprotein showed a significant reduction in their phagocytic activities. Moreover, when an in vitro blood-brain barrier model was used, agnoprotein treatment resulted in decreased monocyte migration through the endothelial cell layer in response to activated astrocytes. All together, these results have revealed a novel immunomodulatory function of agnoprotein during JCV infection within theCNS and open a new avenue of research to better understand the mechanisms associated with JCV reactivation in patients who are at risk of developing PML.

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Sex differences in voluntary wheel running in the HIV-1 transgenic rat

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Within the HIV-1+ population approximately half of patients self-report apathy and decreased motivation; however the role of sex in these motivational deficits is unknown. In the current study, we examined sex differences in the motivational state of the HIV-1 Transgenic (Tg) rat using voluntary wheel running. Adult HIV-1 Tg female animals (n=10) and F344/N female control animals (n=10) were compared to adult HIV-1 Tg male animals (n=10) and F344/N male controls (n=10). Animals were provided nocturnal access to a running wheel (34cm diameter) for 67 minutes per session for 42 consecutive days. First, we found a significant effect of sex on wheel running distance ($p \leq .05$), with females running greater distances compared to males (females=392.7±35.8 m/session vs. males=128.8±35.8 m/session). Second, we found evidence of a sex effect on maximal running speed (95th Percentile), with females running significantly faster (females=21.1±1.1 m/min, males=12.3 ±1.1 m/min) than their male counterparts ($p \leq .05$). We found no significant differences due to animal genotype on either distance or maximal running speed. Third, we examined if female cyclicity (estrous) had an influence on overall wheel running behaviors and found females in estrus had significantly increased wheel running distances ($p \leq .05$; Estrus=457.7±51.4 m/session vs. non-estrus=364.7±48.6 m/session). These data provide evidence of a pronounced sex effect in voluntary wheel running, independent of HIV-1 transgene. In contrast, our previous microstructural analysis of running bouts found reduced number of running bouts/session in the HIV-1 Tg animals compared to controls, suggestive of a dysregulation of motivation in the HIV-1 Tg rat. Thus, bout analysis of running behaviors may provide a more accurate index of motivation than either running distance or speed measures in the HIV-1 Tg rat and other models of impaired motivation.

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HIV Brain Latency May Directly Suppress Brain Cellular Energy

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Background: We recently showed that proton magnetic resonance spectroscopy (1H MRS) frontal white matter (FWM) brain cellular energy (creatine/H2O) was slowly declining in stable HIV-associated neurocognitive disorder (HAND) independent of MRS-defined inflammation. We therefore aimed to explore this observation further by examining the relationship between creatine and the putative brain latency marker BcL11b, while controlling for the effects of CSF markers of inflammation, neuronal damage, and HIV- Tat protein. **Methods:** 26 clinically stable chronic HIV+ men (plasma/CSF undetectable HIV-RNA <20cp/mL) undertook a lumbar puncture at baseline only, and a 1H MRS scan at both baseline and 18-months later. A standard neuropsychological exam classified HAND (31% unimpaired; 42% ANI; 15% MND, 11.5% HAD). CSF samples were analyzed for BcL11b, neopterin, neurofilament light chain (NFL), using standard assays, and Tat by an in-house sandwich ELISA. Baseline regressions to determine the magnitude of CSF BcL11b effect on brain metabolites were tested controlling for neopterin, NFL and Tat separately. A time and a time*CSF biomarker effects were added for the longitudinal analyses. **Results:** At baseline, a higher CSF BcL11b was associated with lower creatine (β=-.36; p=.06). The adjusted analyses for neopterin (β=-.30; p=.15; effect of neopterin: β =.27), NfL (β =-.39; p=.06; effect of NFL: β =.10) and Tat (β =-.36; p=.07; effect of Tat: β =-.007) showed that the magnitude of this effect remained independent of other CSF biomarkers. Creatine remained stable between baseline (2.96) and follow-up (2.90) (p=.42) but was abnormally decreased compared to age-matched controls. The longitudinal analyses showed no time effects but a persistent, albeit of a lower magnitude, BcL11b impact on creatine, in both unadjusted (β=-.26; p=.06) and adjusted models (β=-.20 - β=-.27). **Conclusions:** HIV brain latency appears to directly depress brain cellular energy independent of inflammation or HIV replication. The mechanism by which this occurs requires further study.

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Interferon Regulatory Factor-8 (IRF8) deficiency is associated with cognitive decline and reduced brain volumes in HIV Infected adults on virally suppressive ART

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Monocyte/macrophage (M/M) activation and HIV persistence may contribute to cognitive deficits that persist in treated, chronically HIV-

infected individuals. The transcription factor, interferon regulatory factor-8 (IRF8), regulates M/M, microglia, and dendritic cell development and function, and controls HIV latency. We recently identified loci in the IRF8 gene as being hyper-methylated in monocytes from HIV-infected individuals with cognitive impairment compared to HIV-infected persons with normal cognition. However, the relationship between IRF8 protein expression and HIV-associated CNS abnormalities is unknown. IRF8 expression on monocyte subsets (classical, intermediate, non-classical) was measured by flow cytometry using available cryopreserved blood from HIV-infected individuals on stable antiretroviral therapy from the Hawaii Aging with HIV-Cardiovascular cohort. Neuropsychological (NP) testing included psychomotor speed, executive function (EF), learning and memory (LM), and working memory (WM). Domain-specific standardized scores (NPZ) were determined and a global NPZ score defined. Regional brain volumes (e.g. hippocampus, cortical white matter) were measured using 3T magnetic resonance imaging, and plasma inflammatory factors (e.g. sICAM-1, MCP-1) by ELISA. Twenty HIV-infected participants, (HIV RNA<50 copies/ml; 84% male; median age 51 years; median CD4 count, 548 cells/mm3) were studied. In non-classical monocytes, lower IRF-8 correlated with worse global cognitive performance (Spearman rho=0.646) and LM (rho=0.536), WM (rho=0.647) and EF (rho=0.605) domains (p's<0.05). In classical monocytes IRF8 was associated with LM (rho=0.556) and WM (rho=0.612), and IRF8 in intermediate monocytes with LM (rho=0.532) (p's<0.05). Lower IRF8 in classical monocytes correlated with reduced hippocampal volume (rho=0.573). IRF8 in intermediate and non-classical monocytes was related to reduced cortical white matter volume (rho=0.509 and rho=0.473; p's<0.05). Lower IRF8 in total monocytes correlated with lower sICAM-1 levels, a cell adhesion molecule (rho=0.777; p<0.0001). Our data revealing IRF8 protein dysregulation in myeloid cells introduces a novel transcription factor that may be active in the neuropathogenesis of HIV-related cognitive decline and serve as a therapeutic target.

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Myeloid cells ablation attenuates Concanavalin A-induced liver injury by suppressing immune responses

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Both of bone marrow (BM)-derived monocytes and tissue-resident macrophages play vital role in the maintenance of tissue homeostasis. Recently, we used our intermedilysin (ILY)-human CD59 (hCD59) mediated cell ablation method (Hu, et al, Nat Med 14, 98-) and documented that the specific elimination of myeloid cells protected against Concanavalin A (ConA)-induced acute liver injury (Feng D., et al. JCI 126, 2321-). ILY, a toxin secreted by Streptococcus intermedius, binds exclusively to the human-specific cell membrane protein, CD59 (hCD59). Once bound, ILY rapidly lyses the cells through necrosis by forming toxin pores. We established a floxed STOP-hCD59 knock-in mouse (ihCD59) in which hCD59 expression only occurs following Cre-mediated recombination. By crossing Cre-inducible floxed STOP-hCD59 transgenic mouse line (ihCD59+) mice with myeloid cell-specific Cre transgenic line, LysmCre+ mice, we generated a double transgene positive mouse (ihCD59+LysmCre+) in which Cre expression drives expression of hCD59 in myeloid cells. The rapid and specific ablation of myeloid cells by the injection of ILY to LysmCre+ihCD59+ mice blocked ConA-induced hepatitis. Previous studies have demonstrated activation of NKT and T cells are necessary events in ConA-induced liver injury. To further define the pathogenic role of myeloid cells and its interaction with other immune cells, we investigated the dynamic changes of immune cells and the relevant cytokines in the ConA-induced hepatitis with or without the myeloid cell ablation. We documented that the myeloid cell ablation suppressed infiltration of T and B cells, inhibited the activation of NKT cells without affecting the total numbers of NK and NKT cells, and decreased the serum level of IFN-gamma, a cytokine mainly secreted NKT cells in the hepatitis. These results indicate that the myeloid cells play a critical role in the maintenance of NKT activation, which contribute to ConA-induced hepatitis.

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Enhanced target-enrichment virus sequencing reveals a complex JC virus population in the brain of PML patients

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Background: Deep nucleotide sequencing enables the unbiased detection of viruses in clinical samples but it is limited by low cost-effectiveness. We developed a novel target-enrichment deep-sequencing platform (ViroFind) for the cost-effective sequencing of viral genomes and we investigated the composition of viral populations in the brain of 5 PML patients and in 18 controls with no known neurological disease. **Methods:** ViroFind comprises 165,433 probes, complementary to viral sequences, which cover the entire genomes of 535 human tropic DNA and RNA viruses. The ViroFind probes are used in a hybridization reaction with nucleic acid libraries from clinical samples to enrich only viral sequences. The output of this reaction is sequenced via an Illumina platform and the sequencing data is aligned against a database of all viral genomes. Mapped sequences are used to analyze viral genomes and identify viral variants. **Results:** Compared to direct deep sequencing, ViroFind enriched the JC virus (JCV) sequences detected per PML brain sample, ranging from 584 viral sequences per 1,000,280 total sequences up to 375,653 viral sequences per 430,842 total sequences. The enrichment of JCV sequences attributable to ViroFind ranged from 33 to 127-fold. Each JCV nucleotide was sequenced at least 10 times (coverage 10X). We identified 24 viral capsid protein VP1 variants and 12 variants were associated with amino acid substitutions. Mutation H66D, likely related with VP1 conformational changes associated with tissue tropism, was observed in 4 of 5 (80%) PML brain samples. Lastly, we detected low level JCV sequences in 10 of 18 (56%) control brains. **Conclusion:** Multiple JCV variants constitute highly complex viral populations in the brain of PML patients. JCV genome fragments can also be detected in the brain of 56% of individuals with no known neurological disease, which suggests that JCV is present at a latent stage in the human brain.

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Machine learning interrogation of clinical and plasma markers identify prognostic models for cognitive trajectory

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The presence and severity of HIV-Associated Neurocognitive Disorders can be described by a Global Deficit Score that considers the number and severity of impairments in neuropsychological testing across seven cognitive domains (executive function, learning, delayed recall, working memory, verbal fluency, speed of information processing, and motor skills). Using longitudinal data (study visits interspersed by six months) from 95 subjects enrolled in the CHARTER study we conducted a

principal component analysis using the difference in T-Score for each domain (five components cumulatively explained 87% of the variance and the first three components contributed to 68% of the total variance). We found considerable variability in the contribution of the different domains to the direction of change. K-means clustering revealed 4 distinct clusters in the biplot of the domain differences. These results suggest that cognitive trajectory in each domain should be examined separately. We next interrogated clinical, demographic (n=210 features), and soluble biomarker (n=1005 features) data using machine learning approaches to determine if longitudinal change in individual domains could be predicted by unique groups of features. The learning method of random forest was used to identify important features that contributed to the models created for change in each of the 7 domains. A total of 182-212 contributing features (depending on the domain) were further examined using classification trees, support vector machines, discriminant analysis, bayesian probabilistic modeling, and k-nearest neighbor algorithms. Models with overall sensitivity, specificity, and accuracy >90% were validated using leave-one-out cross-validation. Final cross-validated models uniquely identified worsening or improvement in each cognitive domain with >70% accuracy. These results demonstrate that machine learning can be used to predict changes in specific domains. This approach to predict cognitive trajectory could lead to a personalized approach for interventions that target specific cognitive deficits in HIV-infected patients.

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HIV-1 gp120 promotes lysosome exocytosis in human Schwann cells

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Schwann cell dysfunction, due to viral proteins gp120 and antiretroviral drugs, has been implicated in HIV-1 associated peripheral neuropathy. Lysosome exocytosis in Schwann cells helps regulate myelination and demyelination. However, virtually nothing is known about the extent to which and mechanisms by which HIV-1 proteins and/or antiretroviral drugs affect lysosome exocytosis in human Schwann cells. We demonstrated here that HIV-1 gp120 concentration-dependently induces lysosome exocytosis as evidenced by increased cell-surface expression of LAMP1 and enhanced the extracellular release of the lysosome-resident enzyme acid phosphatase. HIV-1 gp120 concentration-dependently increased lysosome exocytosis. Inhibition of the exocyst complex with endosidin-2 blocked gp120-induced lysosome exocytosis. HIV-1 gp120-induced lysosome exocytosis was also blocked by chelating cytosolic calcium with BAPTA-AM or blocking P2X4 receptor-regulated calcium channels with Bx430; P2X4 receptors were primarily localized to LAMP1-positive lysosomes. Reconstruction of confocal z-stack acquired images using Imaris software showed that HIV-1 gp120 increased the movement of LAMP1-positive lysosomes from the perinuclear region to the cell's periphery. Additionally, HIV-1 gp120 increased ATP levels in LAMP1-positive lysosomes that were destined for lysosome exocytosis. Our findings suggest that HIV-1 gp120 promotes lysosome exocytosis by activating purinergic P2X4 receptors. Further elucidating the role of HIV-1 gp120 on Schwann cell function could be an important step in understanding the neuropathogenesis of HIV-1 associated peripheral neuropathy.

Supported by P30GM103329, R01MH100972, MH105329, R21DA040519

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Modulation of μ-opioid receptor signaling pathway by astrocyte-derived extracellular vesicles secreted in response to interleukin-1β

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Opioid dependence and deaths due to overdose are an enormous burden to public health in the United States. The prevalence of opioid use is 25-57% in HIV-infected individuals compared to 7% in the general population. In addition to promoting structural and functional changes in the brain reward circuitry, opioids modulate inflammatory responses in the central nervous system. Opioids regulate the production of inflammatory cytokines, and pro-inflammatory cytokines enhance the response to morphine through mechanisms that are not completely understood. We hypothesize that astrocytes exposed to the inflammatory cytokine IL-1β, release extracellular vesicles that contain molecular cargo promoting sensitization of the μ-opioid receptor (MOR1)-signaling pathway in target neurons. Previous work in our laboratory has demonstrated that the cellular microenvironment can alter the packaging of miRNA and protein cargo in astrocyte-derived extracellular vesicles (ADEVs). In this study we provide evidence that the miRNA cargo of ADEVs is modified by exposure to IL-1β with specific reduction in miRNAs-106b-5p (fold change [FC]=-3.03), 218a-5p (FC=-2.5), and 328a-5p (FC=-1.8) compared with constitutively released ADEVs ($p<0.05$). These miRNAs target several mRNAs in the MOR1-signaling pathway including OPRM1 (MOR1), Drd1, Gng3, Adcy7, Kcnj3, Prkx and GABAbR2. Exposure of primary neurons to ADEVs shed in response to IL-1β increased the mRNA and protein expression of OPRM1, Drd1, Gng3, Adcy7, Kcnj3, Prkx and GABAbR2. Similar increases in these proteins were observed when neurons were exposed to artificial extracellular vesicles containing synthetic miRNAs-106b-5p, 218a-5p and 328a-5p. Luciferase reporter assays were used to validate miRNA-mRNA target interactions. These findings suggest that inflammation in the setting of HIV-infection may produce specific reductions of miRNAs secreted in ADEVs that target MOR1 signaling in neurons. The resulting relief of target suppression allows for the upregulation of proteins that regulate addiction pathways in neurons.

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Role of Morphine on NF-κB Promoter Variant of HIV Clade C LTR In the Context of Simian Human Immunodeficiency Virus

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HIV clade C viruses are known to predominate infection worldwide and this is ascribed to its unique biological properties. We recently demonstrated emergence of the 4NF-κB promoter variant strains that acquire an additional NF-κB (3 sites in the wild type & 4 in the variant strains) leading to enhanced transcriptional potency of the viral promoter. We hypothesized that this is due to co-morbidity of opiate-abuse, and potentially influencing HIV LTR polymorphism. Therefore, we sought to understand the effects of opiates such as Morphine on NF-κB promoter-variants. We utilized SHIVAD8-EO virus and engineered 3NF-κB and 4NF-κB sites within the promoter. These molecular clones and parental construct were transfected in 293T cells to produce virus. Viral infectivity was measured by TZM-BL/p27 assays. Rhesus macaque (RM) PBMCs obtained from naïve animals were stimulated with ConA+IL-2 for 72hrs, then pretreated with 1μM Naloxone and 0.1μM Morphine for 4hrs. RM-PBMCs infected with these SHIVs were cultured in media containing IL-2. Morphine and Naloxone was replenished every 24hrs and p27 was measured. The newly

engineered constructs were replication competent in various cell-lines tested. The up-regulation of viral replication upon Morphine exposure of RM-PBMCs correlated with the number of NF-κB sites. Addition of Naloxone reversed Morphine up-regulated replication of 4NF-κB, 3NF-κB and AD8-EO suggesting involvement of the μ-opiate receptor. Significantly, 4NF-κB isolate replicated higher than 3NF-κB or AD8-EO (parental) isolates. Finally, the combinatorial effect of Morphine and viral replication was strongest for 4NF-κB SHIV as compared to 3NF-κB and parental virus. In summary, effect of Morphine and duplication of NF-κB motif may result in intensification of HIV infection in our studies. Future in vivo studies will reveal whether these NF-κB variant viruses increase virus replication in response to Morphine.

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Deep sequencing of TCR β-chain repertoire reveals an TCR expansion in patients with HTLV-I Associated Myelopathy/Tropical Spastic Paraparesis and an immune profile signature in patients with Multiple Sclerosis

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The sequencing of T-cell receptor (TCR) repertoire in the peripheral blood of patients with multiple sclerosis (MS) could be used to investigate in an unbiased manner, the expansion and diversity of T cell responses that may play a role in the disease process. We have combined an unbiased molecular technique to amplify the TCR region using a unique molecular identifier (UMI) barcoding system which that tracks cDNA amplified molecules and eliminates PCR errors and corrects over sequencing of TCR repertoire libraries by high-throughput sequencing (HTS). We observed a significant increase in TCR clonal expansion of circulating T-cells in a control group of patients with HTLV-1 associated myelopathy /tropical paraparesis (HAM/TSP) when compared to MS patients and healthy controls. In addition, correlation of diversity of TCR repertoire and clonal expansion in the peripheral blood was seems in all subjects. While there were unique individual TCR repertoires within both the HAM/TSP and healthy controls groups, no common (public) TCR signature was seen in this virus-associated inflammatory myelopathy. By contrast, a clonal T-cell signature was observed in a group of MS patients. By applying phylogenetic tree analyses, we observed a TCR profile similarity in 67% of these MS patients (23 out of 34). Expansion of the MS cohort including patients in exacerbation of the disease will be needed in order to better characterize the TCR repertoire in MS.

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1918 H1N1 influenza virus replicates in the nervous system of ferrets

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The 1918 Spanish H1N1 influenza pandemic was the most severe recorded influenza pandemic with an estimated 20-50 million deaths worldwide. 1918 H1N1 virus infection has been linked to encephalitis and post-encephalitis Parkinsonism in a minority of patients. Although it is known that influenza A virus infection can result in extra-respiratory tract complications -the most common being neurological disease- the potential contribution of extra-respiratory tissues, including the nervous system, to the pathogenesis of 1918 H1N1 virus infection has not been studied comprehensively. Here, we performed a time course study in ferrets inoculated intranasally with 1918 H1N1 virus, with special emphasis on the involvement of the nervous system. Evidence for active virus replication, as indicated by the detection of nucleoprotein by immunohistochemistry, was observed in the respiratory tract, peripheral and central nervous system, and liver. Infectious virus was detected by virus titration in the olfactory bulb and cerebrum at early time points after inoculation; virus could not be detected in the spinal cord or CSF. By immunohistochemistry, influenza virus antigen in the olfactory bulb could only be detected in very few cells in one ferret at 1 day post inoculation (dpi). In addition, virus antigen was detected in the pituitary gland of ferrets at 1 dpi, the brainstem at 1 and 5 dpi, and sensory neurons in the trigeminal nerve at 5 and 7 dpi. There were no histological lesions observed in the trigeminal ganglion or the CNS. These data suggest that 1918 H1N1 virus was able to enter the CNS via cranial nerves, in particular the trigeminal nerve. Taken together, our data support a possible link between 1918 H1N1 virus infection and CNS disease.

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Presence of HIV-1 RNA in Extracellular Vesicles from HIV-1 cART-treated Cells

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HIV-1 is a complex retrovirus that produces chronic infection affecting approximately 40 million people worldwide and 1.1 million new infections each year. To date, the most effective treatment of HIV-1 is a combination of several antiretroviral drugs (cART), which lowers viral titers and reverses pathology. Here we investigated the effect of cART, including an RT inhibitor and a protease inhibitor, on the content of extracellular vesicles (EVs) released from HIV-1 infected cells. We have previously shown that during infection, EVs contain a non-coding HIV-1 RNA, which has been shown to elicit responses in recipient cells (1-3). In our most recent data, we found altered levels of genomic RNA within EVs while other RNAs, such as TAR RNA, remained unaffected by the addition of cART treatment in both cell lines, primary macrophages, and patient biofluids. Furthermore, we determined possible mechanisms involved in the selective packaging of HIV-1 RNA products into EVs specifically, an increase in EV-associated RNA-binding protein hnRNPA2/B1, a "reader" for methylated RNA. More recent experiments in our lab have shown that several other FDA-approved drugs have the ability to alter the content of exosomes released from HIV-1 infected cells. We will also discuss how these findings on cART-altered EV content can be applied to general viral inhibitors (i.e. interferons) which are used to treatment of HIV-1 and other chronic infections. Additionally, we have found unique mechanisms of ESCRT pathway manipulation, specifically the targeting of the exit protein, VPS4, by antivirals. Collectively, these data imply that when patients are under antiretroviral therapy, they still release EVs which may cause dysregulation associated with neurocognitive and immunological dysfunction.

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Effect of Marijuana on EV Release and Cognitive Behavior

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As of 2016, roughly 18.2 million of the approximately 36.9 million people living with AIDS globally were receiving combined antiretroviral therapy (cART). Despite decades of research and development of this complex drug regimen, which is very effective in the prevention of new infection, cells that are already infected with integrated HIV-1 genome are still able to produce viral RNAs and proteins. These viral products can then be packaged into extracellular vesicles (EVs) and released from the infected cell. EVs, specifically exosomes, produced from HIV-1 infected cells contain viral mRNAs and that incubation of these exosomes with primary cells caused a significant increase in the levels of the proinflammatory cytokines, IL-6 and TNF-β, implicating EVs as a possible mechanism of the chronic inflammation observed in the CNS of HIV-1 patients under antiretroviral therapy1-2. Previous studies have shown that marijuana use in HIV-infected individuals is associated with a lower viral load and high CD4+ T-cell count3. Here we investigated the effect of Marijuana compounds, CBDs and THC, will be shown on exosomal marker proteins, CD63 and ALIX. CD63 levels exhibited a dose-dependent decrease regardless of infection in treated T-cells and myeloid cells. However, ALIX levels remained high in both infected cell types. We will discuss the effect of marijuana on ESCRT pathway and autophagy in relation to exosomal release.

P77

CTCF brackets 5' end of HSV-1 and VZV mRNA transcribed during latency in human trigeminal ganglia

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Herpes simplex virus type 1 and varicella zoster virus are ubiquitous human neurotropic alphaherpesviruses that establish latent infection in trigeminal ganglia following primary infection. Current studies suggest that during latency, much of the viral genome is transcriptionally silenced by posttranslational modifications on core histone proteins and neighborhoods within the virus genome containing these markings are held topologically distinct by chromatin insulators, especially CTCF-binding factor (CTCF). Chromatin immunoprecipitation assays followed by target enrichment and deep-sequencing (ChIP-seq) identified read clusters associated with CTCF consensus motifs enriched over input indicating that CTCF binds distinct chromatin sites on the virus genome. The human TG-generated CTCF-read clusters identified HSV-1 CTRL1 and CTRL2 that had been previously discovered in latently infected mice. Similarly located CTCF occupancies were detected on VZV DNA from human TG. RNA-seq with target enrichment identified a putative spliced VZV transcript mapping in the relic LAT region between VZV ORFs 60 and 61 in productively infected cells. More importantly, in-depth RNA-seq of targeted enriched samples showed a VZV LAT-like transcript in multiple individual human TG mapping antisense to ORF61. Alignment of HSV-1 LAT and Varicella Latency Transcript (VLT) with CTCF-ChIP data showed the candidate promoter for each alphaherpesvirus latency transcript bounded by a chromatin insulator element. Our results suggest that control of transcription by CTCF binding is a uniting feature of both HSV-1 and VZV during latency.

P78

Regulation of glutamate transporter, EAAT2 by IL-1β and the impact IL-1β and morphine on hippocampal physiology

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Neuroinflammation mediated by pro-inflammatory cytokines such as IL-1beta induced by HIV-1 infection of macrophages, microglia, and astrocytes in the CNS play a role in the pathogenesis of HIV-associated neurological disorders (HAND). Furthermore, drugs of abuse such as opiates also accelerate the progression of HAND. Our studies show that phosphorylation of NFKB p65 by IL-1beta (10ng/ml) positively regulates miR-146a expression and negatively regulates the glutamate transporter, EAAT2 expression in primary human fetal brain astrocytes (PHFA). In, addition, we show that EAAT2 is a target for miR-146a as evidenced by the observation that binding of miR-146a to the 3'-untranslated region of EAAT2 mRNA results in the reduction of EAAT2 protein levels in PHFA cells. To understand how IL-1beta and morphine play a role in HAND, we studied effects of IL-1beta and morphine on glutamatergic synaptic activity and membrane properties of CA1 pyramidal cells in mouse hippocampal slices. Slices were preincubated in ACSF, IL-1beta (10 ng/ml), morphine (100 nM) or the combination of IL-1beta and morphine and then whole-cell patch-clamp recordings conducted in voltage-clamp mode to measure excitatory glutamatergic postsynaptic currents or in current-clamp mode to test membrane potential, input resistance and excitability in response to a range of intracellular current pulses (0-160 pA). Preliminary findings indicate that, individually, morphine and IL-1beta increase cell excitability, with greatest effects produced by the combination of IL-1beta and morphine. Additional concentrations of each compound are currently being tested to more fully explore these effects. These data support the hypothesis that IL-1beta and morphine, singly and in combination, block hippocampal EAAT2 transporters, producing a build-up of synaptic glutamate that increases excitability of hippocampal CA1 pyramidal neurons.

P79

Modulation of Glucose-6-phosphate dehydrogenase expression in HIV-1 infected macrophages

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Monocyte-macrophages are an important reservoir for HIV infection. Identification of novel cellular proteins is critical in understanding the mechanism of HIV-1 replication in monocytes/macrophages. Our studies demonstrate upregulation of Glucose-6-phosphate dehydrogenase (G6PD) expression during HIV-1SF162 infection of monocyte/macrophages. Reactivation of HIV-1 in U1 cells, a macrophage model of latency by suberoylanilide hydroxamic acid (SAHA) and cocaine also induces G6PD expression and activity. Since HIV-1 macrophages are long-lived, our observation suggests that activation of G6PD promotes G6P metabolism through glycolysis that fuels glucose towards energy production. Our studies also demonstrate enhanced levels of GSH and NADP/NADPH in reactivated U1 in comparison to the uninfected parental U937 cells. These observations suggest that HIV-1 modulates pentose-phosphate pathway (PPP). Upregulation of PPP promotes the reduction of NADP+ to NADPH(H+), a cofactor necessary for antioxidant glutathione (GSH) regeneration from its disulfide form (GSSG) for attenuation of oxidative stress, and also provides the nucleotide pool required for sustained HIV-1 replication. Our studies demonstrate that HIV-1 hijacks the macrophage glucose metabolism pathway to create an environment that is not only advantageous for viral replication and biogenesis, but also for the long-term survival of infected macrophages.

P80

Impact of macrophage activation on Alzheimer Disease pathogenesis in APP/PS1 mice

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Alzheimer disease (AD) is a chronic neurodegenerative disorder characterized by progressive loss of memory and cognition resulting from neuronal loss associated with extracellular deposition of amyloid β (Aβ) plaques and intra-neuronal neurofibrillary tangles. Numerous studies indicate that age associated low grade neuroinflammation coincides with increased incidence of AD. We hypothesize that with increased age, changes in activation of brain infiltrating macrophages promote Aβ deposition and exacerbate neuroinflammation. To test this hypothesis, we used B6C3-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax mice (APP/PS1), a transgenic mouse model of AD, which exhibits temporal progression of Aβ accumulation and associated memory loss. Mononuclear brain cells isolated from 3, 6, 12, and 18 month old APP/PS1 mice or wild-type littermates were analyzed by flow cytometry to determine absolute numbers and activation phenotypes of microglia, infiltrating macrophages, and lymphocytes. We found that CD86+CD45(hi)CD11b(+) infiltrating proinflammatory macrophages increased in number with age. Notably, proinflammatory macrophage numbers were significantly greater in APP/PS1 mice compared to age-matched wild-type littermates. Additionally, the CD206+:CD86+ macrophage ratio in APP/PS1 mice decreased from 50:1 at 3 months to 0.5:1 at 18 months of age. Microglial (CD45intCD11b+) activation measured by CD86 and MHC-II expression was not altered. Transcriptomic analysis of differentially expressed genes at 4 and 12 months of age in APP/PS1 mice showed enrichment of several genes involved in immune responses. Moreover, there was marked reduction in M2 macrophage specific transcripts at 12 months of age in APP/PS1 mice. These findings indicate that changes in macrophage activation with age may influence AD pathogenesis in APP/PS1 mice. Studies are underway to develop murine models to specifically deplete peripheral macrophages without altering microglial constitution in the brain. The impact of reconstitution with M2 macrophages on AD pathogenesis will be investigated in these animal models. These studies may provide insights into immune processes, in particular role of macrophages, in AD pathogenesis.

P81

Particulate Matter 10 and 2.5 (PM10/PM2.5) exposure induces JC polyomavirus replication in the human subjects

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BACKGROUND: Reactivation of latent Human Polyomaviruses (HPyVs) occurs when the immune system is impaired. Air pollution exposure, including particulate matter (PM), is a major problem worldwide and has been linked to many diseases. Studies assessing the relationship between PM10/PM2.5 exposure and HPyVs replication are lacking. **METHODS:** A Chemical Transport Model (CTM) was used to estimate daily PM10 concentrations at municipality resolution, whereas monitoring stations (MS) were used to estimate daily PM2.5 concentration of 50 healthy adult subjects. For each subject, a fast urine sample was collected, and multiplex Real Time PCRs were conducted to quantify HPyVs (JCPyV, BKPyV, MCPyV, HPyV6, HPyV7 and HPyV9) loads. Zero-Inflated Negative Binomial (ZINB) regression was used to model the data as it contained excessive zeros. Covariates were chosen by stepwise selection. **RESULTS:** HPyVs DNA was detected in 54% (median 87.6*105 copies/ml) of urine samples. JCPyV was the most prevalent (48%) with a median viral load of 126*105 copies/ml (range: 5.8*105 - 9.8*108 copies/ml). Considering HPyVs overall, in the count-part of the ZINB model, every unitary increase in PM measured 2 days before urine collection (PM Day -2) was associated to an increase in HPyV DNA load (PM10: +4.1%, p-value=0.003; PM2.5: +3.9%, p-value=0.005). In the zero-part the only significant predictor was age, whereas for year there was a 8% increase in HPyVs DNA detection probability. Focusing on JCPyV DNA load, the effect was confirmed in the count-part for PM Day-2 (PM10: +4.0%, p-value=0.007; PM2.5: +8.2%, p-value=0.01). In the zero-part the only significant predictor was PM10 measured 5 days before urine collection (+3%, p-value=0.03). **CONCLUSIONS:** Environmental levels of PM10/PM2.5 can affect the replication of HPyVs which are urinary excreted after the exposure. Our findings emphasize the need of conducting studies assessing the influence of the air pollution exposure to the risk of viral reactivation into the host.

P82

Radiation-resistant subpopulation of JC virus induced medulloblastoma cells does not require the expression of viral tumor antigens for the maintenance of transformed phenotype

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JCV is the etiologic agent of the fatal demyelinating disease of the brain, progressive multifocal leukoencephalopathy (PML) that is seen primarily in HIV-AIDS patients. JCV expresses several tumor antigens from a single transcript via alternative splicing. JCV tumor antigens can inactivate tumor suppressor proteins, inhibit cell cycle regulators, trigger genomic instability, and eventually lead to cellular transformation. In addition to the PML, JC virus has also been shown to cause a variety of tumors in experimental animals. Inoculation of JCV into several experimental animal models, including hamsters, nonhuman primates, and transgenic mice, results in a variety of tumors depending on the animal type, age, and site of inoculation. There is little known regarding the characteristics of JCV induced tumors. Here we analyzed the possible impact of radiation on transformed phenotype by utilizing a mouse medulloblastoma cell line (BSB8) obtained from a mouse transgenic for JCV tumor antigens. Our results suggest that radiation induces apoptosis in the majority of the BSB8 cells. Interestingly, a small subset of BSB8 cells survives and shows radiation resistance. We cultured these cells and named as BSB8-RR. Secondary radiation of BSB8-RR cells revealed that they were no longer sensitive to the radiation. Further analysis of the transformed phenotype of BSB8-RR cells with the original BSB8 cells showed that BSB8-RR cells form significantly higher numbers of colonies under anchorage dependent and independent conditions. Moreover, BSB8-RR cells show a reduced rate of non-homologous end joining (NHEJ) but increased activity of homologous recombination (HR). More interestingly, knock out studies of JCV tumor antigen by CRISPR/Cas9 approach reveals that unlike BSB8 cells, BSB8-RR cells no longer require the expression of viral tumor antigens. These results suggest that ionizing radiation may eliminate the need of viral tumor antigen expression for the maintenance of transformed phenotype in a small subset of medulloblastoma.

P83

Sequencing and bioinformatics analysis of viral integration in the HIV-1 transgenic rats

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The HIV-1 transgenic (HIV-1Tg) rat was created in 2001. It carries gag-pol-deleted HIV-1 genome and express 7 of 9 HIV-1 proteins. The viral transcripts are expressed in various tissues including brain, lymph nodes, thymus, liver, spleen and kidneys. We have characterized this animal as a valuable rodent model to mimic people living with HIV (PLWH) receiving combination antiretroviral therapy (cART). However, there is no report about sequencing and bioinformatics analyses conducted to determine how actually the modified HIV-1 genome(s) are integrated into the rat genome. In this study, DNA samples were extracted from liver of 10 rats (19-20 months of age), 6 of which HIV-1Tg rats (male, n=3 and female, n=3) and 4 F344 control rats (male, n=2 and female, n=2). Using these DNA samples, the insertion sites of HIV-1 viral genome and estimated head-to-tail copy numbers in HIV-1Tg rats were determined using deep sequencing and bioinformatics analyses. The results showed that the HIV-1 DNA sequence was integrated at two independently chromosomal sites, the one located on 18546018 of rat chromosome 10 and the other one located on 88507092 of rat chromosome 13. For both insertion sites, the sense strand of HIV-1 was located in the forward strand of the rat reference (Rn6) genome deposited by the University of California Santa Cruz (UCSC) database. There were multiple DNA rearrangements in both ends of insertion site on chromosome 10 were identified. A total of 27 copies of HIV-1 DNA that were connected by the 5'-and 3'-ends of HIV-1 was identified. Based on these information, gender basis analysis will be carried out.

Partially sponsored by HD043680, MH106392, DA013137, DA026356, DA036175, NS100624 and AA023172.

P84

Upregulation of TREM2 in microglia by oleamide, a sleep-inducing supplement in human

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TREM2 plays an important role in phagocytosis and in the CNS, TREM2 is expressed highly and exclusively by microglia. It has been shown that TREM2 deficiency reduces the microglial response to amyloid plaque pathology. Therefore, upregulation of TREM2 may be beneficial in different neurodegenerative disorders. This study underlines the importance of oleamide, a sleep-inducing supplement in human, in increasing the expression of TREM2 in microglia. Oleamide dose- and time-dependently increased the mRNA expression of TREM2 in mouse BV-2 microglial cells. Similarly, oleamide also upregulated the level of TREM2 in mouse primary microglia. Consistent to the well-defined role of TREM2 in phagocytosis, oleamide also increased phagocytic activity of microglia. Next, we examined the effect of oleamide on microglial activation. While oleamide suppressed the expression of proinflammatory molecules such as inducible nitric oxide synthase (iNOS) and IL-1β in LPS-stimulated microglial cells, the level of anti-inflammatory M2 subtype markers (e.g. IL-10, IL-13, ARG1, and TGF-β) increased under the same condition. Together, these results suggest that oleamide may be useful in suppressing microglial inflammation and supporting microglial phagocytosis.

This study was supported by a grant from NIH R01AG050431.

P85

Interaction of Drugs of Abuse and HIV - Studies from the NNTC

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As a network of four nationwide clinical sites, the National NeuroAIDS Tissue Consortium (NNTC) has recognized a great opportunity for researchers to access samples, data, and samples with correlating data concerning HIV and drug abuse. In addition to the 2,925 participants in NNTC, of whom 2,212 have drug abuse parameters diagnosed through the Composite International Diagnostic Interview (CIDI) and/or the Psychiatric Research Interview for Substance and Mental Disorders (PRISM), the NNTC now contains data from the CHARTER study of 1,608 participants, of which 1,578 have CIDI and/or PRISM assessments. Furthermore, numerous urine toxicology assessments are also available. These data, as well as biospecimens, obtained from the participants have already been used to contribute to over 30 drug abuse related research publications. These studies have revealed interesting facets of the interactions between drugs of abuse, HIV, and neurocognitive function. The NNTC makes available drug abuse as well as other psychiatric, neuropsychological, neurocognitive, and neuromedical data available to qualified requestors, as well as a broad ranges of specimens from the participants, including plasma, CSF, and tissues. Here we will present the validity and advantages in using the NNTC resources to further the study of drug abuse within the HIV research community.

P86

Targeting Astrocyte HIV-1 Proviral Reservoirs in HAND

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Although antiretroviral therapy (ART) has greatly reduced the incidence of HIV-associated dementia (HAD), nearly 50-70% of HIV-1 infected individuals develop HIV-associated neurocognitive disorders (HAND). Between 5-20% of astrocytes harbor HIV-1 provirus, and they do not actively propagate viral infection. However, it is well established that astrocytes produce viral proteins, which alters their function and aggravate HAND pathogenesis. Moreover, in vivo it is difficult to distinguish latently infected astrocytes from healthy cells. Thus there is a great need to identify latently infected astrocytes and develop strategies to target this specific population. We hypothesize that HIV-1 proviral reservoirs alter astrocyte function in conjunction with unique gene expression patterns that could serve as biomarkers and facilitate targeted therapy. A doubly labeled fluorescent reporter Red/Green-HIV-1 (R/G-HIV-1) was used to visualize viral promoter (LTR) activity in primary human astrocytes. LTR activity of pseudotyped R/G-HIV-1 infected astrocytes decreased over a period of 21 days. Astrocytes with active (mCherry+/GFP+) and silent (mCherry+/GFP-) LTRs were enriched using fluorescence activated cell sorting. Astrocytes with silent promoter activity were devoid of late viral proteins such as p24, indicating a functionally silent HIV-1 LTR. Vorinostat, an HDAC inhibitor, reactivated silent HIV-1 LTR in pseudotyped R/G-HIV-1-infected astrocytes. This suggests R/G-HIV-1 could be used as a relevant model of latency in astrocytes since it mimics virus reactivation in inflammation leading to viral protein expression. Our data indicates astrocytes harboring R/G-HIV-1 provirus exhibited decrease of glutamate clearance ability and cell proliferation, as well as, significantly increased glial fibrillary acidic protein (GFAP), interluekin-1β (IL-1β) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) mRNA levels. This demonstrates harboring R/G-HIV-1 provirus alters function and disrupts astrocyte-mediated homeostasis. We propose that identifying biomarkers for astrocytes harboring HIV provirus and therapeutic gene editing will eliminate proviral gene expression and improve physiological function compared to infected cells.

P87

Drugs of abuse as drivers of epigenetic change in HIV-1 infection

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HIV-associated neurocognitive disorders (HAND) impact nearly one-half of HIV infected patients. There is considerable evidence in the literature that HIV positive substance abusers are at greater risk for HAND and generally have a heightened pathology compared to non-drug abusers. The ordered packaging of eukaryotic DNA into nucleosomes and the subsequent modification of these nucleosomes is critical to the control of all gene expression. These modifications result in an increase or decrease in the affinity of the histone proteins for DNA or the recruitment of other proteins to the DNA. It is this outcome that ultimately controls transcription of a given gene. Indeed, epigenetic control of HIV-1 transcription underlies the establishment and maintenance of latency. During this latent state, multiple factors associate with the viral LTR and repress transcription. Among these factors are the histone deacetylases (HDACs), RUNX1 other chromatin modifying proteins that alter the chromatin structure of the integrated viral promoter to prevent access and binding by positive transcription factors. We hypothesize that changes in chromatin modification driven by drugs of abuse have a role to play in the long term detrimental effects seen in drug abusers and are a confounding factor in latency reversal. We have addressed this hypothesis by examining the effect of two classes of abused substances on HIV-1 latency and epigenetic changes in the nucleus. Our data shows that exposure to opiates renders cells resistant to the activating effects of HDAC inhibitors. Further, we show that clinically prescribed benzodiazepines suppress RUNX1 activity and can be used to reactivate latent HIV-1. Using a novel high-resolution microscopy technique we have imaged epigenetic changes in cells exposed to opiates and benzodiazepines. Our data suggest that substances of abuse may be both affecting HIV-1 transcription and having an effect on the chromatin state of the host cell.

P88

Immunophenotypic characterization of CSF B cells in virus-associated neuroinflammatory diseases

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Intrathecal antibody synthesis is a well-documented phenomenon in infectious neurological diseases as well as in demyelinating diseases, but little is known about the role of B cells in the central nervous systems. We examined B cell and T cell immunophenotypes in CSF of subjects with chronic virus infection and/or neuroinflammatory diseases including HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), HIV-1 infection, multiple sclerosis (MS) and progressive multifocal leukoencephalopathy (PML) compared to healthy normal donors (NDs). Antibody secreting B cells (ASCs) were elevated in HAM/TSP patients, which were significantly correlated with intrathecal HTLV-1-specific antibody responses. High frequency of ASCs was also detected in patients with relapsing-remitting multiple sclerosis (RRMS) and a subset of PML patients. While RRMS patients showed significant correlations between ASCs and memory follicular helper CD4+ T (Tfh) cells, CD4+CD25+ T cells were elevated in HAM/TSP patients, which were significantly correlated with ASCs and HTLV-1 proviral load. In CSF of PML patients, CD25 as well as PD-1 were also significantly elevated in CD4+ T cells compared to NDs, but frequency of Tfh cells were varied. The subset analysis of PML patients demonstrated that Tfh cells were increased in the CSF of PML patients with HIV-1 infection while majority of PML patients showed decreased Tfh cells in the CSF. These results highlight the importance of the B cell compartment and the associated inflammatory milieu in subjects with chronic virus infection and/or neuroinflammatory diseases where virus-specific antibody production may be required to control viral persistence and/or may be associated with disease development.

P89

The California serogroup of orthobunyaviruses differ in neuroinvasion and neurovirulence

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The California serogroup (CSG) of Orthobunyaviruses comprises a number of closely related mosquito-borne viruses including La Crosse (LACV), Tahyna (TAHV), Jamestown Canyon (JCV), Inkoo (INKV), and Snowshoe Hare (SSHV) viruses. JCV and INKV are the most closely related, followed by LACV, SSHV, and TAHV. All five viruses have been reported to cause neurological disease in humans, although the incident rate and age of patients vary between viruses. To determine if disease incidence varied in mice, we evaluated neuroinvasion via intraperitoneal (IP) inoculation and neurovirulence via intranasal (IN) inoculation into weanling, adult, and aged mice. Following IP inoculations in weanling mice, LACV, TAHV and SSHV were neuroinvasive, while JCV and INKV were not. None of the viruses caused neurological disease in adult or aged mice following IP inoculation. In contrast, following IN inoculation in adult and aged mice, LACV, TAHV, JCV, and SSHV caused neurological disease in 100% of mice, indicating they are neurovirulent. INKV caused neurological disease in only ~25% of mice. Analysis of virus replication and cell death in a neuronal cell line revealed that INKV and JCV had slower replication rates and caused less cell death in neurons compared to LACV, SSHV, and TAHV. Together, these results suggest that shared genetic factors between LACV, SSHV, and TAHV may contribute to their neuroinvasive and neurovirulent phenotypes in mice and high infectivity in vitro as compared to JCV and INKV. Elucidating the viral factors involved may be important for identifying potential therapeutic targets for the treatment of viral encephalitis.

P90

Multiple cocaine-stimulated pathways regulate HIV gene expression and replication

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Illicit drug users are a high risk population for infection with the Human Immunodeficiency Virus (HIV). A strong correlation exists between prohibited drugs use and an increase rate of HIV transmission. Cocaine is one of the most widely abused drugs in the United States, which both impairs the normal functioning of brain cells and also activates HIV expression in central nervous system (CNS). Cocaine accelerates HIV replication by altering specific cell-signaling and epigenetic pathways. We have elucidated the underlying molecular mechanisms through which cocaine exerts its effect in myeloid cells, a major target of HIV in the CNS. We noted that cocaine strongly stimulates mitogen activated protein kinase (MAPK). MAPK stimulation later leads to the activation of mitogen- and stress-activated kinase 1 (MSK1). MSK1 subsequently

catalyzes the phosphorylation of histone H3 at serine 10 (p-H3S10), and p65 subunit of NF-κB at 276th serine residue (p-p65276). We demonstrate that a short-term (acute) cocaine treatment promotes HIV-1 transcription by activating both nuclear factor-kappa B (NF-κB) and MSK1. However, during longer-term or chronic cocaine treatment MSK1 is the main facilitator of HIV1 transcription. These events enhance the interaction of NF-κB with histone acetyltransferases (HATs). Subsequently, acetylated core histones, along with p-H3S10, supports the development of an open/relaxed euchromatin structures at HIV LTR. MSK-1-induced p-H3S10 also facilitates the recruitment of positive transcription elongation factor b (P-TEFb) at LTR and thus, promotes the elongation phase of HIV transcription, a prerequisite to generate complete genomic transcript of HIV. Results are also confirmed in primary monocyte derived macrophages (MDM). Overall, our study provides detailed insights into cocaine-driven HIV-1 transcription and replication.

P91

HIV-infected primary human monocyte derived microglial cells can be targeted for HIV cure

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Background: Microglia, the resident immune cell of the central nervous system (CNS), are instrumental in maintaining homeostasis. Microglia are permissive to HIV infection and are considered the major reservoir in the CNS. Human microglia have been difficult to study in the past, particularly due to the paucity of human tissue. New strategies to eliminate HIV reservoirs include the use of genome editing tools. The CRISPR/Cas9 system excises the HIV provirus from the genome, and has the advantage of being selective and efficient. We show that studies to characterize HIV infection and excision are now possible using a new monocyte-derived microglia (MMG) system. **Methods**: MMG were cultured from primary human monocytes with a combination of chemokines and growth factors. After 10 days in culture, microglia were characterized using antibodies to TMEM119, CD11b and Iba-1, and used for downstream analyses. Microglia were infected with HIV for 5 days, and functional tests for phagocytosis and migration were performed. After 5 days of HIV infection, MMG cultures were transfected with an anti-HIV CRISPR/Cas9 plasmid for 5 days, and p24 was sampled to show efficacy in excision. **Results:** Monocyte-derived microglia have the immunologic markers and morphology of primary microglia. HIV infection significantly impaired microglial phagocytosis and increased migration. MMG were transfected with an anti-HIV CRISPR/Cas9 plasmid 5 days after infection to access HIV excision. Supernatants showed a 55% decrease in p24 when MMG were treated with 2μg CRISPR/Cas9 plasmid DNA. Treated/infected MMG morphology was unaffected compared to that of untreated and HIV-infected scrambled plasmid treated MMG. **Conclusions:** The new MMG culture system allows for the study of HIV infection in a primary human privileged reservoir. The MMG system makes it possible to determine the functional consequences of HIV infection before and after the elimination of virus from microglia.

P92

EEG and MRI evidence for a subcortical contribution to cognitive dysfunction in older men living with HIV

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Cognitive impairment is common in people with HIV, even with viral suppression, but the causes and brain substrates remain uncertain. Stimulus-locked EEG (ERPs) provide information about the time-course of neural activity underlying cognitive processes. There are reports of smaller amplitude ERPs in HIV+ compared to HIV- groups, but it is not clear whether this relates to cognition, nor whether it reflects a specific or general degradation of processing. We evaluated EEG activity evoked by two cognitive tasks, assessing perceptual-attentional (auditory oddball) and action selection (Simon) aspects of executive function. We tested whether the amplitude of the P300 evoked response was related to global cognitive performance assessed with a computerized battery testing attention, memory, and executive functions. We sought evidence that nadir CD4 count contributed to the variation in evoked potential amplitudes. Given prior work showing an association between nadir CD4 and subcortical volume, we explored whether thalamus or caudate volumes measured with structural MRI explained P300 variation. 56 HIV+ older men on cART (virus undetectable in 54/56) drawn from the Positive Brain Health Now study underwent high-density EEG and MRI. Thalamic and caudate volumes were estimated from a non-linear registration of theT1-weighted MRIs to the MNI-152 atlas. P300 amplitudes evoked by the oddball and Simon tasks both related to variation in cognitive performance. However, nadir CD4 count only explained the P300 amplitude in the oddball task. Thalamic volumes were correlated with the oddball P300 amplitude, while caudate volumes were correlated with the Simon P300 amplitude. The Simon and oddball tasks emphasize different aspects of executive function, and rely on distinct circuits. Nonetheless, we find evidence that P300 amplitudes evoked by either task relates to variation in overall cognitive performance in older, cART-treated men. However, nadir CD4 count related only to the oddball P300, perhaps reflecting underlying thalamic volume loss.

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Recovery of thin spine density is essential for rescuing cognitive function in a rodent model of HAND: role of the chemokine CXCL12 and the Rac1/PAK pathway

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HIV-associated neurocognitive disorders (HAND) continue to affect approximately 50% of HIV+ individuals despite the introduction of antiretroviral therapy (ART). The loss of dendritic spines, the main sites of excitatory input on neurons, is a major pathological hallmark of HAND and is tightly correlated with neurocognitive dysfunction in these patients. The chemokine-receptor pair, CXCL12/CXCR4, plays a critical role in dendritic spine homeostasis and is known to be disrupted during HIV infection. Thus, we hypothesized that restoration/enhancement of this pathway would ameliorate both structural and cognitive deficits in HIV+ individuals. We report that intracerebroventricular administration of CXCL12 reverses dendritic spine loss and cognitive dysfunction in HIV-Tg rats, a rodent model of HAND in the post-ART era. CXCL12 specifically increased the number of thin spines, which are associated with learning and plasticity, in layer II/III pyramidal neurons of the medial prefrontal cortex (mPFC). In these same animals, this increase in thin spines directly correlates to significant improvements in cognitive flexibility, a task mediated exclusively by the mPFC. Next, we shifted to primary rat cortical neuronal cultures to explore the mechanisms at play. Identical to what we had observed in vivo, administration of CXCL12 increased the density of transient thin spines. This occurs by activation of the GTPase Rac1 and its downstream mediators (PAK1, LIMK1, and cofilin) in a CXCR4-, Gi-dependent manner. Importantly, activation of this pathway was associated with a shift in the F-actin/G-actin ratio, favoring the stabilization of transient thin spines. Inhibition of Rac1

completely blocked the ability of CXCL12 to increase dendritic spine density in vitro. Ongoing studies are currently investigating the role of Rac1 in CXCL12-induced spine and behavioral changes in vivo. Our findings have therapeutic implications not only for HAND but other neurological disorders characterized by dendritic spine deficits and CXCR4 dysregulation, including schizophrenia and Alzheimer's.

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Targeting mitochondria to reduce HIV-induced neurotoxicity

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HIV infects over 1.4 million people in the USA and over 34 million people worldwide. Approximately 50% of HIV-infected individuals will suffer from neurological disorders. Mitochondrial dysfunction has long been associated with HIV-induced neurotoxicity, yet we have no promising therapeutic strategies to target and protect mitochondria during HIV infection. Our group has identified that during HIV infection, the processes of mitochondrial dynamics (fission, fusion, and transport of mitochondria, and recycling by mitophagy), mitochondrial biogenesis (generation of new mitochondria), and energy production via oxidative phosphorylation are impaired. Alterations in these pathways are associated with cognitive and sensory deficits in humans and in transgenic rodent models of HIV-induced neurotoxicity. Neuropathological and molecular analyses revealed these deficits are also associated with reduced mitochondrial DNA, elongated and damaged mitochondria, accumulated mitochondria near neuronal soma, reduced neuronal integrity, and neuroinflammation. Recently, we've identified several promising therapeutic strategies to promote mitochondrial homeostasis in the context of HIV-induced neurotoxicity. In the GFAP-Tat mouse model, we have successfully used a muscarinic antagonist to reduce symptoms of neuropathy via induction of mitochondrial biogenesis. Following treatment, sensory neuron fibers increased in number and size, mitochondrial protein quantity was restored, and neuroinflammatory markers were decreased. In vitro, pretreatment with a synthetic cannabinoid reversed mitochondrial dysfunction and inflammatory gene expression in astroglial and myeloid lineage cells. Moreover, neurons were damaged when treated with conditioned media from astroglia exposed to HIV relevant stimuli. However, pretreatment with a cannabinoid reduced mitochondrial dysfunction and inflammatory gene expression in the astroglia, and prevented neurotoxicity mediated by astroglial CM. Taken together, these data suggest that therapeutic strategies that promote mitochondrial homeostasis may reduce neurotoxicity in HIV-infected patients.

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Amyloid beta and HIV promote a pro-inflammatory phenotype in brain macrophages that may be reversed by cannabinoids

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Up to 50% of living HIV-infected subjects on cART will suffer from a degree of neurocognitive impairment (NCI). Age-related markers, such as amyloid beta accumulation, are increased in brains of HIV-infected patients, however, it is unknown if HIV-induced neurotoxicity shares mechanisms with other age-related diseases, such as Alzheimer's disease (AD). We investigated the levels of age- and AD-related markers in postmortem brains of HIV+ decedents. Specifically, we studied the role of myeloid-lineage cells in the brain, in promoting HIV-induced amyloid beta deposition and inflammation. In brain tissues of HIV+ subjects with NCI we found reduced levels of the immunomodulatory receptor, triggering receptor expressed on myeloid cells (TREM2), and increased levels of amyloid beta protein. Conversely, proinflammatory cytokines TNFa and IL1B were increased in brain tissues from patients with NCI. In vitro, in peripheral blood monocytic cells (PBMC), amyloid beta, IL1B, and gp120 reduced expression of TREM2, increased expression of HLA DR, and increased HIV replication as measured by p24 ELISA. Interestingly, pretreatment of PBMCs with a synthetic cannabinoid reduced proinflammatory markers IL1B and HLADR, and increased TREM2. Together, these data suggest that HIV induces a proinflammatory microglial phenotype in the brain and increased amyloid beta deposition. However, pretreatment with a cannabinoid promotes an anti-inflammatory phenotype in PBMCs. These data suggest that HIV+ subjects may experience increased rates of AD-like neuropathology, and that cannabinoids may serve as a therapeutic avenue to attenuate these changes.

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Imaging flow cytometry as a novel epigenetic tool to detect post-translational modifications and extracellular histone release induced by alcohol abuse

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Previous literature has demonstrated extracellular histone release to be increased and associated with poor outcomes after traumatic injury, to induce inflammation and play a major role during organ injury. However, extracellular release of histones to the periphery during alcohol abuse and whether they play a major role during alcohol-induced inflammation have not been elucidated yet. Our lab has developed a novel method using single cell imaging flow cytometry to detect post-translational modifications in human monocyte-derived dendritic cells (MDDCs) and to elucidate the role of histone modifications during alcohol abuse. In the current study, we have evidence of the effect of alcohol drinking on the release of extracellular histones in human plasma and our results confirm the presence of circulating histones in plasma from alcohol users, and surprisingly, a significant increase of circulating histones in female drinkers. Our findings, for the first time, demonstrate the presence of extracellular histone proteins in human plasma from alcohol drinkers and a gender-specific effect on the extracellular release of histones. In summary, the detection of post-translational modifications and extracellular histones may serve as a promising tool to measure the inflammatory consequences of substance abuse and even serve as a biomarker for substance abuse disorders.

Partially supported by R00AA021264, startup funds from the Department of Immunology, Herbert Wertheim College of Medicine, Institute of NeuroImmune Pharmacology at FIU, and FIU Office of Research and Economic Development.

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Regulation of PINCH as a therapy for Tau-mediated neurodegeneration

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Accumulation of hyperphosphorylated Tau (hpTau) is a pathological hallmark of numerous neurodegenerative diseases including Alzheimer's disease (AD), frontotemporal dementia (FTD), chronic traumatic encephalopathy (CTE), epilepsy and HIV encephalitis (HIVE). It is in this context that we are studying the molecular signaling between Tau and a newly discovered protein involved in neuronal injury, called PINCH. PINCH (Particularly interesting new cysteine histidine) is a non-catalytic 5 LIM domain only adaptor protein involved in protein recruitment, assembly of multi-protein complexes and subcellular localization, cell polarity and migration. PINCH is expressed at high levels during development, but its expression is nearly undetectable after birth in healthy brain. In CNS

diseases with a tauopathy component including AD, CTE, epilepsy, HIVE, FTD, PINCH is robustly recalled in neurons. Importantly, PINCH and Tau interact at the molecular level, are detectable in complex in neurons in the brain, cerebrospinal fluid (CSF) and in blood of patients with neurodegenerative diseases, in animal models of neurodegeneration and in neurons in vitro. Our in vitro and in vivo data show that PINCH binds directly to Tau to form a complex with the ubiquitin ligase protein, CHIP. These data suggest that PINCH binds to hpTau and interferes with heat shock machinery to prevent the proteolytic degradation of aberrant Tau. Silencing PINCH expression as well as excising the PINCH gene decreases levels of hpTau in human primary neurons and in Tat transgenic mice. Moreover, PINCH-mediated regulation of kinases (AKT, GSK3β) and phosphatases (PP1) contributes to downstream hpTau levels. Taken together, these data show that PINCH is an attractive target for regulating levels of hpTau.

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Baricitinib Reverses HIV Associated Neurocognitive Disorders in a SCID Murine Model

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Background: HIV associated neurocognitive disorders (HAND) occurs in up to half of HIV+ individuals, even with cART. Chronic CNS inflammation contributes to HAND and HIV encephalitis (HIVE). Baricitinib is a Jak/STAT inhibitor approved in the EU and Japan for rheumatoid arthritis, demonstrating potent inhibition of IL-6, D-dimer, CRP, IL-1α/β, TNF-α, IFN-α/β, and other pro-inflammatory cytokines. A SCID murine HAND model was used to evaluate the ability of baricitinib to 1)cross the blood-brain-barrier (BBB), 2)reverse behavioral abnormalities and cellular markers associated with HAND and HIVE. **Methods:** B6.CB17-Prkdcscid/SzJ (SCID) male mice were injected intracranially with 105 HIV-infected or uninfected human macrophages. Uninfected controls, HAND (saline), HAND+low dose baricitinib; 10 mg/kg, and HAND+ high dose baricitinib; 50 mg/kg were evaluated (n=9 per group). Baricitinib groups received subcutaneous qd drug starting on day 6 after first object recognition test (ORT). Brains were homogenized and p24+ human macrophages (CD163), activated microglia (MHCII+/CD45+), and astrogliosis (GFAP) were quantified (flow-cytometry). To measure BBB penetration, baricitinib was administered to uninfected mice prior to quantification in brains after perfusion (LC-MS/MS). **Results:** On day 13-post infection, low and high dose baricitinib completely reverse behavioral abnormalities conferred by HIV, demonstrating ORT similar to uninfected controls ($p<0.01$ compared to HAND mice, t-test). Low and high dose baricitinib significantly reduce HIV-induced activated microglia (MHCII+/CD45+), astrogliosis (GFAP), and CD163+/p24+ human macrophages ($p<0.01$ and $p<0.05$ compared to HAND mice; t-test). **Conclusions:** ORT abnormalities in HAND mice are reversed by low and high baricitinib. Cellular markers of HAND and HIVE were significantly reduced by both low and high dose baricitinib, without apparent toxicity. These data demonstrate that baricitinib crosses the BBB and has substantial and meaningful effects on important HAND pathological indices. Baricitinib could be used adjunctively with cART in subjects with HAND. Our studies provide impetus for Phase I/II trials of baricitinib in persons with HAND.

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Exosome-Mediated Soluble Insulin Receptor Secretion Is Associated With Hand

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Previously, we found that plasma soluble insulin receptor (sIR) levels were significantly higher in HIV-seropositive women when compared with controls and were associated with the presence and severity of HAND. However, the mechanisms responsible for the secretion of sIR to the plasma of these patients are unknown. Exosome-mediated release is one of the major mechanisms responsible for the secretion of soluble full-length receptors and viral proteins from infected cells. In this study we investigated if sIR, HIV-1 tat, and Reactive Oxygen Species (ROS) in plasma exosomes were associated with HAND. Seventy six (76) HIV-seropositive women stratified by cognitive status (NC=32, ANI=8, MND=27, HAD=7) and 29 controls without history of diabetes were evaluated. Exosomes were isolated from plasma by ultracentrifugation (100,000xg) and afterwards incubated with aldehyde/sulfate beads. Exosome-coated beads were incubated with CD63-Alexa-647 and Rab-5b-PE antibodies, then permeabilized using the BD Cytofix\Cytoperm Kit, and incubated with anti-IRβ-FITC antibody, anti-Tat-FITC antibody, or ROS detection reagent. Samples were analyzed using a FACSAria flow cytometer. Our results indicated that: (1) HIV-seropositive women had significantly increased levels of the percentage of exosomes with: sIR ($p=0.001$) and ROS ($p=0.005$) and increase levels per exosome of: sIR ($p<0.001$), , ROS ($p=0.001$), and HIV-1 tat ($p=0.003$) compared to controls; (2) When stratified by HAND, a significant association was observed in the percentage of exosomes with sIR between controls and MND, sIR levels per exosome between controls and HAD, sIR levels per exosome between normal and HAD, and ROS levels per exosome between controls and MND. (4) No significant differences were observed in age between HIV-seropositive patients and controls. Our study provides evidence that sIR and ROS in exosomes are increased in HIV-seropositive women. The exosomal sIR secretion may have a role in the pathogenic process of insulin resistance and cognitive impairment in HIV-seropositive population.

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Reduction in cystatin B, cathepsin B, and its interacting partner APOC2 in CD14high/CD16low monocyte subpopulation decreases with HAND severity

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Previous studies using our Hispanic women cohort indicated that intracellular cathepsin B and cystatin B levels are elevated in CD14+ monocytes of HIV+ women with HIV associated dementia (HAD). We also found that cathepsin B protein partners SAPC and APOC2 are increased in HIV infected macrophage supernatants, however their association with neurocognitive disorders (HAND) remains to be elucidated. In this study we investigated if the expression of these proteins in monocyte CD14high/CD16high subpopulations are associated with HAND. Cystatin B and cathepsin B interacting protein partners (APOC2, APP, SAPC) and receptor (IGF2R) were analyzed in CD14+ resting (CD16-) and activated (CD16low and high) monocytes from 59 HIV+ Hispanic women and 10 HIV seronegative controls. Nine (9) progressors and ten (10) non-progressors for HAND were also compared for predictive value of HAND using the outcome from subsequent visits. Peripheral blood

monocytes were immunolabeled for CD14 and CD16 surface markers. For detection of intracellular protein levels, cells were permeabilized using the BD Cytofix/Cytoperm kit, incubated with antibodies for cystatins B and C, cathepsin B, APOC2, APP, SAPC, and IGF2R followed by fluorescence labeled secondary antibodies. The mean fluorescence intensity of each protein in CD14/CD16 subsets was determined by flow cytometry. A significant reductions in asymptomatic compared to normal cognition were obtained in CD14high/CD16low in CATB, CSTB, and IGF2R and in CD14high/CD16high in SAPC. For APOC2, there were no differences by progression for CD14high/CD16high at first (p=.214) or second (p=.191) visit or for CD14low/CD16high at first (p=.477) or second (p=.391). Patients who progressed had significantly lower expression of APOC2 (p=.034). These results indicate that APOC2 decrease with progression in CD14high/CD16low monocytes of Hispanic women cohort. Future studies will be conducted in other cohorts to determine the feasibility of using APOC2 as a biomarker for progression to HAND.

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Zika virus infection and Guillain-Barré syndrome in Bangladesh: a prospective case-control study

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Objective: Previous studies associated Guillain-Barré syndrome (GBS) to outbreaks of Zika virus (ZIKV) infection. In Asia, ZIKV is known to be circulating endemic but the associated risk with Guillain-Barré syndrome is unknown. We assessed whether endemic ZIKV infection is associated with the development of GBS. **Methods:** A prospective case-control study was conducted from 2011-2015 in two GBS referral centers in Dhaka, Bangladesh. 418 patients and 418 healthy family controls were included in the study. Patients were diagnosed with GBS prior to inclusion according to established diagnostic criteria. Detailed information on the epidemiology, clinical presentation, electrophysiology, diagnosis, disease severity and clinical course was obtained during a follow-up of 1 year using a predefined protocol. **Results:** The prevalence of ZIKV neutralizing antibodies was not significantly increased in patients with GBS compared to healthy controls (OR 1.83, p=0.23, 95% CI 0.68-4.96). In patients with GBS antecedent ZIKV infection was associated with higher age and more frequent cranial, sensory, and autonomic nerve involvement compared to GBS patients with Campylobacter jejuni, the predominant antecedent infection in GBS worldwide. Nerve conduction studies revealed that ZIKV infections were associated with a demyelinating subtype of GBS, while Campylobacter jejuni infections were related to an axonal subtype. **Interpretation:** No association was found between ZIKV infection and GBS in Bangladesh. GBS following ZIKV infection was characterized by a distinct clinical and electrophysiological subtype compared to C.jejuni infection. These finding implicate that in endemic regions ZIKV may trigger a GBS subtype but the risk is low.

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HIV-1 Tat dysregulates Mitochondrial Bioenergetics and Quality Control in Neonatal Cardiomyocytes

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Cardiovascular problems in human immunodeficiency virus (HIV)-infected patients have been leading causes of morbidity and mortality even though the viral load is controlled with antiretroviral therapy. Among the proteins encoded by HIV, Tat is a transcriptional transactivator which plays an important role in activation of HIV gene expression and has been reported to play an important role in development of cardiomyopathy. Considering the role of mitochondria in supplying the main energy demand of cardiac excitation-contraction coupling, we have investigated the impact of HIV-1 Tat on mitochondrial homeostasis by evaluating changes in mitochondrial bioenergetics and mitochondrial quality control in the presence of Tat in primary cardiomyocytes. Our results demonstrate that HIV Tat significantly suppresses mitochondrial oxidative phosphorylation, ATP production and mitochondrial calcium uptake. In addition, Tat expression leads to significant accumulation of mitochondria and reactive oxygen species. Taken together, our results demonstrate that HIV Tat by impacting the quality of mitochondria dysregulates cardiac homeostasis.

P103

Heme oxygenase-1 polymorphism associates with neuroimmune activation and neurocognitive impairment in HIV-infected individuals

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We previously demonstrated that expression of the anti-oxidative and anti-inflammatory protein heme oxygenase-1 (HO-1) is decreased within the brains of HIV+ subjects and that this HO-1 loss correlates with increased neuroimmune activation and neurocognitive dysfunction. To determine a potential protective role for HO-1 against HIV neurocognitive impairment, we analyzed a common HO-1 promoter region (GT)n dinucleotide repeat polymorphism that regulates HO-1 promoter activity (shorter (GT)n repeats associate with greater transcription). We determined HO-1 (GT)n polymorphism allele repeat lengths in both an HIV autopsy cohort from the NNTC (n=554) and in a neurocognitively-characterized, living cohort from CHARTER (n=600). HO-1 (GT)n repeat lengths in both cohorts ranged from 13 to 44 repeats with a trimodal distribution of peaks at 23, 30, and 39. In the autopsy cohort, HIV+ subjects with short alleles (<27 repeats) had a significantly lower risk of HIV-encephalitis (p=0.04, OR=0.62). Furthermore, in HIV+ subjects without encephalitis, the presence of a short allele correlated significantly with lower brain RNA expression of type I interferon response genes (ISG15, MX1) and T-lymphocyte activation markers (CD38 and GZMB). In the clinical cohort, HIV+ subjects with short alleles had significantly lower risk of functional impairment as determined by a neuropsychological and clinical diagnosis of minor neurocognitive disorder (MND; p=0.047, OR=0.63). The significance of this decreased risk in functional impairment increased when subjects with comorbidities contributing to neurocognitive decline where excluded (p=0.01, OR=0.54). In both cohorts, the presence of a short allele did not correlate with plasma or CSF viral loads or CD4 T-cell counts. Our data suggest that the presence of shorter HO-1 (GT)n alleles, through increased HO-1 promoter activity, might provide neuroprotection against developing neurocognitive impairment by downregulating neuroimmune activation. Therapeutic strategies that induce HO-1 expression may decrease HIV-associated neuroinflammation and the risk for development of HIV neurocognitive impairment.

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Novel elvitegravir nanoformulation approach to suppress the viral load in HIV-infected macrophages

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Monocytes serve as sanctuary sites for HIV-1 from which virus is difficult to be eliminated. Therefore, an effective viral suppression in monocytes is critical for effective antiretroviral therapy (ART). This study focuses on a new strategy using nanoformulation to optimize the efficacy of ART drugs in HIV-infected monocytes to improve HIV treatment outcomes. Poly (lactic-co-glycolic acid) (PLGA)-based elvitegravir nanoparticles (PLGA-EVG) were prepared by nano-precipitation technique. The physicochemical properties of PLGA-EVG were characterized using transmission electron microscopy, dynamic light scattering, and Fourier-transform infrared spectroscopy. Cellular uptake study was performed by fluorescence microscopy and flow cytometry. All in vitro experiments were performed by using HIV-infected monocytic cell lines U1 and HIV-infected primary macrophages. Elvitegravir quantification was performed through LC-MS/MS. HIV viral replication was assessed by using p24 ELISA. We developed a PLGA-EVG nanoparticle formulation with particle size of ~47 nm from transmission electron microscopy and zeta potential of ~6.74 mV from dynamic light scattering. These nanoparticles demonstrated a time- and concentration-dependent uptakes in monocytes. PLGA-EVG formulation showed ~2 times higher intracellular internalization of EVG than control group (EVG alone). PLGA-EVG nanoparticles also demonstrated superior viral suppression over control for a prolonged period of time. PLGA-based EVG nanoparticles increased the intracellular uptake of EVG, as well as enhanced viral suppression in HIV-infected macrophages. We are now in process of using in vitro blood-brain barrier (BBB) and to determine the ability of our nanoparticles to cross the BBB. Our next step is to synthesize CD-14 antibody conjugated EVG nanoparticles and use in vivo mouse model to optimize our PLGA-EVG nanoparticles and develop the potential use in HIV treatment.

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Gemfibrozil, a lipid lowering drug, increases Nurr1 in dopaminergic neurons via PPARα

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Parkinson's disease (PD) is the devastating neurodegenerative disease of ventral midbrain, which is characterized by the progressive loss of dopaminergic (DA) neurons. Mutation of nurr1 genes had been shown to be involved in the loss of DA neurons and eventually the development of parkinsonian pathologies. However, the molecular mechanism to upregulate the expression of Nurr1 has been poorly understood. Our mRNA analyses followed by different immunoassays clearly indicated that PPARα agonist gemfibrozil is strongly upregulated the expression of Nurr1 in wild-type, but not in ppara-null DA neurons suggesting PPARα might be involved in the upregulation of Nurr1. Moreover, identification of conserved PPRE element in the promoter of nurr1 gene followed by chromatin immunoprecipitation analysis, PPRE luciferase assay and manipulation of nurr1 gene by viral transduction of different ppara plasmids confirmed that PPARα is indeed involved in the expression of Nurr1. Finally, our in vivo work confirmed that WT, but not PPARα mice gavage fed gem for 14 days increased Nurr1 protein levels in the nigra. Our current study identifies PPARα is a novel regulator of Nurr1 and Nurr1-mediated protection of parkinsonian pathologies.

SThis study was supported by a merit award from the Department of Veteran Affairs (1I01BX003033) and a grant (AT006681) from National Institutes of Health.

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Guanylate Binding Protein 5 Inhibits Herpes Simplex Virus Type 1 in Astrocytes

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Guanylate binding protein 5 (GBP5) is an interferon-inducible cellular factor that belongs to the superfamily of guanosine triphosphatase (GTPase). GBP5 plays an important role in cell-intrinsic defense against intracellular pathogens, including the viruses. We thus examined whether GBP5 has the ability to inhibit herpes simplex virus type 1 (HSV-1) infection of human astrocytes (U373). We observed that U373 cells express GBP5, which was inversely correlated with HSV-1 replication. To knock out GBP5 in U373 cells significantly increased susceptibility of the cell to HSV-1 infection. Mechanically, the knock out of GBP5 resulted in the down-regulation of IFNs (IFNα, IFNβ, IFNλ1, IFNλ2/3) and antiviral IFN-stimulated genes (ISGs, MxA, MxB, OAS1, OAS2 and ISG56). These observations provide the first experimental evidence that GBP5 inhibits HSV-1 through the regulation of a number of cellular antiviral factors in the astrocytes. Further investigations are necessary to determine whether GBP5 has potential as a therapy target for HSV-1 infection.

P107

IFN-λ1 enhances TLR3 signaling of human intestinal epithelial cells mediated anti-HIV activity

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IFN-λ has been shown to have antiviral activity against a broad spectrum of viruses, including HIV. We previously revealed that human intestinal epithelial cells (IECs) express functional toll-like receptor 3 (TLR3), the activation of which releases antiviral factors that inhibit HIV infection of macrophages. In this study, we examined the effect of IFN-λ on TLR3 signaling of IECs in the context of induction of the anti-HIV interferon-stimulated genes (ISGs). We demonstrated that IFN-λ was able to upregulate the expression of TLR3 and activate TLR3 signaling by Poly I: C, producing a number of antiviral ISGs (ISG15, ISG56, GBP5, and Viperin). In addition, we found that exosomes released from IFN-λ-sensitized and Poly I: C-stimulated IECs contained the antiviral ISGs and the HIV restriction microRNAs (miRNA-28 and miRNA-29a, b, c). The exosomes with the antiviral factors could be taken up by macrophages, resulting in HIV inhibition. These findings indicate that IFN-λ enhances TLR3 signaling of IECs-mediated antiviral immune response, which may have a key role in the gastrointestinal (GI) innate immunity against HIV infection.

P108

HIV-1 Nef causes Mitochondrial Dysfunction by inhibition of autophagy, mitochondrial biogenesis and oxidative phosphorylation

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Treatment with antiretroviral therapy (ART) has significantly improved the life expectancy of HIV-1 positive patients, and converting a once acute deadly disease to a chronic illness. However, a surviving HIV-1+ population whose viral load is controlled by ART, exhibits a higher rate of cardiac abnormality, and heart failure. Our recent study showed that the HIV-1 protein, Nef, is present in cardiac tissue and in the serum of HIV-1+ patients. Nef is an accessory factor that is known to be involved in the viral replication and cell survival. Deletion of Nef compromises viral persistency. We investigated the role of Nef in the regulation of mitochondrial quality control and function using a primary cell culture model of

neonatal rat ventricular cardiomyocytes. Our initial study shows that Nef causes accumulation of deformed mitochondria through inhibition of mitochondrial quality control by reducing autophagy flux of cells. Mitochondrial fusion and fission regulatory gene expression are also dysregulated in the presence of Nef protein. In addition, we found that Nef causes inhibition of mitochondrial oxidative phosphorylation, reduction of mitochondrial membrane potential and enhanced production of mitochondrial reactive oxygen species. Moreover, Nef-treated cardiomyocytes show a reduced level of PGC-alpha expression, a key regulator of mitochondrial biogenesis. Altogether, our study shows that HIV-1 Nef causes cardiomyocytes abnormality through blockage of mitochondrial quality control and inhibition of mitochondrial function.

P109

Antiretroviral therapy lead to cardiotoxicity through inhibition of autophagy and activation of ER stress

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Due to the introduction of highly active antiretroviral therapy (HAART) regimes, HIV-1 related mortality has significantly decreased and AIDS patients have a longer life expectancy. Although, HAART decreases mortality, the use of antiretroviral drugs has been associated with several adverse effects in different organs and, cardiomyopathy remains one of the leading causes of heart failure in AIDS patients. However, the effect of antiretroviral drugs on cardiomyocytes is poorly understood. In this study, we tested the role of HAART in cardiomyocyte protein quality control using rat primary cardiomyocytes as a model system. Our findings suggest that cardiomyocytes treated with HAART in combination or alone show dysregulation of autophagy and causes accumulation of ubiquitinated protein in the cells. Dysfunction of the cellular PQC leads to endoplasmic reticulum stress (ER stress) and activation of the unfolded protein response (UPR). Mechanistically, we found that HAART treatment causes increased ER stress through upregulation of ER stress associated proteins, ATF6 and CHOP. These studies have revealed the molecular mechanism of HAART induced cardiotoxicity and may lead to the development of a novel strategy for the treatment of cardiomyopathy in the post HAART era.

P110

BAG3: a critical players of nuclear proteostasis and gives protection to nucleus during proteotoxic stress

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Protein quality control (PQC) plays a critical role in maintaining the balance of protein synthesis and protein turnover, and inhibits the accumulation of misfolded, non-functional aggregate proteins in the cytoplasm. BAG3 is a co-chaperone of protein that, in association with HSP70, is actively involved in maintaining cellular proteostasis through assisting protein folding and degradation of unfolded protein through the autophagy pathway. Recent findings indicate that some nuclear envelope proteins also undergo degradation via the autophagy process called nuclear autophagy. In this study, we found that during proteasomal stress, expression of the BAG3 protein is upregulated in cytosol as well as in the nucleus. Immunocytochemistry studies also show that during proteasomal stress, BAG3 is found to be co-localized with the nuclear membrane and forms cytosolic micronuclei with Lamin B and nuclear DNA. Further, any alteration in the level of BAG3 has an impact on the turnover of Lamin B in cells. Reduced expression of BAG3 causes inhibition of cellular PQC and accumulation of the nuclear autophagy marker protein Lamin B in the cytoplasm, and also changes the size and shape of the nucleus. Our results show that overexpression of BAG3 protects the nucleus during proteasomal stress and changes the morphology of the nucleus, likely due to the apoptotic process. Results from protein-protein studies show that BAG3 forms complex with HSP70 and Lamin B through the BAG3 domain. Altogether, our study suggests that BAG3 is a crucial player of nuclear PQC and therefore it could be a novel strategy to protect the nucleus during proteotoxic stress.

P111

De-acidification of endolysosomes induces iron to be released and causes mitochondrial dysfunction

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Mitochondria dysfunction and ROS production play important roles in healthy aging and in age-related neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, and HIV-associated neurocognitive disorders (HAND). Because ferrous iron is a key factor for the generation of reactive oxygen species (ROS) via the Fenton reaction, and because intracellular and intra-mitochondrial iron originates from the endocytosis of ferric iron bound to transferrin, we explored the extent to which and mechanisms by which iron released from endolysosomes is an early and upstream event of mitochondria dysfunction and ROS production. We demonstrated, in U87 glioblastoma cells, that endolysosome de-acidification with bafilomycin A1, chloroquine, and HIV-1 gp120 increased the release of iron from endolysosomes and this resulted in decreased iron levels in endolysosomes, increased levels of iron in cytosol, and increased levels of iron in mitochondria. Endolysosome de-acidification induced with bafilomycin A1, chloroquine, and HIV-1 gp120 increased ROS levels in cytosol and mitochondria and the overproduction of ROS was blocked by chelating iron with deferoxamine. Mechanistically, we demonstrated that endolysosome-resident two-pore channels were involved in iron release from endolysosomes upon de-acidification, and that mitochondrial permeability transition pores were involved in iron uptake into mitochondria. Our findings suggest that endolysosome de-acidification and iron release from endolysosomes play important and early roles in mitochondrial dysfunction including ROS production. Our findings further suggest that chelating endolysosome iron and/or endolysosome acidification might be potential adjunctive therapeutic strategies in alleviating mitochondria dysfunction, preventing neurotoxicity, and enhancing therapeutic outcomes of neurodegenerative diseases and HAND.

Supported by P30GM103329, R01MH100972, MH105329 and R21DA040519.

P112

Sex differences in HIV-1 transgenic and F344 rats on three behavioral assessments

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Human immunodeficiency virus (HIV) proteins in the central nervous system of people living with HIV (PLWH) could contribute to the known neurocognitive impairments. In last two decades, several rodent models have been developed for study of the molecular and cellular mechanisms underlying HIV-associated neurocognitive disorder. The non-infectious HIV-1 transgenic (HIV-1Tg) rat model mimics PLWH receiving combined anti-retroviral therapy. We tested male and female HIV-1Tg rats and F344 controls for locomotor activity (LMA) in an open field, as well as for spatial learning and memory performance in the modified Morris Water Maze (mMWM) (an aversive task) and the Hole Board (HB) (an appetitive task). The LMA test started when the animals were 1 month old and continued each month except when the mMWM and HB tests were conducted at 4 and 7 months, respectively. The female rats traveled further and had more center entries at 3, 5, and 6 months. The mMWM test replicated our previous findings that HIV-1Tg rats take longer to find the escape platform. In addition, the HIV-1Tg rats swam along irregular, circular, and disordered paths, whereas the F344 rats swam directly to the platform. No significant sex difference was found in performance on the mMWM test. The HB test revealed a sex difference in the strategy used to search for sugar pellets hidden in 4 of the 16 holes. Nevertheless HIV-1Tg rats of both sexes showed poorer performance than F344 rats, demonstrating that learning deficits in HIV-1Tg rats occur in both aversive and appetitive spatial learning tasks. Taken together, these results of LMA, mMWM and HB assessments confirm learning and performance deficits in HIV-1Tg rats in aversive and appetitive tasks and suggest that sex is a moderating factor for the cognitive and behavioral performance of the effects of the persistent presence of HIV-1 proteins.

P113

Studying involvement of interferon regulatory factor 7 in cell growth using CRISPR/Cas9 gene editing

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Innate immunity is the first line of host defense. It triggers various immune responses. Interferon regulatory factors (IRFs) are critical transcription factors that regulate transcription of type I interferons (IFNs) and IFN-stimulated genes (ISGs) in the antiviral responses. IRF7 is the master regulator of type I IFN-dependent immune responses which mediates biological functions including cell proliferation. However, the mechanisms underlying IRF7-mediated cell growth are still largely unknown. HIV-1 transgenic (HIV-1Tg) rat is to mimic people living with HIV treated with combined anti-retroviral therapy. We previously reported that expression of IRF7 is significantly elevated in the HIV-1Tg rat's striatum, prefrontal cortex, and hippocampus areas which are highly vulnerable to the adverse effects of HIV infection. We also found that expression of IRF7 was increased in C8-D1A astrocytes given polyinosinic-polycytidylic acid (poly I:C), a synthetic analog of double-stranded RNA to mimic virus infection. We hypothesize that IRF7 is involved in virus mediated biological functions including cell growth. Using CRISPR/Cas9 technique, IRF7 gene was edited in a fast-growing cell line, human embryonic kidney 293FT (HEK293FT) cells. Flow cytometry, qRT-PCR analysis and Western blotting analysis have confirmed decreased expression of IRF7 at both mRNA and protein levels in the IRF7-edited HEK293FT cells. Using Cell Counting Kit-8 assay, we have also shown, in comparison to the wild-type cells, the IRF7-edited cells showed growth retard. In line with slow cell growth, the apoptosis indicators, Bax, Bcl-2 and Caspase 3, were significantly increased suggesting the involvement of IRF7 in apoptosis leading to cell growth retard. Interestingly, expression of cell cycle markers, PCNA, Cyclin B1 and Cyclin D1, were also significantly upregulated in the IRF7-edited cells compared to the wild-type cells. Our ongoing studies are to use the IRF7-edited HEK293FT cells to investigate molecular mechanisms underlying IRF7-mediated cell growth and the virus infection mediated biological functions.

P114

Monocyte-derived exosomes alter their characteristics upon exposure to cigarette smoke condensate and protect against cytotoxicity and HIV-1 replication

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Cigarette smoking is highly prevalent in HIV-1 patients, resulting into higher rates of morbidity and mortality. Smoking is known to exacerbate HIV-1 pathogenesis, especially in monocytes, likely through the cytochrome p450 (CYP)-mediated oxidative stress pathway. Exosomes have been shown to alter HIV-1 pathogenesis through inter-cellular communication. These are nano-vesicles secreted by many cells and are packaged with proteins and miRNA. However, the exact role of exosomes in smoking-mediated HIV-1 pathogenesis is unknown. In this study, we investigated the effect of Cigarette Smoke Condensate (CSC) on the characteristics of monocyte-derived exosomes and their influence on HIV-1 replication. Initially, we characterized the physical properties of exosomes. We showed that CSC reduced total protein and antioxidant levels in exosomes derived from HIV-1-infected and uninfected macrophages. The exosomes from CSC-treated uninfected cells exert protection from cytotoxicity and viral replication in HIV-infected macrophages. However, exosomes derived from HIV-infected cells lost their protective capacity. The results suggest that exosomal defense is more pronounced during the early stages of HIV-infection, which diminishes at latter phase. Furthermore, exosomes from uninfected cells demonstrated a CSC-mediated upregulation of catalase, while a decrease in the levels of catalase and PRDX6 in exosomes derived from HIV-infected cells. These results suggest a potential role of antioxidant enzymes (AOE), which are differentially packaged into CSC-exposed HIV-1-infected and uninfected cell-derived exosomes, on HIV-1 replication of recipient cells. Furthermore, we investigated the role of cytokines and found that exosomes package significantly lower amount of pro-inflammatory cytokines while higher level of TNF-α, IL-8 and RANTES are in CSC-exosomes. Besides, proteomics can reveal more insight about exosomal protein content. Overall, our study suggests a potential role of exosomal CYP, AOE and cytokines in smoking mediated cytotoxicity and HIV-1 pathogenesis. Upon further investigation, these components may be utilized as novel therapeutic strategy through exosome mediated delivery.

P115

HIV disease and neurocognitive performance linked to medial prefrontal cortex volume

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Background: In the era of combination antiretroviral therapy (cART), the prevalence of HIV-associated neurocognitive disorders (HAND) remains high, and older age is associated with an elevated risk. With a rapidly aging populace, there is an increasing demand to further understand the neural mechanisms for HAND diagnosis and treatment. Using voxel-based morphometry, we examined differences in gray matter volume (GMv) related to HIV-status, neurocognitive performance, and depression. **Methods:** High resolution T1-based MPRAGE images were acquired from 101 participants (41-69 years old, 54 HIV+, 47 male). None of the participants had a diagnosis of symptomatic cognitive impairment. All HIV+ participants were on antiretroviral therapy and 79.6% had undetectable viral load and 77.8% had normal CD4 T cell count (>500). Neurocognitive performance was assessed with a full battery in a subset of participants (n=33, 26 HIV+). Depression scores were obtained for a subset of participants (n=61, 41 HIV+), and there was no significant difference between patients and controls. The software package SPM12 was used for MRI data analysis. A threshold of $p<0.001$, uncorrected, 100 contiguous voxels, was used unless otherwise specified. **Results:** Older age was associated with GMv reduction across brain regions. By contrast, HIV-disease was associated with reduced GMv in the ventromedial prefrontal cortex (vmPFC) and occipital cortex. Exploratory analyses ($p<0.01$, uncorrected, 100 contiguous voxels) revealed that lower cognitive performance was associated with reduced GMv in frontal cortex, including lateral prefrontal cortex, anterior cingulate cortex, and vmPFC. GMv at these clusters did not correlate with depression scores. **Conclusions:** Brain atrophy - especially in prefrontal area - was present in HIV+ adults despite controlled viral load and immune recovery on cART. The correlation between poorer neurocognitive performance and lower GMv in frontal cortex but not subcortical GMv suggests that post-cART HAND might be driven by neural injury in cortical rather than subcortical regions.

P116

MicroRNA 301: a pivotal regulator of Japanese Encephalitis virus infection

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Introduction: Japanese encephalitis virus (JEV) is the most leading cause of viral encephalitis in the Asia-Pacific region. Although neurons are the primary target cells of JEV infection, bystander damage caused by pro-inflammatory cytokines released from activated microglia is also a vital concern in JEV-induced neuronal death. MicroRNAs (miRNAs) play a very vital role in establishing viral infections by fine-tuning the existing host regulatory pathways, making the environment favourable for viral propagation. Prominent influence of miRNAs on virus infection thus demands investigation regarding its role in JEV infection. **Methods:** While neuronal (HT22)/microglial (CHME3) cell line was used for in vitro studies, JEV infected BALB/c mouse was used as in vivo model. miRNA expression was assessed by qPCR and in situ hybridization. miRNA expression was modulated by means of miRNA mimics and inhibitors following their transfection into the cell line. Vivo-Morpholino was administered intracranially to alter miRNA expression in mouse model. **Results:** miR-301a was observed to be significantly enhanced upon JEV infection in neurons and microglia. Neuronal miR-301a inhibited type I interferon by targeting interferon regulatory factor 1 (IRF1) and suppressor of cytokine signalling 5 (SOCS5). On the other hand, inhibition of JEV induced miR-301a restored IFN-β expression and thus prohibiting viral propagation Similar observations were found to be reproduced consistently when miR-301a was inhibited in vivo by administration of Vivo-Morpholino. In JEV-infected microglia, miR-301a induced NF-κβ activation by targeting NF-κβ repressing factor (NKRF) and augmented inducible nitric oxide synthase, cyclooxygenase-2, and pro-inflammatory cytokine expression. **Conclusions:** In addition to enhancement of type I interferon signalling, inhibition of mir-301a also culminates into reduced bystander damage to neurons as a result of reduced microglial activation. Role of mir-301a in immune regulation and neuronal death thus makes it a potential target as future antiviral strategy against JEV infection.

P117

Cathelicidin-derived antimicrobial peptides suppress Zika virus through direct inactivation and interferon pathway

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Zika virus (ZIKV) is a neurotrophic flavivirus that is able to infect pregnant women and cause fetal brain abnormalities. Although there is a significant effort in identifying anti-ZIKV strategies, currently no vaccines or specific therapies are available to treat ZIKV infection. Antimicrobial peptides, which are potent host defense molecules in nearly all forms of life, have been found to be effective against several types of viruses such as HIV-1 and influenza A. However, they have not been tested in ZIKV infection. To determine whether antimicrobial peptides have anti-ZIKV effects, we used nine peptides mostly derived from human and bovine cathelicidins. Two peptides, GF-17 and BMAP-18, were found to have strong anti-ZIKV activities and little toxicity at effective doses in Vero cells. We further tested GF-17 and BMAP-18 in human fetal astrocytes, a known susceptible cell type for ZIKV, and found that GF-17 and BMAP-18 effectively suppressed ZIKV regardless of whether peptides were added before or after ZIKV infection. Interestingly, inhibition of type I interferon signaling resulted in higher levels of ZIKV infection and partially reversed GF-17-mediated viral suppression. More importantly, pretreatment with GF-17 and BMAP-18 did not affect viral attachment but reduce viral RNA early in the infection course. Direct incubation with GF-17 specifically reduced the number of infectious Zika virions. In conclusion, these findings suggest that cathelicidin-derived antimicrobial peptides suppress Zika virus through direct inactivation and via the interferon pathway. Strategies that harness antimicrobial peptides might be useful in halting ZIKV infection.

P118

Comorbidities and HAND in HIV Infection - Findings from the NNTC

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Individuals living with HIV with access to antiretroviral therapy (ART) are living longer, healthier lives than ever before. The disease profile is increasingly recognized as that of a complex chronic illness. In addition to complications related to HIV, research has shown that people living with HIV are at higher risk for developing chronic, non-HIV related conditions at younger ages than HIV negative individuals. These conditions include infections such as Hepatitis C, diseases of the cardiovascular system, kidneys, and liver, and metabolic disorders including diabetes and dyslipidemia. CHARTER and NNTC comprise multi-site studies aimed at characterizing and investigating the neurological consequences of HIV infection. The now integrated CHARTER and NNTC research database represents an important resource for investigating the presentation of HIV-Associated Neurocognitive Disorders (HAND) as well as non-HIV related comorbidities, as it is comprised of over 4,000 well-characterized participants representing a broad cross-section of the clinically relevant HIV+ population. Here we explore the presentation of common chronic non-HIV related comorbid conditions within and across the NNTC and CHARTER cohorts. Treatment status, disease severity, and other factors within the population

are related to patterns of comorbid conditions within the cohort. The NNTC database, accessible to the research community, is a rich resource to investigate neurological and other morbidities in those infected with HIV. In addition, specimens, ranging from biofluids (blood, CSF) to tissues (brain, lungs, heart, lymph nodes) are available for research.

P119

Presence of HIV-1 Tat in cerebrospinal fluid despite antiretroviral therapy

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HIV remains a chronic infection with potential long-term deleterious consequences, chronic inflammation and neurocognitive impairment. Evidence suggests that ongoing low-level viral replication and/or production of specific viral proteins in the presence of antiretroviral therapy (ART) play a role. There is a driving need in HIV research to control sources of residual productive or partial viral replication and to provide alternative therapies that fill the gaps left by currently available treatments. The HIV Trans-Activator of Transcription (Tat) is a neurotoxic and pro-inflammatory viral protein that is also essential for optimal HIV transcription. Tat is not directly targeted by current therapies and may be produced even in the presence of ART. We sought to determine whether Tat is present in cerebrospinal fluid (CSF) of individuals on suppressive ART and, if so, whether CSF Tat is biologically active. Tat protein was detected in 32% (19/59) of patient CSF samples as determined by ELISA. Furthermore, Tat plasma concentration increased in 4 out of 5 individuals after initiation of therapy, indicating that Tat was not inhibited by ART. Similarly, exosomes from 34% (11/32) of CSF samples were strongly positive for Tat protein and/or TAR RNA (31%; 10/32), with 2 samples positive for both Tat and TAR. Exosomal Tat protein from 4/6 samples retained transactivation activity in a CEM-LTR reporter assay, which indicates that at least a fraction of Tat in CSF is functional. Antisense oligonucleotides (ASO) complementary to Tat mRNA effectively reduced Tat protein levels and inhibited viral release from HIV-transfected cells in vitro, as measured by ELISA and product enhanced reverse transcriptase (PERT) assay, respectively. These findings confirm that both Tat and viral RNA continue to be produced in individuals otherwise controlled on ART, and highlight a need for new therapies that target Tat.

P120

Plasmacytoid dendritic cells and T cells from HIV+ donors differ in their sensitivity to Δ9-Tetrahydrocannabinol compared to healthy donors

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Stimulation and maintenance of T cell populations is partially mediated through cytokines. Two of the key cytokines in T cell activation during HIV infection are Interferon-α (IFNα), which plays a role in the peripheral control of HIV infection, and IL-7, a key homeostatic cytokine for T cells. Δ9-Tetrahydrocannabinol (THC), the primary psychoactive compound in marijuana, can exacerbate disease progression by suppression of interferon secretion and T cell function. Additionally, many HIV patients utilize medicinal cannabinoids. The objectives of these studies were to determine: 1) whether THC impairs the secretion of IFNα from plasmacytoid dendritic cells (pDC); 2) whether THC suppresses IFNα-mediated activation of T cells; and 3) how pDC and T cells from healthy and HIV+ donors compare in their response to stimulation and THC. IFNα secretion and phosphorylation of pIRF7, the master regulator of IFNα secretion, were induced by CpG-ODN and IFNα secreting or pIRF7+ pDC were enumerated by flow cytometry. T cells were stimulated using recombinant human IFNα using levels of pSTAT1 as an indicator of IFNAR signaling. The expression IL-7Rα was used as a marker of downstream IFNα activation as the IL-7Rα gene promoter contains an interferon sensitive response element (ISRE). Surface bound IL-7R, IL-7-induced pSTAT5, and T cell proliferation were determined by flow cytometry. Levels of pIRF7 and secretion of IFNα was suppressed by THC regardless of HIV status, but pDC from HIV+ donors were more sensitive to THC-mediated suppression compared to healthy pDC. Conversely, while THC suppressed IFNα-mediated signaling, expression of IL-7Rα, IL-7-induced pSTAT5, and proliferation of T cells from healthy and HIV+ donors, T cells from HIV+ donors were less sensitive to THC-mediated suppression compared to healthy T cells. Collectively, these results support THC as an immunosuppressant and reveal the consequence of chronic HIV infection on pDC and T cell sensitivity to THC.

P121

FAAH Inhibition Attenuates the Neurotoxic Microglial Response to HIV-1 Tat Protein

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The HIV-1 virus affects microglial immune responses which likely play a paramount role in the development of chronic neuroinflammatory conditions and neuronal damage found in HIV-1 associated neurocognitive disorders (HAND). The HIV-1 transactivator of transcription (Tat) protein is detectable in the brains of AIDS patients on combined antiretroviral therapy (cART), and can produce neurotoxic effects directly through neuronal pathways and indirectly through microglial proinflammatory action. Interventions with neuroprotective or anti-inflammatory activity are in high demand to stop these chronic neurodegenerative processes. Drugs targeting the degradative enzymes of endogenous cannabinoids show promise in reducing pain and inflammation with minimal side effects in rodents. We previously demonstrated in a murine prefrontal cortex (PFC) neuron culture model that inhibiting the degradation of anandamide (one of two major endogenous cannabinoid ligands) using the catabolic enzyme inhibitor PF3845 blunts the direct Tat neurotoxic effects. In the present study we assessed the effects of PF3845 on Tat-induced proinflammatory responses in microglial cells. Cultured murine microglia were incubated with Tat and/or PF3845. After 24 hours, microglial conditioned media was collected and applied to PFC neuron cultures at different dilutions and neurotoxicity was assessed using live cell Ca2+ imaging. Fura-2AM was used to visualize dynamic changes in neuronal [Ca2+]i during exposure to medium derived from Tat-treated microglia. Medium from microglia exposed to Tat significantly increased neuronal [Ca2+]i levels compared to control medium. Importantly, medium derived from microglial cultures pretreated with

PF3845 and Tat showed significant downregulation of [Ca2+]i in PFC neurons compared to Tat treatment alone, indicating an attenuated microglial response. These data suggest that targeting FAAH expressed in microglia may be useful in treating neuroinflammation in HAND and other neurocognitive diseases.

P122

Synthesis and Characterization of a Hydrophobic and Lipophilic Rilpivirine Prodrug Nanoparticle

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Modern antiretroviral therapy (ART) proficiently suppresses replication of human immunodeficiency virus (HIV), while concurrently restoring immune function and providing an overall reduction in co-morbid conditions. These advancements, while significant are limited by the inability of ART to penetrate cellular and anatomical reservoirs, thereby allowing HIV to persist within ART-adherent patients. Long-acting ART injectables, administered on a monthly or bi-monthly basis, are gathering momentum for the treatment and prevention of infected or virus susceptible people. Specifically, long-acting formulations of Cabotegravir and Rilpivirine (CAB and RPV-LA) are currently under phase III clinical evaluation. Contemporaneously, our laboratory has developed long-acting slow effective release ART (LASER-ART), which utilize encapsulated prodrugs to appropriately tune drug release while improving the pharmacokinetics (PK) and tissue penetrance. To this end we have synthesized a hydrophobic prodrug of RPV (MRPV), utilizing a bioreversable hemiaminal bond to attach a 14-carbon fatty acid. MRPV was formulated into stable nanoformulation (NMRPV) and provided efficient intracellular drug uptake and antiretroviral efficacy in HIV-1 challenged monocyte-derived macrophages (MDM). Additionally, NMRPV administered to BALB/cJ mice by a single intramuscular (IM) dose of 40 mg/kg yielded detectable plasma RPV concentrations up to 56 days. MRPV provided the stage to further synthesize a library of RPV prodrugs, specifically tuned in an effort to achieve optimal drug release, pharmacokinetic profiles and biodistribution. We posit that a hydrophobic pro-drug of RPV encapsulated within a poloxomer-based LASER-ART has the potential to substantially improve the half-life, efficacy, and biodistribution of RPV.

P123

Amyloid precursor protein processing can be altered with molecular tools containing the raft targeting protein US9 from Herpes Simplex Virus: Implications for HAND

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Despite effective antiretroviral treatment, cognitive dysfunction is present in up to 50% of individuals infected with HIV. Importantly, this dysfunction- collectively known as HIV associated neurocognitive disorder (HAND)- can be enhanced in the aged population, which represents a sizeable and growing proportion of the HIV+ community. Converging clinical and preclinical evidence suggest that specific biochemical and structural changes in neurons may similarly underlie cognitive deficits in both HAND and aging (normal and pathological). For example, both HIV proteins and aging increase plasma membrane ceramide content, which can profoundly alter membrane signaling events by differentially segregating membrane proteins and driving their internalization. As a result, membrane ceramide can amplify inflammatory signaling (IL1-beta, TNF-alpha) and processing events (Amyloid Precursor Protein, APP, to Amyloid-beta) that are frequently triggered by infection and CNS injury, and are strongly implicated in both HIV and age-related cognitive dysfunction. To elaborate on the pathophysiologic implications of these membrane alterations, our group has generated and characterized a series of chimeric fusion proteins harboring portions of the non-amyloidogenic APP-cleaving enzyme ADAM10 fused to the raft protein US9 from Herpes Simplex Virus. These proteins localize to membrane rafts where catabolic ceramide generation is thought to promote APP processing to Amyloid-beta. Here we provide evidence that US9-ADAM10 fusion proteins alter APP processing by BACE1 in vitro. Additional findings from our ongoing work to elucidate the impact of HIV proteins (in the presence and absence of these fusion proteins) on indices of neuronal injury and APP processing in vitro and in vivo will also be presented.

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The antiretroviral medicine lamivudine increases neuronal excitability by altering activity of voltage-sensitive Ca2+ and K+ channels in pyramidal neurons of rat medial prefrontal cortex

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Combination antiretroviral therapy (cART) suppresses HIV replication, improves immune function, and prolongs life of HIV+ patients. But the prevalence of HIV-associated neurocognitive disorders (HAND) occurs in ~50% of HIV-infected patients despite cART. Recent studies reveal that many antiretroviral drugs (ARVs) could induce neurotoxicity, including but not limited to decreased dendritic processes, neuronal shrinkage, and mitochondrial dysfunction. Very little is known about the impact of ARVs on neuronal activity in the brain regions that are susceptible and vulnerable to HIV as to potential mechanism(s) exploited by ARVs to alter neuronal activity. Lamivudine is a nucleoside reverse transcriptase inhibitor (NRTI) that is commonly-prescribed to HIV+ patients in cART regiment to suppress HIV replication; but frequently induces neuro/excitotoxicity. Here we assessed the effects of lamivudine on altering firing activity of glutamatergic pyramidal neurons in the medial prefrontal cortex (mPFC, a key regulator of neurocognition) using whole-cell patch-clamp recording in rat brain slices. We found that lamivudine in bath, at concentrations (0.25-40μg/ml, or 1-175μM) comparable to those found in the cerebral spinal fluid (CSF) of HIV+ patients in cART including this NRTI, significantly increased firing of mPFC neurons (evoked by moderate stimuli that mimicked physiological excitatory inputs). The increased firing was concentration-dependent, which was associated with a significantly-enhanced Ca2+ influx through voltage-gated Ca2+ channels (by 0.25μg/ml, or 1.1μM lamivudine), and a significantly-reduced activity of voltage-gated K+ (Kv) channels and inwardly-rectifying K+ (Kir) channels. Both changes in Ca2+/K+ channel activity contribute to depolarize membrane potential, thereby facilitating neuronal firing. Nevertheless, higher concentration of lamivudine (40 μg/ml, or 175 μM) appeared to decrease Ca2+ channel activity in some mPFC neurons. Collectively, these novel findings indicate that lamivudine increases mPFC neuronal excitability by enhancing Ca2+ channel activity and reducing Kv/Kir channel activity; and suggest that chronic NRTI treatment in vivo could potentiate HIV-induced neuro/excitotoxicity in the mPFC.

P125

Sensitization to morphine's anti-nociception in C57BL/6J mice having binge exposure to ethanol

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Binge drinking can elevate the blood ethanol (EtOH) concentration (BEC) to > 80 mg/dL/17.4 mM, causing gut leakage, raising endotoxin in the circulation, and contributing to enhanced activity of neuroimmune signaling pathways. Morphine abuse and drinking are intertwined molecularly, cellularly, and systemically. Morphine exerts its actions mainly on the seven transmembrane G-protein-coupled mu opioid receptors (MOR).

We reported that inflammatory cytokines upregulate expression of MOR. We hypothesized that neuroinflammation is involved in binge drinking-induced upregulation of MOR, contributing to modulation of morphine's anti-nociception. C57BL/6J (B6) mice were treated with or without 3-d binge ethanol (EtOH, 5 g/kg/d, 42% v/v, i.g.), and the anti-nociceptive effect was evaluated using hot plate tests at 24 h after the last EtOH injection with or without a cumulative subcutaneous dose (0, 0.1, 0.3, 1.0, and 3.0 mg/kg) of morphine at intervals of 30 min. The response curve of the mice given binge EtOH was shifted to the left, indicating enhanced sensitization to morphine's anti-nociception. We then investigated the gene expression profile of MOR and some inflammatory molecules at 2 min, 5 h, or 24 h after the first EtOH dose and at 24 h and 48 h after the third EtOH dose after binge EtOH in the nucleus accumbens (NAc) and the striatum (STr), which projects to the NAc. Expression of MOR and pro-inflammatory NLR family pyrin domain containing 3 (NLRP3) mRNA in the STr was significantly elevated at 5 h after the first EtOH injection and then gradually declined to the basal level, as shown by qRT-PCR, while expression of anti-inflammatory NLRP12 did not change significantly. As downstream effectors of NLRP3 inflammasome action, mRNA expression of interleukin (IL)-1β and IL-18 also was elevated in the NAc. Binge EtOH-induced inflammation in the brain may contribute to upregulation of MOR and enhanced sensitization to morphine's anti-nociception.

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Modulation of glial cells of adolescent mice given binge treatment with ethanol

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Brain homeostasis is a relatively stable equilibrium between interdependent brain molecular and cellular components maintained by signals that stimulate supportive and defensive responses. Glial cells, including astrocytes and microglia, are important to maintain brain homeostasis. Astrocyte is the major source of energy storage and metabolic regulator. Microglia are immunocompetent and phagocytic cells in the brain. Binge alcohol intake causes an energy deficit and changes the expression of AMP-activated protein kinases (AMPKs), the master molecules for brain energy homeostasis. Energy deficit creates an imbalance of pro-inflammatory and anti-inflammatory molecules. We hypothesized that binge exposure to ethanol (binge EtOH) modulates glial cells and their function. Adolescent C57BL/6J mice were treated with 5 g/kg/d 42% v/v EtOH, and sacrificed at 2 min, 5 h, or 24 h after the first EtOH injection and at 24 h and 48 h after the third binge EtOH treatment. The gene expression of neuron marker (Rbfox3, Eno2), astrocyte marker (GFAP), microglia marker (Cd68, Itgam), energy-sensor, redox and purinoceptor-7 (AMPK, Trx-1, TXNIP, and P2X7), in nucleus accumbens (NAc) and striatum was analyzed using qRT-PCR. Compared with the group at 2 min after EtOH treatment, Rbfox3, Eno2 and GFAP in the striatum didn't show significant change after binge exposure to EtOH, while microglial marker Cd68 significantly decreased at 5 h and 1 d after treatment. Expression of P2X7 was significantly decreased in the groups at 5 h, 24 h after 1st EtOH injection and 24 h and 48 h after the third injection. Immunoreactivity of Iba-1, a microglia marker, within striatum was elevated given 1-day binge EtOH and decreased in the group given 3-day treatment. Taken together, our data suggest that binge EtOH may encourage glial activation via either direct or indirect effects of energy metabolism in a time-dependent manner.

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Disruption of the HERV-Kenv gene by SaCas9/gRNAs, and mechanisms involved in amyotrophic lateral sclerosis and cancer

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Human endogenous retroviruses (HERVs) are increasingly shown to have effects on the host, inducing gene instability, recombination, superantigenic stimulation, immunosuppressive factors, interactions with growth-controlling genes, etc. The HERV-W and HERV-K families were linked to neurological diseases, due to the neuro-immune-pathogenic properties of the env protein. HERV-W is associated to multiple sclerosis (MS),and HERV-Wenv transgenic mice develop MS-like alterations. HERV-K, which is activated in several human tumors, is activated also in motor-neurons of patients with amyotrophic lateral sclerosis (ALS); HERV-Kenv transgenic mice develop ALS-like motor dysfunctions, suggesting HERV-Kenv as a possible target against ALS, a no-therapy disease. To this end, we used the CRISPR/Cas9 gene-editing strategy as a potential tool for HERV-Kenv elimination, and to test the expression of ALS-associated mutant genes, as the TDP-43 A382T-encoding allele and the C9ORF72 repeat expansion, which are present in >40% of ALS cases from Sardinia, Italy. The Cas9 from Staphylococcus aureus (SaCas9) system was chosen, since its efficiency is similar to that from Streptococcus pyogenes (SpCas9), but it is shorter, and suitable for both basic research and future therapeutic applications that require the adeno-associated virus (AAV) delivery vehicle. Several guide RNA (gRNA) targeting conserved domains of the HERV-Kenv gene were designed; two were selected after testing on LNCaP cells, whose HERV-K transcription profile is known. The results indicate that HERV-Kenv-targeted SaCas9/gRNAs successfully disrupt the gene, both alone or in combination, as evaluated by DNA sequencing, and by disappearance of env transcripts and proteins. When the system was tested for molecular mechanisms linked to ALS pathogenesis, we found that the specific suppression of HERV-Kenv interferes with important regulators of cell expression, involved in signaling, RNA-binding and alternative splicing, as TDP-43 and SF2/ASF. These findings suggest that HERV-K is not a bystander, and may be involved in mechanisms linked to ALS pathogenesis and cancer.

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Development of long-acting emtricitabine

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Nucleoside reverse transcriptase inhibitors (NRTIs) are a key component of many antiretroviral therapeutic (ART) regimens. However, all available NRTIs exhibit short half-lives requiring, at least, daily administration to maintain effective antiretroviral drug levels. Consequently, this has resulted in treatment failures and the emergence of viral resistance due, in measure, to patient adherence. To facilitate long-acting NRTIs, the first hurdle was to convert hydrophilic formulations to hydrophobic lipophilic crystals to affect cellular depots and alter the drug's half-life to improve the pharmacokinetic drug profile. To this end, we modified native emtricitabine (FTC) by esterification with palmitoyl chloride to form palmitoyl-FTC (PFTC). The modified chemical structure was confirmed by 1H-NMR and FTIR spectroscopy. PFTC was encapsulated with poloxamer 407 by high pressure homogenization to generate a PFTC nanoparticle (NPFTC). Analysis revealed a size of 350 nm, a negative charge, a polydispersity index of 0.3 and a drug loading capacity of 70%. NPFTC uptake, retention, and antiretroviral efficacy in primary human monocyte-derived macrophages (MDM) showed up to or greater than 10-fold improvements in each parameter for NPFTC compared to the unmodified drug. Exposure of MDM to NPFTC prior to challenge with HIV-1ADA resulted in complete protection for up to 15 days by assays of HIV-1 p24 staining and reverse transcriptase activity in culture fluids. PK and biodistribution analysis of NPFTC following a single intramuscular dose equivalent to 45 mg/kg of FTC in Dawley Sprague rats led to sustained plasma drug levels for two weeks. This long-acting NPFTC formulation has the potential to prolong the half-life of FTC and to provide improved antiretroviral responses.

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Overexpression and Activation of Colony-Stimulating Factor 1 Receptor in the SIV/Macaque Model of HIV Infection and NeuroAIDS

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Colony-stimulating factor 1 (CSF1) regulates the proliferation, survival, and differentiation of macrophages through the receptor tyrosine kinase CSF1R. Levels of CSF1 in the cerebrospinal fluid are elevated in HIV patients on ART with cognitive impairment. However, little is known about the expression of its receptor CSF1R in the brain. In the present study, using the simian immunodeficiency virus (SIV)/macaque model of HIV infection, we investigated whether CSF1R is expressed on brain macrophages and microglia and whether it is upregulated and activated after lentivirus infection in vivo and contributes to development of encephalitic lesions. We examined, using multi-label, semi-quantitative immunofluorescence microscopy, the protein expression level and cellular localization of CSF1R in brain tissues from uninfected (n = 3) and SIV-infected adult macaques with (SIVE, n = 4) or without (SIVnoE, n = 3) encephalitis. In the normal uninfected brain, CSF1R was detected only in microglia and brain macrophages but not in neurons, astrocytes, or oligodendrocytes. Microglia constitutively expressed CSF1R at low levels and its expression was largely unchanged in SIVnoE and SIVE animals. Brain macrophages including perivascular macrophages (PVMs) expressed higher levels of CSF1R, compared to microglia. Interestingly, we found significantly increased expression of CSF1R on the infected PVMs and lesional macrophages in the brain with SIVE. Using phosphorylated CSF1R at Y723 and phosphorylated signal transducer and activator of transcription 5 at Y694 as markers for CSF1R activation, we found selective activation of CSF1R signaling in infected brain macrophages. We also found colocalization of CSF1R and its ligand CSF1 in PVMs and lesional macrophages in the brain of macaques with SIVE. These findings are very useful for developing a specific approach targeting infected brain macrophage, with several brain-penetrant CSF1R inhibitors now available, for elimination of CNS macrophage reservoirs, while not depleting resting uninfected microglia and PVMs that show no CSF1R activation.

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A meta-analysis of brain atrophy associated with HIV disease and HIV-associated neurocognitive disorders (HAND)

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Background: Gray matter (GM) atrophy is often observed in HIV in the cART era, but the specificity of regions affected in HIV remains elusive. Here, using a novel colocalization-likelihood estimation (CLE) meta-analysis technique, we studied two questions: which region(s) is consistently affected in HIV+ adults; and which region(s) is more likely associated with neurocognitive impairment. **Methods:** In the novel meta-analysis, we first converted the reported local GM atrophy in each study to one of seven clusters: frontal, temporal, occipital, parietal, insular, limbic, and cerebellum. The converted clusters were then used in three separate analyses: I) controls versus HIV+ adults without neurocognitive impairment (11 studies, 368 HIV+, 350 HIV-); II) controls versus HIV+ adults, regardless of neurocognitive performance (25 studies, 1022 HIV+, 862 HIV-); III) HIV+ adults with HAND versus HIV+ adults without HAND (six studies, 134 HIV+/HAND+, 115 HIV+/HAND-). For HIV+ adults in these studies, after excluding those with primary infection (n=121), 84.1% were on cART, and 81.9% with controlled plasma viral load. False discovery rate (FDR) was used to correct for multiple comparison. **Results:** GM atrophy happens more often in the frontal lobe in HIV+ adults compared to other brain regions (Analysis-I: $p<0.001$, $q<0.001$; Analysis II: $p=0.013$, $q=0.090$). The reduced specificity in Analysis-II is likely due to more widespread brain atrophy in HIV+ adults with neurocognitive impairment. Analysis-III revealed that the limbic system is the only region consistently associated with HAND ($p<0.001$, $q<0.001$), especially the basal ganglia ($p<0.05$, $q>0.1$). **Conclusions:** Across studies, frontal lobe is the most commonly affected brain region in HIV. The high prevalence of frontal atrophy might underlie the frequent occurrence of executive deficits in the cART era. Further neurocognitive impairment is associated with more widespread brain atrophy, especially in the limbic system, which consistently differs between HIV+ adults with HAND versus those without HAND.

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INHIBITORY CONTROL DEFICITS IN TAT TRANSGENIC MICE USING THE GO/NO-GO TASK

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HIV-1 associated neurocognitive disorder (HAND) is a mild neurocognitive disorder characterized by working memory, attention, and inhibitory control deficits. The viral protein, HIV-1 Transactivator of transcription (Tat) is the likely agent of damage to the fronto-striatal-thalamocortical (FSTC) circuits which primarily connect the prefrontal cortex to the striatum and other basal ganglia structures. Normal functioning for working memory, attention, and inhibitory control are largely dependent on these circuits, in particular the prefrontal cortex, so Tat is likely precipitating these deficits in individuals with HAND. The current study used Tat transgenic mice to establish and investigate an animal model of inhibitory control deficits related to HAND. Tat+ and Tat- mice were trained on the Go/No-Go task over the course of several months. Mice were trained to nosepoke for reinforcers before learning to discriminate between two stimulus arrangements. In the first arrangement, Go, mice were trained to nosepoke for reinforcement, and in the second arrangement, No-Go, mice were trained to withhold their nosepoke to receive reinforcement. The number of correct omissions from No-Go trials and the incorrect omissions from Go trials were compared to yield an index of inhibition, P-Inhibition. Additionally, the number of premature and perseverative responses were gathered as an index of impulsivity. Testing revealed a main effect of sex on P-Inhibition scores with females demonstrating significantly less inhibitory controls than males. Additionally there was significant interaction between sex and genotype. Specifically, Tat+ females demonstrated significantly less inhibitory control than Tat+ males, there were no significant differences between Tat- males and females. Finally, there was a significant main effect of sex on premature responses; female mice performed significantly less premature responses than male mice. These results indicate that for all genotypes of the Tat transgenic mice, female mice show poorer inhibitory control but also less impulsivity than their male counterparts.

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Highly enriched c-type lectins on myeloid cells provide potential therapeutic strategy to ameliorate neuroinflammatory diseases

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The potential of myeloid cells, in particular 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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Intranasal Nanodelivery of Oxytocin to Treat Drug Addiction in HIV Patients using CRISPR Gene Editing

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Drug abuse is associated with serious medical and health consequences including neurotoxicity, dysregulation of the endocrine-metabolic system, impairment of immune function and neuro-behavioral alternations. It has been shown that endogenous Oxytocin (OXT) delivery inhibits the development of acute/chronic morphine tolerance and attenuate the symptoms of morphine withdrawal in a dose-dependent manner. Repeated morphine ingestion upregulates mu-opioid receptor and leads to suppression of OXT production, which eventually causes the development of tolerance and physical dependence. The aim of this work was to develop the OXT-Cas-9/gRNA nanoformulation (NF) to treat the morphine abuse effects in HIV-1 infected condition. The NF was prepared by simultaneous spray (SS) novel technique using nontoxic PEI-modified transfecting agent [P(SiDAAr)5P3]. The NF was characterized and optimized for different parameters such as effects of pressure, distance, nozzle aperture, N/P ratios and time of incubation with respect to transfection efficiency. Also, the effects of OXT-Cas-9/gRNA plasmid spraying with respect to damage analysis, P(SiDAAr)5P3 condensation ability, cell uptake, cytotoxicity and NF efficacy studies were performed in neuronal cell lines± HIV-1 infection. Results showed that volume mean diameters of the polyplex prepared by SS (0.3 mm nozzle, N/P-5, air pressure- 5 bar with 20 ml/min airflow rate) was 175±5 nm in size with +20 ± 2.0mV surface charge. Further, no degradation of SS sprayed OXT-Cas-9/gRNA was observed compared to non-sprayed plasmid control. NF showed high cell uptake, with a transfection efficiency of >70±12%. P(SiDAAr)5P3 polyplex also exhibited lower cytotoxicity (> 92% cell viability) compared to standard jetPEI polyplex (> 68% cell viability) tested in neuronal cells after 48 hr treatment. Thus, the developed NF is nontoxic in nature and safe for in-vivo use. Currently, we are developing the intranasal aerosol NF and will test the delivery efficacy in a mice model to help develop personalized nanomedicine aimed at drugs of abuse treatment.

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Zika virus, in a strain specific manner, induces human fetal astrocyte cell death through β-catenin inhibition

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Zika virus (ZIKV) has been known to infect humans for 60 years in Africa with relatively few clinical implications. However, over the past ten years there have been several large outbreaks of ZIKV in Asia and subsequently the Americas. In newborns, the congenital Zika virus syndrome, is associated with significant neurologic abnormalities including microcephaly (small brain). We initiated an investigation to address differences in CNS pathogenicity between Asian lineages (recent epidemic) vs. African lineage ZIKV strains on human fetal astrocytes (HFAs). To determine the impact of ZIKV lineage-specific strains on viability of HFAs, we infected HFAs with four strains of ZIKV virus at an MOI of 0.3 and at day seven post infection measured cell viability. We found infection with ZIKV Asian strains (PRVABC59 and FLR) reduced cell viability significantly more than ZIKV African strain (IBH 30656). Asian strains induced 50% cell death by three days post infection (d.p.i), whereas African lineage mediated only 10% cell death at the same time point. We show that Asian lineage PRVABC59 and FLR downregulates β-catenin signaling, a pro-survival pathway that maintains central nervous system function pre-and post-development, through western blot and qPCR. Western blot showed a significant inhibition of β-catenin by three d.p.i as well as reduced protein expression of downstream effector Axin 2. Similarly, mRNA expression of β-catenin and Axin2 is reduced by in HFAs by Asian lineage ZIKV infection. In contrast, African lineage ZIKV had no impact on β-catenin signaling on HFAs. Overexpression of β-catenin in the form of a plasmid containing constitutively active β-catenin (pABC) protected astrocytes from ZIKV mediated cell death. These results were reproducible in both human fetal primary astrocytes (HFAs) and human adult astrogliomas (U138MG). Our findings suggest that Asian lineage ZIKV has evolved to inhibit β-catenin in astrocytes, leading to their heightened pathogenesis in the CNS.

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A human model for VZV vasculopathy using cadaveric cerebral, aortic and pulmonary arteries ex vivo

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VZV is latent in >90% of the world and reactivates in 50% by 85 years of age to produce zoster (shingles). Upon reactivation, VZV can also spread transaxonally to infect intracranial, extracranial and systemic arteries with subsequent infiltration of immune cells, which secrete soluble factors that contribute to pathological vascular remodeling to promote stroke, giant cell arteritis and aortitis (VZV vasculopathy). Mechanistic studies of VZV vasculopathy have been hindered since VZV is an exclusively human virus. Herein, we developed a human cerebral (CA), aortic (AA) and pulmonary artery (PA) explant model that can be infected with VZV. Specifically, CAs, AAs and PAs were obtained <24 hours postmortem and mock- or VZV-infected. At 9 days post-infection, immunohistochemical analyses revealed VZV infection in all vascular beds analyzed, predominantly in the adventitia that was accompanied by a thickened intima. Conditioned supernatants revealed a 5 to 50-fold induction of IL-8 and/or IL-6 in all vascular beds, which recapitulates elevated IL-8 and IL-6 seen in cerebrospinal fluid from VZV vasculopathy patients and in VZV-

infected primary human vascular cells in vitro. In a parallel experiment, neutrophils were added to mock- and VZV-infected cadaveric cerebral arteries and 2 days later, immunofluorescence analyses revealed neutrophils infiltrating in the VZV-infected arteries producing IL-8 but not in the mock-infected arteries. Overall, (1) VZV-infected cadaveric cerebral, aortic and pulmonary arteries have increased IL-8 and/or IL-6 contributing to a proinflammatory arterial environment that can ultimately damage vascular integrity; (2) VZV-infected cerebral arteries producing IL-8 promote neutrophil infiltration; and (3) our functional model can enhance our understanding of the inflammatory response and vascular damage that produces VZV vasculopathy.

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Neurocognitive decline and dysregulation of Astrocyte-TIMP-1 in a Tat-transgenic mouse model

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Despite antiretroviral therapy, HIV-associated neurocognitive disorders (HAND) persist in 60-70% of patients. In the brain, HIV-1 non-productively infects astrocytes, which produce and release HIV-1 proteins such as transactivator of transcription (Tat). Tat induces neuronal death and inflammation by direct and indirect mechanisms. During HAND, elevated matrix metalloproteinases (MMPs) aid ECM breakdown facilitating disease progression; whereas, tissue inhibitors of MMPs (TIMPs) impede their activity. Astrocyte TIMP-1 is an inducible protein and its levels increase during acute neuroinflammation, a response that fails likely under chronic exposure. TIMP-1-mediated neuroprotection is predominantly independent of MMP inhibition. However, little is known about Tat regulation of astrocyte TIMP-1 expression. We hypothesize that HIV-1 Tat downregulates astrocyte TIMP-1 and induces inflammatory changes that contribute to neurocognitive decline. A doxycycline-inducible, glial fibrillary acidic protein (GFAP) promoter-restricted HIV-1 Tat mouse model (GT-Tg) was used to investigate astrocyte-associated disease mechanisms. Neurocognitive decline was assessed using a battery of behavior tests in GT-Tg and wild-type (WT) mice. GT-Tg mice had higher anxiety and lower initiation latency in elevated plus maze and locomotor activity tests, respectively. While GT-Tg mice swam faster in Morris water maze, latency and pathlength were comparable to WT. Discriminated reversal test and novel object recognition did not differ significantly between GT-Tg and WT mice. Subsequently, mouse brains were harvested to evaluate gene and protein expression. Although TIMP-1 gene expression was elevated in GT-Tg verses WT mice, it negatively correlated with Tat expression consistent with human astrocytes chronic responses. Gene and protein expression for other inflammatory biomarkers and GFAP were evaluated, and also correlated with Tat expression. Collectively, our data from GT-Tg mouse model confirmed that TIMP-1 dysregulation is associated with neurocognitive decline in the context of HAND suggesting replenishing TIMP-1 levels could be used as a novel therapeutic option.

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Role of Altered Iron Transport in HIV-1 Latency in Human Microglia

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Latent HIV reservoirs in brain macrophages and microglia drive chronic immune activation and neurocognitive impairment and are not eradicated by antiretroviral therapy. The role of microglial iron homeostasis, which is important for HIV transcription and replication, in HIV latency is largely unexplored. An unbiased shRNA library screen for factors involved in HIV latency using rat microglia (CHME-5/HIV) cells revealed that iron-transport proteins regulate HIV silencing. Here, we determined changes in iron-regulatory protein expression associated with HIV-1 latency in immortalized human microglia (hμglia) and investigated the role of iron in maintaining latency. Expression of transferrin, transferrin receptor (TfR), H-ferritin, ferroportin (Fpn), Hfe, and Dmt1 was quantified and compared by Western blot analysis of lysates from hμglia bearing a latent HIV construct tagged to the GFP reporter (HC69) versus lysates from uninfected hμglia (C20). Changes in iron-transporter expression were assessed after overnight incubation of hμglia with hepcidin, which degrades Fpn and increases cellular iron, the iron chelator deferoxamine (DFO), known latency-reversing agents (TNF-α or polyI:C), or ferrous ammonium citrate (FAC). HIV latency was assessed by immunofluorescence staining. Fpn knockdown was achieved by a 48-hr incubation with Fpn (sense) siRNA. HC69 differed from C20 cells in having near-absent H-ferritin ($p<0.001$) and higher TfR and Fpn expression (both $p<0.05$); FAC treatment induced H-ferritin, but failed to suppress TfR expression in HC69 cells. Hepcidin and TNF-α induced Hfe, which regulates TfR-mediated iron uptake, only in uninfected (C20) cells. Bright green immunofluorescence following hepcidin or FAC treatment of HC69 cells showed HIV reactivation; Fpn silencing decreased cell viability and induced H-ferritin expression. Interestingly, Fpn knockdown in CHME-5/HIV cells strongly reversed latency. These results suggest that microglia iron-mediated signaling enhances HIV expression, with the result that cells with defects in the pathway are enriched in latent proviruses. Ambient iron exposure robustly reverses HIV latency in these cells.

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Bio-distribution and bio-safety assessment of magneto-electric nanoparticles in non-human primate

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To develop a human compatible brain delivery method and to investigate personalized nanomedicine for neuro-HIV/AIDS treatment, our research group has demonstrated magnetically guided delivery of magneto-electro nanoparticles (MENPs ± 25 nm), drug nano-carriers exhibited on-demand release on ac-magnetic field stimulation, to the brain of a baboon (Papio hamadryas) using MRI instrument as a navigation tool. The MENPs presence in the brain was confirmed by post injection MRI brain image analysis as a function of contrast. However, this is very challenging to assess bio-distribution and bio-safety of MENPs in relation with big animal like baboon, an obstacle for developing effective personalized nanomedicines to manage CNS diseases. In this research, for the first time, we explored Raman spectroscopy to assess the presence of MENPs in the brain and evaluate MENPs effects on tissue constituents. The spectral analysis confirmed the presence of MENPs (600 to 800 cm-1) and their clear discrimination from biomolecules or signatures of life, as evident through characteristic vibrations in proteins, nucleic acids, lipids, which were dominant in 800-1800 cm-1. These findings suggest

that MENPs at the interface of tissue do not exhibit cell-damage confirming bio-safety of MENPs for the living being. Thus we proposed MRI as a potential navigation tool for the brain delivery of magneto-electric therapeutic nanoformulations to treat brain diseases and, Raman spectroscopy as an analytical tool for rapid assessment of bio-distribution and bio-safety in-vivo.

Supported by NIH grants namely R01DA042706, R01DA027049, R01DA 034547, R01DA037838, and R01DA040537.

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Role of caspase-1 in HIV-associated atherosclerosis

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Human immunodeficiency virus (HIV) cardiovascular disease and its complications, including heart attack and stroke, is now a leading cause of mortality in HIV+ patients. Extensive clinical evidence suggests well-controlled HIV infection with or without combined antiretroviral therapy (cART) (eg. HIV+ patients or HIV+ elite controllers) accelerates atherogenesis. In patients on cART, HIV infection not only activates immune cells (macrophages), but also triggers an array of molecular pathways, such as caspase-1 activation and its cleavage products IL-18 and IL-1β. Understanding mechanisms of HIV-associated atherogenesis will help to better design and develop novel therapeutics for treatment/prevention. The exact pathogenesis of HIV-associated atherosclerosis has not been investigated due to the lack of a mouse model. Here, we present the development and characterization of a new mouse model for HIV-associated atherosclerosis, and its use in understanding the atherogenic role of caspase-1 in HIV infection. By crossing Tg26 mice (Tg26+/-), which contain a transgene encoding the HIV genome with Apolipoprotein E deficient mice (ApoE-/-) on a C57BL/6J background, we introduced Tg26 mice to hyperlipidemia conditions to induce atherosclerosis. We demonstrate that 1) Tg26/ApoE-/- develop an accelerated atherogenesis with normal renal function, 2) HIV expression increases activation of caspase-1 in circulating monocytes and vasculature of Tg26/ApoE-/-, and 3) Tg26/ApoE-/- have significantly higher levels of IL-1β in the serum after 8 weeks on high fat diet compared to ApoE-/- mice. Importantly, in the serum of 153 HIV+ vs. 67 HIV- patients with coronary atherosclerosis determined by computed tomography coronary angiography, IL-18 levels are higher and significantly correlate with the presence of plaque, and macrophage inflammatory markers. Together, these results not only shed light on the atherogenic role of caspase-1 activation in HIV infection but also indicate that Tg26/ApoE-/- mice provide a new mouse model for investigating HIV-associated atherogenesis.

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Blood Brain Barrier Integrity After Contact with Pre-Exposure Prophylaxis Antiretroviral Drugs Plus a CCR5 Inhibitor

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Background: Various in vitro blood-brain barrier (BBB) models exist which mimic HIV-1 entry into the central nervous system (CNS). Pre-exposure prophylaxis (PrEP) (tenofovir+emtricitabine) targets HIV reverse transcriptase and is >90% effective in preventing HIV infection. Since CCR5 is important in HIV infection and immune signaling, combining PrEP with CCR5-inhibitor maraviroc (MVC) could reduce HIV-1 transmigration into the CNS. We hypothesized: A)Commercially-available primary human brain microvascular endothelial cells (HBMVEC) would exhibit similar integrity and expression of BBB proteins compared to the well-characterized hCMEC/D3 cell line; B)In combination with human astrocytes (HA), BBB bilayers would exhibit high trans-endothelial electronic resistance (TEER) and low permeability; and C)Drug-effect on the BBB would alter presence of efflux pump P-glycoprotein (P-gp) in the HBMVEC layer. **Methods:** Primary HBMVEC and hCMEC/D3 were grown to confluence and characterized for BBB proteins: platelet cell adhesion molecule-1, occludin, zona occludens-1, Factor VIII, intercellular adhesion molecule-1, and P-gp. Comparison of growth rate and integrity of monolayers were assessed by TEER. HBMVEC and HA were then co-cultured on opposite sides of matrix-coated trans-well inserts. BBB integrity was assessed by TEER and permeability with Evan's Blue Albumin. Effect of PrEP+/-MVC on HBMVEC was assessed. **Results:** BMVEC monolayers exhibited higher TEER than hCMEC/D3 with similar expression of BBB proteins. BBB bilayers exhibited TEER >150 Ω/cm2 after six days of culture. PrEP+/-MVC had no impact on growth rate of BMVEC monolayers and no quantifiable difference in expression of P-gp. **Conclusions:** A BBB bilayer model with HBMVEC and HA was established. Neither PrEP alone, nor in combination with MVC, affected P-gp expression, contrary to some literature assessing combined antiretroviral therapy. Findings indicate that PrEP+/-MVC may not upregulate efflux of drug out of the CNS. Future assessment of PrEP+/-MVC influence of non-HIV-infected and HIV-infected monocyte trafficking into the CNS could be tested with the BBB model.

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Enabled Bioimaging Facilitate Tests of Macrophage Targeted Rod-Shaped Nanoparticles for Antiretroviral Drug Biodistribution

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Thernanostic tests for multifunctional nanoparticles allow simultaneous drug carriage and diagnostics. Multifunctional bioimaging allows quantification of drug content in HIV target tissues. To improve upon particle stability and sensitivity for bioimaging tests we developed a bismuth sulfide nanorod (BS NR) platform optimized by creation of a dual-mode nanoprobe. The nanoprobes employ near-infrared fluorescence imaging and single photon emission computed tomography (SPECT/CT) to assess antiretroviral drug particle biodistribution in animals. The rod shape plays a significant role in therapeutic delivery processes, such as rapid, increases macrophage internalization leading to facilitate viral reservoir distribution. To further facilitate this task, a highly stable method for intrinsic radiolabeling of BS NRs with 177Lu (177Lu-BS NRs) was developed for encasements. 177Lu-BS NRs radiolabeling stability in saline and plasma were confirmed. Structural characterization of BS NRs was performed by powder XRD. Morphology and atomic configuration were evaluated by high-resolution transmission electron microscopy (HR-TEM), Scanning transmission electron microscopy (STEM) and selected area electron diffraction pattern (SAED). Chemical compositions of the BS NRs were evaluated by inductively coupled plasma mass spectrometry (ICP-MS), Energy-dispersive X-ray spectroscopy (EDX) and X-ray fluorescence (XRF) spectrometry. 177Lu-BS NRs cytotoxicity, cellular uptake by NIR imaging, retention and anti retroviral efficacy of BS NRs with nanoformulated rilpivirine (NRPV). Biodistribution were studied by SPECT/CT scans were preformed at 0, 1, 2, 5 and 8 days after injection in mice. The SPECT/CT images demonstrated that BS NRs distribute in the RES over time. Ex vivo tissue analysis by TEM and gamma counting showed high uptake in RES organs and lymphatic tissue confirming the SPECT/CT imaging. These results demonstrate the potential of 177Lu-BS NRs in the development of multimodal imaging agents for monitoring therapy during treatment.

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Macrophage Targeted Rod-Shaped Nanoparticles Facilitate Bioimaging Analysis of Antiretroviral Drug Biodistribution

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Thernanostic tests for multifunctional nanoparticles allow simultaneous drug carriage and diagnostics. Multifunctional bioimaging allows quantification of drug content in HIV target tissues. To improve upon particle stability and sensitivity for bioimaging tests we developed a bismuth sulfide nanorod (BS NR) platform optimized by creation of a dual-mode nanoprobe. The nanoprobes employ near-infrared fluorescence imaging and single photon emission computed tomography (SPECT/CT) to assess antiretroviral drug particle biodistribution in animals. The rod shape plays a significant role in therapeutic delivery processes, such as rapid, increases macrophage internalization leading to facilitate viral reservoir distribution. To further facilitate this task, a highly stable method for intrinsic radiolabeling of BS NRs with 177Lu (177Lu-BS NRs) was developed for encasements. 177Lu-BS NRs radiolabeling stability in saline and plasma were confirmed. Structural characterization of BS NRs was performed by powder XRD. Morphology and atomic configuration were evaluated by high-resolution transmission electron microscopy (HR-TEM), Scanning transmission electron microscopy (STEM) and selected area electron diffraction pattern (SAED). Chemical compositions of the BS NRs were evaluated by inductively coupled plasma mass spectrometry (ICP-MS), Energy-dispersive X-ray spectroscopy (EDX) and X-ray fluorescence (XRF) spectrometry. 177Lu-BS NRs cytotoxicity, cellular uptake by NIR imaging, retention and anti retroviral efficacy of BS NRs with nanoformulated rilpivirine (NRPV). Biodistribution were studied by SPECT/CT scans were preformed at 0, 1, 2, 5 and 8 days after injection in mice. The SPECT/CT images demonstrated that BS NRs distribute in the RES over time. Ex vivo tissue analysis by TEM and gamma counting showed high uptake in RES organs and lymphatic tissue confirming the SPECT/CT imaging. These results demonstrate the potential of 177Lu-BS NRs in the development of multimodal imaging agents for monitoring therapy during treatment.

P143

HTLV-1 infection and neuropathogenesis in the context of Rag1-/-γc-/- (RAG1-hu) and BLT mice

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To date, the lack of a suitable small animal model has hindered our understanding of Human T-cell lymphotropic virus (HTLV)-1 chronic infection and associated neuropathogenesis defined as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The 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 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 confirmed reconstitution of Rag1 and BLT mice with human immune cells. Following HTLV-1 infection, proviral load (PVL) was detected in the blood of Rag-1 and BLT hu-mice as early as 2 weeks post-infection (wpi) with sustained elevation in the subsequent weeks followed by Tax expression. Additionally, infection was compared between adult and neonatal Rag1 mice with both PVL and Tax expression considerably higher in the adult Rag1 mice as compared to the neonates. Establishment of peripheral infection led to lymphocytic infiltration with concomitant Tax expression and resulting myelin disruption within the central nervous system of infected mice. In addition, up-regulation in the expression of several immune checkpoint mediators such as programmed cell death-1 (PD-1), T-cell Ig and ITIM domain (TIGIT), and T cell Ig and mucin domain-3 protein (Tim-3) were observed on CD8+ T cells in various organs including the CNS of infected hu-mice. Collectively, these studies represent the first attempt to establish HTLV-1 neuropathogenesis in the context of Rag-1 and BLT hu-mice as potential novel tools for understanding HTLV-1 neuropathogenesis and testing of novel therapies such as immune checkpoint blockade in the amelioration of chronic HTLV-1 infection

P144

Endolysosome iron restricts HIV-1 Tat-mediated HIV-1 LTR transactivation

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HIV-1 Tat is a key virulent factor for HIV infectivity and has been shown to contribute to HIV/AIDS disease progression and the development of HIV-1 associated neurocognitive disorders. Secreted from HIV-1 infected cells, HIV-1 Tat enters CNS cells via receptor-mediated endocytosis and where it accumulates in endolysosomes. Following its accumulation in endolysosomes, HIV-1 Tat escapes endolysosomes by poorly understood mechanisms and enters the nucleus where it activates the HIV-1 LTR promoter. Iron has been shown to affect HIV-1 replication and iron chelators have been used as adjunctive therapeutic agents in HIV+ individuals. Endocytosis is the first step for the uptake of ferric iron bound to transferrin and once in endolysosomes it is changed to ferrous iron. Accordingly, we determined the extent to which ferrous iron in endolysosomes affects Tat-induced HIV-1 LTR transactivation in U87MG cells. We demonstrated that chelating endolysosome iron, but not chelating cytosolic iron, enhanced Tat-mediated HIV-1 LTR transactivation. Furthermore, loading cells with ferric or ferrous iron restricted Tat-mediated HIV-1 LTR transactivation. Our findings suggest that endolysosome iron restricts the escape of HIV-1 Tat from endolysosomes, decreases its access to the nucleus, and restricts HIV-1 LTR transactivation. (Supported by P30GM103329, R01MH100972, MH105329 and R21DA040519)

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Insulin Stimulates the β-oxidation Fatty Acids in Astrocytes for use as an Energy Source

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There is considerable evidence from tissue culture and small animal studies that suggests preserving cellular bioenergetics protects neurons in multiple models of neurodegenerative disease. Based on the actions of insulin to regulate energy metabolism, we reasoned that the intranasal administration of insulin may facilitate rapid glucose utilization and force the brain to use alternative sources of energy. The intranasal administration of insulin in mice reduced striatal glucose levels from 41.7±6.1 to 14.9±1.4 mM ($p<0.02$), and increased levels of several fatty acid species by 2-3 folds in multiple brain regions, suggesting that insulin promoted rapid glucose

utilization, and fatty acid uptake and/or synthesis. Whole genome sequencing of hippocampus showed that insulin modified the expression of 10 distinct genes associated with the β-oxidation of fatty acids. We next exposed cultured neurons and astroglia to insulin, and found that only astroglia responded to insulin by increasing glucose uptake, accumulating long chain fatty acids, and upregulating the expression of genes involved with β-oxidation. Oil-red O staining confirmed that fatty acids and neutral lipids accumulated in the cytosol of astrocytes as lipid droplets. Time course studies provided evidence that fatty acids in astrocytes were desaturated, elongated (within 3h) and utilized as an energy source (within 12h) following exposure to insulin in low glucose conditions. Insulin increased mitochondrial total oxidative capacity, suggesting that the β-oxidation of fatty acids in astrocytes increased cellular energy production. These data suggest that the intranasal administration of insulin may be neuroprotective by promoting astrocytic β-oxidation that produces approximately 15-times more ATP compared to the oxidation of glucose.

P146
ROLE OF GLIAL CCR5 IN HIV-1 TAT AND OPIATE-MEDIATED NEUROTOXICITY AND BEHAVIORAL PHENOTYPE

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HIV-1 persists in certain CNS populations, despite advances in antiretroviral therapy. Infected cells release viral proteins, such as transactivator of transcription (Tat) and chemokines such as CCL5, generating a neuroinflammatory loop. This creates the basis for sublethal and lethal neuropathology that manifests as a spectrum of HIV-mediated CNS impairments, called HIV-associated neurocognitive disorder (HAND). Opiates exacerbate these neurological effects, which we hypothesize is due to converging actions on the CCL5-CCR5 axis by Tat+morphine, leading to chronic glial activation that damages neurons. We performed repeated measures studies on mixed glia and neuron co-cultures obtained from C57Bl6/J and/or CCR5 knockout mice, treated with Tat±morphine for 72 hours. Morphine worsened Tat-induced neurotoxicity in wild-type co-cultures; substitution of CCR5-null glia eliminated the interactive effects of Tat+morphine, but substitution of CCR5-null neurons did not. These results suggest that glial, not neuronal, CCR5, is a convergence point for interactive effects of Tat and morphine that damage neurons. Additional experiments treating with MOR antagonist naloxone, or CCR5 antagonist maraviroc, confirmed each receptor's role in mediating Tat±morphine toxicity. Surprisingly, in co-cultures with CCR5-deficient glia, morphine entirely protected neurons from Tat toxicity. This may reflect an imbalance of neurotrophic factors, particularly BDNF and its neurotoxic precursor proBDNF, whose levels are altered in HIV+ and drug-using patients. Behavioral tests of anxiety, motor, and cognitive function, three areas of neurologic decline seen in HAND, were performed in inducible Tat-transgenic mice given maraviroc via oral gavage. Tat-mediated impairment was observed in the Barnes Maze, a measure of spatial memory, and was ameliorated by maraviroc. Similar behavioral tests in Tat-transgenic X CCR5 knockout mice gave equivocal results, suggesting that long-term CCR5 deficits result in compensatory effects. Overall, both in vitro and in vivo studies support the hypothesis that glial CCR5 plays a central role in driving Tat+morphine-mediated neuronal damage. Support-DA034231 (PEK/KFH).

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TREM2 Expression Increases with SIV Encephalitis in a Macaque Model of HIV CNS Disease

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Microglia upregulate TREM2 (Triggering Receptor Expressed on Myeloid cells 2) expression during the course of several neurodegenerative diseases, most notably Alzheimer's Disease (AD). TREM2 knockout mouse models of AD suggest that increased TREM2 expression is detrimental at disease onset but beneficial at later disease stages, highlighting the need for additional study of the role TREM2 plays in chronic CNS diseases, including HIV-induced CNS disease. Microglia require TREM2 signaling to assume a specific damage-associated phenotype. Consequently, TREM2 expression may represent a generalized microglial response to CNS injury. The potential role of TREM2 in HIV CNS disease has not been described. Our studies examined the role of TREM2 using the SIV/pigtailed macaque model of HIV CNS disease. In macaques, we showed that both parenchymal microglia and perivascular macrophages in the brain express TREM2 using immunohistochemistry and in situ hybridization. TREM2 immunostaining was increased in macaques with SIV encephalitis (SIVE), both in terms of the number of positive cells and the intensity of signal. Similarly, TREM2 mRNA expression, as measured by qPCR, was significantly increased in pigtail macaques with SIVE compared to uninfected controls in both cortical grey and white matter ($P = 0.02$). Interestingly, treating SIV-infected macaques with suppressive antiretroviral treatment (ART) restored TREM2 mRNA expression to levels seen in uninfected control animals ($P > 0.99$). This is in contrast to what we have previously shown with colony stimulating factor 1 receptor (CSF1R) expression in this same model, where CSF1R expression remains elevated with ART. Although both TREM2 and CSF1R signal through DAP12 and play similar roles in microglial survival, this contrast in expression suggests a differential role in chronic inflammation. These findings demonstrate that CNS TREM2 is induced in active SIV infection and suggest that TREM2 and CSF1R may play different roles in HIV CNS disease.

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PROTEOMIC AND CYTOKINE PROFILING OF EXOSOMES DERIVED FROM THE PLASMA OF HIV PATIENTS AND SUBSTANCE ABUSERS

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Background: Though there are biomarkers to diagnose HIV and alcohol/nicotine-induced toxicity, there is a paucity in the literature on specific protein/cytokine interactions that enhance toxicity in HIV-positive drug abusers. Unfortunately, the complexity of body fluids often hampers protein/biomarker discovery. Exosomes (~100 nm) are emerging as a novel diagnostic biomarkers as they package and transport diverse biological cargo such as proteins, mRNA, miRNA, and small molecules from a cell/tissue to distant cells. Therefore, we aim to study the specific proteins/cytokines which are altered in exosomes of both HIV patients and alcohol/tobacco abusers. **Materials and methods**: Exosomes were isolated from plasma samples of healthy individuals (n=10), HIV patients (n=5), chronic smokers (n=11), chronic drinkers (n=5), HIV-positive drinkers (n=3), and HIV-positive smokers (n=4) by using a validated plasma exosomal isolation kit. Quantitative proteomic and cytokine profiling were done using validated kits. **Results:** A total of 8 cytokines were measured and all were present in exosomes of healthy subjects. Interestingly, most of the studied cytokines (IL-6, IL-1B, MCP-1, IL-8 and IL-10) were not detectable in the HIV group, however this was not statistically significant. Proteomic analysis revealed the presence of 563 proteins among all groups. Of those; 15, 48, and 10 proteins significantly differed between control and chronic drinkers, HIV patients, and chronic smokers, respectively. Bioinformatic analysis of significant proteins using Chilibot software revealed the inhibitory relationship between retinol-

binding protein 4 (RBP-4) and transthyretin (TTR) in chronic drinkers. Afamin (AFM) was found to have both stimulatory and inhibitory interactions with other significant proteins in HIV patients based on the available information in the literature. **Conclusion:** Exosomal cytokine levels varied between healthy and other groups but it was not significant whereas few exosomal protein levels significantly varied between different groups. Profiling of exosomal proteins will be useful to study the HIV and drug abuse comorbidities

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Potential role of plasma and monocyte-derived exosomes in tobacco- and HIV-mediated cell-cell interaction

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Smoking is known to exacerbate HIV-1 pathogenesis, especially in the viral sanctuary sites monocytes, however, its underlying mechanism in largely unknown. Exosomes are small extracellular vesicles (<200 nm) that carry specific proteins and miRNA, are known to alter HIV-1 pathogenesis through cell-cell communication via delivery of these components. However, the role of exosomes in smoking-mediated HIV-1 pathogenesis is unknown. In this study, we investigated the effect of cigarette smoke condensate (CSC) on the characteristics of monocyte-derived exosomes and their influence on HIV-1 replication. Initially, we demonstrated that CSC reduced total protein and antioxidant capacity in exosomes derived from HIV-1-infected and uninfected macrophages. The exosomes from CSC-treated uninfected cells showed a protective effect against cytotoxicity and viral replication in HIV-1-infected macrophages. However, exosomes derived from HIV-1-infected cells lost their protective capacity. The results suggest that the exosomal defense is likely to be more effective during the early phase of HIV-1 infection and diminishes at the latter phase. Our further analysis suggest a potential role of exosomal antioxidant enzymes, catalase and PRDX6, in CSC-mediated effects on HIV-1 replication. In addition to monocyte-derived exosomes, we showed that plasma exosomes from healthy individuals contain high level of tobacco-metabolizing cytochrome P450 (CYP) enzymes; CYP1A1, 1B1, and 2A6. The plasma exosomes also carry major drug-metabolizing enzyme, CYP3A4, which is also known to metabolize many antiretroviral drugs. We further showed that the abundance of plasma CYPs is higher than hepatic CYPs,. These findings are intriguing and suggest an important role of plasma CYP enzymes in tobacco- and HIV-1-mediated effects in cellular processes through cell-cell communication. Overall, these results suggest a potential role of plasma and monocyte-derived exosomes in tobacco- and HIV-1-mediated cell-cell interactions, and has clinical significance with regards to their use as potential biomarkers and carriers for therapeutics.

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CD4+ T lymphocytes in Parkinson's disease have a complex phenotypic and functional Th1 bias: cross-sectional studies of Th1/Th2/Th17 and regulatory T cells in antiparkinson drug-naïve and -treated patients

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Parkinson's disease (PD) affects about 10 million people worldwide, and only symptomatic treatments are available to relieve brain dopaminergic neurons loss. Neurodegeneration is the consequence of neuroinflammation in turn influenced by peripheral adaptive immunity, and in particular by CD4+ T lymphocytes. CD4+ T cells may be however proinflammatory, such as T helper (Th) 1 and Th17, and antiinflammatory, such as Th2 and the regulatory T cells (Treg). To what extent the different CD4+ T cell subsets are imbalanced and their functions dysregulated in PD remains largely an unresolved issue. We performed two cross-sectional studies in antiparkinson drug-treated and -naïve PD patients, and in age- and sex-matched healthy subjects. The first study examined circulating Th1, Th2, Th17, and the second study circulating Treg. Number and frequency of circulating CD4+ T cell subsets were assessed by flow cytometry and their functions were studied in ex vivo assays. Complete clinical assessment, blood count and lineage-specific transcription factors mRNA levels in CD4+ T cells were independently assessed in both studies. Results show that PD patients have reduced CD4+ T cells, due to reduced Th2, Th17 and Treg. Naïve CD4+ T cells from peripheral blood of PD patients preferentially differentiate towards Th1, with increased production of interferon-gamma and tumor necrosis factor-alpha, which is insensitive to Treg inhibition. This Th1-biased immune signature occurs in both drug-naïve patients and in patients on dopaminergic drugs, suggesting that current antiparkinson drugs do not affect peripheral adaptive immunity. The complex phenotypic and functional profile of CD4+ T cell subsets in PD patients supports a role for peripheral adaptive immunity and in particular for CD4+ T cells in PD, and represents a target for the preclinical and clinical assessment of novel antiparkinson therapeutics targeting the immune system.

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Affects of antiretroviral drugs on endolysosome pH and calcium

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Currently, around 40-70% of HIV-1 positive individuals are affected by HIV-associated neurocognitive disorder (HAND) even those in whom viral replication is effectively controlled by antiretroviral drug therapy (ART). The mechanisms underlying HAND pathogenesis are not well understood, but calcium dyshomeostasis and dysfunction of organelles such as endolysosomes and mitochondria continue to be implicated. Emerging evidence indicates that some ART drugs are neurotoxic. Because endolysosomes are acidic organelles that contain readily releasable stores of calcium and because others and we have shown that deacidification of endolysosomes causes an efflux of calcium from endolysosomes, here we determined the extent to which ART drugs affect endolysosome pH and calcium release in rat primary cultured neurons. For these studies, we tested two ART drugs in combination (efavirenz and dolutegravir) and compared results with two other ART drugs in combination (abacavir and tenofovir disoproxil fumarate). We found that efavirenz and dolutegravir added in combination significantly deacidified endolysosome pH, while abacavir and tenoforvir disoproxil fumarate acidified endolysosomes. Furthermore, the ART drugs that deacidified endolysosomes (efavirenz and dolutegravir) but not the ART drugs that acidified endolysosomes (abacavir and tenofovir disoproxil fumarate) increased the release of calcium from endolysosomes. Collectively, these data suggest that ART drug-induced neurotoxic effects are propagated by endolysosome deacidification and the release of calcium.

This work was supported by P30GM103329, R01MH100972, R01MH105329.

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HIV-1 Tat alters acute morphine induced changes in excitability in dopamine receptor type-2-expressing striatal medium spiny neurons

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Despite the advent of combination anti-retroviral therapies (cART), 30-50% HIV-infected individuals still exhibit neurocognitive disorders indicating that even with these therapies HIV can still incur significant changes to neuronal function. In the striatum exposure to the HIV-1 protein transactivator of transcription (Tat) has been shown to have neurotoxic effects which are exacerbated by co-exposure to opiate drugs. This effect is of special relevance because HIV-infected individuals have a higher risk for developing neuropathic pain which is commonly treated with opiates. Although recent work from our lab has begun to explore the effects of HIV-1 Tat on the physiology of medium spiny neurons (MSNs), the effects of acute opiate treatments on the physiology of MSNs exposed to HIV-1 Tat is unknown. To explore these two effects we crossed our transgenic murine HIV model: a doxycycline-inducible GFAP-driven HIV-1 Tat model with a Drd2-eGFP expressing line and examined physiological changes using whole-cell patch clamp recording of MSNs in a striatal slices. A recent study from our lab using a similar set up showed that exposure to HIV-1 Tat for two weeks dysregulates the firing rate of Drd2-expressing MSNs. In the current study protocol we first recorded the stimulated "baseline" response then perfused on morphine (500nm) for five minutes and then recorded a second stimulated "morphine modulated" response in both Tat+ and Tat- samples. Our preliminary data from Drd2-expressing MSNs indicate that, compared to controls, the Tat+ group exhibits increased firing as well as reduced responsiveness to acute morphine exposure. Overall, our preliminary data support the previous findings indicating that HIV-1 Tat disrupts the excitability of Drd2-expressing MSNs and also reveals that HIV-1 Tat may alter the intrinsic physiological response of striatal MSNs to morphine exposure in neuroAIDS.

The support of NIDA grants R01 DA018633 and R01 DA034231 is gratefully acknowledged.

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Age, AIDS, and Apathy: Sex-dependent synaptodendritic alterations in the HIV-1 transgenic (Tg) rat

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The rise of combination antiretroviral therapy (cART) has increased life expectancy of HIV+ patients with HIV-1 associated motivation dysregulation, presenting as the neuropsychiatric consequence of apathy, affecting 30-60% of this aging population. Apathy is one of the most frequent behavioral changes associated with diseases affecting either the prefrontal cortex or the basal ganglia. We hypothesized that morphological alterations to medium spiny neurons (MSNs) within the nucleus accumbens (NAc) may underlie apathy observed in patients who have undergone cART, as these structures modulate motivation and goal-directed behavior. Previous studies found synaptodendritic alterations in female HIV-1 Tg rats, but sex as a biological factor has yet to be investigated; moreover, no experiment has targeted MSN spine alterations in aged HIV-1+ individuals. Thus, we presently investigated synaptodendritic alterations in both male and female HIV-1 Tg rats at advanced age. Brains from intact HIV-1 Tg and control F344/N rats (n=13-15 per group) were extracted at 18-20 months of age, ballistically labeled with indocarbocyanine dye, and 3-D imaged using confocal microscopy. Dendritic spines from neurons were quantified and classified using Neurolucida 360 neuron analysis software. Preliminary analyses revealed sex differences were more robust in HIV-1 Tg rats on all measures, and females differed more than males as a function of transgene. Female and HIV-1 Tg rats had shorter dendritic spine backbone lengths compared to male [$X2(15)=2043$, $p\leq0.0001$] and control rats [$X2(12)=1167$, $p\leq0.0001$], respectively. Male and control rats had smaller spine head diameters compared to female [$X2(10)=1126$, $p\leq0.0001$] and HIV-1 Tg rats [$X2(10)=414$, $p\leq0.0001$], respectively. Collectively, these results demonstrate the roles of HIV and biological sex in alteration of reward circuit MSNs in advanced age. Further, the present data implicate gonadal hormones in HIV-1 associated apathy (operationalized by goal-directed behaviors) and suggest synaptodendritic complexity as a key target for testing HIV-1 therapeutic interventions and cure strategies.

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The role of the blood-brain barrier in Zika virus infection

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The blood-brain barrier (BBB) selectively regulates the cellular exchange of macromolecules between the circulation and the central nervous system. The BBB is formed by brain microvascular endothelial cells (BMEC) joined by tight junction (TJ) proteins. Here, we hypothesize that Zika virus (ZIKV) infects the brain via disrupted BBB and alterations of TJ proteins. To assess this hypothesis, BMECs were infected with a Honduras strain of ZIKV (R103451) at MOI 0.01 and MOI 0.1 and assessed for TJ protein levels. At 1-day post infection (dpi), claudin-5 expression was significantly downregulated and ZO-1 expression was significantly upregulated in ZIKV-infected cells at MOI 0.01, compared to uninfected controls. After 2 dpi, claudin-5 protein levels continued to be significantly downregulated but ZO-1 levels returned to control values. No statistically significant changes were observed for occludin at 1 or 2 dpi. Next, BMEC permeability was assessed using a transwell system. Briefly, primary human BMEC were grown to confluency and infected with ZIKV at different MOI. After 2 dpi, media in the apical compartment of the transwell was replaced with media containing fluorescently-tagged dextran (20kDa). After 2h of incubation, the fluorescence intensity in the basal compartment was measured. No differences in permeability between ZIKV-infected and uninfected cells were observed at MOI 0.01 and 0.1. However, a statistically significant increase in permeability was observed when BMEC were infected with ZIKV at MOI 1 and MOI 10. Overall, these results suggest that ZIKV may cause BBB disturbances by altering TJ expression. These events may contribute to the dissemination of the viral infection in the CNS.

Supported in part by the Florida Department of Health 7ZK09 and NIH R01MH098891.

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Prevalence of Salivary Human Herpesviruses in Pediatric Multiple Sclerosis Cases and Controls

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Multiple sclerosis (MS) is a multifactorial disease of unknown origin. The current paradigm is that disease develops in genetically susceptible individuals, influenced by environmental factors. Viral infections are long thought to have a role in MS onset and/or pathogenesis. Epstein-Barr virus (EBV) and Human Herpesvirus 6 (HHV-6) have particularly strong associations with the disease. Both viruses are typically acquired during childhood, decades before MS presents. However, in patients with

pediatric MS, the temporal window between viral acquisition and disease onset is shortened, which may provide insights into the association of herpesviruses and [adult onset] MS. Many studies associating EBV with pediatric MS are based on seroprevalence. The present pilot cross-sectional study compared the frequency of salivary EBV and HHV-6 viral DNA between pediatric MS patients (n=32) and age-matched controls (n=42), as saliva is easily collected from pediatric populations. Compared to our previously generated adult data, pediatric cohorts harbor significantly less salivary HHV-6 viral DNA; there was a similar, though non-significant trend for EBV. This suggests that viral levels may increase with age, perhaps due to re-infection and/or host factors controlling viral replication. Pediatric MS patients did not differ from pediatric controls in the frequency or magnitude of salivary viral shedding. During the assessment of EBV positivity by ddPCR, distinct fluorescence profiles emerged that correlated with mutations in the target amplicon, lmp-1. These mutations were present at a relatively high frequency in the pediatric control cohort, and at even higher frequencies in the adult cohorts. However, none of these mutations were evident in pediatric MS patient samples, suggesting differential host immune control of EBV in this pediatric MS cohort. This observation should be extended to larger pediatric and adult cohorts, with more comprehensive sequencing of EBV, and perhaps other herpesviruses that are associated with adult onset MS.

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HIV-1 Tat and Morphine Limit Antiretroviral CNS Levels Despite Increasing Blood-Brain Barrier Leakiness

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Poor brain penetration of antiretroviral (ARV) drugs likely contributes to HIV persistence. Opiate abuse contributes to the severity of HIV-associated neuropathogenesis. In vitro data suggests opiate-HIV effects on blood-brain barrier (BBB) disruption. However, the simultaneous assessment of HIV-opiate interactive effects on BBB integrity and HIV drug concentrations within brain tissue has not been studied. This study examined effects of the HIV-1 regulatory protein, Tat, and morphine on two different measures of BBB integrity/function; paracellular leakiness of dextrans and ARV drug penetration into the brain. The Tat transgenic mouse model, which conditionally expresses HIV-1 Tat1-86 in a GFAP-driven manner (Tat+) was used, with appropriate Tat-controls. To evaluate paracellular leakiness, mice received morphine (or not) for 5 days. Transcardial injections of various sized (10, 40, and 70 kDa) labeled dextrans were performed and dextran brain content was measured via spectrophotometer. To evaluate effects on drug concentrations within the brain, an additional set of mice was used. All mice received dolutegravir /abacavir /lamivudine ± morphine. ARV concentrations in brain and plasma were quantified via LC-MS/MS. After 14 d of Tat induction, Tat exposure (in the non-morphine group) resulted in increased 10 kDa dextran within the brain ($p < 0.05$). Additionally, morphine exposure, irrespective of Tat status, significantly increased brain content of the larger (40 and 70 kDa) dextrans (both $p < 0.05$), consistent with BBB damage. ARV concentrations were also altered in morphine exposed mice. Within striatum, there were significantly lower concentrations of dolutegravir and abacavir and lower dolutegravir concentrations within hippocampus of morphine-exposed mice (all $p < 0.05$). Lamivudine accumulation was unaffected in all conditions. These findings suggest that Tat and morphine alter BBB integrity and function, resulting in altered concentrations of antiretroviral drugs within the brain.

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Preferential Transmigration across the BBB of HIV-infected CD14+ CD16+ Monocytes and Potential Therapeutic Targets to Prevent the Continued Viral Seeding of the CNS in the ART era

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HIV Associated Neurocognitive Disorders (HAND) affect ~50% of HIV-infected people despite antiretroviral therapy (ART). HAND is mediated in part by the entry of HIV-infected CD14+CD16+ (HIV+CD14+CD16+) monocytes into the CNS, establishing and reseeding CNS viral reservoirs. CD14+CD16+ monocytes transmigrate across the blood-brain barrier (BBB) into the CNS to chemokines such as CCL2, a chemokine elevated in the CSF of HIV-infected individuals even with ART. This is mediated by the expression of CCR2, the only known CCL2 receptor, on monocytes. Viral reservoirs release viral proteins and cytokines that mediate neuroinflammation and neuronal damage, leading to HIV neuropathogenesis. Others have shown that HIV DNA copies/106 PBMC, and specifically within CD14+CD16+ monocytes, are higher in HIV-infected individuals with HAND compared to those with normal cognition. We previously demonstrated that CD14+CD16+ monocytes preferentially transmigrate across an in vitro human BBB model to CCL2, and that HIV+CD14+CD16+ monocytes have a selective advantage in this process compared to uninfected but HIV-exposed (HIVexpCD14+CD16+) monocytes. We also showed that HIV+CD14+CD16+ monocytes have increased junctional proteins JAM-A and ALCAM, even in the presence of ART, providing a selective advantage for the preferential transmigration of these monocytes. Importantly, we now show that even with ART, HIV+CD14+CD16+ monocytes preferentially transmigrate across the BBB to CCL2 compared to HIVexpCD14+CD16+ monocytes. We also demonstrate that CVC, anti-JAM-A, and anti-ALCAM antibodies, block the preferential transmigration across the BBB to CCL2 of HIV+CD14+CD16+ monocytes with ART. Thus, we propose that ongoing entry of HIV+CD14+CD16+ monocytes into the CNS, even in the presence of ART, contributes to the maintenance of reservoirs contributing to HAND. CCR2, JAM-A, and ALCAM may be potential therapeutic targets for HAND to block the preferential entry of HIV+ CD14+ CD16+ monocytes into the CNS in the ART era.

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Intra-individual variability in neurocognitive performance: no influence due to HIV status or self-reported effort

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Introduction: HIV-associated neurocognitive disorders (HAND) are estimated to affect approximately 50% of infected individuals at any one time. Dispersion, a type of intra-individual variability in neurocognitive test performance, has been identified as a potential behavioral marker of HAND; however, the specificity of dispersion to HAND, its utility as a predictor of later decline, and the influence of participant effort when taking neurocognitive tests remains unclear. **Methods:** Data were

collected from 1023 (482 HIV-, 541 HIV+) participants enrolled in the Multicenter AIDS Cohort Study (MACS). Dispersion was calculated based on the standard deviation of an individual's test scores within a single assessment. Effort was determined using the Visual Analogue Effort Scale. The predictive utility of dispersion was examined in a subgroup of 131 participants who were evaluated twice, 2 years apart. **Results:** Contrary to our hypothesis, dispersion was not influenced by effort. Instead, poorer neurocognitive ability and African American race were the sole predictors of dispersion. Dispersion was not associated with HIV status and was not predictive of later neurocognitive decline. **Conclusions:** Our results indicate that dispersion is a valid indicator of neurocognitive dysfunction; however, it is not specific to HIV and is not predictive of changes in neurocognitive status.

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Perinatally acquired HIV infection and epigenetic age acceleration in South African adolescents

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Background: Recent studies demonstrate infection with the Human Immunodeficiency Virus-1 (HIV) is associated with accelerated aging effects according to a highly accurate epigenetic biomarker of aging known as epigenetic clock. However, it not yet known whether epigenetic age acceleration can already be observed in HIV+ adolescent infected perinatally (PHIV+). **Methods:** The Illumina EPIC array was used to generate blood methylation data from 200 PHIV and 48 uninfected (HIV-) adolescents aged 9 to 13 years old. The epigenetic clock software and method was used to estimate two measures of epigenetic age acceleration and to impute blood cell counts. **Results:** HIV is associated with biologically older blood in PHIV+ adolescents according to both measures of epigenetic age acceleration. One of the measures, extrinsic epigenetic age acceleration, is negatively correlated with measures of cognitive functioning (executive functioning, working memory, processing speed) and brain stem volume. Multivariate models suggest that brain stem volume changes mediate the association between extrinsic epigenetic age acceleration and cognitive functioning measures. **Conclusions:** Overall, our results indicate that epigenetic age acceleration in blood can be observed in PHIV+ adolescents and that these epigenetic changes are associated with cognitive functioning and brain stem volume.

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Recent Cannabis Use in HIV infection is Associated with Reduced Markers of Inflammation in CSF

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Background: In HIV infection, microglia are chronically activated and may contribute to neuroinflammation and neurological dysfunction. Type 2 cannabinoid receptors (CB2R) regulate microglial activation and their expression is increased after brain injury. Many HIV-infected individuals use cannabis, resulting in exposure to the CB2R ligands tetrahydrocannabinol and cannabidiol. Based on known actions of CB2R, we hypothesized that recent cannabis use would be associated with reduced markers of immune activation and inflammation in cerebrospinal fluid (CSF). **Methods:** Prospective, observational study of HIV+ and HIV- individuals. IL-16 and CRP in CSF were measured using immunoassays. Participants reported estimated days since last cannabis use. **Results:** Participants were 36 HIV+ and 21 HIV- volunteers; mean (SD) age 45.4 (14.5), 10 women, 8 African American, 21 Hispanic/Latino. HIV+ participants had a mean CD4 of 699; 94% were on cART and 86% were virologically suppressed (plasma HIV RNA <50 copies). The median (range) days since last cannabis use was 304 (1, >1000). HIV+ individuals had nonsignificantly higher CSF IL-16 and CRP levels than HIV-. Taking all participants together, more recent use of cannabis was associated with significantly lower CSF levels of IL-16 ($r=0.593$; $p=.0003$) and CRP ($r=0.359$; $p=0.015$). This effect was numerically present in both HIV+ and HIV- participants; it approached significance in HIV+ only. CD4 and virologic suppression were not associated with CSF IL-16 or CRP levels. **Conclusions:** This analysis is consistent with a short-term anti-inflammatory effect of cannabinoids in the CNS. It should be replicated in a larger, independent cohort. CNS anti-inflammatory effects may be mediated through the CB2R on immune cells including microglia or indirectly, for example, via cannabis alterations in gut microbiota composition, improved gut barrier function, or reduced microbial translocation of pro-inflammatory bacterial products.

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Human Endogenous Retrovirus K envelope protein causes nucleolar dysfunction by activating p53- signal pathway

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About 8% of the human genome is composed of retrovirus-like sequences called human endogenous retrovirus (HERVs), yet their function is poorly understood. Recent studies have shown that HERVs may play critical roles in both pathological and physiological conditions. Our group found that HERV type K (HERV-K) subtype HML-2 was activated in the brain of patients with amyotrophic lateral sclerosis (ALS). Overexpression of HERV-K envelope protein (Env) derived from the consensus sequence caused neurotoxicity in a cell culture system and motor neuron specific degeneration in a transgenic mouse model. In this study, we further studied the potential mechanism of HERV-K Env induced neurotoxicity. We found that HERV-K Env caused nucleolar dysfunction in the transgenic mouse as determined by microarray analysis and confirmed by qPCR. Further studies found that the signal peptide (SP) of HERV-K Env was translocated into the nucleolus. SP directly interacted with nucleolar proteins as determined by co-immunoprecipitation, and reduced the production of 45S pre-mRNA, indicating nucleolar dysfunction. In addition, SP activated p53 signaling pathway in a luciferase reporter assay. Using human neurons differentiated from hNPC as a model, we confirmed that SP upregulated p53 and its downstream target proteins, causing activation of apoptotic pathways. SP also reduced the production of 45S pre-mRNA. Consistent with our in vitro experiments, p53 protein expression was significantly increased in the brain of both ALS patients and HERV-K Env transgenic mice. In conclusion, HERV-K Env may induce neurotoxicity by causing p53-dependent nucleolar dysfunction.

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Region-specific expression of HIV mRNA in the HIV-1 Transgenic rat

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The HIV-1 transgenic (Tg) rat has been widely used as a model to investigate HIV-1 associated neurocognitive disorder (HAND) in HIV-1 positive individuals. The HIV-1Tg rat expresses 7 of 9 HIV-1 proteins, with the exception of gag and pol, under the control of the natural promotor region. Although the HIV-1 provirus is present in all cells, the actual HIV-1 protein expression pattern in the HIV-1 Tg rat brain is still unclear. In the current experiment, we used a highly sensitive innovative RNA in situ detection technology (RNAscope) to determine the HIV-1 mRNA expression in four brain regions: medial prefrontal cortex (mPFC), nucleus accumbens (NAc), hippocampus (HIP), and substantia nigra (SNR) in HIV-1 Tg rats. Furthermore, we also used immunohistochemistry staining (IHC) of Iba1, GFAP, and MAP2 as specific cell-type markers to identify microglia, astrocytes and neurons, respectively, in all four brain regions. We found that HIV-1 mRNA was differentially expressed in the HIV-1 Tg rat brain. Specifically, the most abundant HIV-1 mRNA expression was located in the cerebral cortex. The mPFC and NAc regions had high levels of HIV-1 mRNA expression, whereas the HIP and SNR had low levels of expression. Interestingly, the striatum had high levels of HIV mRNA-RNAscope signal. Combined with the IHC staining with cell-specific markers for microglia, astrocytes and neurons, we found that microglia appeared to be the major expressing cell type in the HIV-1 Tg rat brain. In summary, although the HIV-1 provirus is located in all cells, only particular brain regions and specific cells actively expressed the HIV-1 proteins. Identifying the region-specific and cell-specific expression of HIV mRNA in the HIV-1 Tg rat offers valuable insight, which is beneficial for study HIV-1 associated neurocognitive disorder in HIV-1 positive patients.

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Extracellular vesicle miRNA-23a-mediated loss of pericyte coverage at the blood-brain barrier: Implications for morphine-mediated neuroinflammation

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Opioids such as morphine are the most potent and efficacious drugs currently available for pain management. Both in vitro and in vivo studies have demonstrated that morphine potentiates neurodegenerative effects of HIV in the central nervous system. Impairment of the blood-brain barrier (BBB) causes transmigration of monocytes and leukocytes into the brain, contributing, in turn, to neurodegeneration and neuroinflammation. Astrocyte-pericyte cross-talk is critical for maintaining the structure of the BBB, the underlying mechanisms by which morphine-exposed astrocytes regulate pericytes at the BBB, however, remains unclear. Previously, we identified EVs released from morphine- and HIV protein-exposed astrocytes can shuttle miRNAs to neurons, leading to neuronal dysfunction. The current study was aimed at examining the effect of EV-miRNAs released from morphine-exposed astrocytes in mediating pericyte loss at the BBB. Extracellular vesicles (EVs) were purified from control and morphine-exposed astrocytes using the standard differential ultracentrifugation technique followed by characterization using western blot, transmission electron microscopy, AchE assay, atomic force microscopy and NanoSight analyses. Functional endpoint included assessment of pericyte migration using Boyden chamber and wound healing assays. Our data demonstrated that morphine-exposed astrocytes induced the expression and secretion of miR-23a in the EVs, which was taken up by pericytes, leading, in turn, to induction of pericyte migration. This involved down-regulation of PTEN, a target of miR-23a in pericytes. Furthermore, the effect of miR-23a in decreased pericyte coverage was also assessed using the brain endothelial-pericyte 3D co-culture model. Additional validation of decreased pericyte coverage was also demonstrated ex vivo, wherein lesser PDGFβR+ pericytes were found to co-localize with CD31+ brain endothelial cells in microvessels isolated from morphine-administrated mice. In conclusion, our findings indicate that morphine mediated dysregulation of miRNA expression in the brain involves cell-cell communication via the extracellular vesicles, leading in turn, to pericyte loss at the BBB.

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Unfolded protein response regulator ATF6b contributes to HIV-induced neurotoxicity

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Synaptic injury, neuronal dysfunction, and damage in patients with HIV-associated neurocognitive disorder (HAND) on suppressive antiretroviral therapy are partially driven by immune activation and chronic inflammation in response to soluble factors released by HIV-infected and/or activated macrophages as well as a low level of HIV replication in central nervous system (CNS) reservoirs. Majority of these mediators as well as many of the comorbid conditions can induce the ubiquitous unfolded protein response (UPR) in endoplasmic reticulum (ER). Based on our previous results showing UPR activation in post-mortem tissue from HAND patients in vivo, we expanded our investigation to determine the contribution of one of the regulators of UPR in the ER, ATF6b, to HIV-induced neurotoxicity in vitro. Following cleavage by site-1 and site-2 proteases (S1P and S2P), ATF6b translocates to the nucleus for transcriptional induction of ER resident chaperones, apoptotic genes, and secretory pathway regulatory genes. We found that infection of primary human monocyte-derived macrophages (MDMs) with HIV led to the nuclear translocation of the cleaved, thus active, ATF6b (N-ATF6b), which could be blocked by S1P inhibition. We also observed that blocking nuclear accumulation of N-ATF6b in macrophages by S1P inhibition led to the attenuation of death of primary rat cortical neuroglial cultures exposed to supernatants from HIV-infected MDMs (HIVMDMs). Furthermore, siRNA-mediated ATF6b knockdown in primary human MDMs led to attenuated production of HIV p24 and reverse transcriptase activity over 15 days in vitro as well as attenuated HIVMDM-mediated neurotoxicity. Preliminary data suggest ATF6b might be associated with endogenous antioxidant response modulation in MDMs. These findings suggest that ATF6b might be contributing to sustained HIV replication and subsequent release of neurotoxic factor from infected MDMs, impacting outcomes in several cell types within the CNS of patients with HAND and illustrate ATF6b as a novel endogenous target during HIV infection.

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Creation of Long Acting Phosphorylated Abacavir Prodrug Nanoformulations

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Antiretroviral therapy (ART) restricts replication of human immunodeficiency virus type one (HIV-1) but fails to eradicate infection. Despite suppression of viral load in plasma during combination ART, the virus persists within CD4+ memory effector T lymphocytes in lymphoid tissues that include the gut, lymph nodes, spleen and the central nervous system. Treatment interruptions lead to poor virologic control and emergence of drug resistance HIV strains. To improve ART usage, our laboratory developed first generation long-acting slow effective release ART

(LASER ART) through myristoylation of the parent ART compounds to generate prodrugs that were encased into poloxamer 407. For abacavir (ABC), prodrug (MABC) and its nanocrystals (NMABC) provided limited intracellular level of active drug metabolites (CBV-TP) for two weeks or less. To overcome this limitation, three lipophilic and hydrophobic monophosphorylated ABC prodrugs (M1ABC, M2ABC and M3ABC) were made through PROdrug and nucleoTIDE (ProTide) technology to facilitate intracellular and tissue delivery of pre-activated nucleoside analogs. Chemical compositions of the synthesized prodrugs were assessed by proton nuclear magnetic resonance (1H-NMR), fourier-transform infrared spectroscopy (FTIR) and mass spectrometry (MS). Loading of M1ABC into PLGA polymers was achieved by thin film hydration to form nanoparticles (NM1ABC) with a size of 250 nm. P407-coated nanocrystals of M2ABC (NM2ABC) and M3ABC (NM3ABC) were prepared by high-pressure homogenization with a size of 388 and 340 nm, respectively. Cell uptake, retention and antiretroviral activities were assayed in monocyte-derived macrophages (MDM). NM3ABC exhibited improved MDM drug uptake and prolonged cell retention compared to ABC or first generation NMABC. The results paralleled ARV antiretroviral activities as evidenced by protection of MDM against HIV-1 challenge up to 15 days after NM3ABC treatment as measured by HIV-1 reverse transcriptase activity and p24 staining. These results demonstrate early promise for development of potent, long-acting phosphorylated ABC formulations for treatment and prevention of HIV-1 infection.

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Off-target evaluation of LTR targeted anti-HIV CRISPR/Cas9 therapy

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Despite effective antiretroviral treatment (ART), long-term chronic HIV-1 disease is characterized by the retention of the HIV-1 provirus in an integrated form within the genomes of host cells resulting in a persistent/latent infection. Recently, CRISPR/Cas9 has been used as a gene editing technology to excise or inactivate the integrated genome. The long terminal repeat (LTR) regions represent appealing CRISPR/Cas9 targets because the symmetry between the 5' and 3' LTRs may facilitate complete excision of the proviral genome. This is complicated by the genetic variability of HIV within and across patients within a given viral subtype, and across distinct phylogenetic subtypes. Furthermore, due to the promiscuity of CRISPR/Cas9 binding, additional research is required to investigate possible off-target effects on the human genome. These potential problems were investigated using the CRISPRseek tool designed by Zhu and coworkers, as it employs a position-specific mismatch penalty system that reflects the promiscuity of the spCas9 system. Overall, very little of the human genome is similar enough to the HIV LTR, with an average of 5.29% +/- 0.91% of each chromosome being within 4 mismatches of the HIV LTR. Results have shown that certain regions of the LTR are more problematic when designing potential spCas9 targets than others. However, when using recommended cutoffs (Score $\geq$ 70, gRNA efficacy $\geq$ 0.50, fraction of PAM sequences = 1.00) the probability of off-target effects have been shown to be limited to only five potential locations, four of which target noncoding DNA. While high-probability off-target effects have been associated from HIV Cas9 excision utilizing bioinformatics approaches, they are few in number and can be easily be avoided with proper gRNA design. These studies will be critical to facilitate the rational design of HIV-1-targeted CRISPR/Cas9 therapies to avoid off-target effects.

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HIV infection suppresses TLR3 activation-mediated antiviral immunity in monocytic-lineage cells in brain

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Monocytic-lineage cells in the central neural system (CNS), including microglial cells, perivascular macrophages and brain resident macrophages, assume the main responsibility for innate immunity against viral infection in the CNS, including HIV. However, during the HIV infection, these cells constitute the major target and reservoir of the viruses in brain. The apoptosis of HIV-infected cells released viral RNA could lead to the activation of Toll-like receptor pathway in immune cells and then elicit a second round anti-viral response. In this study, a newly developed novel human cell model of latently HIV-infected microglial cells (HC69.5) and HIV-infected primary monocytes derived macrophages were used to examine whether HIV infection would compromise TLR3 activation-mediated intracellular innate immunity. We observed that HIV infection in either microglial cells or macrophages inhibited the expression of the full-length TLR3 and cleaved TLR3, a functional receptor required for the efficient downstream signaling activation, and decreased the phosphorylation of interferon regulatory factors (IRF3 and IRF7), resulting in the downregulation of type I and type III interferon (IFN). Moreover, IFN-triggered JAK/STAT pathway activation was also suppressed in these HIV-infected cells, leading to a reduced expression of IFN stimulated genes. These data provide a previously unrecognized mechanism of how the HIV facilitates its persistent infection in the CNS.

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A protease inhibitor from Soybean is a potent inhibitor of Herpes simplex virus type 2

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The Bowman-Birk inhibitor (BBI), a protease inhibitor derived from soybean, has been extensively studied in antitumor, anti-inflammation research. We recently reported that BBI has anti-HIV-1 property in primary human macrophages. Because HSV-2 infection has been suggested to play a vital role in facilitating HIV-1 sexual transmission, we here examined whether BBI has the ability against HSV-2 infection. We demonstrated BBI could potently inhibit HSV-2 infection of human endocervical epithelial cells (END1/E6E7). This BBI-mediated HSV-2 inhibition was partially through blocking HSV-2-induced NF-κB and p38 MAPK pathway activation. In addition, BBI treatment of END1/E6E7 cells or Vero cells abrogated HSV-2-induced cellular protein degradation or accumulation through dysregulation of the ubiquitin-proteasome system (UPS). Furthermore, BBI could activate JAK-STAT pathway and induce the expression of several antiviral interferon-stimulated genes (ISGs). These observations indicate that BBI may have therapeutic potential as a natural inhibitor for the prevention and treatment of HSV-2 infection.

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A novel rapid cell ablation model for eliminating one or two maker-labelled cell populations in mice-a model for studying the pathogenesis of human diseases

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Loss-of-function (LOF) studies using conditional targeted cell ablation have been widely used *in vivo* to study cell functions and interactions, tissue repair and differentiation though to date they can only target a single labelled cell population. Therefore, there is an unmet need to develop a tool that is able to specifically eliminate subset cells that require more than one marker to appropriately identify in animals for LOF studies. To this end, we propose to generate an intermedilysin (ILY)-mediated subset cell ablation model by targeting cell subsets with two markers. ILY, a toxin secreted by *Streptococcus intermedius* (SI), exclusively binds to the human cell membrane protein CD59 (hCD59), but not to CD59 of any other species. Once bound, ILY rapidly and potently lyses the cells. Taking advantage of these features, we recently established a Cre-inducible floxed STOP-*hCD59* transgenic mouse (*ihCD59*) where hCD59 expression only occurs after Cre-mediated recombination (Feng D, et al. JCI 2016). To further advance this tool towards subset cell ablation, using the well-established Cre-loxP and Flp-Frt systems, we have successfully developed a double inducible mouse strain: CAG-floxP-STOP-floxP-Frt-STOP-Frt (LSL-FSF)-hCD59 (*DihCD59*). To investigate whether Cre and Flp-mediated combination are required to induce hCD59 expression in *DihCD59*, we crossed *DihCd59* with *ROSA29Flp*$^{+/-}$, a germline Flp expressing strain, and *Foxp3CreER*$^{+/-}$, a Foxp3 (Treg marker) promoter-controlled Cre expressing strain to generate the triple transgene positive mice (*DihCD59*$^{+/-}$*ROSA26Flp*$^{+/-}$*Floxp3CreER*$^{+/-}$). We documented that hCD59 expression in Treg depends on both Cre and Flp-induced recombinational events to remove the two Floxed STOP cassette in triple transgene positive compound mice but not Cre or Flp-induced single recombination. ILY injection resulted in ablating the hCD59 expressing Treg cell population but not any other cells. Together, these results document we have successfully generated a novel rapid cell ablation model for eliminating one or two maker-labelled cell populations in mice.

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Neuroinflammatory mechanisms of morphine -induced hyperalgesia

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Morphine is commonly used to manage pathological pain such as HIV-associated pain. However, recent clinical evidence indicates that chronical use of morphine in fact exacerbates pain state. The mechanism by which morphine exacerbates pain pathogenesis is unclear. We have used the morphine-induced hyperalgesia (MIH) mouse models to investigate the molecular and cellular processes contributing to MIH development. Our data suggest that critical role of glial reaction and the expression of pro-inflammatory cytokines in the spinal dorsal horn. We further determined the contribution of Wnt signaling pathways in the control of the morphine-induced neuroinflammation. Our findings may provide insights into the pathogenesis of MIH.

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Role of PERK, an unfolded protein response kinase, in tau phosphorylation in HIV-induced neurotoxicity

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HIV infection is associated with cognitive impairments termed HIV-Associated Neurocognitive Disorders (HAND) in approximately 50% of the patients, with the more severe forms of HAND affecting daily functioning. One possible hallmark of HAND that has not been extensively examined is insoluble tau aggregates, which result from aberrant phosphorylation of the microtubule-stabilizing tau (p-tau), and contribute to pathogenesis and cognitive impairment in various diseases. Studies suggest that there are increased p-tau levels in HAND patients compared with healthy controls. However, mechanisms of tau phosphorylation and aggregation dynamics that contribute to HAND pathogenesis remain unclear. Unfolded protein response (UPR), a homeostatic mechanism that can be detrimental when aberrantly activated, was shown to be increased in tauopathies as well as HAND. Particularly, activation of the PERK arm of the UPR was shown to correlate with increased p-tau levels, suggesting a potential role for PERK in tau hyperphosphorylation. Given activation of the PERK pathway in the CNS of HAND patients, we hypothesize that PERK is a mediator of neurotoxicity in HIV-induced neurotoxicity through alteration of tau phosphorylation. Twenty-one-day-old primary embryonic rat cortical neurons were treated with HIV-infected monocyte-derived macrophage media (HIV/MDM) or NMDA with or without 1-hour pretreatment with the pharmacological PERK inhibitors GSK2606414 and GSK2656157. Western blotting and MAP2 staining were performed to determine tau phosphorylation, UPR activation, and neurotoxicity levels. Both HIV/MDM and NMDA treatment led to a concentration-dependent increase in p-tau levels, detected by the AT8 antibody, compared with the untreated or Mock-treated cultures. Preliminary data suggest that PERK inhibition might be further increasing p-tau levels. Furthermore, neurotoxicity assays suggest that inhibition with GSK2606414 does not rescue HIV/MDM-induced toxicity. These results warrant further analysis of the PERK pathway and its downstream effectors in mediating tau pathology and neurotoxic effects in the context of HAND.

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HIV-1 Tat regulates glucose uptake in human neuronal cells and exosomal IRS-1 serine/tyrosine phosphorylation levels

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Previously, we found that HIV-1 Tat induces the secretion of soluble insulin receptor (sIR) from human neuronal cells. However, the impact of these actions in the mechanisms of glucose metabolism employed by neuronal cells remains unknown. In this study we investigated if Tat regulates insulin-induced glucose uptake in human neuronal cells by modulating cell-associated and exosomal IRS-1 serine/tyrosine phosphorylation. Human neuronal cells (SH-SY5Y; 5x105 cells) were cultured in presence of Tat (25-200nM) for 24hrs and then exposed to insulin (100nM) for 30min. Glucose uptake was measured by incubating cells with 2-NBDG fluorescent glucose (10μM) and then analyzed by flow cytometry. Exosomes were isolated from SH-SY5Y cell culture supernatant by ultracentrifugation (100,000xg) and then incubated with aldehyde/sulfate beads overnight at 4 °C. Exosome-coated beads were incubated with CD63-Alexa-647 and Rab-5b-PE antibodies for 1hr at 4 °C. Then, exosomes were permeabilized using the BD Cytofix\Cytoperm Kit and incubated with anti-IRS-1-tyrosine or anti-IRS-1-serine antibodies followed by PerCP-Cy5.5 or FITC-secondary antibody. All samples were analyzed using a FACSAria flow cytometer. Our results demonstrated that: (1) HIV-1 Tat significantly reduced ($p<0.05$) insulin-induced glucose uptake in neuronal cells in a dose-dependent manner; (2) the levels of IRS-1 tyrosine phosphorylation significantly decreased and IRS-1 serine phosphorylation significantly increased in neuronal cells; (3) the ratio between serine/tyrosine phosphorylation increased with Tat in neuronal cells demonstrating a significant change of insulin signaling associated with insulin resistance; (4) the levels of IRS-1 tyrosine phosphorylation

significantly increased and IRS-1 serine phosphorylation significantly decreased in neural-derived exosomes. Our results support that HIV-1 Tat reduces glucose uptake and impairs insulin signaling in neuronal cells. The effects of Tat in glucose uptake may be associated with a dysfunctional secretion of phosphorylated IRS-1 in exosomes. The decrease in glucose uptake could be associated to higher binding of insulin to sIR in the culture medium stimulated by Tat.

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Effect of Human Endogenous Retrovirus Virus-K (HML-2) on astrocyte activation and function

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Astrocytes are not the most direct or highly productive targets of retroviral infections yet they play vital roles in viral pathogenesis. Excessive astrocyte activation, characterized by increased GFAP expression, release of pro-inflammatory cytokines/chemokines and/or dysregulation of glutamate uptake and release, occurs in both human immunodeficiency virus (HIV) and human T-cell lymphotropic virus (HTLV-1) infections. Our laboratory recently showed that another retroviral element, human endogenous retrovirus K (HERV-K) subtype HML-2, is upregulated in a subset of amyotrophic lateral sclerosis (ALS) cases and that expression of the HERV-K envelope protein is sufficient to cause motor neuron disease in a transgenic mouse model. Furthermore, the astrocyte activation marker GFAP was upregulated in the brains of transgenic mice compared to littermate controls, which mimics the reactive astrogliosis observed in ALS. We therefore investigated whether HERV-K directly contributes to astrocyte activation, apoptosis and/or dysregulation as previously described for other retroviral infections. Human fetal astrocytes (HFA) were either transfected with a plasmid that encoded HERV-K genome or HERV-K env, exposed to supernatants from HERV-K transfected neurons, or co-cultured with HERV-K-expressing neurons. Expression of GFAP and excitatory amino acid transporter 2 (EAAT2) were determined by western blot and immunocytochemistry. GFAP was not upregulated in response to HERV-K expression or exposure in any context; in contrast, HERV-K moderately diminished GFAP protein levels compared to control. EAAT2 expression was unaffected by HERV-K expression in astrocytes. Therefore, it is likely that the astrocyte activation observed in HERV-K env transgenic mice is not a direct result of astrocyte exposure to env and may instead be a bystander effect that is secondary to the neuronal injury and loss observed in this model.

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Regulation and perturbation of Myocyte enhancer factor-2 activity in Adult T-cell leukemia/lymphoma (ATLL)

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HTLV-1 is a complex human retrovirus, an etiologic agent in causing malignant and intractable T-cell neoplasia. About 5% of the infected population progress to acquiring a more aggressive form of non-Hodgkin's lymphoma (NHL), termed as Adult T-cell leukemia/lymphoma (ATLL). MEF-2 transcription factors are a family of genes that are involved in distinct functions in various tissues. There are four isoforms of MEF-2, 2A, 2B, 2C, 2D in mammals and its mutations are implicated in cancer. Published data from our lab showed that inhibition of MEF-2A leads to the reduction in HTLV-1 viral replication and associated T-cell transformation. Following that, we studied expression levels of different MEF-2 isoforms in a variety of HTLV-1-infected cell systems utilizing advanced techniques such as Prime Flow and WES, for RNA and protein, respectively. We have identified a consistent increased expression of MEF-2C, both at the RNA level and the protein level, among multiple infected cell lines. Furthermore, we noticed post-translationally modified expression of MEF-2C in the immunoblot analysis. MEF-2 is held transcriptionally silent by class II HDACs. Interestingly, a selective class IIa HDAC inhibitor MC1568 provided indications for the induction of autophagy exclusively in HTLV-1-infected ATL cells. So far, we have carried out dose and time-dependent treatments of MC1568 and noticed the accumulation of autophagy marker LC3-II as well as removal of viral gene products, TAX and HBZ. We also observed selective cytotoxicity induced in the ATL cell lines in viability assays that will be further confirmed under in vivo setting with detailed investigations on the underlying mechanism of MC1568 and its derivatives.

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HIV Envelope Mediated Hippocampal Synaptoxicity

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HIV enters the brain during the early stages of infection and because this tissue is somewhat refractory to antiretroviral therapy, it acts as a reservoir. This can lead to a range of neurocognitive dysfunctions called HIV-associated neurocognitive disorders (HAND) with HIV-associated dementia (HAD) being the most severe. HIV is unable to infect neurons but by shedding its toxic proteins, can cause direct and indirect damage to these cells and induce gliosis and inflammation. Prolonged neuronal damage in the hippocampus and pre-frontal cortex inflicted by HIV may be associated with the observed cognitive decline and memory deficits experienced by individuals with HAND. Previous studies have implicated HIV envelope glycoprotein (gp-120) mediated synaptic injury via indirect pathways involving HIV co-receptor signaling, but the detailed mechanisms are not currently known. We are interested in a cytokine-like protein called osteopontin (OPN), which is elevated in HAND and is secreted by macrophages, glial cells, and neurons. We are currently exploring the putative mechanistic role of OPN in gp-120 mediated synaptic/dendritic injury using rat hippocampal primary cultures. In our in vitro model, we found impaired dendritic arborization in developing neurons exposed to gp-120. However, this was reversed by OPN. We also observed impaired synapse formation quantified by diminished anchoring of pre- and post-synaptic proteins. We postulate that this phenotype could be attributed to lower translocation of crucial synaptic proteins to synaptosomes. In this regard, we observed a reduced number of PSD-95 puncta in dendrites of gp120 exposed hippocampal cultures. Interestingly, the addition of OPN also reversed this phenotype. Moreover, we observed changes in the levels of specific extracellular matrix proteins, which anchor synaptic proteins and found that this phenotype was modulated by OPN. Our findings suggest that HIV neurotoxins may negatively impact the formation of functional synaptic connections and that OPN can counter this activity.

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Development and in vitro validation of double edge CRISPR/Cas9 mediated anti-HIV-1 platform.

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To increase therapeutic potential of developed in our laboratory CRISPR/Cas9 mediated virus sterilization strategy we combined it with loss-of-function mutation of human CCR5 gene, a critical HIV-1 entry co-receptor. We used similar approach like in our HIV-1 eradication study: a pair of gRNAs (called here CCR5-A and CCR5-B) to target 5' and 3' regions of, in this case, human CCR5 gene located on chromosome 3.

Simultaneous CRISPR/Cas9 mediated cleavage at the targets sites A and B led to massive, 768bp long deletion of the CCR5 gene (Δ768) and loss of CCR5 expression in transfected HEK 293T cells. Next, using InFusion cloning strategy we combined in single SaCas9-AAV (pX601) delivery vector two pairs of gRNAs targeting both: HIV-1 genome (LTR1+GagD) and human CCR5 gene (CCR5-A+CCR5-B) creating pX601-HC plasmid. Efficacy and safety of the vector was tested in transfection followed by puromycin selection of single cell clones in TZM-bl reporter cell line. Δ768 mutation was detected in total six single cells clones. Additionally several Cas9 specific InDel mutations was detected at CCR5-A target site and LTR1 target site, present in integrated LTR-luciferase/β-gal reporter sequence. What is the most important, all clones carrying CCR5 Δ768 mutation were resistant to infections by R5-tropic HIV-1BAL-GFP and HIV-1JR-FL. To rule out any unwarranted off target effects, the single cell clones carrying confirmed by sequencing, triple on target CRISPR/Cas9 mediated cleavage (at target sites LTR1, CCR5-A, CCR5-B) together with two control (pX601-no gRNA) clones were screened by PCR followed by Sanger sequencing for the presence of any InDel mutations at the total 12 top predicted/possible off target sites in the human genome. No any mutation was detected. Our double edge anti-HIV-1 CRISPR/Cas9 mediated platform provides unique and potentially powerful gene editing based curative strategy to treat HIV/AIDS.

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Targeting SIVmac239 genome with CRISPR/Cas9 gene editing technology

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SIV infection of non-human primates provides an invaluable model to study curative therapies for HIV/AIDS. In our previous studies, we successfully utilized CRISPR/Cas9 gene editing to excise HIV-1 genome from latently infected human cells. To test our approach in a preclinical setting, we developed similar strategy for SIV. A pair of gRNAs targeting SIVmac239 LTR and gag gene regions was selected with the highest on target and lowest off target cleavage scores. Successful SaCas9/2xgRNA mediated cleavage should result in complete removal of the full length viral genome (in case of simultaneous cleavage at 5' and 3' LTRs) or generation of transcription/replication defective, truncated viral genomes (cleavage at 5'LTR-gag or gag-3'LTR sites). The protospacer regions for both gRNAs were cloned into single SaCas9-AAV vector (pX601) and then tested by transfection in HEK 293T cells infected with EcoSIVmac239eLuciferase/VSV-g reporter virus. PCR amplification of targeted 5'LTR-gag region of SIV genome revealed the presence of truncated 464bp long product in treated cells. Sanger sequencing of the purified short amplicon confirmed CRISPR/Cas9 induced double cleavage/end-joining event and removal of 1015bp long 5' segment of SIVmac239 genome spanning enhancer and core regions of viral promoter and start codon of gag gene. This resulted in a significant drop (>50%) in reporter virus expression. To examine if our SIV-specific SaCas9/2xgRNA platform can protect cells from new infections, HEK 293T stably overexpressing E-MLV receptor were transfected with pX601-LTR/Gag-SIV plasmid and then infected with EcoSIVmac239eLuc reporter virus. Expression of CRISPR/Cas9 rendered treated cells resistant to new infections. The resistance was a direct result of CRISPR/Cas9 mediated cleavage of viral genome, confirmed in PCR and Sanger sequencing. Here, we have developed a gene editing system for SIV, paving the road for future in vivo studies, which allows us to test our approach to eradicate virus in a translational relevant rhesus model.

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HIV-1 Nef dysregulation of the Wnt signaling pathway has a causal relationship to colorectal cancer

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With the advent of antiretroviral therapy (ART), patients with HIV are living longer. As the patient population ages, the inherent risk of developing cancer has also increased. Numerous studies have examined the relative risk of HIV infection and colon cancer. One study indicated that the average age of developing colon cancer in infected patients was 48 as opposed to 60 in non-infected patients. Moreover, the stage of colon cancer diagnosed in infected patients was more advanced compared with uninfected individuals. During primary HIV infection, mucosal CD4+ T cells were preferentially depleted in the gastrointestinal (GI) tract, and this tissue remains an area of reduced T-cell mediated immune response during HIV disease. Additionally, HIV infection is associated with proinflammatory cytokine release, resulting in chronic inflammation in the GI tract. Inflammation has been shown to contribute to the increase in HIV-associated GI cancers, including colorectal cancer. Colorectal cancers (90%) result from dysregulation of the Wnt signaling pathway. The canonical Wnt/beta-catenin pathway functions in part to direct cell proliferation, therefore, it is clear why dysregulation of this pathway could lead to tumorigenesis. The HIV-encoded protein negative regulatory factor (Nef) has also been shown to bind to a beta-catenin complex in T cells. One study has suggested an interaction between Nef and beta-catenin via a number assays including co-immunoprecipitation. What has not been demonstrated is which gene products of the Wnt pathway are specifically upregulated in the presence of Nef. Understanding the mechanisms behind how HIV may modulate the Wnt signaling pathway will be crucial to better understand the link between HIV and cancer. Therefore, these studies will proceed to examine how HIV-encoded Nef modulates the Wnt signaling pathway by interacting with the beta-catenin complex in T cells causing accelerated colon tumor outgrowth in the presence of background mutations in critical cancer control genes.

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Beta 2-ADRENOCEPTORS INHIBIT NEUTROPHIL EXTRACELLULAR TRAPS IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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Polymorphonuclear neutrophils (PMN) are the first line of defense against invading microorganisms and contribute to the orchestrated response after tissue invasion by pathogens through a number of different mechanisms, including migration into tissues, oxidative metabolism, production of proinflammatory mediators, degranulation and release of proteolytic enzymes (such as elastase and metalloproteases), and formation of neutrophil extracellular traps (NET). The present study investigated the role of beta-adrenoceptors (AR) in the functional modulation of isolated human PMN, with particular regard to NET formation. PMN were obtained from venous blood of healthy subject and the ability of AR ligands to affect NET production (assessed either as microscopic evaluation or as reactive oxygen species

(ROS) generation), release of elastase and cell migration was investigated in cells cultured under resting conditions or after activation with N-formyl-met-leu-phe (fMLP), lipopolysaccharide (LPS), or interleukin (IL)-8. NET production was increased by fMLP, LPS, and IL-8, and release of elastase was increased by fMLP. Adrenaline (A) inhibited all these responses and its effect was blocked by the beta2-AR antagonist ICI-118,551 but not by the beta1-AR antagonist CGP-12177 or by the beta3-AR antagonist L-74,8337. ROS generation induced by fMLP and migration induced by IL-8 were inhibited by A and by the β-AR agonist isoprenaline (ISO). A- and ISO-dependent inhibition were blocked by ICI-118,551 but not by CGP-12177 or L-74,8337. The expression of beta 1-, beta2-, and beta3-AR on PMN was confirmed by real time PCR, microscopy, and flow cytometry, and the effect ability of fMLP, LPS, and IL-8 was characterized. Results suggest that adrenergic modulation of human PMN function is exerted essentially through beta2-AR, and raise the possibility to exploit beta2-AR as targets for novel agents modulating innate immunity and inflammation.

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Morphine alters an in vitro blood-brain barrier model

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Many of the pathological observations made in human immunodeficiency virus type 1 (HIV-1)-associated neurocognitive disorders (HAND) have been attributed to compromise of blood-brain barrier (BBB) integrity, and selected viral proteins have been implicated in deregulation of the BBB, including the HIV-1 transactivator of transcription (Tat). In addition, illicit drug use has been shown to be a known confounder of disease in HIV-1-infected individuals. Importantly, opioid abuse within this population has been shown to enhance disease progression in multiple ways, including enhanced incidence and severity of neurocognitive impairment including dementia, as compared to non-users. Studies have suggested that exposure to both HIV-1 Tat protein and μ-opioids disrupted BBB homeostasis and permeability in primary cells, including an increased pro-inflammatory state, as well as augmented cellular transmigration, and enhanced 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 morphine exposure on BBB compromise. Changes in mRNA transcripts of tight junction proteins (TJP) were observed throughout the course of exposure. Differences in TJP expression and localization were also observed at the protein level following cellular fractionation and western immunoblot analysis. These studies demonstrate that exposure to morphine compromised BBB integrity by inducing alterations in molecular expression at both the mRNA and protein levels. In addition, a chemokine gradient was shown to be established with the chemokine monocyte chemoattractant protein 1 (MCP-1) being directionally secreted within this polarized cell system. Interestingly, pre-treatment with naloxone, a known MOR-1 antagonist, abrogated morphine's effects on BBB chemokine gradient generation, implying that the effects on the BBB may be mediated in a morphine/MOR-1-dependent manner.

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Verbal and spatial working memory among drug-using HIV-infected men and women

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Working memory (WM), the executive capacity to maintain and update memory representations, is critically dependent on the integrity of dorsolateral prefrontal cortical (DLPFC)-striatal circuitry. WM impairment is a signature deficit among HIV+ substance dependent men but has not been well studied among HIV+ women. We compared WM performance among 360 HIV+ and HIV- male and female SDIs to investigate potential sex-specific effects or interactions with HIV serostatus on this critical executive function. The study sample consisted of 114 HIV-infected and 246 EIA-verified HIV-uninfected substance dependent individuals (SDIs), including 122 men and 238 women, with a history of cocaine (83%), opioid (35%) or alcohol (77%) dependence but verified abstinent at testing. 79% of HIV+ SDIs were virally suppressed, with a median CD4 count of 497. Participants were administered computerized spatial and verbal versions of the n-back task, a working memory measure with known sensitivity to neuroAIDS. Subjects were required to monitor a continuous series of letters and update mentally the stimulus identity (verbal) or location (spatial), with a maximum load of 2 back. Subjects also completed measures of addiction severity, and psychiatric comorbidity. The four groups were comparable in racial composition; alcohol, cocaine and cannabis history; and comorbid psychiatric disorders. Response accuracy was analyzed with a Sex x HIV Serostatus analysis of variance. HIV+ SDIs performed the spatial ($p = .02$, $d = .22$) and verbal ($p = .02$, $d = .25$) WM tasks significantly less accurately compared with HIV- SDIs. Recent studies of visual memory and decision making have shown that profiles of neurocognitive impairment are not identical among HIV+ men and women. However, WM deficits are prominent among HIV-infected SDIs and are not sex-specific. The majority of participants' substance dependence was in sustained remission, indicating that WM deficits could not be attributed to non-specific effects of drug use.

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Structural changes and synaptic protein dysregulation in the CA3 region of the hippocampus are induced by HIV-1 Nef

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Alterations in soma, dendrites, and axon of a neuron as well as dysregulation of proteins and receptors can cause impaired function and be indicative of neurotoxicity. The microtubule associated protein-2 (MAP2) is a neuron-specific cytoskeletal protein that plays an important role in the development of processes, synaptic plasticity, and neuronal fate. As well, the postsynaptic density protein 95 (PSD95) is relevant for synaptic plasticity which is a mechanism involved in learning and memory. Changes in dendritic morphology have been associated with a variety of neuropathologies including HIV-1 Associated Neurocognitive Disorders (HAND). The HIV-1 early protein Nef causes learning impairment and has been linked to HAND. Our lab has demonstrated that Nef impairs novel object and location recognition in Nef-treated rats. We are interested in learning how neurons at the CA3 region of the hippocampus, which communicate with the dentate gyrus and are important for memory formation, are affected by Nef. Rat primary astrocytes were transfected with Nef plasmid prior to hippocampal infusions in male Sprague Dawley rats. Naïve rats or rats infused with non-transfected astrocytes were used as controls. Brain tissues were collected, fixed, and MAP2 and PSD95 proteins were quantified via immunofluorescence. Nef-treated rats show decreased expression of MAP2 and PSD95 as soon as one day after surgery when compared to controls. These results support our previous data showing increased astrogliosis and chromatolysis when treated with Nef. Our data suggests that astrocytes and neurons undergo morphological changes and synaptic protein dysregulation in response to Nef. In sum, these results provide a partial mechanism for the cognitive impairments observed in our Nef animal model. Future experiments include the quantification of presynaptic proteins and of the spine type and density ratio to better characterize effects of Nef on synapse dysfunction.

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Host cell-specific TDP-43 proteinopathy and aggresomes in Theiler's murine encephalomyelitis virus infection

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Background: TDP-43, an RNA-binding protein that is primarily nuclear and important in splicing of many mRNAs, is depleted from the nucleus and accumulates in cytoplasmic aggregates, which contain stress granule (SG) markers, in neurons and glia of patients with amyotrophic lateral sclerosis (ALS). Theiler's murine encephalomyelitis virus (TMEV) L protein is known to disrupt nucleocytoplasmic trafficking, however, mislocalization of TDP-43 has not been investigated in TMEV infection. **Methods:** We infected BHK-21, L929, and HeLa cells with wild type and mutant TMEV strains, including DA, DA with a deletion of L (DAΔL), GDVII, and GDVIIΔL. We then performed immunocytochemistry to detect the cellular localization and phosphorylation status of TDP-43 Ser386, and to identify SG markers with antibodies directed against G3BP1, TIA-1, PTB, and eIF3. We also inoculated DA and GDVII strains in weanling SJL mice and assessed the expression pattern of TDP-43 in infected neurons and glia. **Results:** Cytoplasmic mislocalization and Ser409/Ser410 phosphorylation of TDP-43 were observed in each cell line following infection with DA and GDVII. In BHK-21 and L929 cells, but not in HeLa cells, TDP-43 was tightly aggregated in a juxtanuclear location as a component of the aggresome, which could be disrupted by nicodazole. The aggresome also contained TMEV VP1 capsid protein, double-stranded RNA, and SG markers. In contrast, DAΔL- and GDVIIΔL-infected cells had typical small SGs with no cytoplasmic mislocalization or Ser409/Ser410 phosphorylation of TDP43, and with no aggresome formation. GDVII-infected neurons and glia, but not DA-infected oligodendrocytes had cytoplasmic mislocalization of TDP-43 with Ser409/Ser410 phosphorylation. **Conclusion:** Our findings show that TMEV infection causes TDP-43 proteinopathy, suggesting that disruption of mRNA splicing and other key cellular processes by TDP-43 mislocalization may induce cellular dysfunction and death. The absence of aggresome formation in HeLa cells may reflect the abortive infection of these cells.

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The role of alpha synuclein in immune modulation during West Nile virus infection

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Introduction: Alpha-synuclein (ASYN) is neuronal protein associated with Parkinson's disease and has no known biological function. We have previously shown that ASYN deletion (ASYN-/-) results in a higher mortality and more severe encephalitis during peripheral murine West Nile virus infection. However, intracranial infection exhibited no difference in mortality or morbidity between ASYN-/- and ASYN+/+ mice. Furthermore, ASYN was shown in the periphery to alter immune cell recruitment in the gastrointestinal tract during norovirus infection. Thus, we hypothesized that ASYN may alter the peripheral immune responses to mice infected peripherally with WNV. **Methods:** We infected ASYN+/+ and ASYN-/- mice subcutaneously with 10^3 PFU of WNV in the footpad and harvested spleens and brains. CD4+ T cells, CD8+ T cells, gamma delta T cells, macrophages, natural killer cells, dendritic cells, monocytes and microglia populations were characterized by flow cytometry. Cytokines and chemokines were measured by ELISA and flow cytometry. **Results:** Preliminary findings reveal an increase in infiltrating monocytes and T cells in the brain of WNV infected ASYN+/+ and ASYN-/- mice when compared to mock. However, there were no differences in frequencies of CD4+, CD8+ and gamma delta T cells when comparing the brains or spleens of infected ASYN-/- and ASYN+/+ mice. This was also true for macrophages, dendritic cells and natural killer cells. Furthermore, production of TNF-alpha, IFN-alpha, IFN-beta, and IL-6 were not different in the brains of infected ASYN+/+ and ASYN-/- mice. **Conclusions:** Our findings suggest that ASYN is not altering the immune responses in mice in the context of WNV and may have a more direct role in innate cellular immune responses to WNV. RNAseq pathway analysis of infected CNS tissue is ongoing to investigate this hypothesis.

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The HIV reservoir in a humanized mouse brain

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The central nervous system (CNS) is a target for a broad swath of microbial pathogens. In particular, the human immunodeficiency virus (HIV) persists and affects disease within the CNS. However, unlike other pathogens virus replicates in accessor perivascular macrophages, microglia, endothelial cells and astrocyte (glial) origins. With the goal of creating a small animal model reflective of human HIV disease in a rodent and to study the origins of HIV glial cross talk we produced NOD/SCID/IL2Rγc-/- transplanted with both human neuroglial progenitor cells (NPC and CD34+hematopoietic stem cells. These animals received intracerebral and intrahepatic cell injections, respectively leading to the repopulation of the mouse brain with human glia and peripheral immunocytes. Human glia were in the periventricular areas, meninges, the olfactory bulb, mid brain and brain stem. HIV-1 infection triggered antiviral responses. In these mice brain sections were evaluated for HIV-1 infection by immunohistology and by RNA/DNAscope. A second model was created for comparative studies of human glial survival by boosting macrophage microglial cell elements. Here immunodeficient Balb/c-Rag2-/-CSF1 (BRG-CSF1) mice carrying human macrophage colony stimulating factor. Flow cytometry and immunohistology revealed the significant number of human CD11b+ cells in the brains of mice up to 6 months of age. These mononuclear phagocytes were principally present in meninges and perivascular spaces. Peripheral HIV-1 infection resulted in robust brain infection. Both mouse models of CNS HIV-1 infection are being employed to study viral persistence and human disease pathogenesis and most notable to identify viral reservoirs and means to contain and ultimately one day eradicate them.

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Astrocyte derived extracellular vesicles following morphine withdrawal alter neuronal dendritic spine morphology and RNA expression

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Individuals with HIV not only have a higher incidence of opioid abuse, but also a greater risk for relapse. One of the greatest barriers in the treatment of opioid dependence is relapse following a period of drug withdrawal (abstinence). Opioid withdrawal results in glial activation and increased expression of pro-inflammatory cytokines. In addition to these inflammatory responses, the brain restructures neural circuits

associated with reward during drug intoxication and withdrawal. The role of glial activation and inflammation in synaptic restructuring during opioid withdrawal is poorly understood. Here we show that morphine inhibits the release of astrocyte-derived extracellular vesicles (ADEVs) by 74+/-12.3%. The withdrawal of morphine resulted in a rebounded increase in the release of ADEVs, 3-fold greater than morphine treatment. Neurons treated with withdrawal-ADEVs displayed an 81+/-0.013%, 90+/-0.035%, 58+/-0.012%, and 80+/-0.032% decrease in stubby, thin, filopodia, and mushroom dendritic spines, respectively. Compared to constitutively released-ADEVs and morphine-ADEVs, withdrawal-ADEVs were enriched 2-fold or greater for various collagen proteins (COL) such as: COL1A1, COL3A1, COL7A1, COL1A2, and COL5A2. RNA sequencing of neurons treated with withdrawal-ADEVs revealed 954 significantly down-regulated genes and over 3000 significantly upregulated genes. Ingenuity Pathway Analysis (IPA) revealed an upregulation greater than 2-fold of the FC-gamma receptor gene and its downstream gene targets such as, tyrosine protein kinase Lyn, GRB2-Related Adaptor Protein 2, Linker for Activation of T-cells, Lymphocyte Cytosolic Protein 2, and Bruton Tyrosine Kinase. Collagen is known to bind to, and activate, the FC-gamma receptor and initiate downstream signaling. The increased collagen proteins in withdrawal-ADEVs and increased gene expression of FC-gamma and its downstream targets suggests an upregulation in FC-gamma receptor signaling in neurons following treatment with withdrawal-ADEVs.

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Neuroinflammation induced by exposure to HIV-1 Tat protein increases voluntary morphine consumption

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The HIV-1 Transactivator of Transcription (Tat) protein activates brain microglia and produces neuroinflammation. Exposure to Tat protein also increases the rewarding effects of cocaine and ethanol. We hypothesized that mice exposed to Tat protein would increase morphine consumption in a two bottle choice (TBC) assay in a neuroinflammation-dependent manner. Western blot analysis confirmed the expression of Tat protein in GT-tg bigenic (iTat) mice, in which brain-selective Tat expression is induced by activation of a doxycycline (Dox) promotor for 7 days. IHC analysis of iTat mouse frontal cortex with Iba1 labeling confirmed Tat-induced activation of microglia after 7 days' Dox exposure. A parallel 7d treatment with Dox (100 mg/kg/d, i.p.) significantly increased voluntary consumption of morphine over saline-treated littermates in iTat mice, effects that lasted up to a week after Tat induction. In contrast, Dox treatment reduced daily morphine consumption in control C57BL/6J and G-tg mice lacking the Tat gene. The magnitude and duration of increased consumption depended on the degree of Tat exposure. Confirming the contribution of neuroinflammation to these effects, daily treatment during the 7-d induction with the anti-inflammatory indomethacin (10 mg/kg/d, i.p.) partially prevented Tat-induced changes in TBC, whereas dexamethasone treatment (0.1 mg/kg/d, s.c.) abolished them. To confirm the involvement of neuroinflammation in Tat-induced increased morphine consumption, the secreted phosphoprotein 1 (Spp1) gene was disrupted in iTat mice to impair microglial activation. These Spp1KO/iTat mice demonstrated equivalent expression of Tat protein to iTat mice after a 7 d treatment with Dox, but no concordant increase in morphine consumption, further implicating microglial neuroinflammation in the observed effects in iTat mice. Overall, these data suggest that expression of HIV-1 Tat protein potentiated the rewarding effects of morphine through the elevation of neuroinflammation, suggesting a biological basis by which HIV infection may increase the vulnerability to opioid abuse.
Supported by R01-DA039044.

P188

Interrelationship among neural mechanisms underlying HIV-1-associated neurocognitive disorders (HAND) in the HIV-1 transgenic rat

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The pathogenesis of HAND in the post-combination antiretroviral therapy (cART) era is multidimensional, and may include neurotransmitter alterations, synaptopathy, and neuroinflammation. Using the HIV-1 transgenic (Tg) rat, which resembles HIV-1+ individuals on cART, potential neural mechanisms, and most importantly, their interrelationship, were examined. First, post-mortem synaptopathy was assessed in layers II-III pyramidal neurons of the medial prefrontal cortex at approximately 20 months of age (HIV-1 Tg: N=19 litters: Male: n=28, Female: n=27; control N=16 litters: Male: n=26, Female: n=20). A selective population shift in dendritic spine morphology, dependent upon presence of the HIV-1 transgene and biological sex, was observed. Male HIV-1 Tg animals exhibited a population shift towards shorter dendritic spines with increased head diameter and increased volume relative to male control animals. In sharp contrast, female HIV-1 Tg animals displayed a population shift towards longer dendritic spines with decreased head diameter relative to female control animals; no significant alterations in dendritic spine volume were revealed in female animals. A significant genotype x sex x bin interaction was observed for backbone length ($F(1,1370)=30.5$, $p\leq0.001$) and head diameter ($F(1,851)=58.2$, $p\leq0.001$), but not volume ($p>0.05$). Second, neuroinflammation was examined using four markers, including NF-κB, IL-1β, IL-6, and TNF-α (HIV-1 Tg: N=20 litters: Male: n=32, Female: n=30; control N=17 litters: Male: n=31, Female: n=30) revealing low levels of neuroinflammation, independent of genotype. No significant genotype ($p>0.05$) or sex ($p>0.05$) differences in gene expression were observed, examined using the 2-ΔΔCt method. Third, an increase in dendritic spine backbone length was associated with increased expression of NF-κB in control ($p\leq0.02$), but not HIV-1 Tg ($p>0.05$), animals. Thus, alterations in dendritic spine morphology, independent of neuroinflammation, may mechanistically underlie neurocognitive impairment in the HIV-1 Tg rat, providing key targets for the development of adjunctive therapeutics in the post-cART era.
Supported by NIH DA013137, HD043680, MH106392, NS100624.

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SEX DEPENDENT PREDICTIVE BIOMARKERS OF THE PROGRESSION OF HIV-1 ASSOCIATED NEUROCOGNITIVE DISORDERS (HAND)

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Although several biomarkers of neural damage (e.g., CSF neurofilament light chain, tau, MRI) have been suggested for HAND, they lack specificity for milder forms of HAND commonly observed in the post-combination antiretroviral therapy (cART) era. In the HIV-1 transgenic (Tg) rat, cross-sectional studies have implicated the utility of prepulse inhibition (PPI) as an innovative biomarker, diagnosing presence of the HIV-1 transgene with >90% accuracy. The predictive utility of early neurocognitive impairments (NCI) for the progression of HAND, however, is a critical knowledge gap. Accordingly, using a longitudinal experimental design, multiple neurocognitive assessments, including prepulse inhibition (PPI; examining temporal processing), habituation of locomotor activity (long-term episodic memory), and signal detection (sustained attention) were assessed in HIV-1 Tg and control rats (Tg; N=20 litters; Male: n=37, Female: n=33; control N=17 litters: Male: n=34, Female: n=33) across the majority of the functional lifespan (Postnatal Day (PD) 30 to PD540). First, from the earliest assessments,

HIV-1 Tg animals displayed profound NCI across all cognitive domains tested. A more challenging signal detection task at PD540, as well as a reversal task tapping flexibility and inhibition, revealed marked HIV-1 associated NCI in the detection of shorter signal durations. Second, early NCI were predictive of the progression of HAND in a sex-dependent manner. For HIV-1 Tg females, auditory PPI at PD30 and visual PPI at PD60 explained 84% of the variance in signal detection accuracy at PD540 ($R2=0.84$, $F(2,8)=15.9$, $p\leq0.004$). However, in HIV-1 Tg males, 96% of the variance in accuracy during the reversal task was explained by locomotor activity at PD30 and PD60, as well as visual PPI at PD60 ($R2=0.96$, $F(3,7)=28.6$, $p\leq0.004$). Thus, early NCI accurately predicts executive function deficits at an advanced age, providing an innovative biomarker for milder forms of NCI in the post-cART era. Supported by NIH DA013137, HD043680, MH106392, NS100624.

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Age by genotype interactions in gp120 mice: p75, Tau accumulation and protection with a p75 neurotrophin receptor ligand

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Neuroinflammation is a driving force for the progression of HIV-associated cognitive disorders with no effective treatments. Using an in vitro model of inflammation we have shown that secreted factors induce a dysregulation of neuronal calcium homeostasis followed by focal beading, a potential substrate for the pathogenic accumulation of proteins including a novel hypophosphorylated form of Tau (Tau-1ir). To better understand the evolution of inflammatory pathology during aging, we examined CNS pathogenesis in gp120 Tg mice from 3-23 months of age. Age-related changes in GFAP immunoreactivity appeared first in gp120 Tg mice at 3-4 months but stabilized at the level of age-matched controls by 12 months. In contrast, the p75 neurotrophin receptor (p75NTR) and Tau-1ir increased progressively with age in both gp120 and WT mice starting at about 8 months and was followed closely by Iba-1 immunoreactive microglia. No staining was seen for traditional markers of Tau or Aβ pathology. Clusters of Tau were enriched in the hippocampus and overlapped significantly with p75NTR immunostaining. Both p75NTR and Tau-1ir deposits peaked at 16 months and then decreased, paralleling the timing for loss of cholinergic fibers in the hippocampus. Treatment with the p75NTR ligand, LM11A-31 (50 mg/kg, po), suppressed the accumulation of active microglia and p75NTR but not Tau-1ir clusters. However, the decrease in Tau-1ir seen in 19-23 month old WT and gp120 mice was prevented by LM11A-31. This correlated with the ability of LM11A-31 to preserve cholinergic fibers in the hippocampus of aged mice. Overall, treatment with LM11A-31 reduced the inflammatory response and expression of the p75NTR. This response was consistent with the ability of LM11A-31 to shift macrophages and microglia in vitro to a less damaging phenotype. The anti-inflammatory and neuroprotective effects of LM11A-31 make it an excellent candidate for use in HIV infected patients at risk for cognitive decline.

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Co-administration of chemokine receptor antagonists with morphine attenuated up-regulation of chemokine and cytokine mRNA expression induced by incisional pain in rats

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Chemokine receptors are expressed on leukocytes, as well as microglia, astrocytes, oligodendrocytes, and neurons in the CNS. Chemokines are involved in pain modulation. Central administration of chemokines produces hyperalgesia and diminishes mu-opioid agonist-induced analgesia in rats. We hypothesized that co-administration of chemokine receptor antagonists (CRA) with opioids would increase the analgesic effect via inhibiting cross-desensitization between chemokine and opioid receptors, as well as possibly inhibiting release of chemokines during pain. Previously, we found that co-administration of maraviroc, a CCR5 antagonist, and AMD3100, a CXCR4 antagonist, with morphine, potentiated morphine's analgesic effect using a standard model for assessing post-surgery incisional pain. The present study used the RT2 Profiler® PCR Array (Qiagen) to assess mRNA levels for a panel of chemokines, cytokines, and other mediators resulting from a) incisional pain and, b) effects of morphine alone and in combination with two CRAs on mRNA levels induced by the incision. Male Sprague-Dawley rats underwent incision of the left hind paw, and mechanical allodynia was measured between 15 and 60 minutes post-surgery. At 25 minutes post-surgery animals received either vehicles, two CRAs plus vehicle, two vehicles plus morphine, or two CRAs plus morphine. At 60 min, rats were euthanized and the draining popliteal lymph nodes were harvested for mRNA extraction. Baseline levels of mRNA for the chemokines and cytokines were established using lymph nodes from rats that did not have surgery. It was found that incisional pain up-regulated mRNA for fourteen immune mediators. Co-administration of two CRAs alone down-regulated mRNA for most of these mediators; co-administration of two CRAs given with morphine gave even stronger down-regulation. This study shows that two CRAs alone, or two CRAs plus morphine, can down-regulate mRNA expression of a panel of unrelated chemokines and cytokines following a painful surgical incision.
Supported by grant W81XWH-15-RTR-TRA from the DoD.

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Examining alterations in the function of HIV-1 Tat derived from neurocognitively impaired patients in the Drexel Medicine CARES Cohort

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While the mortality associated with HIV-1 infection has decreased, the incidence of neurocognitive impairment (NI) has increased; particularly the milder forms. Previous studies have detected the HIV-1 protein Tat within the periphery, CSF, and brain of infected patients, even those adherent to antiretroviral therapy. Current studies seek to identify and characterize predominant genetic variations within Tat that correlate with NI and examine their functional impact on the intracellular (promoter transactivation) and extracellular (secretion, neurotoxicity) functions of the protein. HIV-1 Tat sequences were obtained from PBMCs isolated from patients in the presence or absence of NI in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. Given brain-derived Tat variants from demented patients have previously been shown to be unable to transactivate the HIV-1 LTR, five NI and five non-NI patients were selected based on their global deficit score (GDS). Tat

constructs from both groups of infected patients were synthesized from their predominant PBMC-derived Tat sequences. Results have demonstrated no difference in Tat-driven LTR transactivation between the two groups. Logistic regression models have predicted the top 10 amino acid variants that correlated with the NI status of a given patient. These Tat variants are currently being examined for alterations in transactivation of the viral LTR or host genes and their impact on secretion. Preliminary results have demonstrated that selected amino acid variants of Tat exhibit reduced levels of LTR transactivation. In addition, studies are currently utilizing ELISA-based technologies to examine alterations in the secretion of Tat as well as the detection of Tat within peripheral blood derived from patient samples. These studies will also examine the secretion of selected Tat variants predicted to correlate with NI status within patients in the Drexel Medicine CARES Cohort.

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Tat Controls Transcriptional Persistence of Unintegrated HIV Genome in Primary Human Macrophages

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In HIV infected macrophages, a large population of viral genomes persists as the unintegrated form (uDNA) that is transcriptionally active. However, how this transcriptional activity is controlled remains unclear. In this report, we investigated whether Tat, the viral transactivator of transcription, is involved in uDNA transcription. We demonstrate that de novo Tat activity is generated from uDNA, and this uDNA-derived Tat (uTat) transactivates the uDNA LTR. However, Tat stimulates the uDNA LTR to a less extent (10 - 20%) than it stimulates proviral LTR. In addition, uTat is required for the transcriptional persistence of uDNA that is assembled into repressive episomal minichromatin. In the absence of uTat, uDNA minichromatin is gradually silenced, but remains highly inducible by HDAC inhibitors (HDACi). Therefore, functionally, uTat antagonizes uDNA minichromatin repression to maintain persistent viral transcription in macrophages. uTat-mediated viral persistence may establish a viral reservoir in macrophages where uDNA were found to persist.

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Self-reported insomnia is associated with decreased cognitive function in HIV-seropositive women

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Prevalence of insomnia and sleep disturbances have a higher rate in the HIV-positive population than in the general population. In HIV positive patients, insomnia has been associated with poorer cognitive function. Nearly half of the HIV-positive population suffers from HIV-associated neurocognitive disorders (HAND). In our study, we aimed to study the correlation between self-reported insomnia, using the Athens Insomnia Scale (AIS), sleep disturbances using automated bracelet data, and cognitive performance in a cohort of HIV-seropositive Hispanic women. There was no direct correlation between AIS score and automated sleep recordings, suggesting that these two instruments do not measure the same sleep disorders. However, when categorized as having insomnia or not, there was a significant decrease in automated sleep recordings only in the HIV+ group. A partial correlation correcting for age and depression (Beck's Depression Index score), showed that higher AIS presented correlated with lower speed of information processing in participants classified as having asymptomatic neurocognitive impairment, while in those classified as having symptomatic neurocognitive impairment higher AIS score correlated with decrease executive performance. These results suggest that greater insomnia scores, as self-reported by the AIS, correlates with decreased cognitive function in all HAND categories. Further studies are needed to corroborate this data.

P195

Identifying a role for E2F1 in synaptodendritic damage in EcoHIV-infected mice

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E2F1 is a transcription factor important for the transition from G1 to S phase of the cell cycle; however, its expression has been shown to be elevated in several neurodegenerative diseases including a subset of patients with HIV-associated neurocognitive disorder (HAND). In the CNS of HIV-infected patients with encephalitis, E2F1 exhibits increased expression, and in rodent models loss of normal E2F1 function impairs synaptic health, learning and memory. To study the relationship between E2F1 and HIV-related pathogenesis in mice, we used a chimeric HIV engineered to infect mouse cells, EcoHIV. This virus was constructed by replacing the HIV gp120 coding region with the gp80 coding region of ectopic murine leukemia virus to allow for mouse cell infection while keeping HIV cis-regulatory elements intact. We found that 28 days post intraperitoneal inoculation, EcoHIV-infected mice have viral DNA present in the brain (as previously published) and exhibit impaired spatial learning and memory in the radial arm water maze. Preliminary data also indicate that E2F1 levels are increased in the brains of these mice, and this co-occurs with decreased postsynaptic density protein 95 (PSD-95) expression. These results support our hypothesis that E2F1 plays a role in either the development or persistence of neurocognitive deficits in HIV-infected patients.

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Role of endolysosome deacidification on the effects of antiretroviral drugs on morphology of synaptodendritic spines

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Although antiretroviral therapeutic drugs (ARTs) effectively suppress HIV-1 infection and increase the lifespan of people living with HIV-1, upwards of 50% of HIV-1 infected individuals experience HIV-1 associated neurocognitive disorder (HAND). Clinical symptoms of HAND range from "asymptomatic" (mild) to dementia (severe) and synaptodendritic damage appears to be an important underlying mechanism causing HAND pathogenesis. Emerging evidence indicates that some ARTs are neurotoxic and lead to synaptodendritic dysfunction. We have shown that a subset of ARTs can deacidify endolysosome and thereby affect the structure and function of endolysosomes including lysosome exocytosis. Because changes in lysosome exocytosis can affect dendritic spine plasticity, we determined the extent to which ARTs affected dendritic spines in primary cultured rat neurons. Two ARTs that deacidify endolysosomes (dolutegravir and lamivudine) and one ART (abacavir) that acidifies endolysosomes were tested in parallel with positive control agents that either de-acidify (bafilomycin) or acidify (ML-SA1) endolysosomes. All of the agents that de-acidified endolysosomes (bafilomycin, dolutegravir, and lamivudine) caused the dendritic spines to become more immature with longer and thinner spines. In contrast, agents

that acidified endolysosomes (ML-SA1 and abacavir) caused the dendritic spines to become more mature and mushroom-shaped. Our findings suggest that a subset of ARTs that deacidify endolysosomes affects dendritic spines and thereby might be predisposing individuals to increased prevalence and/or intensity of HAND.
This work was supported by P30GM103329, R01MH100972, and R01MH105329.

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Innate immune response of central nervous system to cytopathic virus infection

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Central nervous system (CNS) though considered as an immune privileged compartment can elicit an elaborate immune response upon the viral infection. We previously established that type-I Interferon (IFN-I) signaling primarily orchestrates all innate immune gene expression and myeloid cell dynamics following a noncytopathic virus infection (Lymphocytic Choriomeningitis Virus) infection in the brain. To gain insight into the role IFN-I following a cytopathic virus infection, we looked at CNS gene response using vesicular stomatitis virus (VSV)-encephalitis model. Global differential gene expression profiling was done to identify the host cellular factors associated with CNS pathogenesis. We observed that VSV infection induced 281 gene changes in WT mice but surprising in the absence of IFN-I signaling, a significantly higher 1,357) number of genes showed noticeable changes. We further performed gene enrichment analysis to identify critical candidates and pathways involved in immunopathology. In the next step, genes involved in immune response and neuron apoptosis were tallied against the protein database to generate meta-network of the proteins (interactome) and were ranked by taking account of the degree centrality. Our results show that in the absence of IFN-I signaling, TNF-α is connected maximally to networked candidates, thus proving to be a key regulator in the neuropathology. Our results indicate that in the absence of IFN-I signaling two major TNF-α signaling pathways drive CNS anti-viral response. (i) TNF-α directly regulates innate immune gene expression and recruits myeloid cells for antiviral action against VSV, and (ii) TNF-α signaling on downstream potentiates IFN-γ release thereby, synergistically activates IRF1-dependent TRAIL-mediated apoptosis of infected neuronal cells

P198

Analysis of HIV-1 Tat Impacts on the Hippocampal Neurons Using Whole Transcriptome Sequencing

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HIV-1 regulatory protein Tat is well-known for its DNA and RNA binding properties, which is largely relying on its cysteine rich domain. For this reason, Tat is hypothesized to adversely impact cellular processes at the transcription level via modulating the transcriptome in the favor of virus replication. We have performed deep RNA sequencing and implemented bioinformatics analysis to assess the quality and severity of this impact. Our analyses show a strong mRNA downregulation event as a result of recombinant Tat protein treatment in hippocampal neurons. This is while Tat protein has selective upregulating effects on particular noncoding RNAs, mainly miRNAs and lncRNAs. Our data has also revealed a data-driven association among the expression of miRNAs and some lncRNAs, signifying the interplay between these transcriptional elements under the virus infection. We have also studied the effects of cocaine in presence and absence of Tat to determine their interactive effects on transcription events. Our results show different combinatorial effects of Tat-cocaine co-treatment on the target families of RNAs as compared to Tat and cocaine alone, implying these collective effects are rather randomly occurring across different signaling pathways.

P199

Electrophysiological Studies of HIV-1 Tat Effects on Neurotransmitter Receptors of Rat Hippocampal Neurons

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HIV-1 infected cells release neurotoxic proteins that are believed to play a major role in the emergence of HIV-associated neurocognitive disorders (HAND) including dementia and memory deficits. Among these proteins, trans-activator of transcription (Tat) protein is known for its contribution to multiple adverse effects in cell survival pathways. In this work, the micro-electrode array (MEA) technology is utilized to study the hippocampal neurons electrical activity under HIV-1 Tat. The negative impacts of HIV-1 Tat are shown to be on the neuronal spiking activities in terms of the firing frequency, amplitude and our proposed measures of network-wide communications. To further investigate the underlying mechanisms of Tat mediated spiking activity attenuation, agonists and antagonists of abundant hippocampal receptors are employed, revealing the adverse effect of Tat through the activation inhibitory receptors and partial inhibition of excitatory receptors.

P200

Biological sex determines the development of HIV-associated neurocognitive deficits

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The prevalence of HIV-1-associated neurocognitive disorders (HAND) remains persistently high in the era of combination antiretroviral therapy, and therefore demands our attention. Mounting evidence suggests that biological sex may selectively moderate the progression of HAND. Here, we investigated sex differences in HIV-associated neurocognitive deficits using the HIV-1-transgenic 26 (Tg26) mice, which express 7 out of 9 HIV-1 viral genes. We observed that adult male Tg26 mice, compared to their wild-type male littermates, show an impaired spatial-reference memory and contextual-fear memory. Interestingly, learning and memory in female Tg26 mice were similar to that of the wild-type female littermates. Consistent with these findings, electrophysiological assessment of hippocampal slices revealed suppression of long-term potentiation in area CA1 of Tg26 males, but not of Tg26 females. Detailed analyses of mechanisms underlying the sex-dependent memory deficits in Tg26 mice revealed a reduction in the hippocampal synapsin 1 levels and in hippocampal neurogenesis. These changes were consistent with reduced expression (mRNA and protein) of brain-derived neurotrophic factor (BDNF) in the hippocampus of Tg26 mice. While therapeutic approaches targeted at inducing the expression of BDNF (a known regulator of synapsin 1 and hippocampal neurogenesis) might be beneficial in the treatment of HIV-associated memory loss, this study stresses on the importance of incorporating sex as a biological variable in HAND.

P201

Altered gene expression in HIV gp120 transgenic mouse brains: Effects of methamphetamine treatment.

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Treatment with anti-retroviral therapy (ART) regimens has resulted in life extensions of decades for HIV-infected individuals. However, the prevalence of HIV-associated neurocognitive disorders (HAND) continues to be

high despite successful ART therapy. Brain astrocytes can harbor provirus and express neurotoxic HIV proteins such as gp120 and TAT, leading to HAND. Neurocognitive decline is exacerbated in individuals who use methamphetamine (METH). To examine the role of METH in HAND, we utilized a transgenic mouse expressing glial fibrillary acid protein (GFAP)-controlled HIV gp120 protein (gp120+). Mice were injected intraperitoneally with either 0.9% saline vehicle or successive weekly escalating doses of 1, 5, 10, or 30 mg/kg METH, and their brains were harvested 7 days post-injection for qPCR, immunohistochemistry and protein analyses. In quantitative PCR experiments, gp120+ mice showed dramatically increased levels of GFAP mRNA, suggesting chronic gp120 expression causes astrocyte activation. However, a qPCR probe designed to distinguish the transgene versus the endogenous GFAP transcripts showed lower levels of GFAP activation, suggesting that some of the GFAP mRNA expression is read-through from the transgene construct. Furthermore, GFAP expression in the rostral portion of the brain (anterior to ~0 bregma) was lower than the caudal portion in the gp120- mice, whereas GFAP was higher in the rostral portion in the gp120+ mice, suggesting enhanced astrocyte activation in the rostral portion of the brain encompassing the striatum and frontal cortex. Gp120+ mice demonstrated increased levels of TIMP1 and IL1beta compared to gp120- mice, further suggesting chronic astrocyte activation. Gene expression levels of excitatory amino acid transporter-2, tyrosine hydroxylase, and dopamine transporter were not changed in gp120+ mice. None of the METH treatments resulted in any changes in gene expression in either mouse group at 7 days post-injection. The data indicate long-term expression of gp120 in brain leads to altered gene expression of neuroinflammatory mediators.

P202

Flow Cytometric Identification of Epstein-Barr Virus Specific Memory B Cells in Normal Donors and Multiple Sclerosis Patients

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of unknown causes, that often leads to disability. One recognized aspect, although not fully understood of MS, is a production of antibodies targeting common viruses with a robust and consistent association of MS patients displaying elevated levels of Epstein-Barr Virus (EBV)-specific antibodies and increased CD8+ T cell response to the virus. EBV infection is associated with multiple human diseases and it is the etiologic agent for acute infectious mononucleosis and a variety of malignancies. The predominant host cells targeted by the virus are B cells and epithelial cells, with B cells being the EBV latent compartment in the human body.We have developed a flow cytometry protocol for the detection of EBV-specific IgG+ memory B-cells to compare B-cell frequencies to EBV in normal donors (ND) and patients with MS.
PBMCs from 16 ND and 14 MS patients were stained with different antibodies directed to B cells and B cell subpopulations and then exposed to EBV glycoproteins, gHgL and gp350 probes that are biotinylated and labeled with streptavidin conjugated to APC or PE fluorochromes. PBMCs from two patients with chronic active EBV infection were used as a positive control. Flow cytometric analysis showed a significant increase in the frequency of gp350-specific IgG+ memory B cells in ND compare to the patients with chronic active EBV infection. Preliminary data show comparable frequencies of gHgL-specific and gp350-specific IgG+ memory B cells in ND and MS patients.The flow cytometric strategy and analysis used in this study is not exclusive to EBV/MS, and could be adapted to evaluate frequencies of disease-specific B-cell for other antigens.

P203

Low dose aspirin ameliorates experimental allergic encephalomyelitis via interleukin-11-mediated upregulation of Tregs

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Aspirin (acetylsalicylic acid) is one of the most widely-used analgesics, and experimental allergic encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS), the most common human demyelinating disease of the CNS. This study underlines the importance of low dose of aspirin in suppressing the disease process of EAE in mice. Oral administration of low dose of aspirin suppressed clinical symptoms of adoptively-transferred relapsing-remitting (RR) EAE in female SJL/J mice and chronic EAE in male C57/BL6 mice. Aspirin also inhibited the encephalitogenicity of MBP-primed T cells. Accordingly, aspirin inhibited perivascular cuffing, suppressed inflammation and blocked demyelination in the CNS of EAE mice. From the immunomodulatory side, aspirin upregulated Foxp3 and suppressed EAE via regulatory T cells (Tregs). Consistent to the suppressive activity of Tregs towards autoreactive T cells, aspirin suppressed the differentiation of Th17 and Th1 cells and shifted the balance towards a Th2 response. Interestingly, aspirin increased the transcription of IL-11 via activation of CREB and thereby upregulated and/or protected Tregs. Accordingly, neutralization of IL-11 negated Treg-upregulating effect of aspirin and abrogated aspirin-mediated protection of EAE. IL-11 alone was also sufficient to upregulate Tregs and protect mice from EAE. These results identify a new mode of action of aspirin and suggest that oral administration of low dose of aspirin may be beneficial in MS patients via IL-11-mediated upregulation of Tregs.
This study was supported by a merit award (I01BX002174) from Veteran Affairs.

P204

Alcohol and antiretrovirals modulate transporter in polarized macrophages

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Alcohol consumption, which is prevalent in the HIV+ population, decreases the efficacy of antiretroviral therapy. Macrophages are able to be polarized to the pro-inflammatory M1 or the anti-inflammatory M2 phenotypes. Drug transporters are expressed differently between the two subsets. We hypothesized that ethanol and the antiretroviral drug lopinavir (LPV) differentially alters clinically relevant drug transporter expression in macrophage subsets. We found higher expressions of influx transporter OATP 1A2, OATP 3A1 and the efflux transporter MRP1, and lower expressions of PGP and BCRP in the M1 phenotype compared with the M2 cells. Ethanol and ethanol+LPV treatments decreased the expressions of OATP 1A2 and OATP 3A1 while increased PGP and BCRP levels. Accordingly, the MRP1 and PGP specific inhibitors MK 571 and elacridar significantly decreased viral replication in the M1 phenotype treated with LPV and ethanol+LPV, and in the M2 phenotype treated with ethanol+LPV respectively. LC-MS data showed an increase of intracellular elvitegravir with an addition of PGP inhibitor elacridar in the M2 phenotype, consistent with altered PGP expression between M1 and M2 macrophages. By controlling one of the signaling pathways NF-κB, the expression level of influx and efflux transporters is modified. Our findings may provide a potential strategy to overcome the subtherapeutic drug concentration in macrophages.

P205

Immunogenicity of novel MHC Class I epitopes to confer protective immunity against chronic HTLV-1 infection

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Human T-cell leukemia virus type 1 (HTLV-1) infects approximately 20 million people worldwide. While 90% are asymptomatic, 5% develop severe diseases including adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). No vaccine against HTLV-1 exists, and screening programs are not universal. However, patients with chronic HTLV-1 infection have high frequencies of HTLV-1-activated CD8+ T cells, and the two main HLA alleles (A2, A24) are present in 88% of infected individuals. We thus utilized an immunoproteomics approach to characterize MHC-I restricted epitopes presented by HLA-A2+, A24+ MT-2 and SLB-1 cell lines. Unlike traditional motif prediction algorithms, this approach identifies epitopes associated with cytotoxic T-cell responses in their naturally processed forms, minimizing differences in antigen processing and protein expression levels. Out of nine identified peptides, we confirmed six novel MHC-I restricted epitopes that were capable of binding HLA-A2 and HLA-A24 alleles and used in vitro and in vivo methods to generate CD8+ T cells specific for each of these peptides. MagPix MILLIPLEX data showed that in vitro generated epitope-specific CD8+ T cells secreted IFN-γ, granzyme B, MIP-1α, TNF-α , perforin and IL-10 when cultured in the presence of MT-2 that is a HTLV-1 infected cell line. Degranulation assay confirmed cytotoxic response through surface expression of CD107 on CD8+ T cells when cultured with MT-2 cells. A CD8+ T-cell efficacy assay indicated significant antiviral activity of CD8+ T cells specific against all identified peptides. These epitopes are thus candidates for a therapeutic peptide-based vaccine against HTLV-1, and our results provide preclinical data for the advancement of such a vaccine.

P206

Different mechanisms implicated in ZIKV-induced inflammatory molecules in human microglial cells

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Zika virus (ZIKV) is an emerging mosquito-borne flavivirus pathogen responsible for neurodevelopmental complications like microcephaly. Goal of the present study was to understand underlying mechanisms mediating ZIKV infection in glial cells. Study was performed in primary human microglial cells infected with three different strains of virus (including the African lineage: MR766 and the Asian: R103451 and PRVABC59), at different time-points (24, 48, 72 and 96 hours post infection). Infectivity as measured by immunofluorescence and plaque assay showed differential infectivity and increased propagation rates in glial cells infected with ZIKV. Notably, PRVABC59, had high potential to infect and replicate in microglia and showed significant decrease in cell viability after 48 hours of infection. We also investigated the inflammatory molecules secreted by microglia after indicated time-points and detected about two fold increased secretion in the chemokines RANTES, IL-8, MCP-1 and the cytokine IL-6 with all three strains of ZIKV. Albeit, the expressions of inflammatory molecules by each viral strain were time dependent. We measured potential mechanism(s) mediating inflammation and showed increased expression levels of Toll Like Receptor (TLR)-3 with R103451 and PRVABC59, and increased expression of TLR-4 with MR766 and R103451. We also observed decreased expression levels of TLR-5 with R103451 and PRVABC59. Since ZIKV induced the TLR pathway, and recent evidence suggested a link between TLR and the induction of the autophagy pathway, we studied the modulation of autophagy pathway by ZIKV using a RFP-GFP-LC3 reporter assay. LC3, a significant marker for autophagy pathway, was differentially upregulated by three strains of ZIKV in human microglia. Overall, these findings link two innate immunity defense systems, TLR signaling and autophagy. Ongoing studies in our lab will provide further evidence whether ZIKV-induced TLR has the ability to stimulate autophagy and whether this pathway can be used to treat ZIKV infection.

P207

Elucidating molecular mechanisms of TAAR1-dependent astrocyte regulation during HAND and METH exposure

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In 2012, the National Survey on Drug Use and Health (NSDUH) estimated 1.2 million people in the U.S. reported the use of methamphetamine (METH) that year, with 133,000 new users age 12 or older. METH is a highly addictive substance that leads to an imbalance in dopamine and norepinephrine release causing euphoric effects. Long term METH use has been linked to many central nervous system (CNS) abnormalities including deficits in memory, executive function, anxiety and depression. METH use is associated with risky sexual behavior, lowered inhibitions and increased likelihood for acquiring HIV. METH abuse exacerbates the onset of HIV-associated neurocognitive disorders (HAND) and promotes a neurotoxic environment increasing oxidative stress and excitotoxicity. Our lab previously identified trace amine associated receptor 1 (TAAR1) as a novel stimulatory G protein coupled receptor in primary human astrocytes. The expression of TAAR1 is modulated by METH and HAND-relevant stimuli. We hypothesize that TAAR1 regulates astrocyte intracellular signaling during HAND and METH exposure. This study used a physiologically relevant model of extended METH exposure and low level HIV-associated activation which mimics conditions in HIV+ METH users. We transfected human astrocytes using a TAAR1-GFP tagged overexpression construct to study TAAR1 dependent regulation. First, exogenous TAAR1 expression and intracellular localization were characterized. Next, TAAR1 localization with HAND and METH exposure were assessed. Finally, signaling downstream of TAAR1 was measured via cyclic AMP (cAMP) activation, Ca2+ signaling, protein kinase C (PKC) activity, excitatory amino acid transporter 2 (EAAT2) function and cellular proliferation. We used a TAAR1 selective antagonist, EPPTB, to inhibit activity and investigate TAAR1-dependent astrocyte function. Our study aims to delineate therapeutically targetable mechanisms that regulate astrocytes during neuroinflammation in HAND and METH exposure proposing astrocyte TAAR1 as a potential target to combat neurocognitive decline.

P208

Astrocytic metabolic switch is a novel etiology for Cocaine and HIV-1 Tat-mediated neurotoxicity

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Calcium (Ca2+) dynamics and oxidative signaling are fundamental mechanisms for controlling mitochondrial bioenergetics and cell function in the central nervous system (CNS). Within the CNS, astrocytes serve as a major energy reserve for neurons and these energy pathways are disrupted during

HIV infection and cocaine use. The use of cocaine increases the risk for becoming infected with HIV and a significant number of cocaine abusing individuals are HIV+. Moreover, cocaine use in the presence of HIV infection exacerbates HIV-associated neurocognitive disorders (HAND). The contributions of changes in astrocytic metabolism to the neuropathology during HIV infection and cocaine use have not been established. Our data show that the HIV protein Tat and cocaine induce a metabolic switch from glucose to fatty acid oxidation in astrocytes thereby limiting lactate transport to neurons. Furthermore, mechanistic analyses revealed increased Mitochondrial Ca2+ Uniporter (MCU)-mediated Ca2+ uptake in astrocytes exposed to Tat and cocaine due to oxidation of the pore forming subunit, MCU. Since our data suggest that mitochondrial oxidation is dependent in part on MCU-mediated Ca2+ uptake, we targeted MCU to restore glycolysis in astrocytes to increase extracellular lactate levels. Knocking down MCU in astrocytes prior to Tat and cocaine exposure prevented metabolic switching and protected neurons in vitro. These findings identify a distinct and novel molecular mechanism underlying the neuropathogenesis related to HIV and cocaine use.

P209

Alternative Dopamine Signaling in Macrophages Mediates Increased HIV Infection

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Drug abuse is an important comorbidity in HIV, and all drugs of abuse elevate dopamine within the CNS. Our data demonstrate that elevated dopamine increases HIV entry into macrophages. Macrophages and other myeloid cells are the primary targets for HIV within the CNS, and infection of these cells is central to the development of neurocognitive dysfunction in HIV-infected individuals. Therefore, understanding how dopamine mediates the changes in HIV entry in macrophages is necessary to understand and treat NeuroHIV. We detected transcripts for all five dopamine receptor subtypes in macrophages, with significantly more DR5 present. We examined dopamine-mediated increases in cAMP as a readout for canonical dopaminergic signaling and neither dopamine nor the D1-like agonists SKF-38393 and A68,930 increased cAMP. In contrast, the β-adrenergic receptor agonist isoproterenol produced a dose-dependent increase in cAMP, indicating Gαs and adenylate cyclase are active in these cells. Dopamine was also unable to increase in PKA phosphorylation, confirming the cAMP signaling pathway was inactive. However, dopamine increased PKC phosphorylation as well as Ca2+ flux, indicating activation of the alternative PLC-PKC-Ca2+ pathway. This effect was blocked by pretreatment of the Gq/11 inhibitor YM-254890, suggesting dopamine signaling in these cells may be mediated through DR5. We finally examined whether this pathway was mediating the dopamine effect on HIV by blocking Ca2+ or PKA. Inhibiting calcium release with Dantrolene abrogated dopamine-induced increases in HIV entry, whereas inhibition of PKA with H89 had no effect. These findings suggest dopamine signaling in macrophages occurs primarily through an alternative signaling pathway where DR5 couples to Gq/11, and indicate this pathway mediates the effect of dopamine on HIV entry. Further, these studies indicate the pathway by which dopamine mediates its effects in macrophages is distinct from that seen in neurons, and may serve as a specific target for new therapies.

P210

TEM-assisted evaluation of drug nano-carrier biodistribution in the brain

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In order to develop personalized nanomedicine for the treatment of neuroAIDS, we have developed various nanoformulation in our research group using magnetoelectric nanoparticles (MENPs) as a drug-nanocarrier. The MENPs are core-shell nanoparticles which exhibited biocompatibility (50 ug to brain cells, in vitro) and on-demand release of selected drug on applying ac-magnetic field thorough electromagnetic coil. The ac-magnetic field stimulation also caused delivery of MENPs into brain cells via nano-electroporation (NEP) leading to improve efficacy of selected drug bound with MENPs. The NEP was confirmed using focused ion beam and TEM studies. We have demonstrated magnetically guided delivery of MENPs (10mg/kg) to brain of mice. The presence of MENPs in the mice brain was studies using TEM and validated by STEM. The outcome of this research revealed that MENPs were present in all cell types including neurons, astrocytes, microglia, and are also observed in smooth muscle cells, endothelial cells and blood cells without affecting cells/tissues morphology. The crystallinity and elemental composition of MENPs were also evaluated by electron diffraction and EDS, and results confirmed that MENPs did not lose their integrity during the complex process of crossing blood-brain barrier. Nanoparticles were uniformly distributed in brain cells, and are able to reach nucleus.

P211

Role of macrophage dopamine receptors in mediating cytokine production and neuroinflammation

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Drug abuse is an important comorbidity in HIV infection, and has been linked to exacerbation of HIV-associated Neurocognitive Impairment (HAND). All drugs of abuse increase extracellular dopamine in the CNS, and our lab has shown that dopamine can increase HIV infection of primary human macrophages, as well as increase the production of inflammatory cytokines. This suggests that elevated dopamine could enhance the development of HIV-associated neuropathology, and is corroborated byother studies showing correlations between dopaminergic dysfunction and the development of HAND. However, the precise mechanism(s) by which elevated dopamine could exacerbate the progress of HAND, particularly in regard to neuroinflammation, remain unclear. To define the connection between the dopaminergic dysfunction and HIV-associated neuroinflammation. we examined the correlation between the expression of HIV-associated inflammatory mediators and expression of dopamine receptors. Our data show that dopamine treatment of human macrophages induces production of inflammatory mediators including IL-1β, IL-6, CCL2, CXCL10, and CXCL9. In addition, dopamine reduces production of the anti-inflammatory cytokine IL-10 in response to LPS stimulation. Increased production of IL-1β, IL-6, and CXCL9, and inhibition of LPS-induced IL-10 production correlates with the expression of D1-like dopamine receptor, which we show to be expressed at significantly higher levels than other dopamine-receptor subtypes in human macrophages. Moving forward we are focused on elucidating the role of dopamine in mediating or suppressing cytokine production under different inflammatory conditions. Preliminary data suggests that these effects may be exacerbated by HIV infection, and also suggests that polarization of macrophages into classically activated (M1) and alternatively activated (M2a and M2c) subtypes alters IL-6 production in response to dopamine. Overall, these data will provide more understanding of the role of dopamine in the development of HAND, and may suggest new molecules or pathways that can be useful as therapeutic targets during HIV infection.

P212

Multimodal Investigation of Corticostriatal Circuit Dysfunction in Treated HIV Infection

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Purpose: There is broad evidence of early and enduring striatal effects of HIV infection, with neuropathological studies showing macrophages, microglia and viral proteins there. Metabolic findings include basal ganglia hypermetabolism in early stages of infection, followed by later hypometabolism. Impaired voluntary movement and decreased processing speed in HIV infection could reflect basal ganglia dysfunction. The central purpose of this multimodality MRI study was to determine whether combined measures of striatal structure, inter-regional intrinsic functional connectivity and motor behavior could provide an integrated account of corticostriate circuit dysfunction in HIV infection.Methods: We studied 113 seropositive and 88 seronegative participants, ages 23-73. First, regional gray matter volume (GMv) was estimated using tissue class segmentation of T1-weighted brain images. Voxel-wise regression was used to explore effects of serostatus, age, and substance use on GMv. Next, effects of serostatus, age, and drug use on pegboard performance were modeled. Finally, we used resting state fMRI in 53 of these participants to investigate changes in corticostriatal network functional connectivity related to HIV infection.Results: HIV infection was associated with bilateral decreases in striatal volume, controlling for age and substance use ($p<0.05$ FWE-corrected). Motor performance decreased with age and positive serostatus ($p<0.01$). While HIV infection did not affect intrastriatal functional connectivity, negative cortical striatal correlations between the caudate and multiple extrastriate visual area activities were higher in the seropositive group ($p<0.01$). Conclusions: HIV infection may have a direct striatal effect that is evidenced by striatal GMv loss that does not reverse with anti-retroviral therapy. This structural damage is associated with altered corticostriate circuit function, which, in turn, causes reductions in speeded motor performance. This hypothesis can be tested in future studies examining how the dynamic performance of corticostriate circuits, studied under task conditions know to modulate striatal neural activity, are altered in HIV infection.

P213

Evaluation of MMP-9 and ERK in colon barrier dysfunction in meth self-administering HIV-1 transgenic

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Gut barrier (GB) pathology is common in HIV-infected individuals and methamphetamine (meth) use is prevalent in the HIV-infected population. However, HIV-induced GB pathology in the context of meth use is unknown. We revealed that HIV-1 transgenic (Tg) rats that self-administer meth have reduced expression of GB junction proteins, zonula occludens-1 and claudin-1, and increased colon leakiness (Persons et al, 2018). Mechanisms underlying this GB dysregulation are unclear. Matrix metalloproteinases (MMP) are critical mediators of barrier integrity and function. In the brain, the MMP-9 subtype degrades tight junction proteins and promotes blood brain barrier breakdown. MMP-9 is transcriptionally regulated by the mitogen-activated protein kinase (MAPK) signaling pathways. In the brain, both HIV-1 proteins and meth engage MMP-9 and MAPK signaling cascades. To indicate whether MMP-9 is involved in GB pathology induced by HIV-1 proteins and/or meth, we examined colon samples taken from meth self-administering (SA) HIV-1 Tg and non-Tg rats (0.02-0.04mg/kg/0.05ml iv infusion) 2h/day for 21 days; cumulative meth intake was 4.5 ±0.3mg/kg and 5.2±0.5mg/kg, respectively. Controls were saline-yoked. One day following the last operant session, colon samples were harvested. Levels of MMP-9 and a component of the MAPK cascade, extracellular-regulated kinase (ERK), were evaluated by immunoblotting. A two-way ANOVA revealed a genotype effect for MMP-9 and the ratio of phosphorylated (activated) ERK to total ERK (pERK/ERK), but no meth effect. Post hoc analysis of relevant planned contrasts revealed differences between saline Tg and saline non-Tg rats. However, there was a consistent non-significant trend for MMP-9 and pERK/ERK to be increased by meth in Tg rats. These findings suggests a role for ERK and MMP-9 in GB pathology induced by HIV-1 proteins. We predict that with greater meth exposure, effects of the stimulant would also become apparent.

P214

Lipocalin-2 deficiency limits alterations of neurotransmission-related gene networks in a transgenic model of HIV-induced brain injury

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People living with human immunodeficiency virus-1 (HIV-1) suffer brain injury and a wide-range of cognitive impairments known as HIV-associated neurocognitive disorders (HAND). The pathological mechanisms leading to HAND are incompletely understood. Transgenic (tg) mice expressing HIV envelope protein gp120 in their central nervous system (CNS) provide a model of HIV-associated brain pathology. These gp120tg mice display features observed in neuroHIV patients; including astrocytosis, microgliosis, and decreased synaptic connections and dendrites.Our recent studies of gp120tg mouse brains showed lipocalin-2 (LCN2) as one of the most upregulated genes. LCN2, an acute phase protein, has been shown to play a role in behavior and cognitive function, neuronal excitability, microglial activation and as an autocrine mediator of reactive astrocytosis. To gain insight into the role of LCN2 in HIV-induced brain injury, we cross-bred gp120tg mice with a genetic knock out of LCN2 (LCN2ko). The resulting four genotypes (wild-type, gp120tg, LCN2ko, and LCN2ko x gp120tg) were analyzed using RT2 Profiler™ PCR arrays, which measure expression changes of genes related to the dopaminergic, serotonergic, GABAergic, and glutamatergic neurotransmission. Expression of gp120 was associated with significant alterations in all neurotransmission systems and some of the changes were ameliorated or reversed in the absence of LCN2. Ingenuity Pathway Analysis (IPA) was used to identify biological networks affected by the changes in gene expression and predicted significant alterations in biological and functional networks consistent with neuronal injury in gp120tg and also functional alterations in LCN2ko animals. However, the differential gene expression pattern in LCN2ko x gp120 animals indicated close to normal activity or deactivation of biological pathways altered in gp120tg and LCN2ko animals, which suggested that LCN2-deficiency ameliorated neuronal damage in gp120tg brains.Therefore, a better understanding of the role of LCN2 in pathological mechanisms underlying HIV-Induced brain injury may help to identify new therapeutic targets and approaches.

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Treatment of La Crosse Virus (LACV)-induced neuronal apoptosis with FDA approved drugs

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La Crosse Virus (LACV) is an Orthobunyavirus transmitted by mosquitos, that can induce severe pediatric viral encephalitis. Unfortunately, there are no therapeutic treatments or vaccine for LACV-induced neurological disease and only palliative care can be provided. Recently, we

have conducted a panel study with the National Center for the Advancement of Translations Sciences (NCATS) to screen of more than 3,500 FDA approved compounds to determine if any of these compounds could inhibit LACV-induced death of neurons using the human neuronal cell line SHSY5Y. The initial screen revealed 38 potential compounds, which were subsequently narrowed to four potential compounds for further in vitro and in vivo testing. Of these four compounds, only Rottlerin inhibited LACV-induced apoptosis in multiple neuronal cell lines (N2a, C17.2 and SHSY5Y) and primary murine neurons. Rottlerin had potent antiviral activity against LACV (EC50, 0.16-0.38 μg/ml) with maximum inhibition of cell death at concentrations between 0.3-0.75 μg/ml. Rottlerin treatment reduced virus production by up to 3 logs in culture supernatant at different time interval. Time kinetics study indicated that Rottlerin may inhibit at early stage of virus multiplication/replication. In vivo toxicity study showed that Rottlerin was safe up to 20 mg/kg i.p. route. In vivo protection experiments are ongoing to determine if Rottlerin can inhibit pathogenesis in LACV-infected mice. Collectively, these studies indicate that Rottlerin is an effective inhibitor of LACV replication and neuronal apoptosis.

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Image Guided SPECT/CT and MRI Tests Predict Drug Biodistribution and Efficacy of Long acting Slow Effective Release Antiretroviral Therapy

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Despite significant advances antiretroviral therapies (ART) viral eradication has not "yet" been achieved. Improved drug delivery through the development of long acting slow effective release ART (LASER ART) to sites of viral persistence can accelerate this goal by the development of multifunctional theranostic nanoformulated drug particles. To these ends we have developed a tri-modality imaging particle for magnetic resonance (MR), single-photon emission computed tomography (SPECT/CT) with fluorescence histologic validation to assess the biodistribution of rilpivirine (RPV) when formulated into a nanoaprticle. The particle designed using a "core-shell" composed of europium (Eu3+), 111Indium (111In), and cobalt-ferrite encased in a lipid shell encapsulated polycaprolactone (PCL) core. Thee synthesized particles were 250 nm in size and were stable over three weeks. Particle uptake, retention, cytotoxicity and antiretroviral efficacy were evaluated in human monocyte-derived macrophages. The drug encased particle demonstrated potent macrophage uptake and coordinate antiretroviral efficacy over native drug. Superparamagnetic properties were readily demonstrated during its use as a T2-weighted MRI contrast agent (r2 = 732 mM-1s-1). Biodistribution in male BALB/cJ mice injected with particles at a concentration of 2 mg/kg iron corresponded to what was observed with 550 μCi 111In. MRI scans were performed with or without SPECT/CT imaging at times 0, 4 hours and 2 and 5 days were followed by animal sacrificed and organ collection. MRI, SPECT/CT and transmission electron microscopic (TEM) analysis revealed particle accumulation in liver, spleen and lymph nodes. Such triple-modality particles can be used to non-invasively track antiretroviral drugs tissue distribution with a substantive sensitivity and spatial resolution.

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DNA Methylation and Aging in HIV Infected Individuals with Methamphetamine Use

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Background: Aging related pathophysiologic processes such as cardiovascular disease, immunosenescence and neurocognitive decline have been shown to develop prematurely in HIV-infected individuals at an accelerated rate. Furthermore, methamphetamine use may also accelerate aging. DNA methylation patterns correlate with chronological age, and may be used as a biomarker of accelerated aging. Here, we determined age based on DNA methylation in individuals with HIV infection and methamphetamine use. **Methods:** Frontal lobe brain samples were obtained from the National NeuroAIDS Tissue Consortium. Clinical and sociodemographic variables were obtained from assessments performed during life. Genomic DNA was extracted from brain tissue samples from 30 HIV-uninfected individuals (HIV-), 8 HIV-infected individuals with history of methamphetamine use (HIV+METH+), and 9 HIV-infected individuals without history of methamphetamine use (HIV+). Methylation profiles were measured by Illumina Infinium MethylationEPIC arrays, and methylation age was calculated using epigenetic clock software developed by Horvath (http://labs.genetics.ucla.edu/horvath/htdocs/dnamage/). Bioinformatics and statistical analyses were performed using R statistical software. **Results:** Analysis of all brain samples together demonstrated a strong correlation ($r=0.61$, $p<0.001$) between methylation age and chronological age at death. When analyzed separately, methylation age remained strongly correlated with chronological age (HIV+METH+: $r=0.74$, $p=0.034$; HIV+: $r=0.85$, $p=0.003$; HIV-: $r=0.57$, $p<0.001$). However, HIV+METH+ subjects had evidence of accelerated methylation aging (Slope=0.75) compared to HIV+ subjects (Slope=0.67) and followed by HIV- subjects (Slope=0.51). **Conclusions:** HIV-infected individuals showed evidence of accelerated aging compared to uninfected individuals when using DNA methylation as a biomarker. HIV-infected subjects with history of methamphetamine use demonstrated even more acceleration of aging than individuals not using methamphetamine. Determination of DNA methylation aging may provide quantitative insight into the effects of HIV infection and comorbidities on aging.

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HIV Infection with Methamphetamine Use Alters DNA Methylation and Mitochondrial Genetics Implicated in Neurological Processes

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Background: HIV-infected individuals experience accelerated aging that consequently may result in neurodegeneration. Methamphetamine use, prominent in HIV-infected populations, has also been associated with a rapid progress of HIV-associated neurocognitive disorders. Somatic mutations in mitochondrial DNA (mtDNA) accumulating with age have also been implicated in the pathogenesis of neurodegenerative disorders. Here, we evaluated methylation changes, which regulate gene expression and

occur in HIV infection, methamphetamine use, and their relationship with mitochondrial genetic defects. **Methods:** We evaluated the methylation profiles from frontal lobe brain tissue samples from 30 HIV-uninfected individuals, 8 HIV-infected individuals without history of methamphetamine use, and 9 HIV-infected individuals with history of methamphetamine use. Brain tissue samples and clinical and sociodemographic data were obtained from the National NeuroAIDS Tissue Consortium. Genomic DNA was extracted and methylation analysis was performed using the Illumina Infinium MethylationEPIC arrays. We quantified levels of mtDNA and the relative proportion of mtDNA carrying the "common deletion" in brain tissue by droplet digital PCR. Statistical analyses were performed using R statistical software and annotation of significantly methylation profiles was performed using Metascape. **Results:** We found 2892 methylated sites that were significantly different among the three study groups ($p<0.005$, Kruskal-Wallis test). These sites showed enrichment in processes associated to regulation of axonogenesis ($q<0.001$), mitochondrion organization ($q=0.002$), axon guidance ($q=0.008$), and cerebellum morphogenesis ($q=0.023$) among others. Furthermore, when looking at significant correlations between the methylation profile and the relative abundance of the common deletion (Spearman, $p<0.005$), there were 3614 probes that were enriched for processes involved in regulation of nervous system development, behavior and dendrite development ($q<0.001$ for all). **Conclusions:** Methylation profiles in brain tissue revealed dysregulation of neurological processes and mitochondrial dynamics suggesting that HIV infection and methamphetamine use may share similar mechanisms of accelerated aging via mitochondrial processes.

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Repurposing maraviroc, an antiretroviral drug, for Parkinson disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder in human. Despite intense investigation, no effective therapy is available to stop the progression of this disease. It is becoming clear that both innate and adaptive immune responses are active in PD. Accordingly, we have seen marked increase in RANTES (regulated on activation, normal T cell expressed and secreted) and eotaxin, chemokines that are involved in T cell trafficking, in vivo in the substantia nigra pars compacta (SNpc) and the serum of PD patients and hemiparkinsonian monkeys. Since RANTES and eotaxin share a common receptor CCR5, we examined the efficacy of maraviroc, an inhibitor of CCR5 and an FDA-approved drug against HIV infection, in hemiparkinsonian monkeys. First, we found infiltration of both CD4+ and CD8+ T cells into the SNpc of hemiparkinsonian monkeys. However, oral administration of low dose of maraviroc reduced the infiltration of T cells into the nigra, attenuated neuroinflammation and decreased α-synucleinopathy. Accordingly, maraviroc treatment protected both the nigrostriatal axis and neurotransmitters, and improved motor functions in hemiparkinsonian monkeys. These results suggest that maraviroc and other CCR5 antagonists may be helpful for PD patients.
This study was supported by grants from the National Institutes of Health (NS083054 and NS064564).

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The CCR5-CD11d-CD99L2 axis in the Pathogenesis of HIV Distal Sensory Neuropathy

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Distal sensory neuropathy (DSN) is the most common HIV-associated peripheral neuropathy, affecting ~13-60% of patients despite cART. HIV DSN pathogenesis has not been completely elucidated. Sequence analysis and infectious recombinant viruses containing peripheral nerve-derived C2V3 sequences indicated a predominance of CCR5-dependent and macrophage tropic HIV-1 virus in peripheral neuropathy autopsy specimens. Our objective is to elucidate the determinants and pathogenic mechanisms of HIV DSN. We observed high basal human blood-nerve barrier (BNB) CCR5 ligand expression, implying HIV+ CCR5+ monocytes may "hijack" constitutive mechanisms to access peripheral nerves. In a cohort of 36 HIV+ and 17 HIV- individuals, mean expression of CD14+ CD16+ monocytes (13.8% vs 4.0%) and CD14+ CD16+ CCR5+ monocytes (18.8% vs 9.6%) was increased in HIV+ patients' peripheral blood mononuclear leukocytes (PBMLs) by flow cytometry. No difference in mean % CCR5 expression in CD3+ CD4+ (19.0% vs 18.7%) or CD3+ CD8+ (28.5% vs 25.0%) T-cells was observed; however, this was associated with a mean 191% and 230% increase in CCR5 mean fluorescent intensity respectively. Using RNA sequencing to compare relative transcript expression, we determined a significant 22.3-fold increase in CD11d (αD integrin) expression in HIV+ PBMLs compared to HIV- controls. Flow cytometry demonstrated CD11d expression on 100% of CD14+ CD16+ CCR5+ leukocytes. We also ascertained CD99L2 transcript expression by the human BNB as well as by HIV+ and HIV- leukocytes, implying a possible homophilic role in paracellular diapedesis. Indirect immunohistochemistry performed on HIV DSN patient sural nerve biopsies showed endoneurial CCR5+ expression on CD68+ monocytes, and CD3+ CD4+ and CD3+ CD8+ T-cells, as well as CD11d and CD99L2 expression on infiltrating/ infiltrated leukocytes. Using a flow-dependent human BNB model, 100 ng/mL of a function neutralizing mouse anti-human CCR5 IgG1 antibody significantly reduced HIV+ leukocyte trafficking under basal conditions in vitro, suggesting a plausible therapeutic approach in HIV DSN.

P221

The Neurokinin-1 Receptor - a modulator of CD16- and CD16+ monocyte properties.

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Circulating human monocytes consist, primarily, of 85-90% CD16- and 10-15% CD16+ cells. In HIV, the CD16+ subset can increase up to 20-60%. CD16+ monocytes have greater HIV infectivity than CD16- monocytes in vitro and they transmigrate to the CNS, where they contribute to neuroinflammation. We demonstrated previously that, in HIV infected individuals, elevated plasma levels of substance P (SP), the Neurokinin-1 Receptor (NK1R) preferred ligand, occur and that SP/NK1R signaling alters monocyte differentiation. In order to assess the effect of NK1R signaling on monocyte differentiation we performed an RNA sequencing study. We treated freshly isolated human blood monocytes with SP and sorted them into CD16- and CD16+ subsets. SP treatment led to the differential expression of 36 genes in CD16- and 11 genes in CD16+ monocytes. In both monocyte subsets, the majority of the upregulated genes were pro-inflammatory associated, including IL1α, IL1β, IL6, CCL3 and CCL4 (FDR adjusted p-values <0.001). Some anti-inflammatory genes were also upregulated, including SOCS3 and TNFAIP3 (FDR adjusted p-values <0.001 and <0.05, respectively). Gene-set enrichment analysis showed that in SP-treated CD16+ compared to SP-treated CD16- monocytes, genes from the KEGG peroxisome pathway are upregulated, as well as the pathway for valine, leucine and isoleucine degradation. Alternative splicing analysis indicated that SP treatment of monocyte subsets led to splice variants implicated in apoptosis. Specifically, in CD16- monocytes the VMP1, SOD2 and FOS genes, and in CD16+ monocytes the CASP1, CARD16 and CARD17 genes.Thus we identified genes and pathways, previously unrecognized

in the context of monocyte SP treatment. SP-treated monocytes have the potential for a robust inflammatory response, increased oxidative stress production and altered apoptosis. We will study these pathways in order to understand their contribution to immunomodulation and HIV neuroinflammation.
Supported by NIH U01MH107276, P30MH097488, P01MH105303 and R01AI106509.

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CHRONIC ALCOHOL TREATMENT MODULATES GPR55 EXPRESSION THROUGH EPIGENETIC PATHWAYS IN HUMAN MONOCYTE-DERIVED DENDRITIC CELLS (MDDC).

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The endocannabinoid system (ECS) plays a key role in the mechanisms underlying alcohol dependence. In particular, CB2 agonists have been shown to have potential for the treatment of neuro-inflammation and lately, novel cannabinoid receptors such as GPR55 are emerging as potential targets of substance abuse research. Our lab has demonstrated that acute alcohol or binge drinking increases histone deacetylases while chronic alcohol use has differential epigenetic effects by enhancing histone (H4) quantity and acetylation (ac) at the histone (H)4 lysine(K)12 site (H4K12ac). Therefore, in the current study, we developed two novel screening tools using single cell imaging flow cytometry and matrix assisted laser desorption ionization-fourier transform-ion cyclotron resonance mass spectrometry (MALDI-FT-ICR MS) to detect post-translational modifications (PTMs) in human MDDCs due to chronic alcohol exposure. Our results demonstrate that in vitro chronic alcohol exposure of MDDCs modulates total histone quantity and induces a significant increase in acetylation at H4K12 (H4K12ac). In addition, the alcohol-induced increased in H4K12ac is significantly inhibited by the synthetic cannabinoid agonist, JWH-015, and there is H4K12ac enrichment at the GPR55 locus in MDDCs exposed chronically to alcohol. Moreover, treatment with the Tip60/HAT inhibitor, NU9056, blocked alcohol-induced H4K12ac, modulating the effect of EtOH on inflammatory cytokines/chemokines, suggesting that H4K12ac may be playing a major role during inflammation induced by alcohol abuse.
Acknowledgements: Partially supported by NIAAA award # R00AA021264. Startup funds: Department of Immunology, INIP, Office of Research and Economic development, HWCOM, FIU. TP is partially funded by Presidential Fellowship, partially by Biomedical Science Ph.D. program, HWCOM, FIU. Additional funds for reagents were provided by NIH/NIGMS R25 GM061347.

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Combined effects of Methamphetamine and EcoHIV on the function of mouse neural progenitor cells

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Although pathological conditions following HIV-1 infection have been dramatically reduced by antiretroviral therapy (ART), many patients including methamphetamine (METH) abusers, are still suffering from HIV-1-induced neurocognitive deficit. Therefore, understanding the molecular synergy between METH and HIV-1 is critical to manage the health outcomes of HIV-1-infected patients with neurocognitive deficit. This study was based on a chronic exposure to METH (5 days with escalating doses at 3 hrs intervals), followed by EcoHIV infusion into C57BL/6J mouse brain. EcoHIV, a derivative of HIV-1, contains a substitution of envelope protein gp120 with that of gp80 derived from murine leukemia virus-1 (MuLV-1), which infects only murine cells. Experimental mice were sacrificed to collect brains either 2 or 4 weeks after EcoHIV infusion. For preserving cognitive function throughout life, maintaining an intact pool of functional neural progenitor cells (NPCs) may be crucial for regenerating functionally active new neurons. To evaluate the effect of METH/EcoHIV on the function of NPCs, BrdU was i.p. injected to mice and BrdU-positive cells were counted in the subventricular zone (SVZ) and hippocampal dentate gyrus (DG). Compared to the BrdU-positive cell numbers from the control group, the numbers of SVZ NPCs from METH and/or EcoHIV-treated groups were significantly increased. When ex vivo SVZ NPCs were exposed to EdU (5 μM) for 2 h, the number of EdU-positive cells were significantly increased in the SVZ NPCs from METH and/or EcoHIV-treated mice compared to those from control group. When neural differentiation was induced for 2 weeks, the SVZ NPCs from METH/EcoHIV-treated mice showed delayed differentiation compared to those from control mice. Altogether, our results suggest that comorbidity of METH and EcoHIV induces alterations of NPCs functions, which may accelerate disease progression.

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HIV NeuroAids

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Understanding HIV CNS Dysfunction and various Co morbidities can be a very challenging aspect to define, seeing that HIV Associated Neurocognitive Disorders (HAND), varies, based on recent studies presented, it shows some of the HIV's population are experiencing some kind of Neurological Cognitive impairment disorder, known as NeuroAids or HIV neurodegenerative disorder. During my research I have found various Receptors that play a role in Neurotoxicity and Calcium transport signaling. Although CD4 and Co Receptor Chemokine are key Entry Receptors of HIV, there are also other Receptors involved in HIV's etiological damaging pathway. N-Methyl D-Aspartate (NMDA) being one, which are Ionotropic Glutamate Receptors, Ligand gated-permeable Ion channel that opens, when bound to a certain chemical messengers, presented within Bacteria, Viruses and various other Organisms based on their chemical makeup and constituents, allowing modifications to occur within the cell and by this, it is a distinctive device for stimulating Glutamate and allowing excess calcium into the cell. Excess calcium can pose a problem at a Cardiac level.Germ line mutation and variations takes place within HIV provirus Integration into the Host genome, making its mark as an Endogenous Retrovirus, containing ALU sequences or what is also called Lines or L1, a neural peptide, which are Neural Recognition Molecules. It is a component of the Signal Recognition Particle (SPR) which is highly conserved with Primates genomes. ALU Insertions have been reported to be found in several inherited diseases and various forms of Cancers, it also shows repetitive elements in the Human genome. L1 proteins associates with the L1 Cam gene mutations, an X Linked Neurological syndrome which leads to CRASH (Syndrome 1 defect) Listed as, Corpus Callosum hypoplasia, Retardation, Aphasia, Spastic Paraplegia and Hydrocephalus.

P225

Potential Role of Exosomal Cathepsins in Ethanol-Induced Neurotoxicity

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Chronic alcohol consumption is known to have a number of deleterious effects throughout the body, especially in the brain. In the central nervous

system (CNS), ethanol can cause oxidative stress and neuroinflammation, exacerbating a number of known neurodegenerative conditions. Cathepsins (CTS) are a family of proteases, primarily found in lysosomes, that can be induced and released from various cell types under conditions of stress or cellular damage, such as in the case of ethanol exposure. High expression of cathepsins in the CNS can be neurotoxic due to excessive and dysregulated proteolysis, and their induction is a potential biomarker of diseases such as Alzheimer's and HIV-associated neurocognitive disorders. Exosomes have been implicated in the pathogenesis of those conditions through the transport of neurotoxic agents such as cathepsins. This is due at least in parts to interactions between lysosomes and the multivesicular bodies responsible for exosome secretion. In this study, we examined whether ethanol exposure induces exosomal expression of two particular cathepsins, CTSB and CTSD, in human macrophages, microglia, and astrocytes, and whether or not exposure of ethanol-modified exosomes to recipient neurons enhances ethanol-induced toxicity. Our preliminary data indicate that ethanol exposure increases human macrophage exosomal CTSB and CTSD expression in a dose-dependent manner. Furthermore, exosomes from ethanol-treated cells enhance toxicity when co-treated with ethanol to recipient neurons and astrocytes. This research may be the first to show a role of exosomal transport of lysosomal cathepsins in alcohol-induced neurotoxicity.

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HIV-1 Tat-mediated upregulation of miR-34a activates NF-κB-mediated microglial inflammation via targeting the 3'-UTR of NLRC5

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Although the advent of combination antiretroviral therapy (cART) has dramatically increased the life expectancy of people living with HIV-1, paradoxically the prevalence of HIV-1-associated neurocognitive disorders (HAND) in people treated with cART, is on the rise. It has been well documented that despite the effectiveness of cART in suppressing viremia, CNS continues to harbor viral reservoirs with persistence of low-level virus replication. This leads to the presence and persistent accumulation of early viral protein, HIV-1 Tat that is a well-recognized cytotoxic agent contributing to glial activation. In this study, we demonstrated that exposure of mouse microglia to HIV-1 Tat both dose- and time-dependently upregulated miR-34a expression while concomitantly also downregulating the expression of NLRC5 (a negative regulator of NF-κB signaling). Using bioinformatics analyses, dual-luciferase, and Ago2 immunoprecipitation assays NLRC5 was identified as a novel 3'-UTR target of miR-34a. Transfection of mouse microglia with miR-34a mimics significantly downregulated NLRC5, resulting in nuclear accumulation of NF-κB p65. In contrast, transfection of cells with miR-34a inhibitor notably upregulated NLRC5 levels. Using both gene silencing and pharmacological approaches to block either NLRC5 or NF-κB, our findings demonstrated that HIV-1 Tat-mediated microglial activation involved subsequent downregulation of NLRC5 with concomitant activation of NF-κB signaling. Reciprocally, inhibition of miR-34a in microglia blocked HIV-1 Tat-mediated microglial activation. In summary, our findings demonstrate a novel mechanism of HIV-1 Tat-mediated activation of microglia via upregulation of miR-34a, leading ultimately to downregulation of NLRC5 expression with a concomitant upregulation of NF-κB signaling. In vitro findings also validated in the frontal cortices, striatum and hippocampus of young 5-month old HIV-1 transgenic rats. Modulation of miR-34a could thus be envisioned as a potential therapeutic approach to ameliorate microglial activation and possibly, aid in future development of epigenetic targets as adjunctive therapeutic modalities for treatment of HAND.

Supported by DA036157, DA043138, DA044586, MH062261.

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ASTROCYTE ACTIVATION AND GROWTH RETARDATION PERSIST IN THE ABSENCE OF VIRUS IN SIV-INFECTED NON-HUMAN PRIMATES

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The central nervous system (CNS) is composed of neurons and glial cells (astrocytes, oligodendrocytes, microglia, and ependymal cells). Astrocytes in particular are integral to normal cognition and many functions of the CNS including homeostasis, metabolism, cellular signaling pathways, and neuronal growth and synapse formation. CNS is a key viral reservoir in HIV and Simian Immunodeficiency Virus (SIV). Active viral infection has been shown within perivascular macrophages and microglia within the brain; however, productive infection within astrocytes is a hotly debated topic. SIV viral DNA has been detected within astrocytes upon postmortem evaluation, while the occurrence of productively infected astrocytes remains open. Astrocytic activation is highly likely to occur from exposure to the virus as well as neighboring virus-infected cells. Anecdotally, astrocytes collected postmortem from SIV-infected macaques are challenging to grow in primary culture. To evaluate astrocytes, frontal lobes were collected from SIV-infected and control macaques immediately postmortem. Microscopically, primary cultures of control astrocytes are composed of phase dark, process bearing cells that arrange into clusters, eventually forming a confluent monolayer over a 14-21-day period. The astrocytes from SIV-infected macaques were progressively decreased in size and abundance, and failed to migrate and form a confluent monolayer. Supernatent was periodically collected from all primary cultures for protein, cytokine, and extracellular vesicle analysis. Viral loads from frontal lobes were detected via qPCR. A cure for HIV is hopefully on the horizon. Therefore, determining mechanisms of astrocyte activation in the absence of virus will be vitally important in amelioration of HIV-associated neurocognitive disorder (HAND).

P228

Coming unglued: vital roles for astrocytes in disease and return to health.

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For too long astrocytes were overlooked as mere glue allowing neurons to function optimally. Thankfully, within the last few years, the true importance of astrocytes has become apparent. We have examined brains of nonhuman primates across the spectrum of age and in multiple disease states including depression, infection with bacteria (Brucella), flaviviruses (Dengue and Chikungunya), and lentivirus (SIV). We have observed that astrocytes become more complex as primates mature from juveniles, through adolescence and peak in early adulthood, before reverting to a more simple form in geriatrics. We also noted that viral infections generally result in atrophy of astrocytes, as opposed to hypertrophy following bacterial infection. Perhaps our most important discovery, however, and one that will be vitally important for cure strategies in HIV, is that astrocyte activation continues even in the absence of productive viral replication in brain. These studies are based on immune activation of astrocytes and advanced morphometrics. Unbiased decision tree algorithms showed that the astrocyte activation in macaques infected with SIV, but without any histological evidence of neuroinflammation was most similar to astrocytes of macaques with depressed behavioral phenotype. Thankfully, our research has also stumbled across a potential mechanism of reversing this phenotype: using naltrexone to treat the macaques with the depressed phenotype. It may be that following eradication of

HIV from the CNS, we could reverse the long-term immune activation of astrocytes using this simple therapy.Ex vivo studies are unravelling underlying pathways in common between the different disease states, including biological function of and biochemical characterization of exosomes. Ultimately, we aim to be able to target astrocyte function as part of cure research.

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Three-State Model of HIV-1 Transcription in T-cells and Macrophages

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Millions worldwide live with HIV/AIDS. Treatment with antiretroviral therapy (ART) yields low viral titers and decreased mortality in HIV-1-infected patients. Despite ART, chronic persistence of latent HIV-1 yields viral products in anatomical reservoirs (e.g. CNS), linked with the development of HIV-1-associated neurocognitive disorders (HAND). This demonstrates that HIV-1 transcription machinery is not entirely suppressed. In order to better understand the dynamics of HIV-1 gene regulation in chronic infection, we designed a three-state model representing latent (LTRR), basal (LTRI), and activated (LTRA) states of transcription. Transcriptional noise inducers (PMA/PHA and irradiation (IR); 1) and suppressors (i.e. F07#13; 2) were used to modulate LTRI, LTRR, and LTRA states (3) on HIV-1-infected T-cells and myeloids. Kinase assay of H1 was used to monitor activation of HIV-1 transcription. Short (TAR) and long (envI and envA) viral RNA transcripts were measured. TAR and envI/A indicated transcriptional dynamics and functional activity. Viral proteins (Pr55, p24, and Tat) from each LTR state were detected. Preliminary mathematical predictions showed relative proportions of TAR to envI/A copy numbers over a period of 10 days. LTRA indicated a dramatic increase in the number of envI copies, while TAR copies increased at a lesser rate in both cell types. At 24 hours, envI peaked in production while TAR levels continued to increase. This is consistent with our previously published data using infected PBMCs (4). Future simulations will modulate Tat-dependent transcription by GSK-3β inhibitor (18BIOder; 5). Collectively, the proposed equations cover the most important aspects of transcription in HIV-1-infected cells.

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Ebola VP40 in Exosomes Can Cause Immune Cell Dysfunction

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Ebola virus can result in severe hemorrhagic fever with high mortality rates. Survivors of infection often suffer from long-term neurological complications including severe joint pain, headaches, seizures, and memory loss. Furthermore, the virus may persist in survivors' CNS, possibly leading to recurrence of illness and potential transmission of the virus. During acute infection, massive death of uninfected bystander T-cells occurs by an unknown mechanism, which may allow for unchecked viral replication. We have previously shown that exosomes from cells infected with HIV-1, HTLV-1 and RVFV are able to transfer viral proteins and noncoding RNAs to naïve recipient cells, resulting in altered cellular activity. Recently, we analyzed three Ebola structural proteins (VP40, GP, and NP) to find that VP40 can exit cells by being packaged into exosomes both in vitro and in vivo. Additionally, cells containing VP40 are enriched in several ESCRT pathway proteins (responsible for the biogenesis of exosomes) inside the cell. Increased exosomal biogenesis in cells containing VP40 is potentially tied to a regulation of cell cycle at the G1/S border. This is supported by our data demonstrating the phosphorylation of EBOV VP40 by Cdk2/Cyclin complexes that function at G1/S. We have also found that in recipient T-cells, exosomal VP40 induces cell death by apoptosis. Moreover, specific RNAi components are downregulated in cells receiving exosomes containing VP40. Collectively, our data indicates that VP40 is packaged into exosomes, which may be responsible for the destruction of the T-cell arm of the immune system, allowing for the virus to replicate to high titers in the immunocompromised host (1,2). Accordingly, this may allow for the virus to escape through the leaky blood-brain barrier to hide within the CNS after patient recovery.

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HIV-Tat can autocleave and form branching fibrillar structures

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HIV-Tat protein is released extracellularly in large amounts from HIV-infected cells and causes activation of lymphocytes, glial cells and neurotoxicity. Atomic force microscopy showed that HIV-Tat protein can rupture to smaller pieces, some of them being of similar dimensions with the theoretically predicted inteins of this protein, which can spontaneously excise themselves out of the protein chain. The HIV-Tat 32-62 piece, which we found to be neurotoxic by itself, was synthetized and used in our experiments. Theoretical predictions indicated that this fragment is unstructured and prone to aggregation. Atomic force microscopy showed, at 32 micrograms/ml concentration, aside of aggregates of various sizes, formation of long fibrillar structures for this peptide. The fibrils are irregular along length and width, with dimensions in the range 0.4 - 1.3 nm for height and 3.6 - 8.7 nm for width, as measured at half height. These fibrils present branching and their bending is characterized by an average persistence length of 80 nm. With increasing concentration, their thickness and length grow and persistence length increases. Infrared and polarized Raman microspectroscopies of the fibers suggested that the side chains and the backbone of the peptide are in highly oriented structure with large beta sheet content, consistent with the amyloid fiber structure. This is different compared to the full-length HIV-Tat, which is alpha helical in bound state and in aggregates. The replacement of C34, F38 and I45 with alanine in the polypeptide chain of HIV-Tat 32-62 led to a similar secondary structure according to circular dichroism, but to significant reduction in aggregation capacity, no fibril formation and preliminary tests indicated less neurotoxicity compared to the original peptide. Our results suggest that the autocleavage of Tat makes possible the aggregation of HIV-Tat 32-62 fragment which leads to neurotoxicity, likely through a different mechanism than the full-length protein.

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Reactive glia promote development of CD103+CD69+ CD8+ T-cells through PD-L1

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Previous work from our laboratory has demonstrated in vivo persistence of CD103+CD69+ brain resident memory CD8+ T-cells (bTRM) following viral brain infection, and that the PD-1: PD-L1 pathway promotes development of these TRM cells within the brain. Although microglial

cells express low basal levels of PD-L1, its expression is upregulated upon IFN-γ-treatment, and microglia have been shown to modulate antiviral T-cell effector responses through the PD-1: PD-L1 pathway. In this study, we show that interactions between reactive glia and anti-CD3-stimulated CD8+ T-cells promote development of CD103+CD69+ CD8+ T-cells through engagement of the PD-1: PD-L1 pathway. These studies used ex-vivo cultures of primary murine glial cells obtained from WT animals along with CD8+ T-cells obtained from either WT or PD-1 KO mice. We found that αCD3-stimulated CD8+ T-cells from WT animals increased expression of CD103 and CD69 when co-cultured with primary murine mixed glial cells. In contrast, significantly reduced expression of CD103 and CD69 was observed on CD8+ T-cells from PD-1 KO mice. We also observed that reactive glia promoted high levels of CD127, a marker of memory precursor effector cells (MPEC), on CD69+ CD8+ T-cells, which promotes development of TRM cells. Interestingly, results obtained using T-cells from PD-1 KO animals showed significantly reduced expression of CD127 on CD69+ CD8+ cells. Additionally, blocking of PD-L1 on microglia resulted in decreased expression of CD103, along with decreased CD127 on CD69+ CD8+ T-cells. Taken together, these results demonstrate a role for activated glia in promoting development of bTRM through the PD-1: PD-L1 pathway.

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Cerebrospinal Fluid (CSF) Galectin-9 is Elevated in HIV Infection and Correlates with Measurements of Monocyte/Macrophage Activation, CNS Injury, and Cognitive Performance

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Despite continuous virological suppression by antiretroviral therapy (ART), the persistence of HIV-associated cognitive disorders (HAND) in HIV-infected persons continues to burden their daily living, hastening the need to better understand the underlying neuropathology. Recent findings demonstrate increased galectin-9 (Gal-9), a soluble lectin with immunomodulatory properties, in cerebrospinal fluid (CSF) and brain tissues was associated with progressive multiple sclerosis and glioma, respectively, illustrating a link between Gal-9 and compromised neuronal function. We previously reported that Gal-9 modulates HIV transcription and is elevated in plasma during acute and treated chronic HIV infection. Here, we investigated the CSF Gal-9 levels in HIV-infected adults [Age(IQR): 53.8(51.0,55.6)] with either detectable (n=15) or undetectable CSF viremia (ART suppressed, n=14), and seronegative, age-matched controls (n=13). Controls from HIV-uninfected volunteers, confirmed by serological testing at study visit, were recruited from the community for comparator research purposes. CSF levels of neopterin and sCD163, markers of monocyte/macrophage activation, and neurofilament light chain (NFL), a neuronal injury marker, were also accessed in HIV-infected individuals. Neurocognitive battery was performed in CSF viremic individuals and summarized as composite z-scores. CSF Gal-9 was elevated in CSF viremic ($p<0.0001$) and aviremic ($p=0.014$) persons compared with controls. In aviremic and viremic individuals, Gal-9 correlated with neopterin (rho=0.63/p=0.024; rho=0.70/p=0.005) and sCD163 (rho=0.62/p=0.025; rho=0.59/p=0.024)]. In the viremic group, CSF Gal-9 correlated with NFL (rho=0.71/p=0.004) and inversely correlated with global cognitive performance (rho=-0.63/p=0.018) and performance in executive function, working memory and concentration, motor skills, and psychomotor speed (all $p<0.05$). Furthermore, utilizing datasets available from the NCBI GEO database, we found Gal-9 mRNA expression higher in white matter brain tissue of individuals with HIV encephalitis compared to seronegative controls ($p=0.016$). Our findings provide a novel link between CSF Gal-9 and HIV infection and further study of this pathway in the pathogenesis of HAND is suggested.

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COS-7-BASED MODEL: METHODOLOGICAL APPROACH TO STUDY JOHN CUNNINGHAM VIRUS REPLICATION CYCLE.

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John Cunningham virus (JCV) is a human neurotropic polyomavirus whose replication in the Central Nervous System (SNC) induces the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). JCV propagation and PML investigation have been severely hampered by the lack of an animal model and cell culture systems to propagate JCV have been very limited in their availability and robustness. We previously confirmed that archetype JCV efficiently replicated in COS-7 cells as demonstrated by the progressive increase of viral load by quantitative PCR (Q-PCR) during the time of transfection and that archetypal regulatory structure was maintained, although two characteristic point mutations were detected during the viral cycle. Hereby we reported an important extension of our previous efforts in defining our reliable model culture system able to support a productive JCV infection. Supernatants collected from transfected cells have been used to infect freshly seeded COS-7 cell line. An infectious viral progeny was obtained as confirmed by Western blot and immunofluorescence assay. During infection, the archetype regulatory region was conserved. Importantly, in this study we developed an improved culture system to obtain a large scale production of JC virus in order to study the genetic features, the biology and the pathogenic mechanisms of JC virus that induce PML.

P235

Cinnamic acid protects the nigrostriatum in MPTP mouse model of Parkinson's disease via the PPARα

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Despite intense investigations, no effective therapy is available for Parkinson's disease (PD), the most common neurodegenerative movement disorder in human. This study underlines the importance of cinnamic acid, a metabolite of cinnamon, in protecting dopaminergic neurons in a mouse model of PD. Starting with behavioral testing we found that cinnamic acid significantly restored motor activity deficit induced by MPTP lesion. Cinnamic acid was found to significantly restore dopamine levels compared to MPTP and vehicle groups measured by HPLC. Western blotting and DAB histology for tyrosine hydroxylase (TH) in the substantia nigra pars compacta had similar findings that cinnamic acid protected TH expression. To determine a possible mechanism for cinnamic acid's protective effect we focused on the PPARα pathway as our lab has recently delineated neuroprotective effect of

PPARα. Using PPARα and PPARβ knockout mice we found that there was no similar protective effect on behavioral deficits induced by MPTP by cinnamic acid in animals lacking PPARα, whereas the PPARβ knockout mice had behavioral recovery implying that cinnamic acid is neuroprotective via the PPARα pathway. Biochemical analysis by HPLC, western blotting and immunostaining revealed similar finding. Taken together, cinnamic acid is neuroprotective in the MPTP mouse model via the PPARα pathway. These results suggest that cinnamic acid may find its therapeutic use in PD.

This study was supported by a merit award from the Department of Veteran Affairs (1I01BX003033).

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IFN-FREE HCV THERAPY IN HIV COINFECTION DECREASES INFLAMMATION AND IMPROVES COGNITION

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Background: Chronic inflammation in HIV/HCV coinfection increases cognitive impairment. HIV replication is typically suppressed to undetectable plasma levels through the use of anti-retroviral therapy (ART). Our objective was to determine how HCV eradication in HIV coinfection impacts monocyte immune activation and cognition. **Methods:** We conducted a longitudinal study with 40 subjects before and after interferon (IFN)-free therapy for HCV: 10 uninfected controls, 9 HCV-infected untreated, 12 HIV-infected ART-treated with undetectable HIV viral loads and 13 HIV/HCV-infected ART-treated subjects with undetectable HIV viral load. Indices for monocyte activation included expression of CD16/CD169 protein and IFN markers, IF127, MX1, CD169 and RSAD2. Plasma levels of activation included neopterin, sCD163 and IL-6. Cognitive impairment was assessed using neuropsychological testing covering 7 domains. Individual test scores were converted to standard T scores. A global deficit score was calculated as a single number representing overall test performance. **Results:** All HCV-infected subjects achieved sustained viral response (SVR) regardless of what IFN-free therapy was used. CD16+ monocytes were significantly decreased by 40% after HCV therapy in the coinfected subjects. Interferon stimulated gene expressions on monocytes, IF127, MX1, CD169 and RSAD2, were decreased in coinfected subjects. Cytokine levels for neopterin, sCD163 and IL-6 were decreased after treatment in both HCV and coinfected subjects. Subjects with HCV infection alone showed a trend toward lowering sCD163, neopterin and LGAL. Global deficit scores decreased (improved) 25% in coinfected subjects with no demonstrated effect in HCV infection alone. **Conclusions:** The new IFN-free treatments for HCV have been successful in achieving SVR. Concomitant with HCV eradication is a decrease in monocyte activation marker CD16 and plasma sCD163 and neopterin but no consistent decrease in the interferon marker CD169 and intracellular LGAL protein levels. These two IFN markers reflect ongoing HIV infection. Cognition was improved after HCV therapy in subjects with HIV coinfection.

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Multiple mutations on human dopamine transporter at tyrosine 88, aspartic acid 206, and histidine 547, preserve its normal transport function and attenuate Tat-induced inhibition of dopamine uptake

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HIV-1 Tat protein directly inhibits the dopamine (DA) transporter (DAT) leading to dopaminergic dysregulation, which has been implicated as a risk factor of HIV-1 associated neurocognitive disorders. We have demonstrated that single point mutations on human DAT (hDAT) at tyrosine88 (Y88F), aspartic acid206 (D206L), and histidine547 (H547A) attenuate Tat-induced inhibition of DA uptake by altering transporter conformational transitions. In addition, compared to wild-type hDAT, Y88F and D206L preserved basal DA uptake, whereas H547A potentiated DA uptake by 196%. The current study evaluated the effects of double (D206L/H547A) and triple (D206L/H547A/Y88F) mutants on basal DA transport, Tat-induced inhibition of DA uptake, and transporter conformational transitions. Compared to wild-type hDAT, the Vmax of [3H]DA uptake and the Bmax of [3H]WIN35,428 binding were not altered in double and triple mutants, while the triple mutant displayed an increase in Km and a decrease in Kd. The double mutant did not alter IC50 values of DA, cocaine, and GBR12909 for inhibiting [3H] DA, while the triple mutant increased IC50 for DA, and decreased IC50 for GBR12909. Importantly, the double and triple mutants attenuated Tat's inhibitory effect on DA transport. Both mutants attenuated zinc-induced increase in [3H]WIN35,428 binding, which was accompanied by altered basal MPP+ efflux in D206L/H547A, indicating changes in conformational transitions. We further determined whether the H547A-potentiated DA uptake is result of alteration of transporter conformational transitions by the substituted cysteine accessibility assay. We generated MTSET-insensitive and -sensitive hDAT mutants, C90A/C360A and C90A/C306A/I159C, respectively. Compared to C90A/C306A/I159C, combination of C90A/C306A/I159C/H547A potentiated the reaction to MTSET-mediated inhibition of DA transport, suggesting a shift to a more outward-open conformation. These results indicate that double and triple mutants generated from Y88F, D206L, and H547A attenuate the inhibitory effects of Tat while preserving normal transporter function, providing insights into identifying targets for improving DAT-mediated dysregulation of dopaminergic neurotransmission.

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Nef expression in astrocytes promotes disruption of tight junctions

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Tight junctions play a crucial role in maintaining the proper physiological functions of the blood brain barrier (BBB), intestines, and other epithelial tissues. HIV+ individuals show disruption of the intestinal lining and up to fivefold increase in permeability. Previous research shows that Nef produced by transplanted transfected astrocytes causes disruption of the BBB. To better understand how Nef causes this damage and how it may affect other epithelial organs both in vivo rat and in vitro human models were exposed to Nef. Primary rat astrocytes expressing Nef or GFP were infused in the right hippocampus of 30-day-old Sprague Dawley rats; two days after surgery, they were sacrificed. Naive rats of the same age were used as a control group. Brain tissues were collected for claudin 5 immunofluorescence and its expression in both hippocampal hemispheres was assessed using Image J software. Rat serum samples were collected for detection of Nef by ELISA. For in vitro experiments, glioblastoma cells (U87) were transfected (pEGFP or pNef) and the supernatants were collected at 48 hours. Human colorectal epithelial cell line (Caco-2), which form tight junctions in culture, were seeded into a six well plate and treated with U87 supernatants and E-cadherin expression was analyzed. Nef-treated animals showed a significant decrease in claudin 5 expression in both hemispheres ($p<0.001$) when compared to controls. Nef protein was detected in the serum of rats infused with astrocytes expressing Nef. The same effect was observed in E-cadherin expression when Caco-2 cells were exposed to Nef U87 supernatants. These results demonstrate that Nef can disrupt tight junctions both in the brain and intestinal

epithelial barriers. Taken together with earlier findings, this data suggests that HIV-infected astrocytes maybe an important source for Nef and may influence HIV co-morbidities in the brain and other organs.
Funding: GM106970, MD007579, GM096955.

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Specific packaging of CYP2E1 in human plasma exosomes and their critical role in cellular communications

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The presence and functional role of cytochrome P450 (CYP) enzymes in human plasma exosomes are yet to be explored. In this project, we studied the relative levels and functionality of various CYP enzymes, especially CYP2E1, in human plasma exosomes. We further studied the effect of plasma exosomal CYP2E1 in mediating ethanol (ETH) and acetaminophen (APAP) induced toxicity. To achieve this goal, we isolated exosomes from plasma obtained from de-identified healthy individuals and two clinically relevant cell lines- hepatic and monocytic cells. We characterized them for their physicochemical properties. We analyzed the relative level of exosomal CYP mRNAs and proteins by Q-RTPCR and western blot analyses, respectively. The CYP activity was measured by using Vivid® assay. The cytotoxicity was measured by LDH assay. We observed that the relative level of CYP enzymes in exosomes is higher than in plasma, suggesting their specific packaging in exosomes. Interestingly, among all the seven CYPs tested, the relative level of CYP2E1 mRNA was >500-fold higher than others, and showed substantial enzymatic activity. We also found that CYP2E1 is expressed relatively higher in plasma exosomes than exosomes derived from hepatic and monocytic cells. Finally, we observed that the plasma exosomal CYP2E1 cargo played a synergistic role in mediating ETH and APAP induced toxicity in a time dependent manner in primary hepatocytes. This is the first report of the specific packaging and circulation of CYP enzymes, especially CYP2E1, in human plasma exosomes.
This work is supported by the National Institute of Health (AA022063 & DA042374 to SK).

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Cardiorespiratory fitness response to high intensity interval training in HIV+ Hispanic women with and without neurocognitive impairment

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High intensity interval training (HIIT) helps improve cardiorespiratory fitness (CRF) in healthy and chronic disease groups. However, such effect has not been tested among HIV+ women with and without neurocognitive impairment (NCI) compared with HIV- women. Purpose: to test the hypothesis that a low-volume (LV) HIIT intervention will improve CRF in HIV+ with and without NCI, and HIV- women. Methods: 20 HIV+ (13 with, and 7 without NCI), and 11 HIV- Hispanic women completed a 6-week, 3-days/week LV-HIIT on a cycle ergometer. Maximal Oxygen consumption (VO2max, as a proxy for CRF) using a cycle ergometer with 25W increments every 2 minutes until volitional fatigue, and blood samples for insulin, glucose, and lipid profiles were evaluated before and after the intervention. During the first 2-weeks, participants completed 8-intervals (1-min intense, 1-min active resting) of cycling at 80% of their HR reserve (HRR). During the last 4-weeks, they completed 10-intervals at 90% of their HRR. NCI was determined with a battery of neuro-psychological testing (7-domains), and daily physical activity (PA) with accelerometers. Kruskal-Wallis tests were used to determine between group differences. Results: CRF improved in all HIV- Hispanic women; while 62% of HIV+ with NCI, and 29% of HIV+ without NCI improved their CRF (P=0.006). No significant between group difference were observed for age, lipid profile, insulin resistance, or daily PA. Except for HR max which was lower in HIV+ (regardless of NCI diagnosis) compared with HIV- participants, other CRF testing variables (load, ventilation, time) were not different between groups. Conclusion: Differences in CRF response to the HIIT intervention could not be explained by PA or metabolic variables. Future evaluation of exercise-induced myokines and circulating micro RNAs, known to influence energy metabolism, cardiac, and vascular responses to exercise, could help explain our results.
Supported by NIMHD S21MD001830, R21MH095524, U54MD007587, R25MD007607, and UM1AI069415.

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Stimulation of tyrosine hydroxylase in dopaminergic neurons by cinnamon and its metabolite sodium benzoate: Implications for Parkinson disease

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Increasing the function of residual dopaminergic neurons in the nigra of patients with Parkinson disease (PD) is an important area of research. Although tyrosine hydroxylase (TH) is the rate-limiting enzyme in the dopamine (DA) biosynthesis pathway, there are no effective drugs/molecules to upregulate TH and increase the production of DA by nigral neurons. This study underlines the importance of cinnamon, a widely-used food spice and flavoring material, and its metabolite sodium benzoate (NaB), a widely-used food preservative and a FDA-approved drug against urea cycle disorders in humans, in stimulating the expression of TH and increasing the level of DA and its metabolites in the CNS. NaB dose-dependently increased the expression of TH in mouse MN9D dopaminergic neuronal cells and primary dopaminergic neurons. Interestingly, oral administration of ground cinnamon increased the expression of TH in the nigra and upregulated the level of DA in striatum of normal mice and aged transgenic mice expressing mutated A53T α-syn. Accordingly, oral feeding of NaB also increased the level of TH and DA in the CNS of normal mice and aged A53T α-syn transgenic mice. Furthermore, the presence of cAMP response element (CRE) in the promoter of TH gene, the activation of cAMP response element binding (CREB) by NaB, and the abrogation of NaB-induced expression of TH by siRNA knockdown of CREB suggest that NaB stimulates the expression of TH dopaminergic neurons via CREB. These results highlight a new property of cinnamon and its metabolite NaB in stimulating the TH-DA pathway, which may be beneficial for PD.
This study was supported by a merit award (I01BX003033) from Veteran Affairs.

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Benzop(a)pyrene in Cigarette Smoke Enhances HIV-1 Replication in Monocytic cells: Potential Role of Cytochrome P450s and Oxidative Stress

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Smoking aggravates HIV-1 pathogenesis and leads to decreased responses to antiretroviral therapy. Here, we aim to find a molecular mechanism that would explain the smoking-induced HIV replication. Benzo(a)pyrene (BaP) is a major carcinogen in cigarette smoke. It requires metabolic activation through cytochrome P450s (CYPs) to exert its toxic effects. We hypothesize that BaP is metabolized by CYP-mediated pathway and produce reactive oxygen species (ROS). Oxidative stress resulting from increased ROS would aggravate HIV-1 replication. Recently, we have shown that the chronic exposure of BaP to

U937 monocytic cells resulted in the significant induction of CYPs (1A1 and 3A4) and AOEs (catalase and SOD1), and increase in ROS and cellular toxicity. We also confirmed these results in human primary macrophages. Next, we examined the chronic effect of BaP in U1 cells. The chronic exposure of BaP resulted in ~4 fold increase in HIV-1 replication. In addition, there was a significant increase in the expression of CYP1A1 at mRNA level as well as increase in its enzymatic activity. Elevated ROS and massive cell death were also observed. We observed decrease in viral replication in BaP-exposed U1 cells upon SiRNA silencing of CYP1A1 and treatment with antioxidants and CYP inhibitors. There was nuclear translocation of NF-kB subunits (p50 and p65) with chronic treatment of BaP. The treatment of specific IKK inhibitors (IKK-16, SC-514) to the BaP-treated U1 cells also reduced the viral replication significantly. Furthermore, we have also shown that treatment of SiRNA and antioxidants/ CYP inhibitors reduced the nuclear translocation of NF-kB subunits. Our results suggest that BaP enhances HIV replication in U1 cells by a CYP-mediated oxidative stress and NF-kB pathway. The results from the present work are clinically relevant as they would help to find a novel therapeutic target to improve drug therapy outcomes in HIV positive smokers.

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Exosomes from Cigarette Smoke Exposed HPV-infected Cervical Cancer Cells Exacerbate HIV-1 Replication in Macrophages

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Smoking and comorbidity with Human papilloma virus (HPV) infection, which lead to cervical cancer, are major factors that contribute to a reduced HIV-1 suppression in HIV-1 patients. There is a high prevalence of smoking (~2-3 fold) and comorbidity of HIV and HPV (~6-fold) among HIV-1 infected population. Here, we explore the underlying mechanism by which HPV alone and in the presence of smoking increases HIV-1 pathogenesis. We propose that exosomes secreted from HPV-infected cervical cells (caski cells) exacerbate HIV-1 replication in HIV-1-infected macrophages, which is further enhanced in presence of tobacco exposure. To test the hypothesis, we treated U1 (HIV-infected monocytic cell line) with the supernatant obtained from caski cells. We observed an approximately 2-fold increase in HIV-1 replication in the treated U1 cells, which was further increased with exposure to tobacco-treated Caski supernatant. We also observed a significant increase in the expression of cytochrome P450s (CYP 1A1 and 2A6), decrease in total antioxidant capacity, and increase in cytotoxicity in caski supernatant-treated U1 cells. Furthermore, the viral replication was reduced when treated with chemodietary agents such as curcumin (20 μM) and curcubitacin-D (0.1 μM), which emphasized the role of specific oxidative stress pathway in HIV-1 replication. We speculate that the supernatants from the caski cells contain exosomes from caski cells. These exosomes carry factors such as HPV viral particles, CYPs, and/or oxidative stress related factors, which are delivered to U1 cells and induce oxidative stress and the subsequent HIV-1 replication. We are in the process of isolating exosomes from supernatant obtained from tobacco constituents-treated caski cells to examine if they contain these oxidative stress inducing factors, which are responsible for HIV-1 replication via exosomes-mediated cell-cell interaction. The results from our study are likely to help identify therapeutic targets to improve the treatment outcomes in women with HIV-HPV co-infection who smoke.

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Implication of HHV-6 and HHV-7 infection in the pathogenesis of neurological disorders

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Background. Human herpesviruses (HHV)-6 and -7, which belong to Herpesviridae family, Beta-herpesvirinae subfamily, Roseolovirus genus, are lymphotropic, immunomodulating and neurotropic viruses, which are associated with several neurological diseases. Aim was to determine implication of HHV-6 and HHV-7 infection in the pathogenesis of fibromyalgia (FM), myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and encephalopathy. **Material and methods.** Peripheral blood of 200 ME/CFS patients, 45 fibromyalgia patients, 150 apparently healthy individuals and autopsy tissue of brain from 57 individuals with encephalopathy as well as 51 individuals without neurologic diseases were enrolled in this study. Various PCR methods and immunohistochemistry were used to detect presence of virus genomic sequences, activity phase, viral load and expression of virus-specific antigens. **Results**. Concurrent HHV-6 and HHV-7 infection markers were detected significantly often in patients with FM (40%) than apparently healthy individuals (4%) ($p<0.0001$), and infection in active phase and higher viral load had only patients with FM. Markers of a persistent HHV-6 infection in active phase had only ME/CFS patients and none of apparently healthy individuals ($p<0.0001$) with significantly higher HHV-6 load among patients with active than latent infection ($p=0.0019$). Persistent HHV-7 infection markers of an active phase were detected in 34% of ME/CFS patients and 8% of apparently healthy individuals ($p<0.0001$) with elevated load in case of severe clinical symptoms ($p=0.0254$). In group of individuals with encephalopathy, HHV-6 and HHV-7 infection markers were detected more frequently than in group of individuals without neurologic disorders. HHV-6 specific antigen expression was observed in brain tissue. **Conclusion.** Significantly more frequent findings of persistent HHV-6 and HHV-7 infection in an active phase with a higher viral load among patients with FM, ME/CFS and presence of HHV-6 in brain tissue of individuals with encephalopathy, compared with apparently healthy individuals, indicate the importance of these infections in pathogenesis of above-mentioned nervous system disorders.

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Nanomedicine-based therapeutic approaches to treat methamphetamine-induced reactivation of HIV latency in the brain

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Methamphetamine (METH) is implicated in exacerbating HIV infection and progression to NeuroAIDS. Chronic METH use results in damage to the blood-brain barrier(BBB), neuronal/glial toxicity involving dopaminergic nerve damage and microgliosis. METH induced toxicity results in increased sensitivity to HIV disease in the Central Nervous system (CNS). Astrocytes the most abundant cell in the CNS and are long- lived such that they become reservoirs for HIV. Therefore, to effectively treat HIV infection in METH abusing individuals therapeutics targeting both METH toxicity and HIV latency reactivation are needed. Here we develop and test the efficacy of two nanocarriers - a liposomal magneto-electro nanoparticle (LipoMENP) and an exosomal-magnetic nanoparticle(ExoMNP). Each nanocarrier is loaded with METH antagonist (SB206553) and SAHA, a HIV latency breaking agent. To determine therapeutic efficacy of LipoMENP-SB-SAHA or the ExoMNP-SB-SAHA were added directly to a METH-treated in vitro model of the blood brain barrier (BBB). Alone METH disrupted the BBB integrity and increased the permeability as measured by transendothelial electrical resistance (TEER) and dextran-FITC transport assay, respectively. Both LipoMENP-SB-SAHA and ExoMNP-SB-SAHA were able to attenuate METH-induced effects on the BBB model. Using an astrocytic HIV latency reporter cell system (U87-RGH), developed in our laboratory

we show that METH alone significantly upregulated the number of reactivated U87-RGH but in the presence of either LipoMENP-SB-SAHA or ExoMNP-SB-SAHA U87 reactivation was reduced by almost 50%. Finally, METH-treated neuronal cells(SH-SY5Y) undergo elevated apoptosis as measured by increased caspase-3 activation. Treatment with ExoMNP-SB-SAHA or LipoMENP significantly reduced METH-mediated caspase-3 activation in SH-SY5Y. Taken together, these results show that the METH-associated BBB damage, neuronal cell apoptosis induction, and HIV latency reactivation can be modulated using nanocarriers. Our study demonstrates the potential of liposomal- or exosomal-based magnetic nanotherapeutics to effectively deliver drugs across the BBB in order to prevent METH-associated pathology in the context of HIV-infection.

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New IRS-1/LC3 nuclear structures and their role in autophagy control and glioblastoma cell survival

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Isulin receptor substrate 1 (IRS-1) is a common cytosolic adaptor molecule involved in signal transduction from insulin and IGF-1 receptors. IRS-1 can also be found in the nucleus following its direct interaction with large T-antigen from human polyomavirus JC. In this study, we report a new finding of unique IRS-1 nuclear structures, which we observed initially in glioblastoma biopsies and glioblastoma xenografts. These nuclear structures can be reproduced in vitro by ectopic expression of IRS-1 cDNA cloned in frame with nuclear localization signal (NLS-IRS-1). In these structures, IRS-1 localizes at the periphery while the center harbors a key autophagy protein, LC3. These new nuclear structures are highly dynamic. Rapidly exchange IRS-1 molecules with the surrounding nucleoplasm, disassemble during mitosis and require growth stimulus for their reassembly and maintenance. In tumor cells engineered to express the NLS-IRS-1 and capable of forming the IRS-1/LC3 nuclear structures, autophagy, induced either by amino acid starvation or rapamycin treatment, was severely attenuated. In this process, IRS-1 nuclear structures sequester LC3 inside the nucleus, possibly preventing its cytosolic translocation and the formation of new autophagosomes. This novel mechanism provides a quick and reversible way of inhibiting autophagy, which could counteract autophagy-induced cancer cell death under severe stress including anticancer therapies.

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Impaired Mitochondrial dysfunction in an Aging HIV patient cohort with HIV-associated neurocognitive disorders (HAND).

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HIV-associated neurocognitive disorders (HAND) includes a spectrum of mild to moderate neurocognitive dysfunction associated with HIV infection. HIV viral proteins are known to cause oxidative damage by increased NO and ROS production that can lead to mitochondrial DNA (mtDNA) mutations and deletions and impaired mitochondrial function. A chronic state of systemic inflammation can induce oxidative stress and cellular bioenergetic dysfunction. We hypothesize that impaired mitochondrial function induces an early senescense and that HAND patients develop a neuropathology that similar to aging but at an accelerated rate of progression. We obtained postmortem HIV brain tissue from National NeuroAIDS Tissue Consortium (NNTC) and stratified samples into 4 groups (n=4/group): Group 1-patients age > 60 yr without HAND; Group 2-patients age > 60 yr with HAND; Group 3- patients age <60 yr without HAND and Group 4- patients age <60 yr with HAND. We measured mtDNA, NOS, TNF-α, IL1-β and TREM2 expression using QPCR and OXPHOS and ROS expression in brain tissue lysates. We observed increased gene expression of neuroinflammatory markers such as NOS, TNF-α, IL1-β, TREM2 and increased mitochondrial dysfunction and oxidative stress in HAND patients (Group 2 & Group 4) as compared to the patients without HAND (Group 1 & Group 3) in both the <60 yr and > 60 yr patient cohorts. Increased oxidative stress in patients with HAND due to increased NOS and ROS levels is attributed to mitochondrial dysfunction which occurs when oxidative stress results in modification in the respiratory chain complex. The resulting neuroinflammation amplifies and promotes further oxidative damage, causing increased neurocognitive deficits in HAND patients. Therapeutics strategies that are focused on mechanisms regulating mitochondrial biogenesis and ROS to restore mitochondrial function as well as mitochondrial ROS production need to be explored for HAND.

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JCV infection of meningeal and choroid plexus cells in patients with progressive multifocal leukoencephalopathy

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Background: JC virus (JCV) can cause a lytic infection of oligodendrocytes and astrocytes in the central nervous system (CNS), leading to Progressive multifocal leukoencephalopathy (PML). JCV variants can also infect and destroy cerebellar granule cell neurons and cortical pyramidal neurons, causing JCV granule cell neuronopathy (JCV GCN) and JCV encephalopathy (JCVE), respectively. Finally JCV can also infect meningeal and choroid plexus cells and cause JCV meningitis (JCVM). However, whether JCV also infects meningeal and choroid plexus cells in patients with PML and other immunosuppressed individuals remains unknown. **Objective:** To determine whether JCV infects meningeal and choroid plexus cells in PML and HIV infected patients with no overt signs or symptoms of meningitis. METHODS: We analyzed archival formalin-fixed, paraffin-embedded brain samples from PML patients as well as HIV-seropositive and seronegative control subjects by immunohistochemistry for the presence of JCV early regulatory T Ag and JCV VP1 late capsid protein. **Results:** In meninges, we detected JCV T Ag in 9/40 (22.5%) HIV+/PML patients and 2/8 (25%) HIV-/PML patients. JCV VP1 protein was detected in 6/40 (15%) HIV+/PML patients and 2/8 (25%) HIV-/PML patients. Neither JCV T Ag nor VP1 protein was detected in meninges of 26 HIV-infected individuals and 17 HIV-negative control subjects. In choroid plexi, we detected JCV T Ag in 1/6 (16.7%) HIV+/PML and not in 1 HIV-/PML case, whereas JCV VP1 protein was not detected in any of those cases. Neither JCV T Ag nor VP1 protein was detected in choroid plexus of 2 HIV-infected individuals and 12 HIV-negative control subjects. **Conclusions:** Our findings suggest that productive infection of meningeal cells by JCV is a frequent occurrence in PML patients, whereas choroid plexus cell may rarely harbor restrictive JCV infection in those patients. These data provide new insight on JCV pathogenesis in the CNS.

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A unique subset of CD8 T cells (CD4DIMCD8Bright T Cells) is negatively associated with HIV content in the brain and positively correlated with neuropsychological function

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HIV invades the brain within two weeks of infection and can lead to a spectrum of neurocognitive dysfunction termed HIV-Associated Neurocognitive Disorders (HAND). We previously showed that a subset of CD8+ T cells, CD4dimCD8bright T cells (DP T cells), is enriched in

anti-HIV responses. In NOD/SCID/IL-2rcγ-/- mice reconstituted with human PBMCs (NSG-huPBMCs) infected with HIV, the percentage of DP T cells inversely correlates with HIV-gag mRNA but not CD8+CD4- T cells. To assess if this correlation is evident in HIV infected patients, we evaluated the relationship between DP and CD8SP T cells from CSF and CSF viral load (VL) in HIV infected patients. This cohort consisted of 40 HIV infected patients. Patients were either on therapy and controlling HIV (HIV RNA < 500 copies/mL; n=11), on therapy and failing to control HIV (>500 copies/mL; n=12), or not on therapy. CSF DP T cells inversely correlated with CSF VL (rs = -0.53565, p≤0.0019) across all groups of HIV infected patients. Conversely, CSF CD8SP T cells positively correlated with CSF VL (rs = +0.55913, p≤0.0011) across all groups of HIV infected patients. We evaluated the relationship between DP and CD8SP T cells and neuropsychological Z -4 score (NPZ-4). NPZ is a composite Z score for a short battery of tests (grooved pegboard, timed gait, finger tapper, and WAIS-R digit symbol) which evaluate memory, psychomotor speed, and executive function. We show that percentage of CSF DP T cells is positively correlated with neuropsychological function (rs=+0.45341, p≤ 0.008) and no such association exists for CD8SP T cells. No such correlation existed with CD4+ single positive T cells. Collectively, these data indicate that a higher frequency of DP T cells and not CD8SP T cells are associated with HIV control in the CNS and better neurocognitive function.

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Inflammation caused by the HIV Nef protein is inhibited by the blockade of beta-adrenergic receptors

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Nef is an early HIV-1 protein produced by latently infected astrocytes. Our model in Sprague Dawley (SD) rats shows Nef causes memory impairment, inflammatory cell infiltration, blood brain barrier (BBB) compromise and peripheral organ inflammation. Here we extend those findings to gain a better understanding of the brain, lung, and small intestine responses to the toxic effects of Nef. In this study we examine the role of astrocyte Nef expression and the activation of the sympathetic nervous system (SNS), which connects the central nervous system with the peripheral nervous system and upon activation it triggers a systemic immune response. We used male and female SD rats and infused them with astrocytes transfected to produce Nef and divided into three groups: Nef, Nef with propranolol, and naive. The beta-adrenergic antagonist propranolol (10mg/kg) was administrated daily one day prior to and two days after surgery. Two days after surgery, the rats were sacrificed and brain, lung, small intestine (SI), and spleen tissues were collected. Tight junctions of the BBB were more prominently detected by immune assay in Nef animals co-treated with propranolol than in the Nef alone group, which showed significant loss of claudin-5. Lung tissue samples appeared healthy in rats co-treated with propranolol when compared with Nef treated animals that demonstrated an increase in immune cell infiltration. Histological analyses showed that rats co-treated with propranolol demonstrated normal structure of the SI with decreased Peyer's Patch diameter when compared to the Nef-only group. Analysis of the splenic leukocytes by flow cytometry demonstrated that Major Histocompability Complex II (MHC-II) is downregulated in rats treated with propranolol when compared with the Nef-treated group. These indirect measurement of the sympathetic activities demonstrated that the pathology caused by Nef can occur through the beta-receptors.

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HIV-infected Cannabis Users Display Lower Levels of Circulating CD16+ Monocytes and IP-10 Compared to Non-using HIV-infected Individuals in a Mid-Michigan HIV Cohort

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A hallmark of HIV infection is chronic immune activation and is believed to be one of the major contributors to HIV-associated neuroinflammation and cognitive decline. Circulating activated monocytes, including those that are CD16+, have been implicated in this neuroinflammation. These activated monocytes become infected with HIV in circulation, can cross the blood-brain barrier and release inflammatory factors, HIV virions and viral proteins. This can lead to HIV infection and activation of brain-resident cells, including microglia and astrocytes, driving an inflammatory environment in the brain. These processes contribute to neuronal dysfunction and death, ultimately promoting cognitive decline, which impacts up to 50% of the HIV-infected population. Cannabis, which possesses immune modulatory and anti-inflammatory properties, is widely used by HIV-infected individuals with an estimated prevalence of 20-37% in the United States and Canada. Here we report that HIV-infected donors using cannabis (MJ+) have lower circulating CD16+ monocytes and plasma IP-10, compared to HIV+MJ- donors. Furthermore, Δ9-tetrahydrocannabinol (THC), a constituent in cannabis, impairs human CD16- monocyte transition to CD16+ and monocyte-derived IP-10 in vitro, suggesting that THC may decelerate peripheral immune processes contributing to HIV-associated neuroinflammation.

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Beta-catenin negatively regulates neuroinflammatory cytokine and chemokine expression in human astrocytes

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HIV invades the brain during acute infection, setting the stage for persistent neuroinflammation despite combined antiretroviral therapy (cART). These events lead to HIV-Associated Neurocognitive Disorders (HAND), which occurs in ~50% of HIV-infected individuals. The cellular and molecular mechanisms driving this neuroinflammation/HAND are not entirely clear. Our lab has been focused on understanding the role of Wnt/β-catenin signaling in HAND. The Wnt/β-catenin signaling pathway is integral to cell survival and proliferation. We have shown that both HIV and inflammatory signals downregulate Wnt/β-catenin signaling in astrocytes leading to neuronal injury. Here, we evaluated the impact of β-catenin on two key proteins (IL-6 and IL-8) associated with neuroinflammation and chemotaxis in context of HIV/HAND. We demonstrate that knockdown of β-catenin in normal human astrocytes (NHAs) significantly induced IL-6 and IL-8 at the transcription and protein levels and conversely, induction of β-catenin significantly downregulated these two molecules. These findings are intriguing given that no role for β-catenin to date is associated with IL-6 and IL-8 regulation. To assess the direct impact of β-catenin on transcriptional activity of IL-6 and IL-8, we conducted a bioinformatics analysis of their respective promoters to test for presence of putative TCF/LEF binding sites. TCF/LEF are transcriptional factors that partner with β-catenin to regulate gene expression. We found a proximal TCF/LEF site located between the -91 and -86 region sandwiched between C/EBP and NF-κB and a distal TCF/LEF site located between the -948 and -943 region on the IL-6 promoter. We also found one TCF/LEF site located between the -175 and -169 region on the IL-8 promoter. These findings suggest that β-catenin regulates inflammation in astrocytes and may do so through direct regulation of IL-6 and IL-8 at the transcriptional level. On-going studies are addressing the mechanism of this regulation and its biologic consequence in HAND.

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Selective loss of peripheral non-peptidergic and peptidergic neurons in SIV infection.

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HIV peripheral neuropathy (HIV-PN) is a common neurological complication of HIV infection, with estimated prevalence as high as 69% of people living with HIV (PLWH). The most common form of HIV-PN, HIV distal sensory polyneuropathy (HIV-DSP) involves the predominant loss of small myelinated and unmyelinated peripheral nerve fibers. The pathophysiologic mechanisms of HIV-PN are not fully understood. Previous investigations using a SIV-infected primate model have shown loss of peripheral axon terminals, measured by intraepidermal nerve fiber density (IENFD), and damage to neuronal bodies in the dorsal root ganglia (DRG), assessed by presence of satellitosis, neuronophagia, and Nageotte nodules. Loss of IENFD and severity of pathology was associated with monocyte inflammatory markers and increased traffic of activated monocytes to the DRG. Here, we investigated changes in three specific DRG neuronal populations during SIV infection; neurofilament 200 (NF200)+ myelinated neurons, isolectin B4 (IB4)+ non-peptidergic neurons and tyrosine kinase A (TrkA)+ peptidergic neurons. Using immunohistochemistry and quantitation of neuronal subsets, we observed a significant decrease in the percent of IB4+ and TrkA+ neurons in the DRG of SIV-infected macaques, compared to uninfected controls. There was no difference seen in the percent of NF200+ neurons. Measurement of neuronal diameters revealed a significant decrease in the average diameter of IB4+ and TrkA+ neurons in SIV-infected macaques, compared to uninfected controls. Additionally, a marked shift in the frequency distributions of neuronal diameter (in 5 mm increments) showed higher prevalence of smaller diameter IB4+ and TrkA+ neurons in SIV-infected animals, compared to uninfected controls. These data show a significant change in the percent and size of non-peptidergic and peptidergic DRG neurons during SIV infection. Our data suggests that SIV infection promotes a loss and atrophy of non-peptidergic and peptidergic neurons, which may lead to disrupted signaling and neuropathic pain seen in HIV-PN.

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Cognitive Profile in Human Immunodeficiency Virus (HIV+)/Hepatitis C Virus (HCV) Co-Infected Hispanic Women

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BACKGROUND: Due to antiretroviral treatment (cART), patient survival has increased, but it has been observed a higher prevalence in Neurocognitive Disorders associated with Human Immunodeficiency Virus (HAND). In the United States, one quarter of patients with HIV+ are co-infected with Hepatitis C Virus (HCV). HIV+/HCV-co-infected women are more susceptible to health complications and mortality in comparison with HIV-mono-infected women. The AIM of this study was to describe the cognitive profile of HIV+/HCV-co-infection in Hispanic women. METHOD: This study is a secondary data analysis collected from the HIV+ women Cohort from the University of Puerto Rico, Medical Science Campus. 43 HIV+ women were recruited. HIV+ women was stratified in: HIV+/HCV-co-infected (n=15) and HIV-mono-infected (n=28) women. Their cognitive performance was stratified in normal cognition (NC) and mild neurocognitive impairment (MND) according to the HAND criteria. Evaluation methods included neuropsychological test (NP), viral-immune profiles, as others. RESULTS: 43 HIV+ women were evaluated between 24 to 59 years old. 35% of our sample of HIV+ women had HCV-co-infection. There were no significant differences ($p<.05$) between the HIV-mono-infected women and HIV+/HCV-co-infected women in age, Depression Scale [BDI-II], CSF Viral, CD4 Nadir, and current CD4. HIV+/HCV-co-infected women showed greater cognitive impairment with a proportion of 80%, in contrast to 72% in HIV+ women. Comparing women in the MND group with NC participants, significant differences were observed in NP performance, especially in Speed Processing ($p=.003$), Memory ($p=.027$), and Motor Skills ($p=.003$) domains. CONCLUSIONS: Studies have demonstrated that HIV+/HCV-co-infection increases the risk of health complications. Therefore, larger studies are required for better assessment of the HIV+/HCV-co-infection prevalence and its effect in cognitive performance, especially in women.

GRANT SUPPORT: Partially supported by: R25MH080661, R21MH095524, U54NS43011, S11NS046278, U54RR026139, P20RR11126, P031S100037, G12MD007600, 2U54MD007587.

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Dimethyl fumarate decreases cathepsin B release from HIV-infected macrophages

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Introduction: HIV-associated neurocognitive disorders (HAND) are prevalent in ~50% of HIV-positive patients, despite antiretroviral therapy (cART). During HIV infection of macrophages, the lysosomal protein, cathepsin B, is secreted and induces neurotoxicity. Cathepsin B is increased in plasma and post-mortem brain tissue from patients with HIV-associated dementia. Oxidative damage increases in HIV-infected patients while antioxidants are decreased in HIV dementia. Dimethyl fumarate (DMF), an antioxidant, has been reported to reduce HIV replication and neurotoxicity mediated by macrophages. We hypothesized that DMF would reduce cathepsin B secretion by preventing oxidative stress in macrophages. Methods. Monocyte-derived macrophages (MDM) were isolated from healthy donors, inoculated with HIV-1ADA, and treated with DMF. Cathepsin B secretion was assessed from HIV-infected MDM supernatants at 12 days post-infection (p.i.) using ELISA. Hydrogen peroxide (H2O2) levels were measured from whole cell lysates and supernatants at day 12p.i. Results. Treatment with DMF reduced HIV replication and cathepsin B secretion from HIV-infected MDM. However, intracellular H2O2 levels were not affected by HIV infection at day 12p.i., and cathepsin B levels did not correlate with H2O2 levels. Although HIV infection did not alter intracellular H2O2 levels, DMF treatment decreased intracellular H2O2 levels in HIV-infected MDM. Conclusions. Thus, our results suggest that cathepsin B secretion from HIV-infected MDM is mediated by a mechanism different from oxidative stress and, thus, the mechanism by which DMF reduces cathepsin B secretion from HIV-infected MDM remains unknown. Therefore, DMF represents a potential strategy against HIV-induced cathepsin B neurotoxicity in HAND.

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The effects of HIV infection and antiretroviral therapies on oligodendrocyte growth and maturation

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Despite combined antiretroviral therapy (cART), HIV-associated neurocognitive disorder (HAND) occurs in 30-50% of HIV-positive patients. Further, white matter (WM) pathologies persist in HAND patients despite effective viral control. The thinning of the corpus callosum and disruption of WM microstructures seen in HIV-positive patients on cART suggest both HIV infection and/or antiretroviral drugs might perturb myelin production and oligodendrocyte growth and maturation. Thus, we hypothesized that HIV infection of macrophages and/or antiretroviral compounds alter oligodendrocyte differentiation, function, and/or survival, influencing HAND persistence in the cART era. To examine the effect of HIV infection on oligodendrocyte differentiation, we treated primary rat oligodendrocyte precursor cells (OPCs) with supernatants from HIV-infected primary monocyte-derived macrophages (HIVMDM) at the time of differentiation into mature oligodendrocytes. Using this model, HIVMDMs significantly inhibited OPC differentiation, which may explain initial WM loss in HIV-infected patients. To gain insight into the persistence of WM changes in the cART era, we previously demonstrated that two protease inhibitor (PI)-class antiretroviral compounds, lopinavir and ritonavir, inhibited OPC differentiation, whereas zidovudine, a nucleoside reverse transcriptase inhibitor (NRTI), did not. We extended these analyses to show that another PI, darunavir, two NRTIs, tenofovir alafenamide and tenofovir, and two integrase inhibitors (INSTIs), elvitegravir and raltegravir. Darunavir, tenofovir alafenamide, and elvitegravir also inhibited OPC differentiation, which was not observed with tenofovir or raltegravir. These findings suggest that further investigation into the effects of HIV and/or first-line antiretroviral compounds is warranted to provide insights into the observed persistent WM changes seen in HAND patients, with implications for their contribution to cognitive impairment.

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Development of Nanodiamond-based anti-HIV drug delivery targeted to the Brain

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Combined antiretroviral therapy (cART) is considered to be widely acceptable therapy for Human Immunodeficiency Virus (HIV-1) infection. However, it does not cure the disease and ineffective to clear the virus from its reservoir organ like the brain. Since, most of the current cART drugs cannot enter the brain through Blood Brain Barrier (BBB) which makes the brain a reservoir organ for HIV. There are many targeted drug design approach that has been investigated towards the brain. However, most of them are yet to establish in the clinics because of the associated toxicity to the neurons. The present study proposes the nanodiamond as an excipient for anti-HIV drug delivery to the brain. Being a nanosized carbon molecule with natural biocompatibility and non-toxic nature, it becomes more efficient drug carrier than other carbon-based materials. Considering its potential and importance, we have characterized unmodified and surface-modified (-COOH and -NH2) nanodiamond for its capacity to load the anti-HIV-1 drug efavirenz and observe it's biological stability in vitro. Nanodiamond was chemically characterized by different surface modification (-COOH and -NH2) in order to load optimum amount of anti-HIV drug efavirenz. The formulation was further tested for cellular toxicity, biocompatibility, and neurotoxicity. Biologically characterized drugs were finally tested for its ability to cross Blood-Brain Barrier to deliver the drug to the brain and its therapeutic efficacy against HIV-1. Our study has established that unmodified nanodiamond conjugated drug formulation has significantly higher drug loading capacity than surface-modified nanodiamond with minimum toxicity. Further, drug delivery profile has shown that it has higher sustained drug release capacity than unformulated drugs with optimum therapeutic efficacy. Therefore, Nanodiamond can be used an excipient for nanomedicine based drug delivery to the brain. The present biological characterizations provide a foundation for further study of in-vivo pharmacokinetics and pharmacodynamics of nanodiamond-based anti-HIV drugs.

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Neuron-microglia interaction in HIV-1 gp120-induced synapse degeneration

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HIV-1 infection of the central nervous system (CNS) can cause synaptic degeneration, which likely underlies the pathogenesis of HIV-related neurological diseases. Our previous studies show significant decrease of synaptic protein in the spinal cord of HIV-1 patients with pain. However, the mechanism by which HIV-1 causes the synaptic degeneration is unclear. We hypothesize that microglia, the major immune resident cells in the CNS, contribute to removing damaged synapses induced by HIV-1 infection. To test the hypothesis, we determined the effects of HIV-1 coat protein gp120 on synapses in primary cortical cultures and mouse spinal cords. By western blotting analysis, we observed decrease of pre- and post-synaptic markers synapsin I and PSD95 following gp120 exposure. By immunostaining, we also observed decreased synaptic puncta. Interestingly, we found that gp120-induced synapse loss was associated with microglial activation, and blockage of microglia abolished the synapse decrease. In addition, we observed that fractalkine (FKN), a chemokine that is specifically expressed in neurons and can regulate microglial activation, was up-regulated in response to gp120 stimulation. Knockout of FKN receptor CX3CR1, which is specifically expressed in microglia, attenuated gp120-induced synapse degeneration. We also found that disruption of Wnt3a/β-catenin pathway blocked gp120-induced FKN up-regulation and synaptic degeneration. Furthermore, we showed that gp120 induces the Wnt3a/β-catenin signaling and FKN up-regulation via NMDA receptors. Our findings collectively suggest that HIV-1 gp120 induces synapse degeneration involves neuron-microglia interaction mediated by Wnt-KFN-CX3CR1 intercellular signaling.

P259

Quantification of SIR full-length levels in the urine of HIV-seropositive women

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Previously, we showed an association between plasma and CSF soluble insulin receptor (sIR) and HIV-associated neurocognitive disorders (HAND) in our cohort of HIV-seropositive women. This receptor has the capacity of sequestering free plasma insulin, thus contributing to the insulin resistance observed in this population. Although plasma and CSF sIR could serve as a biomarker for the presence and severity of HAND, there is a need for a more accessible and less invasive method to monitor sIR changes. In this

study, we investigated if sIR full length levels were present in the urine in seropositive women and their association with HAND and renal function. We determined urine sIR levels using the classical enzyme linked immunosorbent assay (ELISA) from HIV-seronegative controls (n=29) and HIV-seropositive (n=73) women from the Hispanic/Latino Longitudinal Seropositive Women Cohort. In HIV-seronegative participants, urine sIR levels had a negative correlation with age, and a positive correlation with glomerular filtration rate (GFR). A similar correlation with age was observed in the HIV-seropositive group, but no significant correlation was observed with GFR. When controlling for age and GFR, no correlations were observed between urine sIR levels and HAND severity, nor for each of the neuropsychological domains tested. To our knowledge, this is the first study that demonstrates that urine sIR levels can be detected by ELISA in samples of non-diabetic HIV-seronegative and HIV-seropositive participants. Further analysis need to be conducted to determine the plausibility of urine sIR levels as a biomarker for neurocognitive impairment in HIV-seropositive participants.

P260

HIV-1 gp120 protein promotes neuronal deregulation: Mechanisms and Players

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In the absence of HIV-1 infection of neurons, several mechanisms have been proposed for HAND, including indirect inflammatory effects in the CNS and direct effects of viral proteins (e.g. gp120) shed from activated HIV-1-infected cells. The fact that these viral proteins enter the neurons through several pathways suggest the presence of many competing mechanisms that can contribute to HAND, each of which has its advocates. Their relative contributions to clinical disease in vivo remain to be sorted out, and this is an outstanding problem in HIV research. Studies from other neurodegenerative diseases described the cAMP responsive-element binding (CREB)-1 protein as a Key Regulator of the Memory. These studies also showed that loss of CREB protein expression and phosphorylation contributes to the development of neurocognitive impairments such as Spatial and Declarative Memory Alteration. Recently, we have obtained data showing a clear-cut effect of HIV-1 gp120 on CREB protein phosphorylation and function in vitro (primary human and mouse neurons and in the neuronal cell line, SHSY5Y) and in vivo (in mice injected with gp120. Inhibition of CREB functions led to reduction of Mitochondrial energy, biogenesis and movement, and alteration of synaptic plasticity. The effect of gp120 protein was abolished in the presence of Rolipram [activator of CREB]). These findings are exciting for two reasons: (1) A correlation between CREB protein and neurodegeneration has been well-established in the literature, suggesting that this may be a significant contributory mechanism in HAND; and (2) Agents are available which can specifically restore CREB protein levels and function, offering an opportunity to test directly the contribution of this mechanism in vivo in mice. Therefore, Learning Deficit and Spatial & Declarative Memory Impairment that are commonly observed in HIV-1 patients as well as in aged persons can be restored using Rolipram.

P261

Human Polyomavirus, JC virus (JCV), Regulatory Agnoprotein (Agno) Targets Mitochondria and Impairs its Functions

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Agno is one of the critical regulatory proteins of JCV. JCV infects glial cells in the central nervous system in immunocompromised people and causes a fatal brain disease, progressive multifocal leukoencephalopathy. In the absence of Agno expression, JCV is unable to sustain its productive life cycle which is evident from a poor replication of the Agno null mutants. Understanding the molecular mechanisms underlying the functional roles of Agno in JCV life cycle is still at infancy despite much progress has been made in this field. 3D-NMR structure of the full-length Agno was recently determined revealing that this protein contains two alpha-helices (a minor and a major), while the rest adopts an unstructured conformation. The major alpha helix encompasses the Leu/Ile/Phe-rich amino acids with four distinct faces - an aromatic, one hydrophilic and two hydrophobic. Leucine residues on the hydrophobic face play an important role in protein stability and function while charged residues located on the hydrophilic surface of the helix controls its release. Furthermore, molecular targets of Agno in the JCV infected cells are largely unknown. Computer predictions indicated that Agno carries a potential mitochondrial targeting sequence located at its N-terminus region (aa 1-14, MVLRQLSRKASVKV). Initial studies of the mitochondrial targeting led to some intriguing results; First, Agno was found to co-localize with mitochondrial network and appears to disrupt mitochondrial architecture. Second, it is co-fractionated with mitochondrial compartments and interacts with a mitochondrial protein, cytochrome c oxidase, and reduces ATP production. Finally, a significant increase in Ca2+ uptake by mitochondria was observed when Agno was expressed in cells indicating a possibility that this protein deregulates Ca2+ homeostasis. We have just begun to unravel the impact of Agno on mitochondrial functions, which may be used as a model system to understand the molecular mechanisms underlying the progression of certain neurodegenerative diseases.

P262

Structure-based systematic release analysis of the JC virus agnoprotein regions: an important role for the hydrophilic face of the major alpha-helix domain of the protein in release

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JC virus encodes several regulatory proteins from its early and late coding regions including a small basic protein designated as Agnoprotein (Agno). Agno plays important regulatory roles during the viral replication cycle, because, in the absence of its expression, JCV is unable to sustain an efficient life cycle. Previous studies have indicated that this viral protein interacts with various host proteins, arrests cells at the G2/M transition phase and interferes with the chemokine production. The three dimensional (3D) structure of Agno was recently resolved by NMR revealing the two main structural characteristics of the protein - the presence of two alpha-helices (a minor and a major) and the unstructured regions constituting the rest of the protein. Agno was also recently reported to be released from the Agno-positive cells but the mechanism of which has yet to be determined. In this study, we have further delineated the release mechanism by a systematic mutagenesis approach based on its 3D structure. These studies revealed that amino acids, Lys22, Lys 23, Phe31, Glu34 and Asp38, located either on or adjacent to the hydrophilic face of the major alpha-helix of Agno regulate its release and therefore this face was designated as "Agno release surface" of the protein. We then investigated the fate of the "released" Agno by treating the glial cells with a synthetic or recombinant Agnoprotein and demonstrated that it strongly interacts with the cell surface and some of the bound protein may be internalized by cells. Taken together Agno perhaps plays a role in modulating the physiology of the neighboring cells and sets up a friendly environment for the incoming virus for the better viral replication cycle and/or it may have long term effects on the glial cells in the brain for the initiation of various neurological diseases.

P263

Trans-spliced products of JC virus (JCV) late transcripts encode novel proteins detectable in PML patient brain samples

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JC virus was isolated almost a half century ago and yet understanding the molecular mechanisms governing its biology remains highly

elusive. JCV infects the glial cells including, oligodendrocytes and astrocytes, in the central nervous system (CNS) and causes a fatal brain disease, PML, in immunocompromised individuals including AIDS, cancer and MS patients. The genome of JCV generates two primary transcripts from its early and late coding regions and produces several predicted alternatively spliced products mainly by cis-splicing. We now report the discovery and characterization of two additional novel open reading frames (ORF1 and ORF2) associated with JCV late transcripts which are generated by an unusual splicing process called trans-splicing. These ORFs result from (i) the trans-splicing of two different lengths of the 5'-short coding region of VP1 between the coding regions of agnoprotein and VP2 after replacing the intron located between these two coding regions, and (ii) frame-shifts occurring within the VP2 coding sequences terminated by a stop codon. More importantly, ORF1 and ORF2 are capable of encoding 58 and 72 aa long proteins respectively and the expression of ORF1 protein is detected not only in infected cells but also PML patient samples. Each ORF protein shares a common coding region with VP1 and has a unique coding sequence on their carboxy terminus. To understand the functional roles of both proteins during the viral life cycle, we have introduced a stop codon right before VP2 coding region at the junction of the common and unique regions of both ORFs to block the expression of their unique regions in the viral background. Results from the replication studies showed a significant reduction in JCV replication for the mutants compared to wild-type and we are currently in the process further investigating the functional roles of these novel proteins in the JCV life cycle.

P264

Non-Nucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine suppresses Zika virus (ZIKV) in glial cells.

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ZIKV is an emerging virus with important public health consequences. Many people infected with ZIKV will have no symptoms or mild symptoms. However, ZIKV infection during pregnancy can cause a serious birth defect called microcephaly and other severe fetal brain defects. Reverse-transcriptase inhibitors (RTIs) are a class of antiretroviral drugs used to treat HIV infection. We asked whether these classes of anti-retroviral drugs could have any impact on ZIKV replication and gene expression. We analyzed and screened the effect of several NNRTIs on ZIKV propagation. Interestingly, among the drugs used, rilpivirine showed a dramatic reduction in ZIKV copy numbers, a robust suppression in replicated viral genomic RNA, and a significant decrease in viral gene expression. Anti-ZIKV activity of rilpivirine was also assessed in IFNR knockout (IFNR-/-) mice. Two to four month old mice (male and female) were infected with PRVABC59 strain of ZIKV through footpad injections and treaded daily with rilpivirine via intraperitoneal (IP) injections. All mice were monitored daily with bi-daily weight checks and grasp test analysis. Mice were sacrificed based on weight loss, physical, behavior, and motor function deficits. All mice were sacrificed at day 14. The Kaplan-Meier estimate was performed to calculate survivor curves. One mouse died at 7 dpi and two others died at 8 dpi from the ZIKV infected (untreated) group suggesting that ZIKV infection was highly lethal in IFNR-/- mice. On the other hand, all mice from ZIKV infected and rilpivirine treated group were survived suggesting that rilpivirine was capable of suppressing ZIKV pathology in this animal model. We also analyzed ZIKV RNA copies in post-mortem brain tissues by Q-RT-PCR. ZIKV RNA copies were significantly reduced in mice treated with rilpivirine. Our results suggest that rilpivirine possesses a strong anti-ZIKV activity in vitro and in vivo.

P265

Inflammation and Excitotoxicity at the Synapse in HAND : Role of PEBP1

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A significant gap that still remains in HIV associated neurocognitive disorders (HAND) is identifying synaptic protein targets including their modulation by therapeutic drugs to ameliorate inflammatory responses and glutamate excitotoxicity at the synapse. This ongoing study focuses on one such synaptic target phosphatidylethanolamine-binding protein 1 (PEBP1) which we have identified from a recent study and whose reduced expression by HIV (Tat, gp120) and meth was reversed by pretreatment with the anti-inflammatory drug Ibudilast, a phosphodiesterase inhibitor. Based on the synaptic localization of PEBP1, we tested a more causal effect of knock down of PEBP1 using siRNA on mixed cerebrocortical cultures that comprise of differentiating neurons and astrocytes. Knockdown of PEBP1 by siRNA showed a decrease in the expression of the glutamate transporter EAAT2 and a concurrent increase in the vesicular glutamate transporter 1 (vGLUT1) in the lysates by western blot. ELISA on cell supernatants showed an increase in the levels of the proinflammatory cytokines IL6 and TNF-a. Further extending our studies to ascertain how knockdown of PEBP1 impacts dendritic spines that are actin-rich and regulate synaptic function, we found a decrease in the expression of F-actin, the dominant form of actin in spines. These above changes were more pronounced with HIV and meth interaction and were reversed by pre-treatment with ibudilast. Based on the well-established dogma of inflammation and excitotoxicity by HIV and meth, our central hypothesis is that down regulation of PEBP1 by HIV and meth exacerbates inflammatory and excitotoxicty responses thus leading to synaptodendritic injury and ibudilast attenuates these deficits. These in vitro studies are of high significance as we have identified a potential synaptic protein target and an anti-inflammatory drug with a therapeutic efficacy to mitigate aberrations associated with its downregulation at the synapse and will provide a strong foundation to further extend these in vivo.

P266

Trk signalling and sensory neuron fate is impaired in a severe human neuropathy

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Charcot-Marie-Tooth disease type 2D (CMT2D) is a nervous system disorder caused by dominant gain-of-function mutations in the housekeeping gene GARS. GARS encodes glycyl-tRNA synthetase (GlyRS), which covalently link glycine to its correspondent transfer RNAs (tRNAs), thereby charging the tRNA for protein synthesis. The mechanisms underlying selective nerve pathology in CMT2D remain unresolved, as does the cause of the sensory involvement characterising CMT2D. To elucidate the mechanism responsible for the underlying afferent nerve pathology, we examined the sensory nervous system of CMT2D mice. We show that the physiological balance between functional subtypes of sensory neurons is distorted by Gars mutations, leading to sensory defects in peripheral tissues, which correlate with disease severity. CMT2D mice display changes in sensory behaviour, which are present at birth and non-progressive, indicating that sensory neuron identity is pre-natally perturbed and that a critical developmental insult is key to the pathology. Importantly, mutant, but not wild-type GlyRS was shown to aberrantly interact with the entire family of Trk receptors and cause misactivation of Trk signalling, the precise modulation of which is essential for sensory neuron differentiation and development. Together, our findings demonstrate that human neuropathies manifest through malfunctioning of the complex interplay between developmental, maturation and survival programs orchestrated by neurotrophin signalling, which has important implications for the timing of future therapeutic treatments.

P267

Investigating the effects of HIV gp120 internalization on microtubule associated proteins

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Human Immunodeficiency Virus (HIV) is known to cause HIV-associated neurocognitive disorder (HAND). HAND is a complex disease characterized by impaired behavior, memory, motor, and cognition. On a cellular level, it is characterized by neurite pruning and ultimately, neuronal death. Glycoprotein120 (gp120), an envelope protein of HIV, endocytoses into neurons and glia but, interestingly, primarily causes neuronal death. Recently, it has been shown that once gp120 is internalized by neurons, it associates with microtubules, which have a unique role in neurons compared to other cells. In neurons, microtubules aid in vital intracellular transport. These microtubules are stabilized by microtubule associated proteins (MAPs) such as tau. Tau has six alternatively spliced isoforms divided into two categories of 4R and 3R, based on the number of microtubule binding domains. Because of the increased number of domains, 4R binds more tightly than 3R. The ratio of 4R:3R is usually 1:1, but it has been shown that Alzheimer's Disease and Parkinson's Disease have an increase in 4R. We hypothesized that gp120 alters the 4R:3R ratio. To examine this, we used DIV14 rat primary cortical neurons treated with 5nM gp120 for various durations up to 24 hours. Using a western blot analysis, we observed a time-dependent increase in tau 3R and 4R, similar to other neurodegenerative diseases. Furthermore, using PCR analysis of post-mortem human samples with varying levels of neurocognitive severity, there is an increase in 4R as the cognition impairment increased. Future experiments are necessary to examine the functional implications of this ratio shift in tau. Moreover, it would be interesting to see if a similar phenomenon occurs with microtubule associated protein-2 (MAP2) which is primarily associated with dendrites. Overall, this experiment suggests a new avenue of gp120 related neuronal death as well as a starting point for possible biomarker development.

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TLR9 agonist CpG-ODN down regulates Wnt/β-catenin signaling in astrocytes: Implication for gut-brain axis in HAND

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Emerging evidence points to a significant role of the microbiota in neurodegenerative diseases. In HIV infection, microbial translocation driven by gut leakiness contributes to HIV disease progression. The impact of this leaky gut and specifically microbial products on HIV-Associated Neurocognitive Impairment (HAND) is not entirely clear. Microbial products translocated from the gut are recognized by toll-like receptors (TLRs), a family of 10 pathogen recognition receptors (PRRs) expressed in various cell types including resident brain cells. We evaluated here the impact of TLR agonists on Wnt/β-catenin signaling in astrocytes. Wnt/β-catenin is a highly conserved signal transduction pathway regulating genes responsible for numerous cellular processes including cell survival and proliferation and is generally an anti-inflammatory pathway. We show that normal human fetal astrocytes (NHAs) express TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9 by qt-PCR. TLR10 expression on NHAs was undetectable. To assess the effect of these TLRs on β-catenin signaling, NHAs were treated with agonists for TLR2 (Lipoteichoic acid; LTA), TLR4 (lipopolysaccharide, LPS), TLR5 (flagellin), TLR7/8 (ssRNA40), and TLR9 (CpG oligodeoxynucleotide, CpG-ODN). At 24 hours post treatment, β-catenin expression, along with its downstream target Axin2, was assessed by western blot and immunofluorescence. We observed that CpG-ODN significantly down-regulated active β-catenin expression by ~ 80% and Axin2 expression by ~65% in comparison to basal expression in NHAs. LTA, LPS, flagellin, and ssRNA40 had no effects on β-catenin or Axin-2 expression at the doses tested. Inhibition of NF-κB by using Bay 11-7085 reversed the inhibition of active β-catenin by CpG-ODN, suggesting cross-talk between the NF-κB and Wnt/β-catenin pathway in astrocytes. These findings have implications for HIV CpGs contribution to neuroinflammation through the down-regulation of active β-catenin.

P269

Role of WNT signaling in Zika Virus Envelope protein induced quiescence in primary human neural stem cells

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Zika Virus (ZV) infection gathered worldwide attention following large outbreaks in Brazil during 2015-2016 epidemics and is linked to severe clinical condition called microcephaly where fetuses are born with abnormally small brain. ZV directly targets stem-cell niche in brain and alters their properties. To date molecular mechanism of ZV induced microcephaly is poorly understood. We used an in-vitro model of well-characterized primary cultures of human fetal neural stem cells (hfNSCs) to understand molecular mechanism of ZV induced alterations in properties of hfNSCs. We first investigated whether ZV Envelope (E) protein alters proliferation of hfNSCs by inducing quiescence and reduced growth of neurospheres. We analyzed expression of genes involved in proliferation and differentiation of fNSCs. Post ectopic expression of E protein resulted in attenuated expression of genes involved in proliferation (CyclinD1 and WNT2) and augmentation of pro-neural (PTN, SPOCK1 and ROBO2) genes, suggesting E protein induces quiescence in fNSCs and pre-mature neural differentiation. WNT family of proteins are crucial in neurodevelopment, hence we explored its role to gain insights into molecular mechanisms of ZV E protein induced alterations. We found that WNT2 (an important growth stimulator) is significantly reduced in response to E protein. Using various cell biology and molecular approaches we investigated role of WNT2 in E protein induced alteration in fNSCs by modulating WNT signaling. Further we found that miRNA hsa-mir-204 is upregulated in response to E protein and WNT2 is direct target of hsa-mir-204. Upregulation of hsa-mir-204 alone can cause quiescence in fNSCs indicating that it is important for maintaining stemness of NSCs. Our study for the first time reports that ZV E protein alters WNT signaling and role of hsa-mir-204 in post transcriptional regulation of WNT2 and maintenance of stemness in fNSCs.

P270

Investigation of neuronal markers of synaptic function in simian immunodeficiency virus (SIV) infected rhesus macaques

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Although the reduction of viral loads in HIV+ patients with antiretroviral therapy (ART) mitigates AIDS related mortality, HIV-associated comorbidities have increased, such as HIV-associated neurocognitive disorders (HAND). Symptoms of HAND include memory impairment, attention deficit and memory processing and retrieval impairments. These symptoms are in part caused by synaptic dysfunction. Synapsin I (Syn I) belongs to a small family of synaptic proteins, which are required for the proper release of neurotransmitters at the synaptic cleft and play an important role in synapse function and plasticity. Here, we used simian immunodeficiency virus (SIV) infected CD8 depleted rhesus macaques as a model of HIV pathogenesis to examine synaptic function during

neuroAIDS. We used protein lysates and brain sections of the frontal cortices from cohorts of SIV-controls, SIV+ and SIV+ ART treated rhesus macaques to analyze neuronal markers of synaptic function. These experiments revealed a significant increase in the phosphorylation of synapsin I protein in SIV+ compared to SIV- controls without affecting the total synapsin I protein expression. Intriguingly, the higher phosphorylation of Syn I was alleviated in the animals treated with suppressive ART. The analysis of brain sections confirmed higher phosphorylation of synapsin I and detected greater phosphosynapsin I staining around somata of neurons in layer 4/5 of cortex in the SIV+ animals. These findings support the occurrence of specific changes in the synaptic activity during SIV infection, which may be altered during HAND.

P271

Inflammasome activation in brain modulates cell survival during HIV-1 infection

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HIV-1 infection of the brain results in inflammasome activation including induction of caspase-1, IL-1beta and IL-18 although the relationship between brain viral burden and expression of individual inflammasome genes remains uncertain. Inflammatory programmed cell death, pyroptosis, is driven inflammasome activation resulting in myeloid cell death through the pore-forming protein, gasdermin-D encoded by GSDMD. Herein, we investigated inflammasome activation in brains of HIV-infected patients together with examining human microglial responses to HIV-1 infection in conjunction with inflammasome inducer exposure. Analyses of inflammasome transcript levels revealed that IL-1B, IL-18, CASP1, AIM2, GSDMD and NLRX1 expression levels were increased in brains of patients with HIV-associated neurocognitive disorders, especially those with concurrent encephalitis compared to uninfected patients. HIV-1 RNA and DNA levels measured by ddPCR in brain were associated with specific inflammasome genes including IL-1B, CASP1, AIM2, GSDMD as well as the innate immune genes IRF1 and CD163. Immunolabeling revealed AIM2 and GSDMD expression in microglia/macrophages and in neurons. HIV-1 infection of human microglia showed release of IL-1beta together with increased expression of gasdermin-D without cell death. Exposure of HIV-infected microglia to inflammasome inducers reduced cell survival as well as HIV-1 p24 release. In contrast, HIV-1 Vpr exposure to human neurons resulted increased gasdermin-D expression and cell death that was blocked by insulin treatment. The present studies indicate that inflammasome expression is associated with HIV-1 burden in the brain although induction of inflammasome expression by HIV-1 infection does not lead to myeloid cell death unless enhanced inflammasome activation occurs. In contrast, neuronal expression of gasdermin-D results in cell death that was abrogated by the neuroprotective effects of insulin. These findings underscore the divergent outcomes in cell survival following inflammasome activation by HIV-1 infection.

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HIV -Tat induced HIF-1α mediated astrocytic amyloidogenesis: Implications for Alzheimer's Disease

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Increased life expectancy of HIV patients in the post HAART era parallels with increased incidence of HIV-associated neurological disorders and associated comorbidities such as Alzheimer's Disease (AD). Interestingly, there have been reports on HIV-Tat-mediated production of the toxic neuronal amyloid protein and the interaction of the two leading to increased toxicity. In the current study we hypothesized that similar to neurons HIV-Tat could also induce amyloidogenesis in glial cells such as the astrocytes. In our in vitro findings we demonstrated upregulation of AD markers - BACE-1, amyloid precursor protein (APP), Aβ, p-Tau, in HIV Tat-exposed human primary astrocytes (HPA). Increased expression of cellular BACE-1 activity and Aβ-42 in the supernatant fluids of Tat-exposed astrocytes further validated the contribution of astrocytes in the amyloidogenic pathway. Molecular mechanism(s) underlying this process involved upregulation of hypoxia inducible factor (HIF-1α) with a concomitant translocation of HIF-1α to the nucleus, followed subsequently by its binding to the promoter of BACE-1 leading in turn, to increased expression of BACE-1 and consequential generation of Aβ-42 protein via cleavage of APP. Furthermore, overexpression of HIF-1α in astrocytes resulted in increased mRNA and protein expression levels of BACE-1, while gene silencing of HIF-1α resulted in abrogation of Tat-mediated upregulation of BACE-1 & Aβ levels, both intracellularly as well as extracellularly. BACE-1 silencing also abrogated Tat-mediated upregulation of Aβ generation, but failed to exert any effect on HIF-1α, indicating thereby the upstream involvement of HIF-1α in BACE-1 regulation. Further validation in the brains of SIV-infected macaques, revealed brain region specific upregulation of the amyloidogenic components that co-localized with GFAP positive astrocytes, thereby underscoring the contribution of astrocytes in the development of AD like symptoms associated with HIV infection. HIF 1-α can thus be envisioned as a novel therapeutic target for ameliorating Tat-induced astrocytic amyloidogenesis.

Supported by DA036157, DA043138.

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Creation of a Long-Acting Nanoformulated Dolutegravir

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Dolutegravir (DTG) is a potent integrase strand-transfer inhibitor with an excellent resistance profile for treatment of HIV-1 infection. Limitations in current treatment strategies include adherence, drug fatigue, and poor penetrance to viral reservoirs and herald the need for long-acting, slow effective release antiretroviral therapy (LASER ART). LASER ART can improve treatment adherence, and positively affect drug resistance and systemic toxicity patterns. Long-acting prodrugs that reduce systemic metabolism and polarity, and enhance cell membrane permeability and lipophilicity are also of high value. To this end, a modified DTG (MDTG) prodrug was synthesized, thoroughly characterized, incorporated into a poloxamer-nanoparticle platform, and efficacy evaluated. P407 nanoformulations were prepared for both DTG (NDTG) and MDTG (NMDTG) with uniform 234-360 nm particle sizes. NMDTG was rod-shaped, taken up avidly by MDM and demonstrated prolonged cell retention for > 30 days with no cytotoxicity. Native DTG activities were operative for a single day. The results paralleled antiretroviral activities by protection of MDM against HIV-1ADA challenge up to 30 days. PK testing in mice showed that apparent half-life was increased from 61.9 hours for NDTG to 330.4 hours for NMDTG, with blood drug levels above the IC90 (64 ng/mL) for > 8 weeks. At day 28, NMDTG-treated mice had significantly higher drug levels than NDTG-treated mice in all tissues tested and maintained detectable drug levels through day 56. NMDTG-treated humanized mice showed complete protection for three weeks when challenged with virus two weeks after a single dose; confirmed by plasma viral load, semi-nested real-time PCR, and HIV-1 RNAscope. Our data provide evidence that DTG conversion into LASER ART is readily achievable with reductions in dose and dosing intervals that extend to one month or longer and could be employed to improve regimen adherence, limit adverse reactions and drug fatigue, and facilitate drug penetrance into viral reservoirs.

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Morphine alters dopamine receptor type 2-expressing striatal medium spiny neuron physiology following acute exposure to HIV-1 Tat in a transgenic mouse model

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Despite use of combined antiretroviral therapy (cART), HIV-1 viral proteins such as HIV-1 trans-activator of transcription (Tat) can still be expressed in the CNS of HIV patients. HIV-1 Tat has deleterious effects on neuronal morphology and function, which may underlie the development of HIV-associated neurocognitive disorders (HAND) which affect 30-50% of HIV patients. Additionally, opiates can exacerbate the functional and morphological deficits seen in HIV-infected patients and animal models. Recently, using a transgenic mouse with glial fibrillary acidic protein-driven tet-on (doxycycline) inducible CNS expression of HIV-1 Tat bred with a Drd2-eGFP-reporter mouse, we showed that dopamine receptor type 2 (D2)-containing medium spiny neurons (MSNs) in the striatum are selectively vulnerable to 2 weeks of HIV-1 Tat induction. Two weeks of HIV-1 Tat exposure had biphasic effects on D2 firing patterns, decreasing neuronal firing between 200-300 pA, and increasing firing at 450 pA. To further characterize the D2 MSN response to HIV-1 Tat, and to examine the effect of morphine on D2 MSN firing patterns, Drd2-eGFP-reporter/Tat+/- mice were placed on a doxycycline-containing diet for 48 h, sacrificed, and the brains used for slice electrophysiology. D2 MSNs were identified using LED illumination and a filter for green wavelengths. Recordings were taken first in plain extracellular solution. Morphine (500 nM) was added to the solution for 5 min and a second recording was taken. Preliminary data suggest that among Tat- animals, morphine did not alter firing frequency, rheobase, firing threshold, or action potential amplitude. In Tat+ animals, following 48 h Tat induction, morphine increased firing frequencies at 200 pA and larger currents, as well as decreased rheobase. Thus, 48 h of Tat exposure is sufficient to cause alterations in D2 MSN neuronal firing patterns in response to morphine, potentially driving more serious functional changes than would occur with Tat or HIV exposure alone.

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NFE2L2 haplotypes are associated with HIV-associated neurocognitive impairment and intermediate phenotypes

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Background: Overlapping pathogenic processes lead to HIV-1 associated neurocognitive disorders (HAND), including oxidative stress and dysfunctional cellular antioxidant defense systems. Both host genotype and environment influences affect oxidative stress and antioxidant defense. We and others reported on nuclear factor erythroid-2-related factor 2 (Nrf2)-mediated antioxidant response pathway and HAND. In this study, we assessed whether haplotypes of NFE2L2, the gene that encodes Nrf2, affect susceptibility to HAND and associated histopathological markers. **Methods:** Participants from two cohorts were examined separately: 1) Multicenter AIDS cohort (MACS): Global neurocognitive functioning (GNF) was determined for 144 HIV+ and 59 HIV- MACS participants 2010-2014. 2) National NeuroAIDS Tissue Consortium (NNTC). GNF and several histopathological markers (GFAP, Iba-1, HLA-DR and A-beta) in frontal lobe grey matter were quantified for 164 HIV+ individuals. For participants in both cohorts, single nucleotide polymorphisms (SNPs) near/within NFE2L2 (rs10183914, rs13035806, rs16865105, rs1806649, rs2001350, rs2364722, rs2364725, rs2706110, rs2886161, rs3562124, rs6706649, rs6726395, rs7557529) were genotyped via Sequenom MassARRAY. Phenotype-haplotype associations were determined with HaploStats version 1.7.7, which uses generalized linear models, provides omnibus tests and allows for phase ambiguity. Covariates were ancestry (NNTC/MACS), HIV status (MACS) and age at death (NNTC). **Results:** MACS: 14 haplotypes have frequencies >1%; GNF ($p=0.05$) is significantly associated with these haplotypes but HAND is not ($p=0.10$). NNTC: 11 haplotypes have frequencies >1%; GFAP, HLA-DR, and IBA are significantly associated with these haplotypes ($p=0.015$, $p=0.021$, $p=0.019$), but not GNF or A-beta. In the MACS, when examining haplotype effects, alleles at rs2001350, rs6726395, and rs1806649 differ with extremes in GNF, consistent with single SNP analyses in which rs2001350, rs6726395, and rs1806649 are the most significantly associated. These patterns were not evident in the NNTC data. **Conclusions:** The use of haplotype analysis suggests that polymorphisms in or near NRF2 could be involved in HAND and related intermediate histological phenotypes.

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PARALYTIC RETROVIRUS-INDUCED SPONGIFORM NEURODEGENERATION IS PHYSIOLOGICALLY CHARACTERIZED BY GLIAL-MEDIATED MOTOR NEURON DEPOLARIZATION BLOCK

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Retrovirus (RV) infection of the central nervous system in humans and animals is associated with a broad spectrum of cognitive and motor abnormalities. Moreover, human endogenous retroviral (HERV) expression has been implicated as playing a causative role in several sporadic neurodegenerative diseases including multiple sclerosis, amyotrophic lateral sclerosis, and schizophrenia. To understand how RVs mediate neurodegeneration, we have undertaken a comprehensive neurophysiological interrogation of rapid, stereotypic, murine leukemia virus (MLV)-induced motor neuron disease models characterized by progressive non-inflammatory spongiform neuropathology. Our initial studies on the inferior colliculus (IC) demonstrated that glial infection selectively altered neurons characterized by after hyperpolarization firing (rebound neurons; RNs). These interneurons lost rebound firing, became hyperexcitable, and disrupted rhythmic circuits and auditory brainstem responses. Notably, similar but milder changes were observed with a control "non-neurovirulent" RV. To assess glial-mediated motor neuron (MN) changes, we extended these investigations to the vestibular nucleus (VN), a classical motor area in the brainstem. The virus-induced MN alterations observed in the VN included elevated firing thresholds, decreased firing frequencies, large inhibitory post-synaptic potentials (IPSPs), and depolarization block with ramp stimulation that failed to recover on ramp decrease. Importantly, "non-neurovirulent" virus caused similar, but milder MN changes, including threshold elevation, decreased firing frequency at maximum ramp stimulation and depolarization block at very high current ramps (however, with recovery during ramp decrease). These findings establish clear neurophysiological hallmarks for how RV expression within CNS glia can alter the intrinsic properties of MNs, RNs and their associated circuits. Moreover, these findings provide important mechanistic insight into virus-induced clinical manifestations, and set the stage for addressing whether similar neuron/circuit alterations are induced by human RVs or HERVs. In this presentation we will discuss the nature of the specific glial targets involved, and the means by which they precipitate the observed neurophysiological changes.

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Development of a Long Acting Slow Effective Release Phosphorylated Emtricitabine Prodrug Nanoformulation

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Despite the wide spread use of antiretroviral therapy (ART) with notable diminished disease-associated morality and morbidities the human immunodeficiency virus type one (HIV-1) continues to replicate at low levels within CD4+ memory effector T lymphocytes, dendritic cells and monocyte-macrophages in the gut associated lymphoid tissue, lymph nodes, spleen and the central nervous system. Our laboratories have developed second and third generation long acting slow effective release ART (LASER ART) to improve antiretroviral drug (ARV) apparent half life, cell and tissue biodistribution. Our works have demonstrated that this can be achieved through medicinal chemistry and polymer formulations and through affecting drug metabolism through producing hydrophobic and lipophilic nanocrystals. While progress has been made towards developing long-acting hydrophobic nanoformulations, intracellular delivery of hydrophilic phosphorylated active metabolites of nucleoside reverse transcriptase inhibitors has remained elusive. That is until now. In the current work we synthesized a long acting emtricitibine (FTC) through phosphorylation of the parent compound with a lipophilic chlorophoshoramidate. This produced a modified next generation FTC that is named M1FTC. The chemical structure of M1FTC was confirmed by nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR). Poloxamer 407 stabilized nanoformulated M1FTC (NM1FTC) was prepared by high-pressure homogenization and evaluated for human monocyte-macrophage uptake, retention and antiretroviral efficacy. NM1FTC was characterized by an average particle size of 400 nm, negative charge, narrow polydispersity index and loading capacity of greater than 60%. The synthesized NM1FTC particles remained stable for over a month. NM1FTC exhibited enhanced monocyte-macrophage drug uptake when compared to either native drug or to comparative sized FTC nanoparticles. In summary, a long acting NM1FTC that has potential to improve intracellular accumulation of FTC active metabolites for improved antiretroviral activities. Biodistribution and pharmacokinetic profiles of the NM1FTC particles are currently being tested.

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Cerebral amyloid-β plaques link host genotypes to neurocognitive impairment among HIV-infected adults

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Background: Neurocognitive impairment remains common among HIV-infected adults. We hypothesized that host genotypes, age, and cerebral gliosis (as evidence of neural injury in HIV disease) would predict Alzheimer's disease (AD)-related pathology (that is, amyloid-β plaque deposition and neuronal phospho-tau pathology), which would in turn predict neurocognitive impairment. **Methods:** We studied genetic variants reported to be associated with late-onset AD or immune response. We assessed AD-related pathology, microgliosis, and astrogliosis by immunohistochemistry in the frontal neocortex, putamen, basal-temporal neocortex, and hippocampus of HIV-infected adults (age 26-70 years). We used logistic regression to predict regional AD-related pathology, based on host genotypes, age, and gliosis. We used multiple linear regression to predict neuropsychological domain T-scores, based on regional AD-related pathology and gliosis. The Bonferroni correction was applied (α=0.05). **Results:** APOE-ε4, CD33-rs3865444, and age were predictive of amyloid-β plaque deposition in frontal neocortex (n=162; odds ratio (OR) 6.05, 3.26, 1.07; p<0.002, =0.02, =0.004, respectively); IL10-rs1800872, IL1A-rs17561, and age in putamen (n=94; OR 0.10, 0.09, 1.10; p<0.002, =0.004, =0.012, respectively); APOE-ε4 in hippocampus (n=67; OR 4.74; p=0.028); and none of factors tested in basal-temporal neocortex (n=66). CXCL12-rs1801157 was predictive of neuronal phospho-tau pathology in frontal neocortex (n=171; OR 0.35; p=0.036); CR1-rs6701713 and IL6-rs1800796 in putamen (n=97; OR 3.88, 4.60; p=0.028, =0.036, respectively); black race and age in basal-temporal neocortex (n=73; OR 21.05, 1.11; p<0.002, =0.028, respectively); and CD2AP-rs9349497 and age in hippocampus (n=69; OR 0.19, 1.12; p=0.044, =0.012, respectively). Neither microgliosis nor astrogliosis significantly predicted AD-related pathology. Amyloid-β plaque deposition in putamen predicted lower attention domain T-scores (n=86, Beta -0.294, p=0.032). **Conclusion:** In the context of HIV disease, certain genetic variants, black race, and age, but not cerebral gliosis, predicted regional AD-related pathology. The putamen amyloid-β plaque deposition served as an intermediate phenotype linking IL10-rs1800872, IL1A-rs17561, and age to attention impairment among HIV-infected adults.

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Analysis of co-linear genetic variation across two exons of the HIV-1 tat gene from patients of the Drexel CARES Cohort using third generation PacBio sequencing

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HIV-1 mortality has decreased with the prolonged use of suppressive antiretroviral therapy (ART) while the incidence of HIV-1-associated neurocognitive disorder (HAND) has increased. The HIV-1 Tat protein has been shown to cause neurotoxicity and be associated with neuroinflammatory and neurodegenerative CNS disease. We have recently performed studies to identify and characterize predominant amino acid variants within Tat that associate with HAND. To this end, HIV-1 Tat sequences were obtained from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. The initial studies amplified Tat exon I and II separately from patient PBMC populations. The PCR amplicon was then sequenced with Illumina next-generation sequencing. The sequences were translated and aligned to the HIV-1 subtype B reference genome, HXB2, to identify amino acid substitutions. Multiple positional hotspots of high variation in Tat were identified within both the first and second exons. Statistical analyses were applied to the amino acid positions and variants were each associated with a HAND diagnosis. To be able to examine the co-selection of amino acid variants that exist in both Tat exons, PCR primers encompassing both exons were selected. This amplification strategy was used on patient samples yielding 17 samples with the target 3-kilobase Tat DNA fragment. These patient samples were sequenced using PacBio long-read next-generation sequencing

technology which resulted in an average of 15,000 HIV reads per sample. Preliminary results demonstrated that some samples have a single dominant Tat exon I and II sequence while others have many minor variants. Future studies will continue to examine co-selected amino acid variants across both Tat exons.

P280

Morphine synergizes with CCL5 in neuroadaptations of the reward circuity of the rodent brain during conditioned place preference trials

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Opioid addiction, including misuse of prescription pain relievers and abuse of illicit substances, is an exploding national crisis. It is estimated that greater than 2 million individuals suffer from the effects of substance abuse and withdrawal cycles nationwide. Prolonged usage of opioids produces cellular and molecular neuroadaptations that alter reward circuitry in the mammalian brain. Escalating morphine treatments, simulating opioid tolerance and dependence, have been shown to increase CCL5 levels in the rodent cortex, striatum, and midbrain. Much less is known about how chemokines modify the reward circuity during the initiation of drug abuse and how this may predispose the organism to further tolerance and dependence. In the present study, we sought to elucidate the role of two chemokines (CCL5 and CX3CL1) in modifications of the reward circuity during initiation of opioid abuse using the conditioned place preference (CPP) paradigm. We detected a modest increase in the levels of CCL5 in the prefrontal cortex in rats subjected to regular morphine injections when compared to controls. Interestingly, we also observed a decrease in CX3CL1 across multiple brain regions after exposure to chronic morphine. Using an intra-accumbal injection of a lentiviral vector, we overexpressed CCL5 in a second cohort of rats to determine if CCL5 synergizes with morphine during CPP testing. CCL5 lentivirus-injected rats demonstrated higher CPP scores when compared to controls. Together, our results indicate that CCL5 may act to modulate the effects of reward circuits relevant to opioid abuse.

P281

Aprepitant reduces inflammatory markers in HIV infected cART treated and cART naïve adults

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HIV infection is associated with systemic inflammation. Substance P (SP) is an undecapeptide involved in neurotransmission and inflammation. SP effects are mediated via neurokinin 1 receptor (NK1R). Plasma SP levels are elevated in HIV-infected individuals. NK1R antagonists, including aprepitant, have anti-HIV and anti-inflammatory effects. HIV infection is associated with metabolic and pro-inflammatory changes, including activation of tryptophan catabolism via the kynurenine pathway, leading to increase in plasma Kynurenine-to-Tryptophan ratio (KTR). Increased tryptophan catabolism is associated with HIV comorbid conditions and is a potential novel avenue for therapy. We studied 57 HIV infected individuals (45 cART naïve and 12 on a ritonavir containing cART regimen). Aprepitant treatment did not affect viral load. Aprepitant treatment led to a decrease in plasma levels of SP and IL6 and a modest increase in cholesterol levels (total, HDL and LDL) in both cART treated and cART naïve individuals. In cART naïve individuals aprepitant treatment also decreased levels of several pro-inflammatory markers (TNFα, MIP-1α, G-CSF, IL-8, sCD163 and PD-1 expression by CD4+ T-cells). In cART naïve individuals aprepitant treatment was associated with decrease in kynurenine levels but not KTR while in cART treated individuals plasma KTR decreased (-15.8; $p<0.01$). Analysis of plasma levels of 1300 proteins using SOMAscan assay in cART treated individuals identified 176 plasma proteins (56 after FDR adjustment) and several metabolic pathways affected by aprepitant treatment including inflammation, immune response, apoptosis, cell adhesion and lipid metabolism (JCI Insight. 2017 Oct 5;2 epub). Conclusions: Aprepitant was safe and well tolerated. Aprepitant administration changed expression of inflammation associated biomarkers. The pattern of affected molecules is different between cART treated and cART naïve individuals. Aprepitant may have role as an adjunctive therapy to reduce comorbid conditions associated with HIV infection.

Supported by NIH U01 MH090325, P30 MH097488 to SDD and P01 MH105303 to JR and SDD.

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Identifying and characterizing therapeutic antagonists to HIV Tat protein

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The Tat protein of HIV is a small, highly basic, regulatory protein that is the first protein to be produced during viral replication. Tat plays a critical role in driving viral replication by transactivation of the Long Terminal Repeat (LTR) promoter region of the virus, and has long been implicated in the pathophysiology of HIV-Associated Neurocognitive Disorders (HAND). Although Tat has been extensively studied, it remains an elusive target. We have established a Tat-mediated HIV LTR transactivation assay with which we can identify new antiviral agents that target Tat itself. Using this cell line in high throughput screening assays in 384 well plate format, we've screened more than 2000 compounds from the Spectrum Collection to identify therapeutic antagonists to Tat-TAR binding and subsequent LTR transactivation. We have confirmed activity and are completing characterization of the screening "hit" compounds in concentration dependent analyses. Ultimately we will determine their direct binding interactions with HIV Tat, demonstrate HIV antiviral activity and adjunctive neuroprotective efficacy versus Tat exposure in order to provide a novel therapeutic for HIV infection.

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Inducible HIV-1 Tat transgenic mice display a decrease in [3H]Dopamine uptake via the dopamine and norepinephrine transporters in the prefrontal cortex

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HIV-1 Tat protein and cocaine synergistically impair dopaminergic transmission by directly inhibiting the dopamine (DA) transporter (DAT), which has been implicated as a risk factor of HIV-1 associated neurocognitive disorders (HAND). The inducible HIV-1 Tat transgenic (iTat-tg) mouse model

recapitulates many aspects of the neuropathologies-cognitive impairments observed in HIV-infected individuals. Additionally, the prefrontal cortex (PFC) is an important brain region for higher cognitive function, where the norepinephrine (NE) transporter (NET) plays a critical role in reuptake of DA and NE. This study determined the pharmacological profile of DA transmission in iTat-tg mice following 7-day administration of doxycycline (Dox) or saline. The Vmax of [3H]DA uptake via the DAT and the NET in PFC of Dox-treated iTat mice was decreased by 26% and 27%, respectively, compared to their respective saline controls, while no differences in the Vmax was found between Dox and saline treated control C57BL/6J mice. This decrease in Vmax of [3H]DA uptake was not accompanied by changes in total DAT or NET expression. Consistently, HPLC analysis revealed a 268% increase in DA tissue content and a 144% increase in DOPAC in the PFC of Dox-treated iTat-tg mice, relative to saline control mice. In a separate experiment, we performed in vitro patch clamp recordings to measure membrane action potentials (APs) in nucleus accumbens shell medium spiny neurons in iTat-tg mice. Results showed that both acute application of cocaine or cocaine/DA reduced the APs in saline control mice, which was attenuated in Dox-treated iTat-tg mice; however, C57BL/6J mice treated with saline or Dox retained a similar AP response to cocaine or cocaine/DA. These findings add to the evidence that in vitro Tat-induced inhibition of DA transport can be documented in iTat-tg mice, which may be responsible for the neurocognitive deficits observed in HAND.

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IFN-λ4 inhibits HIV infection of macrophages by signaling through IFN-λ R1/IL-10R2 receptor complex

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The recently discovered IFN-λ4 has been found to have antiviral activity against a number of viruses. However, it is unknown whether IFN-λ4 has the ability to inhibit HIV infection/ replication. Here, we show that IFN-λ4 could suppress HIV infection of macrophages, the primary target and reservoir of the virus. Mechanistic studies demonstrated that the IFN-λ4-mediated HIV inhibition in macrophages could be compromised by the antibodies against IFN-λ receptor complex, IFN-λ R1/IL-10R2. In addition, IFN-λ4 treatment of macrophages enhanced the phosphorylation of STAT1, and induced antiviral interferon-stimulated genes (ISGs: ISG56, GBP5, and Viperin). These findings suggest the potential for the use of IFN-λ4 as a therapeutic agent for HIV infection.
Supported by National Natural Sciences Foundation of China (81360258 and 81571962), and in part by the National Institutes of Health Grants (DA022177, DA041302, DA040329 and MH109385).

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Identification of tissue HIV-1 reservoirs in infected humanized mice

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Eradicating reservoirs of HIV-1 infection remains the major obstacle in achieving viral sterilization. Thus, gaining a comprehensive understanding of where and to what extent viral replication occurs in tissue sites of HIV-1 persistence is of immediate importance and especially pivotal during suppressive antiretroviral therapy (ART). Defined primate species tropism of HIV-1 restricts its studies to humanized mice. These models provide insights into the pathobiology and therapy of infection. We used two humanized mouse models of HIV/AIDS to study viral persistence. This includes both human CD34+ hematopoietic stem cells (hu-HSCs) and peripheral blood lymphocytes (hu-PBLs)-transplanted NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ mice. Both models were used to investigate early events in the viral life cycle and the establishment of latency. Twenty hu-HSCs or hu-PBLs mice were injected intraperitoneally with HIV-1ADA at 104 TCID50. Infected animals were randomly separated into 4 groups then sacrificed at day 3, 5, 7 and 14 after infection. Blood and tissues were harvested for immunohistochemistry, RNA-scope and seminested-PCR tests for HIV-1 DNA and RNA. Peripheral CD4+ T cells were reduced by 10% after HIV-1 infection in hu-HSCs mice, but over 20% in hu-PBLs mice. IHC tests showed HIV-1p24 expressing cells only in 14-day infected hu-HSCs mice, but in all infected hu-PBLs mice. HIV-1 RNA and DNA were observed in 14-day infected hu-HSCs mice from spleen lung liver and gut up to 107 copies/106 hCD45+ cells; whereas virus was detected in 2/5 animals (105 copies/ml) at early time points. Infection was observed in all infected hu-PBLs mice (105 copies/106 hCD45+ cells in 3-day group and 108 copies in other time points). RNA-scope confirmed viral replication in the same animals. HIV-1 was detected in both hu-HSCs and hu-PBLs models as early as 3 days after infection and increased with time. Targeting these host factors may provide additional clues on how HIV-1 persistence is established.

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Upregulation of Neuroprotective Superoxide Dismutase 2 by Astrocytes in the SIV/Macaque Model of HIV

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HIV-associated neurocognitive disorders (HAND) remain prevalent despite the use of antiretroviral therapy (ART). Development of HAND is linked to mitochondrial dysfunction and overproduction of reactive oxygen species (ROS) in the brain, therefore, upregulation of antioxidant defenses is critical to curtail neuronal damage. Superoxide dismutase 2 (SOD2) is a mitochondrial antioxidant enzyme essential for quenching ROS and maintaining cellular viability. We hypothesized that SOD2 is upregulated during HIV infection to reduce cellular susceptibility to oxidative injury. Using a simian immunodeficiency virus (SIV)-infected macaque model of HIV, we measured SOD2 expression in cortical gray matter (GM) and white matter (WM). Quantitative PCR (qPCR) showed SOD2 mRNA was increased in both GM (mRNA 7.6 for SIV relative to uninfected) and WM (mRNA 77 for SIV relative to uninfected) during SIV infection. In addition, SOD2 immunostaining quantified by digital image analysis showed enhanced expression in both GM (fold change from uninfected 11.0 for SIV) and WM (fold change from uninfected 60.8 for SIV) in untreated SIV-infected animals. Confocal microscopy evaluation of double immunofluorescence labeling demonstrated that SOD2 primarily co-localized with the astrocytic marker glial fibrillary acidic protein (GFAP) in WM of untreated SIV-infected animals. In contrast to untreated SIV infection, after suppressive ART, CNS SOD2 levels were similar to levels present in uninfected animals with marked reduction in astrocyte SOD2 expression. Together, our data provide evidence that astrocytic expression of SOD2 is enhanced in the brain during SIV infection but this neuroprotective mechanism is not sustained with ART. Whether novel therapies enhancing SOD2 may be useful for delaying the onset or reducing severity of HAND seen in HIV-infected patients on ART can be studied using the SIV model.

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Determining the specificity of anti-HIV-1 gRNAs used for CRISPR/Cas9 cure strategies: Uncovering on- and off-target effects using GUIDE-seq

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The CRISPR/Cas9 gene-editing system has been successful at disrupting viral gene expression and excising the HIV-1 genome from infected cells. However, few anti-HIV-1 gRNAs are able to account for the genetic variation in the viral quasispecies (vQS) of patients. Our in silico analysis has demonstrated only two published anti-HIV-1 gRNAs targeting the LTR can cut >90% of the patient-derived HIV-1 subtype B LTRs from the Los Alamos National Laboratory (LANL) HIV database. Therefore, we have designed broad-spectrum anti-HIV-1 gRNAs to target all of the vQS without having off-target effects. To do this, LTRs from 269 patients enrolled in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort were deep-sequenced. A novel gRNA design pipeline was designed to select for optimal gRNAs and filter out those with predicted off-target binding. These results informed the design of a 4 and 10 selected molecular gRNA targets (SMRT) package. Utilizing an in silico approach, the SMRT-4 and -10 packages were shown to excise all subtype B LTRs from LANL and the CARES Cohort, without any predicted off-target excision. Functional studies have demonstrated that the SMRT-4 gRNAs performed better than other gRNAs in numerous assays, especially at cleaving patient-derived HIV-1 sequences. Studies were then performed to determine if there was off-target excision using genome-wide, unbiased identification of DSBs enabled by sequencing (GUIDE-Seq). This technique will be used to determine if off-target excision events occur with the SMRT gRNAs and others on a whole-genome level with NGS sequence coverage. GUIDE-seq was used to identify off-target excision events by the sequencing off a short 35bp oligonucleotide (linker) that integrates anywhere Cas9 induces a double-stranded DNA break. GUIDE-seq results have indicated that the SMRT gRNAs do not demonstrate off-target excision. These studies will advance a second-generation of CRISPR/Cas9 therapeutics and inform design of novel anti-HIV-1 gRNAs.

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Endocannabinoid, anandamide ameliorates acute lung injury through regulation of Micro-RNAs pathway induced anti-inflammatory response and modulation of both oxygen consumption rate and extra cellular acidification rate

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Staphylococcus Enterotoxin B (SEB) produced by Staphylococcus aureus is a superantigen that activates ~ 30% of T cells. The inhalation of SEB leads to toxic shock and death. SEB is a CDC select agent of bioterrorism. In the current study, we used an intranasal dose of SEB to induce acute lung Injury (ALI) in C57BL/6 mice. We found that treatment with anandamide (AEA) alleviated ALI in SEB-exposed mice. AEA is part of the endocannabinoid system (ECs) and binds to CB1 and CB2 receptors which are expressed primarily on neurons and immune cells, respectively. Next, we examined whether miRNA mediate the protective effects of AEA on SEB-induced ALI. Microarray analysis of lung-infiltrating mononuclear cells revealed 60 up- and 77 down-regulated miRNA in SEB+AEA mice relative to SEB+Veh. Using Ingenuity Pathway Analysis (IPA), We found that both miR30c-5p and miR 194b-5p were downregulated which led to upregulated expression of Suppressor of Cytokine Signaling (SOCS1) leading to suppression of pro-inflammatory cytokine, IFNγ as well as IL-10 genes, both of which are considered as potent inhibitors of inflammation. The miR and target genes were validated by RT-PCR and cytokine expression by ELISA. Flow cytometric analysis showed a significant increase in CD4+IL10+ in SEB+AEA compared to SEB+VEH groups. Furthermore, we determined the metabolic profile of SEB-activated splenocytes treated with vehicle or AEA by performing the glycolysis rate assay (GRA). We found that AEA-treated SEB-activated T cells became quiescent metabolically in comparison with the energetic vehicle-treated SEB-activated T cells. Furthermore, we found a significant decrease in both Oxygen Consumption Rate (OCR) and Extra Cellular Acidification Rate (ECAR) in vehicle-treated SEB-activated T cells in comparison with AEA- treated SEB-activated T cells. Collectively AEA ameliorates ALI through both regulation of miRNA pathway which upregulate SOCS1 and IL-10 expression, leading to an anti-inflammatory phenotype while modulating the metabolic profile.

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Detecting amnestic MCI (aMCI) among HIV+ individuals with high rates of HIV-associated neurocognitive disorder (HAND)

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Background: Older HIV+ individuals are at risk for age-associated neurodegenerative disorders [e.g., Alzheimer's disease (AD) and its precursor amnestic mild cognitive impairment (aMCI)]. Identifying aMCI among HIV+ individuals is challenging given the overlap in neurocognitive and neuropathologic characteristics of aMCI and HIV-associated neurocognitive disorders (HAND). With that said, aMCI is associated with greater cerebral β-amyloid (Aβ) plaque and phospho-Tau pathology, and more amnestic memory deficits (recall and recognition deficits in aMCI versus recall, but not recognition, deficits in HAND). We aimed to develop and test neuropsychological methods of identifying aMCI in HIV. **Methods**: Participants included 100 HIV+ cases (age: 50-70 years) from the National NeuroAIDS Tissue Consortium that had a neurocognitive assessment within a year of death and neuropathological characterization of frontal Aβ plaque and phospho-Tau pathology. We applied an empirical neuropsychological diagnostic approach to identify aMCI (<1.0 SD below demographically-corrected normative mean) on two tests of memory recall and/or recognition (at-least one recognition impairment required) from the Hopkins Verbal Learning Test-Revised and the Brief Visuospatial Memory Test. Differences between the aMCI and HAND diagnostic groups in the presence of frontal Aβ plaque and phospho-Tau pathology were examined. **Results:** There were significantly more aMCI diagnoses in the HAND group (71%) than the non-HAND group (51%, p=.02). Using logistic regression adjusting for age, education and apolipoprotein-E genotype, the presence of phospho-Tau pathology did not differ between aMCI groups; however, Aβ plaques were 3.8 times more likely in the aMCI versus non-aMCI group (p=.02). Aβ plaques or phospho-Tau pathology did not differ between HAND groups. **Conclusions:** The overlap in aMCI and HAND diagnoses suggests that many aMCI cases in HIV+ individuals are undetected because impairment is assumed to be HAND. An aMCI cognitive and biomarker profile may be detected in HIV+ populations by identifying consistent impairment on memory tests, particularly recognition.

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Activity-dependent HIV-induced synaptic excitotoxicity via cGMP-dependent protein kinase II activation in the FIV infection model

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Over half of human immunodeficiency virus (HIV)-infected individuals suffer from HIV-associated neurocognitive disorders (HAND), yet the molecular mechanisms leading to neuronal dysfunction and death are poorly understood. Feline immunodeficiency virus (FIV) naturally infects cats and shares its structure, cell tropism, and pathology with HIV, including wide-ranging neurological deficits. We employ FIV as a model to elucidate the molecular pathways underlying HIV-induced neuronal dysfunction. Among HIV-induced neuron-damaging products, the HIV envelope glycoprotein gp120 triggers elevation of intracellular Ca2+ in neurons, resulting in apoptosis. By quantifying neuronal activity using intracellular Ca2+ imaging in mouse cultured hippocampal cells, we confirmed that the FIV envelope glycoprotein gp95 also elevates intracellular Ca2+ activity. In addition, we revealed that gp95 interacted with the chemokine receptor, CXCR4, and facilitated the release of intracellular Ca2+ by the activation of the endoplasmic reticulum (ER)-associated Ca2+ channels, inositol triphosphate receptors (IP3Rs). This suggests that gp120 and gp95 share a core pathological process in neurons. Significantly, gp95's stimulation of glutamate NMDA receptors activates cGMP-regulated kinase II (cGKII) through the activation of the neuronal nitric oxide synthase (nNOS)-cyclic GMP (cGMP) pathway, which increases Ca2+ release from the ER and promotes surface elevation of AMPA receptors, causing synaptic excitotoxicity. Moreover, we cultured feline hippocampal neurons and confirmed that gp95-induced neuronal excitotoxicity was mediated by CXCR4 and cGKII. These results thus provide a novel cGKII-dependent cellular understanding of synaptic dysfunction conserved in cats and humans.

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Crossing the blood brain barrier with bispecific antibodies

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Background: PGT121, a broadly neutralizing antibody against HIV, showed promise in prolonged suppression of the viral replication in non human primate infected with the Simian HIV virus (SHIV). However, it is unclear if it can cross the blood brain barrier to target viruses in the CNS. As such, the antibody quantities measured in the CSF of monkeys infused with IV PGT121 showed less than 0.2% of the quantities of those in the periphery. To increase PGT121 penetrance into the CNS, we hypothesized that a bispecific antibody with one arm binding HIV and the other arm binding the human transferrin receptor for receptor mediated transmigration will deliver higher quantities of antibodies across the blood brain barrier. **Methods:** Using Fab arm exchange methods, we generated a bispecific antibody with one arm binding PGT121 and the other arm binding the human transferrin receptor. We tested this antibody's neutralization functions by the TZM infected cells. A sandwich ELISA was developed to determine dual binding properties of the bispecific antibody. We infused this bispecific antibody intravenously into 3 healthy monkeys. **Results:** We generated a bispecific antibody PGT121/HTfR, where one arm binds to HIV and the other to the human transferrin receptor for receptor mediated transcytosis across the blood brain barrier. It neutralized majority of the HIV and SHIV strains with one log higher in titer as compared to the parent PGT121. Infusion into healthy rhesus macaques did not cause any significant clinical effects. We measured CSF concentration of the antibodies and demonstrated an increase over PGT121. **Conclusions:** Bispecific with antibody designed for receptor mediated crossing of the blood brain barrier may have potential in treatment of neuro HIV.

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Co-occurrence of PD-1 with 2B4, TIGIT, and TIM-3 on T cells from HTLV-I infected HAM/TSP patients

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Targeting inhibitory receptors is an established strategy in cancer as well as chronic infections and could be applicable for infection-associated neuroinflammatory diseases. HTLV-1 is an etiologic agent of HAM/TSP. There is no effective treatment or vaccine for this infection to date. Despite the high frequency of virus-specific CD8+ T cells, CTL responses are ineffective in controlling HTLV-1 viral loads, suggesting that T cell exhaustion may be a potential mechanism limiting immune control or viral clearance. Previous studies from our group in a Jamaican HTLV-1 cohort showed that PD-1:PD-L1 pathway directly correlated with heightened proviral load, dysregulated dendritic cells' functions, reduced polyfunctional T cells, and reduced MIP-1α expression during HTLV-1 associated oncogenesis and neuroinflammation. Bioinformatics analyses of existing data revealed increased expression of PD-1, TIGIT, and 2B4 in infected patients compared to controls. This was confirmed on HTLV-1-infected subjects from a US based cohort that showed co-existence of PD-1 and TIGIT on both CD4 and CD8 T cells. This co-expression was more pronounced on Tax (11-19)-specific CD8 T cells, the immunodominant epitope of Tax seen in HLA-A2+ patients. These cells also over expressed TIM-3 and 2B4 along with PD-1. More recent data from two cohorts from Brazil and US, showed a higher frequency of CD4 T cells expressing TIM-3 and TIGIT and CD8 T cells expressing TIM-3 and Lag3. Accordingly, we observed a greater expression of PD-L1 alone and in co-occurrence with PVR (TIGIT ligand) on mDCs and pDCs from HAM/TSP patients. Collectively, these results demonstrate involvement of two or more immune checkpoint regulators in defining anti-viral T-cell responses during HTLV-1 chronic infection, which will be extended to a bigger cohort of Brazilian patients. A complete understanding of this will assist in designing an effective blockade strategy to restore the polyfunctionality and cytolytic potential of antigen-specific T cells in HAM/TSP patients.

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HIV-1 TAT-mediated activation of microglia involves mitochondrial dysfunction

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Despite the effectiveness of combination antiretroviral therapy in suppressing viremia, CNS continues to harbor viral reservoirs with persistent low-level virus replication, leading to the accumulation of early viral protein such as HIV-1 TAT. HIV-1 TAT is a well-recognized cytotoxic agent, contributing to glial activation. HIV-1 TAT-mediated activation of microglia has been shown to cause dysfunction and degeneration of surrounding and distant neurons. Based on this premise, we sought to explore the role of impaired lysosomal fusion and defective mitophagy in HIV-1 TAT-mediated microglial activation. Our results demonstrated that exposure of mouse primary microglia to HIV-1 TAT resulted in cellular activation involving altered mitochondrial membrane potential that was accompanied by accumulation of damaged mitochondria. Exposure of microglia to HIV-1 TAT resulted in increased enrichment of mitophagy signaling proteins, such as PINK1, PRKN, and DNM1L, in the mitochondrial fractions. HIV-1 TAT exposure also resulted in increased formation of mitophagosomes, as evidenced by the increased presence of

mitochondria in double-membrane autophagosomes (TEM images), co-localization of mitoDsRed with LC-3 and increased expression of BECN1 and LC-3. Intriguingly, exposure of microglia to HIV-1 TAT also resulted in increased expression of p62, signifying thereby a possible blockade of the mitophagy flux. Also, HIV-1 TAT exposure also resulted in decreased expression of LAMP-2, a lysosomal marker protein and decreased lysosomal fusion with mitophagosomes, leading, in turn, to increased accumulation of mitophagosomes. Interestingly, HIV-1 TAT-mediated activation of microglia was associated with decreased rate of extracellular acidification and mitochondrial oxygen consumption and increased expression of proinflammatory cytokines, such as TNF-α, IL1β, and IL6. HIV-1 TAT-mediated mitochondrial dysfunction and defective mitophagy leading to microglial activation was also validated in vivo in the brains of HIV-1 transgenic rats. In summary, HIV-1 TAT activates microglia by inducing mitochondrial damage and defective mitophagy via impaired mitophagosome-lysosomal fusion.
Supported by DA036157, DA043138, DA044586, MH062261

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Modulating cellular autophagy for controlled drug delivery

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URMC-099, a MLK3 inhibitor was shown to induce autophagy extending macrophage depots of nanoformulated antiretroviral therapy (nanoART). This was due to drug nanoparticle sequestration in endosomes and multi-vesicular bodies (autophagosomes) whihc prolonged drug retention and antiretroviral potency. As a range of autophagy inducers display variant mechanisms in affecting autophagy, comparisons were made for nanoART retention and antiretroviral activities for URMC-O99, Rapamycin (a mTORC1 inhibitor), Clonidine (a mTOR independent imidazoline-1 receptor agonist), 2-Hydroxypropyl-β-cyclodextrin (HBC, a transcription factor EB mediated, Metformin (an AMPK activator mTOR inhibitor) and Desmethylclomipramine (DMC) (a regulator of the autophagic flux and the autophagosome-lysosome). They were administered for testing at doses of 1 μM, 10 nM, 0.5 μM, 20 μM, 0.5 mM and 0.5 μM respectively. Each were studied using the test drug atazanavir (nanoATV) with or without HIV-1 infection of human monocyte-derived macrophages (MDM). Maximum drug retention was obtained by URMC-O99 and clonidine (17 μg versus 10 μg/10^6cells without an autophagy inducer). Western blot analysis of each of the drugs confirmed autophagy. HIV-1 infected cells treated with sub therapeutic nanoATV (1 μM) with each of the autophagy inducers, notably, showed enhanced antiretroviral activity with the exclusion of HBC while URMC-O99 demonstrated the highest activities. These data, taken together, support a novel antiretroviral boost by URMC-O99 and other autophagy inducers that can be utilized to improve therapeutic efficiency in future.

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Withaferin A is a neuroprotective agent: Studies towards neurocognitive disorders

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In this era of antiretroviral therapy, the life span of the HIV-infected individuals have increased leading towards increased neurocognitive dysfunction in nearly 30% of HIV-infected individuals, specifically older people. Deposition of the amyloid-β (Aβ) plaques in the CNS is one the major phenomenon happening in ageing HIV patients. ART suppresses the viral replication, but the neurotoxic protein (Tat) is still produced and results in increased levels of Aβ. In this study we show that in SHAPP cells, Tat increases the secreted Aβ levels. Our Fluorescence microscopy studies show the increased concentration of Aβ40 in Tat (50ng/ml) treated cells as compared to control. We also confirm the dose dependent neurotoxic effects of Tat towards increased Aβ secretion by our flow cytometry studies, where different concentrations of Tat were used and 50ng/ml was the most neurotoxic dose. Additionally, we have studied a potent molecule Withaferin A (WA) which reduces secreted Aβ and induced neurotoxicity. In order to address the issue of less drug bioavailability in the CNS, we have developed WA loaded liposomal nanoformulation (NF) and studied its size, charge and drug binding efficacy. The developed NF had a hydrodynamic size of 599nm and showed 28% drug binding capacity, with no cellular toxicity. We have studied the BBB integrity by measuring the trans-endothelial electrical resistance (TEER) and paracellular permeability using fluorescein isothiocyanate (FITC)-dextran transmigration of the developed NF. Currently we are working on the efficacy studies of the NF towards, AB40 induced neurotoxicity.

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Effect of HIV-1 Tat on the expression of an uncharacterized long non-coding RNA U1 in Rat primary neurons

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Long non-coding RNAs (lncRNAs) are a class of RNAs larger than 200 base-pairs which appear to lack a potential protein coding sequence. The emergence of lncRNAs as key players in a disease etiology placed them in the spotlight to study the pathogenesis of human diseases. HIV-1 Tat protein is a trans-activating regulatory protein, essential for efficient transcription of the viral genome. Little is known about the effect of Tat on the transcriptome and particularly on lncRNAs. Here in, we hypothesized that Tat may modulate host gene regulation by altering the lncRNAs expression in neurons. To test our hypothesis, we treated rat primary neurons with purified recombinant Tat protein for 48 hrs and RNASeq analysis was performed on treated and non-treated cultures. Sequencing and quantifications by bioinformatics approaches revealed global alterations of several key cellular genes as well as up- and down- regulation of multiple lncRNAs. We have selected multiple candidates and designed specific primers for RT-PCR. One of the up-regulated candidate lncRNAs, namely U1, showed two fold increase in Tat treated neurons and revealed a splicing alteration event that caused the formation of a new isoform. Studying thirteen existed QTLs in U1 chromosome region, we found that some of the genes are up-regulated. Meanwhile one of the genes associated with anti-stress response in neurons as an opioid ligand. So, first we are going to detect any possible linkage between U1 and detected differentially expressed genes in QTLs. Then we will figure out if U1 has any regulatory effect on investigated genes. In this point we can conclude that HIV-tat protein can alter the cell global gene expression in rat hippocampal neurons that it may happen by altering some gene regulatory elements.

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HIV and Aging - Studies from NNTC

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With the introduction of effective antiretroviral therapy (ART), the disease profile of HIV has shifted to that of a complex chronic illness. Owing to the increased life expectancies of those living with HIV, a need has arisen to understand what it means to be healthy in the context of HIV infection. Aging in this context is a complex process - involving both the clinical features normally observed in aging and the addition of complicating factors such as the effects of long-term ART exposure and the presence of HIV-associated factors. Much of the research on HIV and aging has focused on virally mediated changes that lead to a compression of the aging process in HIV infected individuals. These changes may result in biological changes that are normally observed in much older individuals. Using both the CHARTER and NNTC clinical research cohorts (studies designed to examine the neurological effects of HIV in the context of broader characterization of the participants), researchers have observed this apparent age advancement in HIV infected individuals as measured through changes to the methylome, prolonged occurrences of astrocyte activation, neurodegeneration, and advanced disease progression. Other investigators have focused on chronic inflammation and cognitive decline. Here we will explore the presentation of aging within and across the NNTC and CHARTER clinical research cohorts, and illustrate the utility of the data and specimen resource of the NNTC, now inclusive of CHARTER.

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Simian Varicella Virus (SVV) DNA in Saliva and Buccal Cells Following Experimental Infection in Rhesus Macaques

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Simian varicella virus (SVV) infection of primates is the counterpart of varicella zoster virus (VZV) infection in humans. We tested the ability to detect SVV DNA in saliva similar to VZV infection in humans. We included buccal cells to assess if it would be better for testing. Five rhesus macaques, intratracheally inoculated with SVV, developed varicella after 1-2 weeks which later resolved. Eight months later, 4 of 5 five monkeys were immunosuppressed. Zoster rash (confirmed by the presence of SVV antigen in skin) erupted within 9-15 weeks. DNA from blood, saliva and buccal cells collected during acute infection, latency and post-viral reactivation were analyzed by PCR. Following SVV inoculation, animals were viremic with 102-103 copies of SVV DNA (per μg of DNA) detected in blood cells from 5 of 5 animals on 7 and 9 days post inoculation (dpi) and 101-102 copies/ug in 4 of 5 animals on 14-56 dpi. Viremia was absent after immunosuppression. At 7 dpi, up to 102 and 103 copies of SVV DNA (per ng of GAPdH DNA) was detected in saliva and buccal cells (4 of 5 monkeys), respectively. At 9 dpi, 101-102 copies of SVV DNA was found in saliva (1 of 5 monkeys) and buccal cells (2 of 5 monkeys). Similar to viremia, SVV DNA copies in saliva and buccal cells decreased during 11-56 dpi. SVV DNA levels were higher in buccal cells than in saliva. Following immunosuppression and reactivation, saliva and buccal cells were negative for SVV DNA. Absence of SVV DNA in saliva or buccal samples at reactivation may be due to the absence of viremia compared to acute infection. Our findings indicate SVV shedding into the oral cavity following acute SVV infection but not after reactivation. Saliva and buccal cells are relevant samples to monitor acute varicella virus infection.

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Antiretroviral-mediated microglial activation involves dysregulated autophagy

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While the advent of combined antiretroviral therapy (cART) has transformed the course of HIV disease, allowing for increased life spans for infected individuals, paradoxically, however, life long dependence on these drugs is often associated with chronic toxicity & neuroinflammation. While the contribution of HIV proteins in mediating neuroinflammation is well studied, contribution by antoretrovirals is much less explored. The current study was aimed at understanding the detailed molecular pathways by which cART exposure could mediate activation of microglia in the CNS. The ARV cocktail used in our study included a combination of two reverse transcriptase inhibitors, Tenofovir & Emtricitabine, and an integrase inhibitor Dolutegravir (each at 5μM concentration). Our results demonstrated that exposure of microglial cells (BV2 & rat primary microglia) to ARV cocktail resulted in time-dependent increase in expression of both ER stress and autophagy markers, such as BIP, PERK, Beclin1, LC3II and p62. This, in turn, was accompanied by a time-dependent decrease in the expression of lysosomal markers, LAMP2 and Cathepsin D. Additionally, lysosomal dysfunction was also validated by assessing activity of Cathepsin D as well as lysosomal membrane permeabilization. These results thus implicated ARV-mediated induction of autophagy, with a block in autophagosome maturation and impairment of autophagy flux. Interestingly, exposure of microglia to ARVs resulted in upregulation of pro-inflammatory cytokine (IL1β, IL6 and TNFα). Taken together, our findings underscore the role of ARVs in mediating neuroinflammation via the ER stress/Autophagy axis. Targeting the autophagy pathway could thus be developed as a potential therapeutic approach to ameliorate ARV-mediated microglial activation and ensuing neuropathogenesis associated with HAND.

Supported by DA044586 (MLG & SB), DA043138 (SB & MLG), MH106425 (S. B.) and MH112848 (S. B.)

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SIGMA-1R ANTAGONIST BD1047 PRIOR TO COCAINE REDUCES CATHEPSIN B SECRETION IN HIV-1 INFECTED MACROPHAGES

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Pathogenesis of HIV-associated neurocognitive disorders (HAND) is mediated through the infiltration of perivascular macrophages into the brain with the secretion of viral proteins, neurotoxic and inflammatory factors. One of these factors is cathepsin B (CATB), a lysosomal cysteine protease that induces neuronal apoptosis, and increases in plasma and cerebrospinal fluid from HIV-1 infected patients (Cantres-Rosario et al., 2013). Cocaine further potentiates CATB neurotoxicity in vitro and in vivo (Zen√≥n et al., 2014). Modulation of sigma-1 (Sig1R) by cocaine increases oxidative species, cytokines and other factors that promote lysosomal disruption. However, the role of Sig1R in CATB secretion and HIV-1 replication in presence of cocaine in macrophages is unknown. We hypothesized that pharmacological modulation of Sig1R would alter CATB secretion from HIV-1 infected macrophages in vitro. To test our hypothesis, monocyte derived-macrophages (MDM) from HIV-1 seronegative donors were isolated, infected with HIV-1ADA, and pretreated with Sig1R antagonist (BD1047) or agonist (PRE-084) prior to cocaine for 3,6,9 and 11 days post-infection (dpi). Treatment of infected macrophages with BD1047 10 μM prior to cocaine decreased infection levels

and CATB secretion when compared to cells treated with cocaine (p24=130 ng/mL vs. 15 ng/mL; 3000 ng/mL vs. 10 ng/mL). Though, when PRE-084 was added prior to cocaine, no significant differences in p24 levels and CATB were found. The effect of Sig1R antagonist was verified in vivo using the HIV encephalitis (HIVE) mouse model. Therefore, BD1047 might be a potential therapeutic for reducing CATB neurotoxicity in HIV-1 infected patients that use cocaine.

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M2 Differentiation of MonoMac-1 Cell Line Induced by M-CSF and Glucocorticoid Pathways

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Monocytes and macrophages have an important role in the pathogenesis of HIV-1 infection,serving as reservoirs of HIV infection and contributing to neuroinflammation.Virus-host interactions appear to contribute to altered immune polarization and immune suppression through altered macrophage polarization.Previous studies have demonstrated an increased percent frequency of CD163+CD16+ monocytes among viremic patients that correlates directly with viral load and inversely with CD4+ T cell count.CD163+CD16+ monocytes likely serve as precursors to alternatively activated (M-2) macrophages,which may contribute to HIV persistence by dampening immune responses. CD163+CD16+ monocytes harbor HIV proviral DNA in HIV infected persons and are preferentially infected by HIV in vitro. In order to develop strategies to reduce this subset therapeutically,we aimed to establish a cell culture model system to test compounds of interest. We investigated the effects of PMA, TNF alpha, IFN gamma, LPS, DEX and M-CSF on expression of CD163 and CD16 by a monocyte/macrophage cell line, MonoMac-1.Using a stepwise approach, cells treated with PMA and PMA+LPS for 3 days,followed by DEX+M-CSF and DEX+M-CSF+IFN gamma and TNF alpha for 4 days, showed increase expression of CD163 and CD16.These affects appear to require the action of dexamethasone and M-CSF on the cognate glucocorticoid and cFMS receptors, respectively, as RU486 and PLX3397 exhibited inhibitory effects.Furthermore,the CD163+CD16+ MonoMac-1 cells induce tryptophan catabolism/IDO activity as demonstrated by an increase in the tryptophan/kynurenine ratio in response to combined TNF alpha and IFN gamma treatment.Our results underscore the contribution of M-CSF and glucocorticoids on myeloid differentiation and, as such, these pathways may serve as viable targets for therapeutic intervention in disease states where monocyte maturation and activation of the kynurenine pathway has been implicated(i.e., HIV induced CNS diseases,cardiovascular disease,atherosclerosis).The culture conditions we present here are designed to provide a basis for screening compounds that may have therapeutic potential in disease states involving altered monocyte/macrophage differentiation.

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Mechanisms of survival of HIV reservoirs: role of glutamate and glutamine

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Immunodeficiency virus (HIV) produces a broad spectrum of central nervous system disorders in at least half of the HIV infected population despite effective ART. Our unpublished data indicate that areas of the brain containing viral reservoirs, integrated viral DNA, even in the absence of significant viral replication, results in bystander compromise. We identify that lately HIV infected macrophages and T cells use glutamine/glutamate as a significant source of energy to produce ATP. In the brain, glutamate is the more abundant neurotransmitter. Thus, viral reservoirs have an unlimited source of energy to survive for extended periods of time. Here we demonstrate that glutamate, glutamine and other TCA intermediates can regulate viral replication and silencing of the virus. Our studies were performed using primary and well-characterized cell lines (HL-60, OM10.1, ACH-2, and A3.1) in the presence and absence of reactivating agents. The viral reactivation was quantified by flow cytometry protocol combined with the fluorescence in situ hybridization (FISH-Flow). We propose that glutamate, glutamine, and other TCA intermediates regulate viral silencing and reactivation. Thus, blocking these pathways could kill viral reservoirs.

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Influenza virus-associated CNS disease: Direct or remote damage

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The most common extra-respiratory tract complications of influenza are diseases of the central nervous system (CNS). However, the pathogenesis of these severe, sometimes fatal, complications has not been studied extensively. To get more insight into the pathogenesis of influenza virus-associated CNS disease, we determined the ability of pandemic 2009 H1N1 virus and highly pathogenic H5N1 virus to enter the CNS, and to induce pro-inflammatory cytokines at different days post inoculation (dpi) in ferrets. In these studies we showed that H5N1 virus, but not H1N1 virus, efficiently entered the CNS via the olfactory nerve, resulting in the development of a severe meningo-encephalitis. H5N1 virus entered the CNS at 3 days post infection (dpi) and infected predominantly neurons, meningeal cells and to a lesser extent glial and ependymal cells. Already at 1 and 3 dpi, pro-inflammatory cytokines (IL-6, IL-8 and TNF) were upregulated in the olfactory bulb and cerebrum in both H1N1 and H5N1 virus-infected ferrets. These cytokines were induced focally in neurons, microglia and endothelial cells and were not associated to the presence of virus antigen. Interestingly, the efficiency of CNS entry varies largely between influenza A virus subtypes, and recent data suggests that specific mutations in the polymerase complex are important for the neurotropic potential of influenza A viruses. Altogether these data show that in a ferret model, which closely mimics influenza in humans, influenza viruses can damage the CNS via two different mechanisms. First, direct via virus entry into the CNS and secondly remote via the local induction of pro-inflammatory cytokines.

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HIV-1 Nef causes astrogliosis and downregulates expression of glutamate transporter 1 in vivo and in vitro

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Astrocytes play a major role in homeostasis and neuronal function by preventing excitotoxicity in the CNS. During HIV infection, astrocytes are characterized for producing early viral proteins such as Nef, which is known to cause neurotoxicity. Glutamate transporter 1 (GLT-1) is a major astroglial transporter involved in the regulation of extracellular glutamate concentration. We previously established that Nef expressed in astrocytes in the hippocampus is sufficient to cause spatial learning impairment, Herein, we investigated astrocytes transfected with Nef to understand the effect on astrocyte morphology and glutamate transport as possible mechanistic components of that learning impairment. Primary rat astrocytes expressing Nef or GFP were infused in the right hippocampus of 30-

day-old male Sprague Dawley rats. Five days after infusion, brain tissues were collected for immunofluorescence staining and the morphology of astrocytes and GLT-1 expression were assessed in different areas of the hippocampus (CA3 and dentate gyrus) using Image J software. In vitro experiments using rat primary astrocytes directly transfected (pEGFP or pNef), or co-cultured with transfected cells (Nef exposed) and cultured for 48 hours were assessed using immunofluorescence. GFAP area fraction was significantly increased ($P<0.05$), while GLT1 expression was significantly decreased ($p<0.05$) in the Nef-treated animals when compared to GFP. In vitro results confirmed the downregulation in GLT1 expression in the exposed astrocytes and in the Nef-transfected astrocytes, being more significant in the latter, when compared to the control. Brain tissue showed no differences in the lengths of processes; however, the number of secondary processes was increased by Nef. The increase of astrogliosis, the number of secondary processes, and the decrease of GLT1 correlate with the learning impairment shown by the Nef treated group. These results suggest an astrocyte dysfunction that affects glutamate uptake at the synaptic cleft leading to neuronal toxicity.

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Modulation of tryptophan catabolism via the kynurenine pathway during SIV infection in rhesus macaques.

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Tryptophan (TRP) is an essential amino acid and precursor for the de novo synthesis of nicotinamide adenine dinucleotide (NAD), as well as serotonin and melatonin, by two different pathways. TRP is catabolized by the kynurenine (KYN) pathway to produce NAD, whereas modification occurs via the methoxyindole pathway to generate serotonin and melatonin. Increased KYN/TRP ratios have been associated with the progression of acquired immune deficiency syndrome (AIDS) as determined by association with lower CD4/CD8 ratios, reduced T cell recovery after initiation or cART, and increased mortality risk. KYN pathway metabolites have been implicated in the pathogenesis of HIV associated neurocognitive disorders (HAND) as well as immune dysregulation, including Th1 CD4+T cell suppression and Treg expansion. We identified an increase in the KYN/TRP ratios in the SIV macaque model with a positive correlation with viral load and soluble CD163 in plasma, with the latter suggesting the role of macrophage activation in this process. In an effort to modulate the K/T pathway, and decrease tryptophan catabolism, we performed a dose escalation study with the NAD salvage pathway precursor, nicotinamide riboside (NR), in SIV infected macaques. NR treatment modulated K/T ratios in SIV infected (where K/T was significantly increased), but not in non-infected animals. NR treatment significantly reduced the mean fluorescence intensity and percent frequency of CD14+/CD16+ monocytes as determined by flow cytometry, in both SIV infected and uninfected animals. This monocyte subset has been implicated in the pathogenesis of HIV infection, HAND, as well as other HIV associated comorbidities. The regulation of the kynurenine pathway appears to be complex, yet an attractive target for HIV therapeutics. Understanding the mechanism by which HIV modulates the KYN/TRP pathway could provide new therapeutic targets for the treatment of HIV, HAND, as well as other comorbid conditions.

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Mitochondria-targeted antioxidative treatment attenuates accelerated senescence of neural progenitor cells induced by antiretroviral drugs

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HIV infection is associated with symptoms of accelerated aging. Compared to non-infected controls, HIV-infected individuals experience accumulated comorbidities 15-20 years earlier. These disorders are likely to be driven not only by HIV itself, but also by the toxicity of antiretroviral drugs used long-term. Therefore, it is of crucial importance to understand the molecular mechanisms by which antiretroviral drugs may contribute to accelerated aging. The aim of this study was to investigate the hypothesis that antiretroviral drugs cause mitochondrial dysfunction that increases reactive oxygen species (ROS) generation and culminates in promoting cellular senescence. However, it is notoriously difficult to deliver available antioxidants such as coenzyme Q10 (CoQ10) to the mitochondria, the site of ROS production. Thus, we applied a mitochondria-targeted nanoparticle (NP)-based strategy to deliver antioxidants to the mitochondria in order to re-establish mitochondrial functions. We conducted proof-of-concept studies based on cultured neural progenitor cells (NPC) as differentiation of these cells into mature neurons is affected during HIV infection and the aging process. Exposure of NPC to an anti-retroviral drug combination, a mixture of Tenofovir, Emtricitabine, Ritonavir, Darunavir, that is recommended for HIV-infected and treatment naïve patients induced mitochondrial ROS generation, mitochondrial membrane hyperpolarization, decreased oxygen consumption, decreased ATP levels, and decreased cell proliferation. These alterations were accompanied by an increase in senescence-associated beta-galactosidase staining, and shortening of telomeres as assessed by the telomere-FISH labeling. Importantly, targeted nanoparticle delivered CoQ10 effectively attenuated these effects. Overall, these results indicate that antiretroviral treatment promotes cellular senescence by causing mitochondrial dysfunction, which can be successfully reversed by including mitochondria-targeted antioxidative treatment.

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Expression of CD32, a marker of HIV latency, is enriched on CD4dimCD8bright T cells

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CD32 is a low affinity IgG Fc fragment receptor commonly expressed on cells such as neutrophils, dendritic cells, monocytes, and B cells. Recently CD32a, a subtype of CD32 receptor, was reported as a marker of HIV latency expressed on ~50% of the latent CD4+ T cell reservoir in the peripheral blood of HIV+ patients. Our lab has identified a unique population of CD4dimCD8bright (DP) T cells, infectable by HIV, that constitutes greater than 55% of CD8+ T cell anti-HIV response and migrates into the CNS. Here we assessed the frequency of CD32 expression on DP T cells in the peripheral blood of HIV+ patients (n=11) and HIV- controls (n=4). We report that CD32 is enriched on DP T cells compared to single positive CD4 T cells among HIV- and HIV+ individuals. In HIV- individuals, mean CD32 percent expression was 73.05% on DP T cells and 22.83% on CD4+ T cells ($p<0.001$). In HIV+ patients, mean CD32 percent expression was 54.40% on DP T cells and 12.79% on CD4+ T cells ($p<0.001$). CD32 expression on both T cell populations was determined in HIV+ patients of varying clinical status (elite controllers (n=4), viremic (n=3), and cART suppressed (n=4)). Viremic and cART suppressed individuals showed significant enrichment of CD32 on DP T cells compared to CD4+ T cells (both $p<0.001$). Elite controllers, while not significant, showed a trend of CD32 enrichment on DP T cells. Difference in CD32 expression on DP T cells was not statistically significant between HIV+ cohorts and HIV-individuals. CD4 count and viral load among HIV+ patients did not correlate with CD32 expression on DP T cells. These data suggest DP T cells are enriched for CD32 independent of HIV status. Ongoing studies include increasing sample size and assessing the relationship between CD32+ DP T cells and HIV RNA/DNA content.

P308

Multiple sclerosis treatment strategy after natalizumab-associated progressive multifocal leukoencephalopathy

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Background: Progressive multifocal leukoencephalopathy (PML) is a known serious complication associated with natalizumab (Nz) and JC virus seropositivity in patients treated for multiple sclerosis (MS) and Crohn's disease. PML-related discontinuation of Nz requires re-establishing maintenance MS therapy, for which there is no recognized standard of care. **Objective:** To determine optimal management for MS after Nz-associated PML. **Methods:** We retrospectively analyzed clinical symptoms, diagnostic methods; survival outcome and MS therapy in 17 patients post Nz-PML at a tertiary care center from 2010-2017. **Results:** Mean age of Nz-PML onset was 43.6 years and occurred on average after 44.5 infusions. Of 17 patients, 16 (94.1%) were alive and 1 suicide took place after 2 years of PML onset. Eight (47%) patients had at least 1 relapse 2 years post PML. Three relapsed within the first year and 5 beyond 1 year post PML. Reactivation of MS after Nz discontinuation was lower than expected in this previously highly active cohort. One of 3 patients treated with maintenance monthly IV corticoids relapsed. Eight patients started other MS therapies post PML on average 26 months after Nz withdrawal, including 3 with dimethyl fumarate (DMF), 3 with glatiramer acetate (GA) and 2 with mycophenolate mofetil (MMF). One relapse occurred on MMF. No relapses were reported with GA and DMF. No recrudescence of PML occurred with these therapies. **Conclusions:** Our findings suggest that treating MS post-Nz PML carries no risk of PML reactivation. PML may play a role in ameliorating the clinical course of MS. DMF and GA appears to be effective MS therapies after Nz-PML. Prospective studies, including larger number of patients are needed to confirm these findings.

P309

Canonical Wnt signaling mediates CD8+ T cell non-cytotoxic antiviral factor activity

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HIV+ long-term non-progressors (LTNPs) and elite controllers (ECs) control HIV load without antiretroviral therapy. While the mechanisms(s) attributed to this natural immunity is not clear, a secreted soluble factor coined the CD8+ T-cell antiviral factor (CAF) is associated with potent HIV control. The identity of CAF has remained elusive for over 30 years. CAF is a heat-stable, small secreted factor (~30kDa) that inhibits HIV transcription. As several biologic and functional characteristics of CAF are reminiscent of our published data of Wnts effects on HIV, we assessed here if CAF activity is mediated by Wnt signaling and whether it is associated with LTNP and EC status. Wnts are a family of 19 secreted glycoproteins that signal through beta-catenin-dependent canonical or beta-catenin-independent non-canonical signaling. We show that CD8+ T cells express all Wnt ligands, albeit with donor variability. CD8+ T-cell conditioned media induced the transcription of beta-catenin downstream targets CyclinD1 and Axin2, indicating that CD8+ T-cell conditioned media express functional Wnts capable of inducing canonical Wnt signaling. Pre-treatment of HIV+ CD8 depleted PBMCs with the canonical Wnt signaling inhibitor Dikkopf-1 (DKK-1), reversed CAF activity by 2.5 fold in comparison to control cultures. Further, addition of a prototypical canonical Wnt inducer (human recombinant Wnt1) inhibited HIV transcription and not reverse transcription or integration, and induced CyclinD1 and Axin2 expression in recipient cells. We found that DKK-1 concentration was significantly higher in HIV+ patients with lower than 500 CD4 count/ul and higher viral loads. Wnts 2B, 3A and 9B expression negatively correlated with viral load, and Wnts2B and 9B expression were significantly higher in ECs and LTNPs compared to viremic patients. Collectively, this data demonstrates that canonical Wnt signaling is responsible for CAF activity. Ongoing studies are using siRNA to knockdown and thus identify specific Wnts responsible for CAF activity.

P310

HIV-1 gp120-mediated deacetylation of tubulin is neurotoxic and is prevented with tubacin, an HDAC6 inhibitor.

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Despite successful antiretroviral drug therapy, a subset of Human Immunodeficiency Virus-1 (HIV) positive individuals still display synapto-dendritic simplifications and functional cognitive impairments. These symptoms are referred to as HIV-associated neurocognitive disorders (HAND). Considerable experimental evidence indicates that HIV proteins, including the envelope protein gp120, can cause neurological damage to a similar extent as the full virus. However, the full mechanism of gp120-mediated neurotoxicity is still unknown. We have recently established that gp120 is internalized and binds with high affinity to class III β-tubulin, a component of neuronal microtubules, through a conserved α-helical motif. Gp120 causes deacetylation of tubulin, a post-translational modification which impairs the functionality of microtubule. We hypothesize that the deacetylation of tubulin caused by gp120 impairs the axonal transport of organelles and essential nutrients for neurons and eventually leads to neuronal cell death. To test this hypothesis, we utilized a pharmacological approach to prevent gp120-mediated tubulin deacetylation which we hypothesized to be neuroprotective. Tubulin deacetylation is mediated by histone deacetylase 6 (HDAC6) and we used tubacin, a potent and selective HDAC6 inhibitor, to establish whether this post-translational modification of tubulin underlies the neurotoxic effect of gp120. We demonstrated that tubacin prevents gp120-meditaed deacetylation of tubulin, as well as gp120-mediated neurite shortening and cell death of primary rat cortical neurons as measured by Hoechst/Propidium iodide staining. Overall, our data suggest that gp120-mediated tubulin deacetylation causing impairment of axonal transport is a novel mechanism for gp120 mediated neurotoxicity.

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Zika virus resistance to the antiviral 2',5'-oligoadenylate synthetase/ribonuclease L (OAS/RNase L) pathway

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Since the 2015 onset of the Zika virus (ZIKV) outbreak, novel modes of transmission, neurological symptoms, and viral persistence within human tissues have been reported, highlighting our poor understanding of ZIKV pathogenesis and host response to infection. Using CRISPR-Cas9 gene editing technology, we evaluated ZIKV replication and innate immune responses in a human cell line with targeted knockout of various host genes to elucidate innate immune pathways important for defense against ZIKV infection as well as those pathways potentially antagonized by ZIKV. Knockout of type I IFN proteins, namely MAVS, STAT1, or STAT2, had no effect on ZIKV replication compared to that of the parental A549 WT cells, suggesting ZIKV inhibition of type I IFN responses during infection. Using a Bioanalyzer microchip assay depicting rRNA degradation as a marker for activated RNase L, we demonstrated that ZIKV activated the antiviral OAS/RNase L pathway, which dramatically reduced ZIKV genomic RNA expression by northern blotting. While increased viral genome

expression in RNase L KO cells compared to WT suggests RNase L-mediated cleavage of ZIKV genome, knockout of OAS or RNase L proteins failed to enhance ZIKV replication compared to that in WT cells. In contrast, both the flavivirus Dengue virus as well as the alphavirus Sindbis virus replicated to significantly higher titers in RNase L KO cells compared to WT, demonstrating strong antiviral function carried out by activated RNase L during infection with other viruses. Future studies will focus on investigation of a probable ZIKV mechanism of RNase L antagonism, highlighted by RNase L failure to limit replication of ZIKV in particular, despite its activation and cleavage of host RNAs.

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B-Arrestin 2 is a Key Regulator of Neuroinflammation During Viral Infection

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B-Arrestins are scaffolding proteins that terminate G Protein-Coupled Receptor (GPCR) signaling through receptor internalization. In addition to this canonical role, B-Arrestins also act independently of GPCRs to facilitate their own unique signaling cascades. In this capacity, B-Arrestins may modulate peripheral inflammatory processes. However, the roles of B-Arrestins in regulating CNS immune responses are not well characterized. In this study, we evaluated the contribution of B-Arrestins to neuroinflammation during viral infection of primary human macrophages. We focused on CCR2 as our model GPCR as it is highly expressed in myeloid cells and its primary ligand, CCL2, is a strong B-Arrestin activator that is associated with many neurological diseases. We determined that CCL2-mediated B-Arrestin activation promoted internalization of the Type I Interferon (IFN) receptor, IFNAR1, which prevented subsequent STAT1 and IRF3 activation. As a result, uninfected and HIV infected macrophages, as well as cells exposed to Toll Like Receptor ligands, did not mount adequate IFN responses. B-Arrestin-mediated IFNAR1 internalization selectively decreased IFN-a, but not IFN-B. There was a functional consequence to inhibiting IFN-a, as downstream IFN-induced cytokines were significantly decreased following B-Arrestin activation. CCR2 blockade restored macrophage Type I IFN responses, indicating that the GPCR was required for the altered IFN signaling pathway. However, CCR2 ligands did not broadly diminish IFN, as CCL7 and CCL8 which do not strongly recruit B-Arrestins,had no effect on IFN. We identified B-Arrestin 2 as the specific arrestin subtype required to abrogate IFN, as siRNA knockdown of this isoform, but not B-Arrestin 1, restored all IFN responses. In summary, these data identify B-Arrestin 2 as a critical regulator of neuroinflammation during viral infection, as it suppresses innate immune responses in uninfected cells prior to viral exposure, and by inhibiting their ability to elicit effective immune responses following infection.

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17beta-estradiol Suppresses Oxidative Stress and Neurotoxicity in Human Macrophage Neuroinflammatory Models

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As women are aging with HIV, studies have suggested that women post-menopausal have lower CD4 counts compared to pre-menopausal women, however, how estrogen signaling plays a role in HIV infection and its associated co-morbidities such HIV-associated neurocognitive disorders is still not clear. 17beta-estradiol, the most active form of estrogen, activates classic estrogen receptors, ER alpha and ER beta and the non-classical estrogen receptor GPER and has been reported to inhibit HIV infection in primary macrophages and peripheral blood mononuclear cells. 17beta-estradiol has also been shown to be protective neurons against HIV proteins, gp120 and tat and suppresses inflammation caused by LPS in human macrophages. However, a mechanistic understanding of how estrogen signaling affects inflammation and neurotoxicity is still needed. Many reports have cited 17beta-estradiol's ability to induce the Endogenous Antioxidant Response (EAR) Pathway via Nrf2 translocation in breast cancer cells, therefore we hypothesized that 17beta-estradiol may be neuroprotective during HIV infection and other inflammatory stimuli, due to its ability to induce Nrf2 translocation, reduce oxidative stress, inflammation and neurotoxin production in human macrophages. To understand this, we stimulated or infected macrophages with LPS or HIV-JAGO, respectively, in the presence and absence of increasing doses of 17beta-estradiol. Whole cell and cytoplasmic lysates, mRNA and conditioned medium were collected at various time points. We found that 17beta-estradiol increased HO-1 expression, a phase II antioxidant gene during LPS treatment and HIV infection in an Estrogen receptor dependent manner. Estradiol also suppressed neurotoxin production from macrophages in an estrogen receptor dependent manner, which was assessed by loss of map2 expression in rat cortical neurons exposed to macrophage conditioned medium. Given these studies, estrogen signaling may reduce oxidative stress and inflammation seen during neuroinflammatory disorders, such as HIV-associated neurocognitive disorders.

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Characterization of a doxycycline inducible and astrocyte-specific HIV-1 Nef transgenic mouse model (iNef)

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Over 37 million people worldwide are currently infected with human immunodeficiency virus type 1 (HIV-1). Introduction of combination antiretroviral therapy (cART) has improved the quality and length of life. While the more severe forms of HIV-associated cognitive and motor dysfunction have become less prevalent in the era of cART, the incidents of minor cognitive and motor disorder have increased. HIV-1 infects astrocytes, which are the most abundant cells of the central nervous system (CNS), a major component of the blood brain barrier, and are involved with maintaining neuronal integrity. Once infected, astrocytes become reactive, characterized by increased expression of astrocyte-specific protein glial fibrillary acidic protein (GFAP). However, those cells do not support productive HIV replication, primarily expressing non-structural viral proteins, such as Tat and Nef. The role of Tat in HIV/neuroAIDS has been extensively studied and HIV-1 Nef is known to play important roles in immune evasion, T-cell depletion, and disease progression. Nevertheless, there is little known about the roles of Nef in HIV/neuroAIDS. To investigate the roles of Nef in HIV/neuroAIDS, we developed a doxycycline inducible astrocyte-specific HIV-1 Nef transgenic mouse model (iNef), in which Nef expression is under the control of both doxycycline and the GFAP promoter. This unique iNef model allows for characterization of the effects of Nef expression on the CNS, independent of HIV-1 infection. We specifically focused on the impact of Nef on two domains that are affected in HIV/neuroAIDS: neuropathology and neurobehavior. Using real-time RT-PCR, immunofluorescent staining, Western blotting, and neurobehavioral batteries, we demonstrated that Nef expression led to astrocytosis, immune activation, compromised neuronal integrity, and changes in cognitive, memory and motor function in Nef-expressing mice. Further investigation is under way to determine the underlying molecular mechanisms.

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CCR2 is not required for Ly6Chi monocyte egress from the bone marrow, but is necessary for migration within the brain in La Crosse Virus encephalitis

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Inflammatory monocyte (iMOs) recruitment to the brain is a hallmark of many neurological diseases. Studies using both inflammatory and encephalitic virus models such as West Nile and herpes simplex virus have demonstrated iMOs must first egress into the blood from the bone marrow through mechanism involving CCR2/CCL2 signaling prior to entering the brain. Thus, it has been widely held that this signaling axis is necessary for iMO recruitment to the brain. Here, we show that during La Crosse Virus (LACV)-induced encephalitis, egress of iMOs was surprisingly independent of CCR2, with similar percentages of iMOs in the blood and brain of heterozygous and CCR2-/- mice following infection. Studies with mice deficient in other chemokines or receptors (CXCL10, CCR5, CCR7) or elements of the innate immune response (MyD88, MAVS), known to be involved in leukocyte or iMO activation and recruitment did not alter iMO recruitment suggesting the mechanism is more complex than simple compensation for the absence of CCR2. However, analysis of other related Orthobunyaviruses, showed that Jamestown Canyon virus also induced CCR2-independent iMO egress to the blood while Tahyna virus did not. Thus, CCR2-independent monocyte recruitment is engaged during infection with more than one virus, but not by all the viruses of the same family. Interestingly, these studies also revealed that CCR2 was necessary for iMO trafficking from perivascular areas to sites of virus infection once iMOs enter the brain. These studies demonstrate that the CCR2-requirement for iMO egress to the blood is not universal for all viruses, but may instead be necessary for trafficking within the brain to areas of damage.

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Viral small RNAs of simian immunodeficiency virus-infected primary astrocytes

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Introduction: The astrocyte, the most abundant CNS cell type, contributes to innate responses and is susceptible to simian immunodeficiency virus (SIV) infection. At least in vitro, primary nonhuman primate astrocytes actively replicate SIV. Since CNS atrocytes could be a potential viral reservoir, and in light of several reports that HIV-infected cells produce small RNA fragments of the HIV transactivation response element (TAR) that may function as microRNAs (miRNAs) or small RNA (sRNA) suppressors of HIV-1 transcription, we studied the small RNA landscape of astrocytes infected or not with SIV. **Methods:** Primary rhesus macaque astrocytes cultured in Lonza astrocyte medium were infected or not with SIV 17E-Fr. Total RNA was extracted at seven days post-infection (mirVana total RNA protocol) and quality controlled. Illumina TruSeq small RNA libraries were prepared and sequenced. Trimmed data were aligned to the macaque genome or human genome, and to the SIV transcriptome. miRNA hairpin structure prediction was performed for the SIV genome and compared with enriched SIV-derived sequences. Several SIV sequences were identified for qPCR validation. **Results and conclusions:** An abundance of random SIV-derived sRNA fragments were identified in infected astrocytes, covering the entire SIV genome. However, several SIV-derived small RNA fragments were highly enriched, some of them associated with hairpin-containing regions. At least one appears to have been derived from an antisense transcript. Interestingly, the SIV TAR has a different structure from the HIV-1 TAR, and perhaps for this reason, we did not observe abundant SIV TAR sRNAs. One "SIV" sRNA was also identified in uninfected cells, and we are currently evaluating this as a possible endogenous retroviral sequence. It remains unclear how or if the identified SIV sRNAs are specifically processed or protected from degradation by secondary structure or protein binding. Additional studies are needed to assess possible cell-specificity and function of SIV sRNAs.

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HIV Tat dysregulates endocannabinoid signaling

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HIV-associated neurocognitive disorders (HAND) affect nearly half of the >37 million people infected with HIV. Infected microglia release neurotoxic agents that induce excitotoxic synaptic injury, resulting in altered neuronal function. The endocannabinoid (eCB) system attenuates excitotoxicity and acts as an on-demand endogenous neuroprotective system. Whether this neuroprotective mechanism is altered in the presence of HIV is unknown. Here, we show that exposure to the potent HIV neurotoxin Tat (transactivator of transcription) impairs the neuroprotective potential of the eCB system. Using patch-clamp electrophysiology in cultured hippocampal neurons, we measured adaptive changes in retrograde eCB signaling following exposure to Tat. We found a significant reduction in the magnitude of the depolarization-induced and metabotropic suppression of excitatory postsynaptic currents (EPSCs) following exposure to Tat (50ng/mL, 24h). These effects were not due to a loss of CB1R function, as indicated by no change in the concentration-response relationship for Win55,212-2 (a cannabinoid receptor agonist) inhibition of EPSCs in Tat-treated cultures. We hypothesize that exposure to Tat impairs the synthetic pathway for the endocannabinoid 2-arachidonoyl glycerol (2-AG). Changes in this neuroprotective mechanism may contribute to synaptodendritic injury in HAND. Thus, drugs that protect or enhance eCB signaling may attenuate the symptoms of HAND.

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Durable control of viral rebound in rhesus macaques by Rev-dependent lentiviral vectors carrying HSV-tk and TRAF6

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Persistence of HIV in anatomic sanctuary sites such as the brain prevents viral eradication. To target HIV reservoirs, we have developed an HIV Rev-dependent lentiviral vector carrying a series of therapeutic genes, such as DT (diphtheria toxin), AnlO (anthrolysin O), HSV-tk (herpes simplex 1 virus thymidine kinase), or human TRAF6. We have tested the Rev-dependent vectors for Rev-dependent selective killing of HIV-infected cells in vitro. Recently, we further tested the feasibility of using the Rev-dependent vectors to target viral reservoirs in a SIV/rhesus macaque model. We assembled viral particles from two SIV Rev-dependent vectors, SIV-HSV-tk-RRE and SIV-TRAF6-RRE, and injected them into SIV-infected rhesus macaques. After the SIV-HSV-tk-RRE vector injection, animals were further treated with ganciclovir (GCV) for two weeks daily to induce the killing of SIV+ cells. Following GCV treatment, cART was terminated in all animals. To further diminish possible residue

viral reservoirs, rhesus macaques were also injected with the SIV-TRAF6-RRE vector, which can directly kill surviving SIV+ reservoir cells. After the SIV-TRAF6-RRE particle injection, all treatments were terminated. We observed durable control of viral rebound with the vector injections. One of the animals had viral load reduced to the threshold of detection by the Rev-dependent vectors after the termination of cART about 11 months ago. Currently, all therapeutic treatments have been stopped for this animal in the past 3 months, and the animal had the viremia controlled at the threshold of detection. Our results suggest that the Rev-dependent vectors can diminish viral reservoirs and prevent viral rebound in vivo. The Rev-dependent vectors have a great potential to be used with cART for treating HIV infection for a functional cure.

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IL-22 inhibits HSV-2 infection of human cervical epithelial cells

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The epithelium is the main entry point for many viruses, but the processes that protect barrier surfaces against viral infections are incompletely understood. Interleukin (IL)-22 is a member of the IL-10 family of cytokines. In recent years, the role that IL-22 plays in antiviral immune responses has been examined in a number of viral infections, including Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Rotavirus, Influenza, and so on. However, there is little information about whether IL-22 in the mucosal innate immunity against herpes simplex virus 2 (HSV-2) infection. In this study, we examined the ability of IL-22 to inhibit HSV-2 infection of human cervical epithelial cells (End1/E6E7 cells). Using RT-PCR, we demonstrated that End1/E6E7 cells express the functional IL-22 receptor complex consisting of IL-22R1 and IL-10R2. We found that treatment of the End1/E6E7 cells with IL-22 induced the expression of IFN-stimulated genes (ISGs: ISG15, ISG56, OAS-1, OAS-2, and Mx2). This IL-22 action of cervical epithelial cells on HSV-2 was mediated through STATs signaling pathway. Further studies showed that IL-22 could enhance the expression of tight junction proteins, including ZO-1 and Occludin, which correlated with integrity of epithelial cells. Collectively, this is the first experimental evidence highlighting anti-HSV-2 activity of IL-22 in human cervical epithelial cells.

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ENDOCANNABINOID-MEDIATED NEUROPROTECTION IN MODELS OF NEUROAIDS

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In the era of combined antiretroviral therapy (cART), human immunodeficiency virus type 1 (HIV-1) is considered a chronic disease with an inflammatory component that specifically targets the brain and causes a high prevalence of HIV-associated neurocognitive disorders (HAND). The endocannabinoid (eCB) system has attracted interest as a target for treatment of neurodegenerative disorders due to its neuroprotective and anti-inflammatory properties. To address this issue, we investigated the neuroprotective actions of the eCBs N-arachidonoylethanolamine (anandamide/AEA) and 2 arachidonoylglycerol (2 AG) against Tat toxicity in vitro and in vivo. Our in vitro findings indicate that direct application of eCB ligands as well as pretreatment with respective highly selective and potent inhibitors (PF3845, MJN110) of their primary hydrolytic enzymes have neuroprotective effects against Tat toxicity. Prefrontal cortex (PFC) neuronal cultures were directly treated with Tat ± eCB and indirectly with Tat ± eCB microglia conditioned media. Increase in eCB signaling results in neuroprotection through the reduction of Tat-induced increases in intracellular calcium, synaptodendritic damage, cell excitability, and neuronal death via a CB1/2R-related mechanism. Using the Tat transgenic mouse model for our in vivo studies significant deficits were noted in the PFC-mediated behavioral Go/No-Go task. Preliminary data demonstrate that transgenic Tat [Tat(+)] mice show less behavioral inhibition and increased impulsivity compared to their wild-type counterparts [Tat(-)], specifically for males. This was consistent with ex vivo recordings from medial PFC slices of Tat mice (males, females) that indicated significant increases in glutamatergic neurotransmission (EPSCs) for Tat(+) slices. Importantly, bath application of PF3845 showed an enhanced decrease in sEPSCs frequency for Tat(+) compared to Tat(-) slices. Overall, results indicate that eCBs AEA and 2-AG elicit neuroprotective actions against Tat-induced toxicity structurally and functionally. Furthermore, our studies suggest that eCB catabolic enzymes should be further examined as promising targets for treatment of neurodegenerative disorders associated with HIV/AIDS.

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Development of Cas9/gRNA based nano-formulation to eradicate latent HIV-1 infection in the brain

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HIV infection persists in several reservoirs in patients despite the availability of highly active antiretroviral medications and brain being one of the important reservoirs. Most of the antiretroviral agents do not cross the blood brain barrier thus establishing the viral reservoir in the brain. Further, the integration of HIV-1 genome to the host genome cause viral latency in the brain. Therefore, the elimination of the integrated HIV genome remains as a formidable task. Although HIV-1 specific Cas9/gRNA has been extensively used to eliminate the HIV viral genome in the peripheral organs, the application of Cas9/gRNA to eliminate the HIV genome in the brain remains as a task because of the impenetrability of Cas9/gRNA across BBB. In this work, we explored magnetically guided delivery of Cas9/gRNA (RNA-directed gene editing) across BBB using magneto-electro nanoparticles (MENP) for recognition and eradication of latent HIV-1 infection. We developed a Cas9/gRNA bound MENP (composed of $BaTiO3@CoFe2O4$) nano-formulation (NF) to deliver across the BBB followed up by on-demand controlled release of Cas9/gRNA to eradicate HIV-infection. MENP-Cas9/gRNA NF was characterized qualitatively and quantitatively to estimate toxicity, drug binding and release mechanism. An optimized NF of Cas9/gRNA (10 μg) MENP (50 μg) was navigated across the BBB under a static magnetic field (0.8 T for 3 hrs.). An external ac-magnetic field (60 Oe for 30 minutes) via electromagnetic coil was applied to achieve on-demand 100% release of Cas9/gRNA from MENP surface. The released Cas9/gRNA retained its biological activity and successfully reduced HIV-LTR expression in human primary microglia cells. Such developed MENP-Cas9/gRNA NF could be used to eliminate latent HIV-1 infection in the brain and can be promoted as future therapy for neuro-AIDS.

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Implication of dysfunctional endolysosomal biogenesis in HIV infection; Evidence from HIV-infected individuals to rodent model of HIV infection

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An increasing amount of neuropathological and experimental evidence suggests that deficits in endolysosomal biogenesis and function contribute to neuronal damage in HIV-Associated Neurocognitive Disorders. The Coordinated Lysosomal Expression and Regulation (CLEAR) network is a central mechanism regulating endolysosomal biogenesis/function. Here we examined the CLEAR pathway in human brain tissues obtained from the National NeuroAIDS Tissue Consortium, and in HIV-infected NOD/SCID/IL2Rγc-/- (NSG-HIV) mice reconstituted with a humanized immune system. Frontal neocortices obtained from autopsy of HIV-infected patients diagnosed (at that time) with minor cognitive-motor disorder (MCMD; n=36) showed increased expression of the master regulator of CLEAR network gene expression TFEB, and several lysosomal hydrolases. Subjects with HIV-Associated-Dementia (HAD; n=29) showed increased expression of TFEB, a negative regulator of the lysosomal TRPML1 channels called transmembrane 55B, the lysosomal transmembrane glycoprotein Lamp1, and several lysosomal hydrolases compared with similar tissues from cognitively normal HIV-infected (CN; n=41) subjects, and HIV negative controls (n=68). We also observed decreased expression of the aspartyl protease CTSD in MCMD and HAD, and decreased expression of TRPML1 and a glucosidase in HAD compared with CN and HIV- controls. Changes in the expression of TFEB, Lamp1 and CTSD were confirmed by immunoblotting and immunohistochemical analyses. We found a similar pattern of disruption in CLEAR network gene expression in NSG-HIV mice that exhibited increased expression of TFEB, Lamp1, and several glycosidases, with decreased expression of lysosomal proteases in the hippocampus and cerebral cortex. These in vivo findings suggest that peripheral infection is sufficient to perturb the CLEAR gene network in brain. Studies are ongoing to determine if restoration of the CLEAR network with the TRPML1 agonist ML-SA1 can preserve neuronal structure in NSG-HIV.

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Characterizing the Role of Cysteinyl Leukotrienes in HIV-1 gp120 Associated Brain Injury

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Individuals infected with human immunodeficiency virus (HIV)-1 often develop HIV-associated neurocognitive disorders (HAND). Studies in our laboratory indicate that p38 MAPK is essential for the neurotoxic phenotype of HIV gp120-stimulated macrophages and microglia, as well as the induction of neuronal apoptosis triggered by macrophage-derived toxins and proinflammatory molecules. Microarray analyses of human macrophages stimulated with HIV envelope protein gp120 and of brains of transgenic mice expressing the viral gp120 under the control of a modified GFAP promotor in astrocytes (gp120tg mice) suggested the involvement of cysteinyl leukotrienes (cysLTs) in HIV neurotoxicity. Leukotrienes (LTA4, LTB4, LTC4, LTD4, and LTE4) are the product of the 5-lipoxygenase (5-LOX) metabolism of arachidonic acid. CysLTs (LTC4, LTD4, and LTE4) are formed by the addition of cysteine derivatives. Two distinct CysLT receptors exist, CysLTR1 and CysLTR2 (Kanaoka & Austen reported a third CysLTR). CysLTs are most notably researched for their role in the pathophysiology of asthma but their role in the brain is largely unknown. We have found that cysteinyl leukotrienes (CysLTs) are released by both HIV-1 infected and gp120-stimulated macrophages, and knockdown of p38 MAPK down-regulates cysteinyl leukotriene synthase (LTC4S). Herein, we describe the characterization of both CysLTR1 deficiency and LTC4S deficiency in gp120tg mice by immunofluorescence staining and RNA analysis.

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CNS CORRELATES OF HIV-ASSOCIATED PERIPHERAL NEUROPATHY AND POSTURAL INSTABILITY

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Persisting disturbances in the treated HIV population include peripheral neuropathy and postural instability. Traditionally, compromise in these functions was associated with HIV-related indices (e.g., low CD4 count), but emerging evidence supports central mechanisms. This study was conducted in 68 controls (29 women, 48.1+/-11.4 years), 59 participants with HIV (20 women, 52.1+/-8.1 years), and 65 participants co-morbid for HIV and alcoholism (HIV+AUD: 22 women, 51.0+/-8.0 years) to investigate CNS correlates of 4 variables: subjective neuropathy (self-report), objective neuropathy (2-point discrimination of the hands and feet), and ataxia (static standing balance, eyes closed). Group differences were significant for scores on subjective neuropathy, objective peripheral neuropathy (2-point discrimination of the hands and feet), and ataxia. For all 4 measures, the two HIV groups performed worse than the control group, but not from one another: these 2 groups were thus combined for evaluation of relationships with physiological, demographic, HIV-related, and brain volume variables. After accounting for a number of significant correlations, enduring statistical relationships were found between subjective neuropathy and a smaller volume of the precuneus, objective neuropathy (feet) and a smaller volume of the parietal supramarginal cortex, and ataxia and a smaller volume of the pons. The relationship between subjective neuropathy and precuneus might have been predicted as the precuneus is involved in reflective self-awareness. The relationship between objective neuropathy (feet) and parietal supramarginal cortex concurs with a study demonstrating that neuropathy is associated with volume of the posterior cingulate: both studies implicate the parietal cortex. Worse performance on ataxia with eyes closed was associated with smaller volume of the pons extending our previous work demonstrated a relationship between compromised postural stability and pontocerebellar volume deficits in HIV. In summary, this study supports the contention that peripheral neuropathy has a central component.

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HIV gp120 increases GABAergic synapses and tonic inhibitory current through pathways that diverge downstream of interleukin-1 receptors

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HIV-associated neurocognitive disorder (HAND) affects about half of HIV-infected patients. HAND is mainly mediated by neurotoxins released from HIV-infected cells, including the HIV envelope protein gp120. Previous studies showed that gp120IIIB impairs excitatory synaptic signaling by potentiating NMDA receptors (NMDARs). Because normal cognitive function requires balanced excitatory and inhibitory neurotransmission, we examined the effects of gp120 on tonic GABA receptor (GABAR)-mediated currents and the number of inhibitory synapses using rat hippocampal cultures. Tonic inhibition was defined as a bicuculline-induced shift in basal GABAR currents. Inhibitory synapses were quantified by counting the number of fluorescent puncta in live cells labelled with a recombinant antibody-like protein targeted to gephyrin, a scaffolding protein at GABAergic synapses. The number of puncta correlated with the frequency of mIPSCs. Treatment with 600 pM gp120 increased tonic inhibition by 4 h and the number of inhibitory synapses by 24 h. gp120 increased both types of inhibition through activation of C-X-

C chemokine receptor 4 on microglia and subsequent release of interleukin-1β (IL-1β). Both tonic and synaptic effects of gp120 were blocked by an IL-1 receptor antagonist. Activation of p38 mitogen-activated protein kinase increased tonic inhibition independent of activation of NMDARs or protein synthesis. Tonic currents were mediated in part by α5-GABARs. The increase in the number of inhibitory synapses required a src family kinase, activation of GluN2A-containing NMDARs and protein synthesis. Increased tonic and synaptic GABA signaling may be a mechanism to compensate for excessive excitatory input induced by gp120. Excess inhibitory tone contributes to cognitive impairment in many neurodegenerative disorders, and this study suggests changes in GABAergic signaling may contribute to network dysfunction in HAND.

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Exosomes from IFNα-treated Hepatic Stellate Cells Transport the Antiviral Factors that Inhibit HCV Infection in Hepatocytes

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Host cell innate immunity plays an important role in controlling hepatitis C virus (HCV) infection. However, HCV has evolved complex mechanisms to evade or counteract host cell immunity. There is little information about whether HCV non-target cells in liver participate in the immune defense against HCV. Here we investigated whether IFN-α treatment of human hepatic stellate cells (HSCs) can produce antiviral factors that inhibit HCV in hepatocytes. We showed that IFN-α-treated HSCs produced a number of antiviral ISGs (ISG15, ISG56, MxA, OAS-1). More importantly, we found that supernatant (SN) from IFN-α-treated HSCs cultures could efficiently inhibit HCV replication in Huh7 cells in a dose-dependent manner. Our further investigation showed that IFN-α-treated HSCs release exosomes with the antiviral ISGs, which could be internalized by the hepatocytes. The depletion of exosomes from IFN-α-treated HSCs cultures diminished the anti-HCV effect mediated by HSCs SN. These observations indicate HSCs-based immune response is an important defense mechanism against HCV immune evasion in liver.

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Human Hepatic Stellate Cells Release Antiviral Factors that Inhibit HBV Replication in Hepatocytes

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There is limited information about the role of hepatic stellate cells (HSCs) in liver innate immunity against hepatitis B virus (HBV) infection. We thus examined whether HSCs can be immunologically activated and produce antiviral factors that inhibit HBV replication in human hepatocytes. We found that HSCs expressed functional TLR-3, activation of which by polyI:C induced IFN-β and the phosphorylation of IFN regulatory factors 3 and 7 (IRF3 and IRF7), the key regulators of IFN signaling pathway. When hepatocytes were pretreated with supernatant (SN) from poly I:C-activated HSCs, HBV replication was significantly suppressed in hepatocytes. This SN action of HSCs on HBV inhibition was mediated through IFN-β, as the antibody to IFN-β could neutralize the anti-HBV effect of SN from poly I:C-activated HSCs. In addition, the treatment with the activated HSC SN could induce a number of IFN-stimulated genes (ISGs: ISG20, ISG54, ISG56, OAS-1, Trim22 and Trim25) and phosphorylation of STATs in hepatocytes. These observations indicate that HSCs may have a previously unrecognized immunologic function, which is involved in liver innate immunity against HBV infection through the activation of IFN-β signaling pathway.

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ONC201 INHIBITS HIV-1 REPLICATION IN HUMAN MACROPHAGES VIA FOXO3A and TNFSF10

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Despite the success of antiretroviral therapy (ART), eradication of HIV-1 from brain reservoir cell types such as perivascular macrophages and microglia remains elusive. Macrophages and microglia express transcription factor FOXO3a and TNF superfamily cytokine TNFSF10, which are known to targets HIV-1-infected macrophages for apoptosis upon activation. ONC201 is a novel and potent small molecule FOXO3a activator capable of inducing TNFSF10. It can cross blood-brain barrier, and has shown antitumor effect in clinical trials. We hypothesize that targeting FOXO3a/TNFSF10 through ONC201 will suppress HIV-1 and improve ART in the CNS reservoirs. Using primary human monocyte-derived macrophages, microglia, and macrophage-tropic HIV-1ADA, we demonstrated that ONC201 dose-dependently decreased HIV-1 replication levels as determined by HIV-1 reverse transcriptase activity assay and Western blots for p24. Consistent with data on HIV-1 replication, ONC201 also reduced integrated HIV-1 DNA in infected macrophages and microglia in two step Alu-based nested PCR. Interestingly, the levels of HIV-1 replication in the infected cells were negatively correlated with ONC201-induced FOXO3a activation and TNFSF10 expression. Blocking TNFSF10 or knockdown of FOXO3a with siRNA reversed ONC201-mediated HIV-1 suppression, suggesting that ONC201 suppresses HIV-1 through FOXO3a and TRAIL. The anti-HIV-1 effect of ONC201 was validated in an in vivo studies, where HIV-1-infected macrophages were intracranially injected into the basal ganglia of NOD/scid-IL-2Rgcnull mice. Daily intraperitoneal injection of ONC201 for 6 days significantly decreased p24 levels in macrophages, suggesting that ONC201 suppresses HIV-1 in vivo. To determine whether ONC201 synergizes with current anti-HIV-1 treatment, we treated macrophages with ONC201 along with reverse transcriptase inhibitor azidothymidine (AZT). Addition of ONC201 increased the potency of AZT and achieved longer viral suppression during viral rebound. Therefore, ONC201 can be a promising drug candidate to combat persistent HIV-1 infection in the CNS.

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Astrocyte activation during reovirus encephalitis

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Reovirus encephalitis was used as a model system to investigate mechanisms underlying astrocyte activation following viral infection of the brain. Reovirus infection resulted in astrogliosis in areas of the brain that are targeted by reovirus, with marked changes in astrocyte morphology and increased expression of glial fibrillary acidic protein (GFAP), as well as additional genes associated with astrocyte activation. Astrogliosis also occurred following reovirus infection of ex vivo brain slice cultures (BSCs) demonstrating that reovirus-induced astrocyte activation can be brought about by factors intrinsic to the central nervous system (CNS). In areas where infection was pronounced, an absence of astrocytes was consistent with activation-induced cell death (AICD) as a mechanism of inflammation control. The presence of activated Bak in astrocytes following reovirus infection of the mouse CNS indicated that activated astrocytes are cleared from reovirus infected brains by Bak-mediated apoptosis. In agreement with previous reports, reovirus antigen did not co-localize with GFAP in infected brains cells suggesting that reovirus does not infect astrocytes. However, interferon (IFN) treatment of primary astrocytes resulted in the up-regulation of GFAP and cytokines that are associated with astrocyte activation, as well as Bak activation. In addition, the ability of media from reovirus-infected BSC to activate primary

astrocytes was blocked by IFN antibodies. These results suggest that IFN, released from reovirus-infected neurons, results in the activation of astrocytes during reovirus encephalitis and that these activated astrocytes are cleared from the CNS by Bak-mediated apoptosis.

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Effectiveness of LIF to modulate the effects of macrophage in HIV-1 Nef neurotoxicity

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Reovirus encephalitis was used as a model system to investigate mechanisms underlying astrocyte activation following viral infection of the brain. Reovirus infection resulted in astrogliosis in areas of the brain that are targeted by reovirus, with marked changes in astrocyte morphology and increased expression of glial fibrillary acidic protein (GFAP), as well as additional genes associated with astrocyte activation. Astrogliosis also occurred following reovirus infection of ex vivo brain slice cultures (BSCs) demonstrating that reovirus-induced astrocyte activation can be brought about by factors intrinsic to the central nervous system (CNS). In areas where infection was pronounced, an absence of astrocytes was consistent with activation-induced cell death (AICD) as a mechanism of inflammation control. The presence of activated Bak in astrocytes following reovirus infection of the mouse CNS indicated that activated astrocytes are cleared from reovirus infected brains by Bak-mediated apoptosis. In agreement with previous reports, reovirus antigen did not co-localize with GFAP in infected brains cells suggesting that reovirus does not infect astrocytes. However, interferon (IFN) treatment of primary astrocytes resulted in the up-regulation of GFAP and cytokines that are associated with astrocyte activation, as well as Bak activation. In addition, the ability of media from reovirus-infected BSC to activate primary astrocytes was blocked by IFN antibodies. These results suggest that IFN, released from reovirus-infected neurons, results in the activation of astrocytes during reovirus encephalitis and that these activated astrocytes are cleared from the CNS by Bak-mediated apoptosis.

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