Abstract Booklet

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Current as of June 1st, 2021

Poster No. 1

Brain immunophilin response to SARS-CoV-2

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Recent reports suggest that up to 50% of the COVID-19 (SARS-CoV-2) surviving patients have neurologic symptoms. We performed a pilot autopsy study on cases banked since April 2010: HIV+ SARS+, and HIV- SARS+. Systemic and specific tissue distribution of SARS-CoV-2 was assessed using digital droplet PCR (ddPCR) and immunohistochemistry (IHC) for the spike RNA and protein. Our preliminary data show that SARS-CoV-2, while undetectable in the serum at the time of autopsy, is present in lungs, liver, spleen, trigeminal ganglia, and the brain. Significantly, in COVID-19 cases brain neuroglial reactivity was associated with a robust immunophilin (IP) response characterized by upregulation of FKBP52 and FKPB51 in the gray matter neuropil and white matter axonal tracts. Both IP are key molecules in the glucocorticoid receptor (GR) translocation to the nucleus and the downstream gene transcription cascade involved in the response to chronic neuroendocrine stress. Additionally, we have immunohistologic evidence that infiltrating brain macrophages, expressing higher levels of immunophilins, may be infected with SARS-CoV-2 resembling the HIV "Trojan Horse hypothesis" invasion of the brain. These data raise the possibility that the brain may become a sanctuary for SARS-CoV-2. Furthermore, we propose that in post COVID-19 surviving patients with chronic neurologic disease the IP co-chaperons responsible for brain GR homeostasis may be suitable targets for neuroprotective interventions using currently FDA approved drugs like Rapamycin and FK506.

Poster No. 2

In silico design of a SHERLOCK-based point-of-care diagnostic for HIV-1 drug resistance

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Recent developments in antiretroviral therapy (ART) have reduced human immunodeficiency virus type 1 (HIV-1) infection from a potent killer to a chronic illness. However, major HIV-1 drug resistance mutations (DRMs) hamper ART's efficacy. Low-resource regions cannot screen for DRMs due to poor infrastructure and high testing costs. To overcome this obstacle, we propose using Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) to act as a rapid and inexpensive HIV-1 DRM diagnostic assay. Here, we present an in silico proof-of-concept using HIV-1 protease. SHERLOCK employs Loop-mediated isothermal AMPlification (LAMP) and a Cas12b-guide RNA (gRNA) complex to isothermally amplify protease sequences and detect any amplified DRM targets, respectively. Different LAMP primers and gRNAs were packaged together to account for HIV-1 genetic variation. DRMs and sequences were collected from the 2019 IAS-USA Drug Resistance Mutations List and Stanford HIV Sequence Database, respectively. LAMP primers were scored by their target diversity and thermodynamic characteristics to select sensitive primer sets. Sequences not targeted by these sets were re-inputted into the pipeline. The best sets from three iterations were combined into a sensitive (70.5%) package. Next, DRM-specific gRNAs were ranked by F3-score and the top 128 gRNAs with ≥95% specificity for each DRM were considered for

packaging. gRNAs were incrementally added to DRM-specific packages until the following ranked gRNA would only provide an additional sensitivity of <1%. All packages were specific (92.2%±4.3%) and a majority (21/24) were highly sensitive (≥90%). DRM-specific packages were then combined into highly sensitive (95.2%±2.1%) drug-specific meta-packages. Future directions entail improving LAMP primer and gRNA robustness and validating package performances in vitro.

Poster No. 3

Human NK Cells target JC Polyomavirus

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Most healthy individuals are persistently infected with the human polyomavirus JC (JCPyV) without significant consequences, yet in immunocompromised hosts, JCPyV can cause an often fatal disease -- progressive multifocal leukoencephalopathy (PML). There are currently no effective therapeutics to prevent or treat PML and novel immune-based therapies are urgently needed. The importance of CD8+ cytotoxic lymphocytes in controlling viral infection has been demonstrated, little is known about natural killer (NK) cell responses against JCPyV. NK cells are effector cells of the innate immune system that play critical roles in defense against viral infections or nascent neoplasms. We hypothesized that specific subsets of NK cells can mediate potent antiviral responses against JCPyV and aim to define the mechanisms crucial in controlling JCPyV by NK cells. To study NK cell responses to JCPyV-infected cells, SVGA cells were infected with the JC SVEdeltaM1 virus and co-cultured with NK cells purified from whole blood. Killing of JCPyV-infected cells by NK cells was determined using flow cytometry quantification of cells expressing the VP1 capsid protein and the Large T antigen regulatory protein in the presence or absence of NK cells. In parallel, NK cell degranulation and cytokine production were measured by intracellular staining in response to JCPyV-infected SVGA cells as well as in response to PBMC pulsed with four sequential pools of overlapping peptides spanning the JCPyV major capsid protein VP1. We observed a 50-60% decrease in JCPyV-infected cells when co-cultured in the presence of NK cells. NK cells also displayed enhanced degranulation and significant IFN-y production against VP1 peptide pools in the absence of detectable T cell responses. Thus, NK cells can recognize and eliminate JCPyV-infected cells, strongly suggesting NK cells may significantly contribute to regulating JCPyV replication and may have the potential to be harnessed for immunotherapies against PML.

Poster No. 4

Extracellular vesicles from HTLV-1 infected cells modulate target cells and viral spread

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The Human T-cell Lymphotropic Virus Type-1 (HTLV-1) is the causative agent of Adult T-cell Leukemia/Lymphoma (ATLL) and HTLV-1 Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Up to 10 million of people worldwide are infected with HTLV-1. We have previously shown Extracellular Vesicles (EVs) derived from HTLV-1-infected cells promote cell aggregation which enhance viral spread through cell-cell contact. In this study, we used differential ultracentrifugation (DUC) to separate EVs from HTLV-1 infected cell supernatants into different EV subpopulations (2 k, 10 k, and 100 k). Through proteomic analysis, we found that differential packaging of viral/host proteins into different EV types. EVs (2 k) subpopulation carries the highest viral/host protein among other EV types. Our Western

blot data showed that the 2 k and 10 k EV populations contained viral proteins (p19 and Tax), and autophagy proteins (LC3 and p62), which may indicate the biogenesis pathway generating each EV population. The data from an angiogenesis assay showed that 2 k and 10 k HTLV-1 EVs caused tubular deterioration, suggesting their potential role in HTLV-1 pathogenesis. HTLV-1 EVs induced expression of cytokines responsible for cell migration and inflammation (IL-8 and IL-6, respectively) in CNS-related cells (i.e., astrocytes, macrophages, and neurons). Additionally, HTLV-1 EVs enhanced cell-cell contact ultimately viral spread in monocytic cell-derived dendritic cells. 2 k and 10 k HTLV-1 EVs caused an increase in proviral DNA and RNA levels in humanized mice tissue (i.e., Blood, Lymph Node, and Spleen), suggesting the important role of these EV subpopulations in HTLV-1 viral spread. In conclusion, our findings suggested that different HTLV-1 EV types promote tissue damage, cytokine expression, and viral spread.

Poster No. 5

Abstract withdrawn

Poster No. 6

Evidence supporting the involvement of Pur-alpha in protein quality control (PQC) and cell survival

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Pur-alpha is a ubiquitous protein which its excess amount prevents the growth of cancer cells and blocks their proliferation; thus, it has been considered as a tumor suppressor. Pur-alpha is known to contribute to the pathogenesis of several diseases including AIDS, FRX, ALS and PML. Identifying the cellular partners of Pur-alpha which can contribute to its role in cell survival and homeostasis is essential to understand the etiology of those diseases associated with Pur-alpha alteration. Bcl-2-associated athanogene 3(BAG3) is the co-chaperone protein and part of PQC. BAG3 has a unique antiapoptotic activity that enhances survival of stressed cells. Here, we hypothesize that Pur-alpha/BAG3 together promote cell survival. Using fibroblasts derived from our animal model, we introduced multiple stressors to the cells, and we studied the consequence in the presence and absence of Pur-alpha. Our observations indicated colocalization and correlation between Pur-alpha and BAG3 under both oxidative stress and heat shock conditions. Interestingly, our results demonstrated that in the presence of Pur-alpha, the cell has a very strong response to stress, however in the absence of Pur-alpha there is no significant alteration in cell homeostasis. These observation on the correlated BAG3/ Pur-alpha alteration turned out to be not cell specific and different cell types show different level of Pur-alpha/BAG3 alteration under the stress. Altogether, our current results suggest a critical relationship between Pur-alpha and BAG3 and how they interact with each other to regulate protein quality control and cell survival.

Poster No. 7

Amyloid transfer via extracellular vesicles of the blood-brain barrier: modulation by RAGE and HIV-infection

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Increased amyloid beta (Abeta) deposition was demonstrated in HIV-infected brains and associated with neurocognitive disorders. The blood-brain barrier was hypothesized to be crucial in the brain's Abeta homeostasis. In addition, extracellular vesicles (EVs) may be important players in Abeta pathology. Therefore, we focused on the role of brain endothelial EVs in the HIV-1 associated Abeta pathology. Exposure to HIV-1 facilitated shedding of EVs from human brain endothelial cells and affected their Abeta cargo. Exposure to HIV and/or Abeta markedly altered surface and total proteome profile of EVs. Interestingly, brain endothelial EVs transferred their Abeta cargo to cells of the neurovascular unit, including neural progenitor cells (NPCs). Mechanistically, we investigated a possible involvement of the

receptor for advanced glycation end products (RAGE) in these events. EVs loaded with Abeta (Abeta-EVs) were readily taken up by NPCs and Abeta partly colocalized with the inflammasome markers ASC and NLRP3 in the nuclei of the recipient NPCs. This colocalization was affected by HIV and RAGE inhibition by a high-affinity specific inhibitor FPS-ZM1. Blocking RAGE resulted also in an increase in EV number produced by brain endothelial cells, decreased Abeta content in EVs, and diminished Abeta-ECVs transfer to NPC nuclei. Interestingly, both Abeta-EVs and RAGE inhibition altered NPC differentiation. Overall, these data indicate that RAGE inhibition affects brain endothelial EV release and Abeta-EVs transfer to NPCs. These events may modulate EV-mediated amyloid pathology in the HIV-infected brain and contribute to the development of HIV-associated neurocognitive disorders. Supported by the National Institutes of Health (NIH) grants MH122235, MH072567 and MH098891 and the Florida Department of Health grant 8AZ24.

Poster No. 8

Morphine, Methamphetamine, and Antiretroviral Drugs Promote Inflammatory Properties in Macrophages and Change Macroautophagy: Implications for HIV Neuropathogenesis

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HIV enters the CNS most often before an individual starts antiretroviral therapy (ART). Infected CNS macrophages release factors that induce neuronal damage and cognitive dysfunction, HIV-associated neurocognitive disorders (HAND), in ~15-40% of people living with HIV (PLWH) despite ART. Opioids and methamphetamine (meth) may exacerbate cognitive dysfunction in PLWH through mechanisms affecting macrophage homeostasis. We have characterized the impact of morphine, an opioid, meth, and common ART regimens on functions of primary human monocyte derived macrophages (MDM) that are dysregulated in HAND. We demonstrated that morphine and/or ART significantly increase phagocytosis of E. coli and latex beads in uninfected MDM, and in HIV-infected MDM, increase phagocytosis of beta-amyloid, which exacerbates neuropathogenesis. Meth appears to decrease beta-amyloid uptake in infected MDM. Morphine, meth, and ART also significantly increase ROS, which can be neurotoxic. Additionally, meth significantly increases ROS during ongoing mitochondrial stress in HIV-infected MDM while morphine does not. Dysregulation of phagocytosis and ROS by morphine, meth, and ART may relate to changes in macroautophagy, a quality control degradative process that, in part, regulates macrophage functions that contribute to HAND. We determined the impact of morphine, meth, and ART on autophagy in MDM by Western blotting for LC3II, a key protein on autophagosomes, and for p62, which mediates selective degradation of protein aggregates, and by confocal immunofluorescence. In uninfected MDM, morphine +/- ART significantly induce autophagy to reduce potential stress yet inhibit p62 mediated autophagy. In HIV-infected MDM, morphine with/without ART, or meth alone, induces autophagy and impairs total flux and p62 mediated autophagy. In response to morphine, meth, and ART, infected cells cannot effectively upregulate autophagy to mitigate drug-induced stress. We will characterize further how substance use impacts HIV neuropathogenesis to develop therapies that alter macroautophagy to restore homeostasis in CNS macrophages in people with HAND with substance use disorder.

Poster No. 9

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Poster No. 10

IFNalpha and beta mediated JCV suppression through C/EBPbeta-LIP isoform

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The polyomavirus JC (JCV) causes the demyelinating disease progressive multifocal leukoencephalopathy (PML). Infection by JCV is very common in childhood after which the virus enters a latent state, which is poorly understood. Under conditions of severe immunosuppression, especially AIDS, JCV may reactivate to cause PML. JC viral proteins expression is regulated by the JCV non-coding control region (NCCR), which contains binding sites for cellular transcriptional factors which regulate JCV transcription. Our earlier studies suggest that reactivation occurs within glial cells due to the action of cytokines such as TNF-a which stimulate viral gene expression. In this study, we have examined the cytokine interferon-alpha (IFNalpha) or beta (IFNbeta) which, in contrast, has a negative effect on JCV transcriptional regulation. We also showed that interferon-a or b induce the endogenous liver inhibitory protein (LIP), an isoform of CAAT/enhancer binding protein beta (C/EBPbeta). Treatment of glial cell line (SVGA) with IFNalpha or IFNbeta increases the endogenous level of C/EBPbeta-LIP in time dependent manner. Furthermore, we could show that the negative regulatory role of interferon a or b in JCV early and late transcription and viral replication is more pronounced in the presence of C/EBPbeta-LIP. Knock down of C/EBPbeta-LIP by shRNA reverse the inhibitory effect on JCV viral replication. Therefore, interferon-a or b negatively regulates JCV through induction of C/EBPbeta-LIP, which together with other cellular transcriptional factors may control the balance between JCV latency and activation leading to PML. This balance may be regulated by proinflammatory cytokines in the brain.

Poster No. 11

Role of microglia specific p38alpha MAPK signaling in NeuroAIDS

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Human immunodeficiency virus-1 (HIV-1) causes severe and progressive neurological impairment in humans known as HIV-associated neurocognitive disorders (HAND). The HIV-1 envelope glycoprotein gp120 is an extracellular protein that is shed from infected cells and interact with distant uninfected brain cells like microglia, neurons and astrocytes. Transgenic mice expressing HIV-1 coat glycoprotein gp120 in brain glial cells (HIVgp120tg) display neuropathological features similar to HIV dementia patients. During inflammation, p38alpha MAPK is known to regulate the biosynthesis of pro-inflammatory cytokines in endotoxin-stimulated monocytes. Thus, p38 MAPK may be an important mediator in the development of HAND and immunodeficiency during HIV-1 infection. To determine the role of microglial p38 signaling in HIV mediated neuronal injury in vivo, we generated p38 floxed CX3CR1 Cre HIVgp120-expressing mice that have a p38 knockout specifically in microglia and macrophages. Brain sections of these mice were stained for neuronal MAP2, synaptophysin and GFAP. Astrocytic GFAP levels remained unaffected by microglial p38 deficiency. However, we observed a significant loss of MAP2 and synaptophysin levels compared to non-tg controls only in HIVgp120tg animals with p38-expressing microglia but not in HIVgp120-expressing brains with microglia-specific p38 knockout, indicating p38 deficiency in microglia protected neurons from HIVgp120 toxicity. Further, we sought to analyze the changes in the microglia transcription profile in HIV glycoprotein expressing mice. We sorted microglia cells from wild type, gp120 tg, microglia specific p38alpha knockout and microglia specific p38alpha knockout expressing gp120 mice for further gene expression analysis using RNA seq. Thus, our model confirms the critical role of microglial p38alpha MAPK in the neurotoxicity caused by the HIV-1 envelope protein. Funding Resources:R01 MH087332, MH104131, MH105330, DA052209 to M.K.

Poster No. 12

Post-acute SARS-CoV-2 infection in non-human primate olfactory system and extended amygdala

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The extent of viral invasion and persistence of SARS-CoV-2 in the brain has not yet been determined. Here, using SARS-CoV-2 infected non-human primates and the RNAscope in situ hybridization technique, the distribution of SARS-CoV-2,angiotensin-converting enzyme 2 (ACE2), and transmembrane serine protease (TMPRSS2)were identified in the olfactory epithelium and pyriform cortex/amygdala from recovering SARS-CoV-2 infected non-human primates. Cells of the olfactory epithelium and pyriform cortex/amygdala exhibited SARS-CoV-2 mRNA and DNA, ACE2, and TMPRSS2. Furthermore, dual-labeling of platelet-derived growth factor receptor beta, and SARS-CoV-2 indicated that pericytes were the cell type to harbor the virus in the pyriform cortex/amygdala. Critically, there was no relationship between viral load, clinical assessment or lung histopathologic score and SARS-CoV-2 mRNA in the pyriform cortex/amygdala.

Poster No. 13

A Human Cerebral Organoid Model for HIV-1 Virus-Host Interactions

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While combined antiretroviral therapy (cART) has reduced the incidence and severity of HIV-related AIDS, up to half of HIV-positive patients must still grapple with neurological complications. These complications, which are gaining prevalence due to longer life expectancy of HIV individuals on cART, can eventually escalate into HIV-Associated Neurocognitive Disorder (HAND). There is an urgent need to develop a more representative human model system to study the molecular and cellular mechanisms underlying HIV-related neuropathology. Here we develop human microglia-containing cerebral organoids (hmCOs) derived from human induced pluripotent stem cells (hiPSC) to investigate how HIV-1 infection leads to microglia dysregulation, persistent inflammation, and neuronal functional changes. Using immunohistochemistry, gene expression, and electrophysiological analyses, we characterized the morphology and functionality of neurons as well as microglial cells in hmCOs. We found that neurons in hmCOs display mature functional and morphological characteristics, including firing repetitive action potentials and forming functional synapses. We also found that microglia successfully integrate and interact with neurons within hmCOs. We infected primitive microglial precursor cells (PMPs) with HIV-1 (JR-FL) and detected productive infection by p24 production in a time-dependent manner. Further, we incorporated HIV-1-infected PMPs into hmCOs and showed the viability and mobilization of HIV-1 infected microglial cells. Our data indicate that human stem cells were efficiently differentiated into microglia precursors and neural progenitor cells that can mature into functional cerebral organoids. We are currently conducting intensive analyses to reveal the impact of HIV-1 infection on microglial and neuronal physiology. A well characterized HIV-hmCO model presents a unique in vitro platform for investigating the underlying molecular and cellular mechanisms associated with HIV-1-human neural cell interactions. We anticipate that this platform will help facilitate new insights into pathogenesis and novel therapeutics for neuroAIDS.

Poster No. 14

Using the Herpes Simplex Virus 1 protein US9 as a novel tool to control APP trafficking and APP beta/alpha-cleavage

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Altered Amyloid Precursor Protein (APP) processing and Amyloid beta (Abeta) production have long been implicated in the cognitive decline associated with age-related neurological disorders such as Alzheimer's Disease (AD) and HIV-Associated Neurocognitive Disorders (HAND). Past attempts to mitigate the neuronal deficits caused by alterations of APP processing have focused exclusively on Abeta. Here, we exploited the molecular properties of the Herpes Simplex Virus 1 (HSV-1) transport protein US9 to generate novel chimeric constructs that target APP trafficking and processing by different pathways. Our approach effectively modulates APP-dependent biochemical steps that lead to neuronal damage and loss, including the phosphorylation of APP, tau, and glycogen synthase kinase 3beta, in addition to controlling APP beta-cleavage. We further demonstrate the release of neuroprotective soluble alphaAPP as a consequence of altered APP trafficking. Notably, these effects rely on the activity of endogenous proteins and constitutive targets in neurons. Overall, these findings introduce a multimodal approach to limit APP misprocessing and its cellular consequences without directly targeting secretase activity, thus lending itself well to the study of fundamental cellular mechanisms of APP processing/trafficking. Furthermore, this strategy offers novel tools that may treat cognitive impairment in age-related pathologies including Alzheimer's disease and HIV-Associated Neurocognitive Disorders.

Poster No. 15

Retroviral Infection of Human Neurospheres and Use of Stem Cell EVs to Repair Cellular Damage

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HIV-1 remains an incurable infection and HIV-associated neurocognitive disorders are reported to affect at least 50% of infected individuals despite cART treatment. Recently, neurospheres derived from iPSCs have been used to model the effects of neurotropic viruses. Here, we report the generation of neurospheres from iPSC-derived neural progenitor cells. Our data suggests these cultures contain microglia-like cells that are permissive to retroviral infection, as well as the induction of cytokines and reversal with Cannabidiol. However, repair of infected cells requires additional treatments. Stem cells have broad therapeutic potential and stem cell therapy has been evaluated for CNS repair. While the exact mechanisms are unknown, it is believed that extracellular vesicles (EVs) mediate many of their functional effects. Because of their small size, stability, and low immunogenicity, EVs hold high therapeutic potential, especially for CNS pathologies since they can cross the blood-brain-barrier. Here, we also report the isolation of high yields of EVs from iPSCs and mesenchymal stem cells. Our EV characterization includes phenotypic (size, tetraspanin expression), biochemical (protein, cytokine, RNA), and functional analysis. Consistent with the literature, our data suggests that stem cell EVs may modulate neuroprotective and anti-inflammatory properties in damaged and/or infected CNS cells. Collectively, this data demonstrates the feasibility of NPC-derived neurospheres for modeling HIV-1 infection and highlights the potential of stem cell EVs for rescuing cellular damage induced by HIV-1 infection.

Poster No. 16

Abstract withdrawn

Poster No. 17

HIV infection and antiretroviral therapy modulates splicing factors in peripheral blood mononuclear cells from HIV-positive patients with cognitive impairment

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Soluble insulin receptor (sIR) levels are higher in the plasma and exosomes from HIV-positive patients under combined antiretroviral therapy, compared to HIV-negative subjects. We have observed insr RNA variants in cells isolated from the cerebrospinal fluid and plasma of HIV-positive patients. Therefore, we hypothesize that the release of the insulin receptor to the plasma, free and in exosomes, may be a result of the action of intracellular splicing factors SF2/ASF and hnRNPA1 generating sIR variants. We first exposed peripheral blood mononuclear cells (PBMCs) from control donors to HIV-1 Tat, and after HIV-Tat immunoprecipitation, we measured the levels of free and Tat-bound splicing factors. Both splicing factors effectively bind to HIV-Tat in Tat stimulated PBMCs ($p<0.001$). In addition, after HIV-Tat removal, the free levels of SF2/hnRNPA1 ratio are higher in Tat-stimulated PBMCs compared to untreated ($p=0.014$). We measured the levels of the splicing factors SF2/ASF and hnRNP-A1 in PBMCs from HIV-positive patients, stratified by cognitive status. In HIV-positive patients SF2/ASF is significantly higher ($p=0.009$), while hnRNPA1 is lower ($p=0.002$) compared to control subjects. In control subjects, splicing factors ratio has a negative correlation with sIR in plasma ($r=-0.45$, $p=0.011$), while this association is not observed in HIV-positive patients ($r=-0.12$, $p=0.360$). In all patients with detectable HIV RNA in plasma, the levels of SF2/ASF have a significant positive correlation with viral load ($r=0.6783$, $p<0.001$). Moreover, in HIV-positive cognitive impaired patients, higher SF/ASF levels correlate with worse executive function ($r=-0.39$, $p=0.002$) and lower Antiretroviral CNS Penetration-Effectiveness (CPE) score ($r=-0.037$, $p=0.041$). When we targeted SF2/ASF with siRNA in PBMCs from HIV-positive patients, the sIR levels positively correlated with hnRNPA1 ($r=0.8572$, $p=0.006$). These results suggest that viral infection and antiretroviral therapy regimen modulate the levels and functions of SF2/ASF and hnRNPA1 splicing factors in PBMCs, which may contribute to cognitive decline in HIV-positive patients.

Poster No. 18

Role of caspase-1 activation in HIV-1 associated atherogenesis

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Atherosclerosis-associated cardiovascular disease (CVD) is a leading cause of mortality among people with HIV (PWH). Macrophages phagocytose oxidized low-density lipoprotein (ox-LDL), transitioning activated macrophages into foam cells. The activated macrophages and foam cells may be associated with release inflammatory cytokines such as interleukin-1beta (IL-1beta) promoting atherogenesis, showing the significant role of NLR family pyrin domain containing 3 (NLPR3) inflammasome. HIV infection and subsequent inflammatory processes in humans accelerate atherogenesis, likely through macrophage activation. Previously, we demonstrated that HIV integration alone is sufficient to accelerate atherogenesis. Here, we hypothesized that chronic HIV infection induces monocyte/macrophage activation via caspase-1 pathway, a downstream of NLRP3 inflammasome formation, contributing to HIV-associated atherogenesis. We investigated HIV-1-associated atherogenesis using a newly established HIV-1 transgenic mouse model on an atherogenic background (Tg26+/-ApoE-/-) and crossed with a caspase-1 knockout mouse (Tg26+/-

/ApoE-/-/casp-1-/-), an in vitro model system for foam cell formation and specimens from PWH. Our results indicate that caspase-1 knockout in Tg26+/-/ApoE-/-/casp-1-/- mouse model significantly decreased foam cell formation, thoracic plaque formation, IL-18 levels in serum and expression of CD36 scavenger receptor compared to Tg26+/-ApoE-/-. Macrophages from PWH on ART have a greater capacity to develop foam cell than those from HIV- controls with higher expression of NLRP3 and caspase-1. We further defined the role of transgenic expression of HIV and caspase-1 knock-out in hematopoietic cells on atherogenesis using bone marrow (BM) transplantation. Apoe-/- mice were reconstituted with BM cells from Tg26+/- Apoe-/- or Tg26+/-/ Apoe-/-/ Casp1-/- donors. After 5 weeks, mice were fed high-fat diets for 10 weeks and spleens, aorta and serum were harvested. There was a statistically significant decrease in foam cell formation and in aortic plaque in animals who received BM from Tg26+/-Apoe_/-casp-1-/-. These results demonstrate that expression of HIV-1 transcripts can drive atherosclerosis through activation of NLRP3 inflammasome and caspase-1 in inflammatory monocytes.

Poster No. 19

The relation of insular metabolite concentrations to chronic neuropathic pain and depression in people with HIV

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Background: Chronic pain and depression frequently co-occur and are highly comorbid in people with human immunodeficiency virus (PWH). These conditions have been linked to low concentrations of neurometabolites gamma-aminobutyric acid (GABA) and N-Acetylaspartate (NAA). Here, we used 1H-magnetic resonance spectroscopy (MRS) to assess the relationship between depressive symptoms and insular metabolite concentrations in healthy controls (HC) and PWH without neuropathic pain (NP-), with pain (NP+), and with pain plus chronic opioid therapy (NPO+).

Methods: Twenty-one PWH (mean age 56 years ±7.99) on stable antiretroviral-therapy and seven HC (58.1 years ±10.3) were recruited. Participants completed the Beck Depression Inventory (BDI), a well-validated self-report inventory for depression in chronic pain patients, and underwent MR brain imaging. 1H-MRS:data were obtained using a PRESS sequence (TE/TR=30/1700ms) with voxel (8cm3) placed in the left insula. Metabolites were quantified in LCModel with respect to unsuppressed water signal.

Results: Significant differences in BDI scores among the four cohorts ($p<0.001$) were identified. Post-hoc pairwise comparisons (Dunnett's test) showed lower BDI scores in HC than NP+ ($p=0.04$) and NPO+ ($p<0.001$). Group differences in insular GABA+ concentration were also observed, with NPO+ demonstrating lower GABA+ than HC ($p=0.024$; Dunnett's test). BDI score was negatively correlated with concentrations of GABA+ ($R=-0.55$; $p=0.023$) and NAA ($R=-0.53$, $p=0.019$) across all PWH.

When subdividing PWH by pain status, post-hoc pairwise comparisons (Tukey-Kramer test) showed higher BDI scores in PWH-with-pain (NP+/NPO+) than NP- ($p=0.0182$) and HC ($p=0.0004$). Significant differences in insular GABA+ were also observed ($p=0.0082$), with PWH-with-pain demonstrating lower GABA+ than NP- ($p=0.023$; Tukey-Kramer test) and HC ($p=0.0225$).

Conclusions: Our preliminary findings support a link between HIV+ status, pain, depression, and neurometabolite levels. The negative correlation between insular GABA+ and NAA concentrations and

BDI suggests that reductions in GABA-mediated inhibitory tone and neuronal integrity may accompany depressive symptoms in PWH. Further work is needed to corroborate these observations.

Poster No. 20

Differential cytokine-induced responses of polarized microglia

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Glial cells govern the activity of brain-infiltrating T-cells through upregulation of programmed death ligand 1 (PD-L1); and previous studies have demonstrated its induction following IFN-Gamma treatment. Here, we examined the role of several pro- and anti- inflammatory cytokines in modulating PD-L1 expression on M1- and M2-polarized microglia. Microglia were isolated from 10-14 d old mice using the Miltenyi adult dissociation kit, followed by CD11b selection. We subsequently assessed PD-L1, MHC-II, Arginase 1 (Arg 1), and iNOS expression using real-time RT-PCR and multi-color flow cytometry. We treated microglia with IFN-Gamma for 24 h, 48 h, and 72 h, which resulted in 83.3%, 94.6 %, and 97% of the cells expressing PD-L1, respectively. Moreover, IFN-Gamma also stimulated expression of MHC-II. We observed 20.8 %, 37.2 %, and 57.5 % of microglia expressing MHC-II at 24, 48, and 72 h post-stimulation, respectively. We did not observe Arg 1 expression after IFN-Gamma stimulation, but observed 48.6% of the cells expressing iNOS at 48 h. However, upon treatment with the anti-inflammatory cytokine IL-4, PD-L1 expression was significantly decreased (17.7 %, 33.9 %, and 38.2 % at 24 h, 48 h, and 72 h post-treatment, respectively) when compared to IFN-Gamma treatment. We did not observe any MHC-II or iNOS expression on IL-4-treated cells. Instead, the microglia were M2-polarized as indicated by expression of Arg-1 (27.7%, 37.3%, and 42.3% at 24 h, 48 h and 72 h, respectively). As a positive control, we also treated microglia with LPS and observed expression of both PD-L1 and iNOS. Finally, when we treated these M2-polarized microglia with IFN-Gamma for 48 h, we observed a significant increase in expression of both PD-L1 and MHC-II. RT-PCR data supported these flow cytometric observations. We are currently studying the effects of various cytokines in modulating responses from both M1- and M2-polarized microglia.

Poster No. 21

Mechanisms of HIV and methamphetamine mediated neuropathogenesis in the ART era

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HIV enters the CNS early after peripheral infection, establishing viral reservoirs that persist despite antiretroviral therapy (ART). One complication of HIV infection in the CNS is HIV associated neurocognitive disorders (HAND) that develop despite ART. Substance use disorder, including methamphetamine use, is a comorbidity in people living with HIV (PLWH). Some studies show that methamphetamine increases neuroinflammation and cognitive disorders in PLWH. To characterize the impact of methamphetamine on HIV mediated neuroinflammation and viral seeding in the ART era, we examined its effect on mature monocyte entry into the CNS and its effect on the human blood brain barrier (BBB). Uninfected/HIV-infected mature monocytes were treated with methamphetamine, and/or ART (Tenofovir and Emtricitabine). Cells were added to a BBB model, and allowed to transmigrate to CCL2 or CXCL12, chemokines elevated in the CNS of PLWH. Our data indicate that methamphetamine increases transmigration of uninfected and HIV-infected cells. Our preliminary data also demonstrate that HIV Tat, present in the CSF of PLWH despite ART, increases transmigration of mature monocytes, with greater transmigration when monocytes are treated with methamphetamine. To characterize mechanisms that contributes to this increased transmigration, we treated mature monocytes with methamphetamine and stained for surface junctional proteins that mediate monocyte entry across the BBB. Methamphetamine increases ALCAM on HIV infected monocytes. These data suggest that methamphetamine may contribute

to increased monocyte transmigration, in part through increased ALCAM on HIV-infected cells. Treatments of the BBB did not affect permeability or junctional proteins indicating that methamphetamine effects are on the monocytes. Our ongoing studies are to identify targets to reduce/eliminate monocyte entry into the CNS, thereby reducing inflammation and viral seeding, contributing to eradication of reservoirs and HAND.

Poster No. 22

EcoHIV infection of rodent microglia activates the microglial NLRP3 inflammasome: Implications for NeuroHIV and cocaine abuse

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The chimeric EcoHIV is known to infect murine immune cells and is useful for the investigation of HIV-mediated immune responses, neuroinfection and pathogenesis in a small animal model. Although emerging evidence indicates the involvement of NLRP3 in HIV-1 and cocaine-mediated glial activation and neuroinflammation, whether cocaine potentiates the effects of HIV involving activation of the NLRP3 inflammasome has not been clearly investigated. To address this, mouse primary microglia (mPm) were infected with EcoHIV/NL4.3 with or without addition of cocaine or mock infected and evaluated for the expression of NLRP3 pathway mediators. Exposure of mPm to both EcoHIV and cocaine resulted in upregulated expression of NLRP3 compared to cells exposed to either EcoHIV or cocaine alone. Exposure of microglia to both EcoHIV and cocaine also increased the expression of mature IL-1beta suggesting increased activation of NLRP3 by the combination of these agents. These studies were validated in mice infected with EcoHIV for 21 days followed by analysis for inflammasome and microglial activation. NLRP3 and IL-1beta were significantly upregulated in the cortical brain region of EcoHIV infected mice compared with control animals. In addition CD11b, a marker of microglial activation was also upregulated in EcoHIV infected mice compared with control animals. Collectively, these findings suggest that both EcoHIV & cocaine could, via their co-operative actions, exacerbate microglial activation, thereby compounding the severity of HIV and cocaine-induced neuroinflammation. The future directions of this study involve exploring the potential of NLRP3 inhibition in alleviating neuroinflammation in the context of co-morbid EcoHIV infection and cocaine administration.

Poster No. 23

Neurocognitive impairment in recovering COVID-19 patients is related to abnormal Kynurenine pathway metabolites and anosmia but not disease/anxiety severity

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Background: COVID-19 is associated with cognitive impairment, the pathogenesis of which is unclear - possibly secondary to disease severity, hypoxia, anxiety/depression. The Kynurenine pathway (KP) is an interferon stimulated tryptophan degradation pathway important in immune tolerance, neurotoxicity and vascular injury. It is abnormal in COVID-19. We hypothesized that cognitive impairment was associated with an activated KP but not the previous factors.

Methods: The current analysis includes 132 COVID-19 patients as part of the ADAPT study, a prospective cohort. Disease severity was assessed by the number of symptoms. Bloods were taken at enrolment (2

months post diagnosis), 3 and 4 months. The KP was assessed by GC-MS and uHPLC. Patients had cognitive testing at 2 months with the Cogstate computerised cognitive battery, psychological assessment and olfaction testing using the NIH toolbox Odor Identification Test. A demographically-corrected composite z-score was created representing global cognitive performance, and then classified as impaired/unimpaired. A mixed effect model (time effect and cognition x time interaction) corrected for disease severity tested whether neurocognitive status was associated with KP products at enrolment, and follow-ups. The model also assessed KP product time dynamics.

Results: 132 patients (mean age=46±15; disease severity: 40% mild, 50% moderate, 10% hospitalised at risk of hypoxia). 17% had cognitive impairment with significantly elevated KP products: KYN ($p=0.0007$), KYN/TRP ratio ($p=0.002$), 3HK ($p=0.001$) and QUIN ($p=0.002$) at 2 months compared to unimpaired cases. These results were also significant when using cognitive performance as a continuous score. Cognitive impairment was correlated with anosmia ($p=0.03$), but not with disease severity, nor hypoxia, nor anxiety/depression. Models for PIC, 3HAA, AA and TRP were not significant.

Conclusions: COVID-19 cognitive impairment is strongly associated with KP activation related excitotoxicity suggesting a causal relationship.

Poster No. 24

Role of EVs and miR-29b in astrocytic COX-2 regulation

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In the current era of antiretroviral therapy chronic inflammation in the brain persists in people living with HIV-1 infection despite undetectable viral loads. Neuroinflammation triggered by early viral proteins such as Tat expressed by latently infected cells might contribute to HIV-1-associated neurocognitive disorders (HAND). In this study we assessed the role of miRNA in extracellular vesicles (EVs) released from microglia exposed to Tat on astrocyte DNA methyltransferase 3B (DNMT3B), cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) synthesis. EVs were isolated from conditioned media from human fetal brain derived microglia treated with HIV-1 protein Tat or left untreated by size exclusion chromatography. EVs were characterized for size distribution and yield by Nanoparticle tracking analysis using a NanoSight NS 500 instrument, and EV protein marker. microRNA analysis revealed increased miR29b levels in EVs isolated from microglia treated with Tat, Human astrocytes were treated with EVs derived from control and Tat exposed microglia and expression levels of DNMT3b, a potential target of miR29b and COX-2 were assessed by western blot analysis. In addition, methylation status in the COX-2 gene promoter region was assessed by methylation-specific PCR and PGE2 levels were measured in conditioned media. The results showed a significant decline in DNMT3b expression and increase in COX-2 expression and PGE2 levels in astrocytes treated EVs derived from Tat exposed microglia. Methylation-specific PCR of COX-2 promoter showed hypomethylation of CpG islands in COX-2 promoter. The results indicate that Tat leads to the upregulation of miR-29b level in EVs, which represses astrocytic expression of DNMT3b and enhances hypomethylation of the CpG islands in COX-2 gene promoter region. This in turn upregulates COX-2 mediated PGE2 synthesis in astrocytes which contributes to chronic inflammation.

Poster No. 25

Endolysosome localization of ERa is involved in neuroprotective effect of 17a-estradiol against HIV-1 gp120-induced neuronal injury

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Neurotoxic HIV-1 viral proteins contribute to the development of HIV-associated neurocognitive disorders (HAND), the prevalence of which is high (30 to 50%) and no effective treatment is available. Given

estrogen is neuroprotective, we determined the extent to which and mechanism by which 17a-estradiol (17a-E2), a natural non-feminizing estrogen, affects HIV-1 gp120-induced neuronal injury. We demonstrate in primary hippocampal neurons that estrogen receptor alpha (ERa) is localized to endolysosomes and that 17a-E2 acidifies endolysosomes. ERa knockdown or over-expressing ERa mutant deficient in endolysosome localization prevents 17a-E2-induced endolysosome acidification. Furthermore, we demonstrate 17a-E2-induced increased dendritic spine depends on endolysosome localization of ERa. Importantly, 17a-E2 protects against HIV-1 gp120-induced endolysosome de-acidification and reduction in dendritic spines, and such protective effects depends on endolysosome localization of ERa. Our studies demonstrate a novel endolysosome-dependent pathway in 17a-E2's neuroprotection, which could lead to the development of novel therapeutic strategies against HAND. (Supported by R01MH100972 and R01MH105329)

Poster No. 26

Effect of Cannabinoids on Extracellular Vesicle Release and Viral Transcription in HIV-1 infected myeloid cells

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As of 2019, approximately 38 million individuals were infected with human immunodeficiency virus type 1 (HIV-1)/AIDS globally. Of the 25.4 million people receiving antiretroviral therapy (ART) approximately 30-50% develop HIV-associated neurocognitive disorders (HAND). Our lab and others have shown that HIV-1 viral RNAs, such as Trans-activating Response (TAR) RNA, are incorporated into extracellular vesicles (EVs) and elicit an inflammatory response in recipient cells. Previous studies have shown that cannabis [Cannabidiol (CBD) and Œî9-tetrahydrocannabinol (THC)] use in people living with HIV-1 is associated with a lower viral load, lower circulating CD16+ monocytes, and high CD4+ T-cell count, possess anti-inflammatory effects suggesting a potential therapeutic application. Our data suggests that CBD and THC treatment results in a significant reduction in the number of EVs released from infected cells possibly via viral transcription inhibition and autophagy activation. EV concentrations from infected cells following treatment with CBD or THC were analyzed using nano-tracking analysis. Changes in intracellular and EV-associated viral RNA were quantified using reverse transcription- quantitative polymerase chain reaction (RT-qPCR) and changes in viral protein levels, EV markers, and autophagy pathway protein expression was assessed using Western blot analysis. These studies are significant in that cannabinoids, particularly CBD, may provide a protective effect by alleviating the pathogenic effects of EVs in HIV-1 and CNS-related infections.

Poster No. 27

Bioenergetic adaptations to HIV infection and antiretroviral therapy

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Primary HIV infection and antiretroviral therapy (ART) are associated with metabolic syndrome which is known to increase morbidity and mortality. Very little is known about the metabolic adaptations that occur in people with HIV (PWH) before and after ART. In this study, we conducted a full metabolomic analysis

of serum from PWH before and up to 24-months after the initiation of ART. PWH were enrolled through the Rakai Uganda Community Cohort Study (n=278), and HIV-seronegative controls (SNC) (n=300) were enrolled from the same geographical region and matched on age and gender. Serum metabolites were detected and quantified using a high-resolution mass spectrometer. The initial analysis focused on substrates of energy metabolism. Pre-ART HIV infection was associated with impaired bioenergetic pathways linked to glycolysis, pentose phosphate pathway (PPP), Krebs's cycle, fatty acid beta-oxidation, and branched-chain amino acid metabolism. After ART initiation, ketone bodies and amino acid metabolism were largely normalized, but fatty acid beta-oxidation, PPP, glycolysis, and lactate production remained abnormal compared to SNC. Our findings suggest that pre-ART HIV infection is associated with increased energy demand. When there is insufficient glucose to satisfy cellular energy requirements, alternative energetic substrates are mobilized. Our data suggest that pre-ART HIV infection increases lipolysis to export fatty acids from adipose and protein degradation from muscle to release amino acids into circulation that can be used as alternative energetic substrates. ART partially normalized gluconeogenesis and fatty acid oxidation as evidenced by a complete normalization of ketone bodies and partial restorations of circulating amino acid and free fatty acid levels. These findings demonstrate that ART is insufficient to completely restore the bioenergetic adaptations associated with HIV infection and suggest the increased prevalence of diabetes, cardiovascular, neurovascular, cancer, and neurodegenerative disease in PWH may involve sustained impairments of cellular energetics despite suppression of HIV replication with ART.

Poster No. 28

Assessment of integrated HIV-1 proviral DNA content in the PBMC compartment of individuals in the absence or presence of neuroHIV

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The integrated human immunodeficiency virus type 1 (HIV-1) virus forms a stable but also transcriptionally silent viral reservoir, which prevents anti-retroviral therapy (ART) from being a curative strategy. Studies have also shown that this viral reservoir contains both replication competent and defective proviral sequences. It has been hypothesized that at least some defective proviruses may still be able to produce viral proteins. These proteins may then have effects on chronic HIV pathogenesis and disease. We have previously studied genetic variation associated with the expression of the vial accessory protein Tat, linking to its functional outcomes. Sequencing results in our laboratory showed an approximate 30 percent failure of amplification of the tat gene region. This could be due to genetic variation at the site of the PCR primers, integrated copies of HIV in the PBMC cell population at a level to low for PCR to detect, or a lack of target due to increased defective provirus. This suggests additional methods were needed to assess HIV-1 provirus in patient samples. We hypothesized that the reason for such differential results could be due to accumulation of replication incompetent defective proviruses. Given these observations, we used a single genome amplification strategy (SGA) of near-full length provirus, to examine the full spectrum of integrated proviruses contained within the PBMC compartment of individuals in the absence or presence of neuroHIV. SGA was performed by either Sanger sequencing or utilizing a third-generation sequencing (TGS) platform such as MinION's nanopore sequencing. Analyses will determine if the ratio of defective to non-defective virus is altered in individuals with neuro HIV and if the complete tat gene is more commonly encountered in the neuroHIV population. Future studies will also examine the spectrum of integrated proviruses in the CNS compartment of HIV-1-infected individuals.

Poster No. 29

Morphine and HIV-1 Nef induce mu-opioid receptor splice variant MOR-1X expression in neurons

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People with HIV (PWH) are most likely to abuse addictive drugs, such as morphine, resulting in development of dependence and addiction. Morphine activates the mu-opiod receptor, a member of the G protein-coupled receptor family. Studies in humans have shown that OPRM1 gene is alternatively spliced into different isoforms, including MOR-1 and MOR-1X. While MOR-1 isoform has been extensively studied, role of MOR-1X isoform needs to be further investigated. Furthermore, multiple studies have shown that HIV infection may play a role in altering pre-mRNA splicing of OPRM1. Our results suggest that the accessory protein Nef of HIV and morphine play an active role in inducing MOR-1X isoform. In neuronal cultures, morphine exposure induces expression of MOR-1X isoform. Interestingly, the expression of MOR-1X isoform is also induced in postmortem brain tissues obtained from PWH, while no changes were detected in MOR-1 expression. We further investigated the in vivo synergistic effects of HIV-1 and morphine in inducing MOR-1X isoform, using F344 and HIV-1Tg rat models. In rats treated with morphine, our result showed induction in expression of MOR-1X isoform in brain regions involved in the reward pathways. Additionally, HIV-Tg rats showed an additive induction of MOR-1X expression, when treated with morphine. To further assess the possible role of HIV secretory proteins in alternative splicing of OPRM1 gene, we analyzed the impact of HIV-1 Tat, gp120 and Nef proteins on alternative splicing of MOR-1X isoform. Interestingly, while the recombinant Tat and gp120 had no effects, treatment of neurons with Nef induced MOR-1X alternative splicing. More interestingly, Nef-EVs synergistically induced alternative splicing of MOR-1X isoform as morphine did in both human and rat neurons. Altogether, our results suggest that HIV-1 may contribute to the enhanced rate of opioid dependence in PWH with Nef protein by amplifying the rate of MOR-1X alternative splicing induced by morphine.

Poster No. 30

HIV-1 Induces Astrocyte Dysfunction in the Brain of Post-Mortem HIV+ Humans and HIV-1 Tg Rats

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Combination anti-retroviral therapy (cART) has improved life expectancy for people living with HIV/AIDS; but that is associated with HIV-1 associated neurocognitive disorders (HAND). Although the mechanism underlying HAND is unknown, it could be related to HIV-induced neuron/astrocyte dysfunction in the brain. Among astrocytes, HIV-1 not only alters cytokine/chemokine secretion, but also disturbs K+ buffering (mediated by inwardly rectifying Kir4.1 channels), glutamate uptake (mediated by excitatory amino acid transporters, EAAT2 in humans/GluT-1 in rodents), and cell-to-cell communication (mediated by gap-junction protein connexin, Cx43), which could cause neurotoxicity. However, it is unknown how and to what extent HIV-1 alters Kir4.1 channel, GluT-1, and Cx43 function in human brains; and that has not been studied. Here we assessed HIV-induced alterations in the protein/mRNA levels of Kir4.1 channels, GluT-1s, and Cx43 in the prefrontal cortex (PFC) from post-mortem HIV+ human brain tissues (44~97-year-old,; HIV+ n=6/HIV- n=4). We found that Kir4.1 channel and GluT-1 protein levels were significantly decreased ($p<0.01$ and $p<0.05$, respectively); but Kir4.1 channel mRNA levels were significantly increased ($p<0.05$), associated with a trend towards a decrease in Cx43 mRNA expression, in HIV+ human PFC. Further, we also evaluated HIV-induced PFC astrocyte dysfunction in 12-month-old (12mo, adult) and 5mo (young adult) HIV-1 transgenic (HIV-1 Tg) rats compared to age-matched non-Tg rats (n=4-6/ea). We

found that there was no significant difference in mRNA levels of Kir4.1 channels, GluT-1, or Cx43 between 5mo Tg and non-Tg rats; but a significantly-reduced GluT-1 (p=0.01), increased Cx43 (p=0.01), and unchanged Kir4.1 channel mRNA levels in PFC of 12mo Tg rats compared to non-Tg rats. Collectively, our novel findings indicate that HIV significantly alters astrocyte function in the human PFC by disrupting Kir4.1 channel, GluT-1, and Cx43 expression. Such astrocyte dysfunctions are likely age-dependent and may contribute to PFC neuronal dysfunction, which could ultimately induce neurotoxicity in the brain.

Poster No. 31

Immunophenotypic characterization in CSF of patients with virus-associated neuroinflammatory diseases

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Acute and chronic viral infection may cause immunological alterations such as chronic activation, immunodeficiency and infiltration of inflammatory cells into the central nervous system (CNS) that underlie the pathogenesis of neurologic disorders. In the ongoing pandemic of a novel coronavirus SARS-CoV-2, neurologic manifestations associated with coronavirus disease 2019 (COVID-19) have emerged such as headache, loss of smell, myalgia and fatigue. To understand the virus-associated immune signature in the CNS of patients with neurologic diseases, we examined the immunophenotypes in CSF cells of subjects with virus infection and/or neuroinflammatory diseases including HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), multiple sclerosis (MS), progressive multifocal leukoencephalopathy (PML), and post-COVID-19 neurologic syndrome (post-COVID), compared to healthy normal donors (ND). The subjects with chronic virus infection, such as HAM/TSP and PML patients, showed characteristic signatures of T cell subsets and expressions of multiple immune checkpoint molecules, PD-1, CD244, TIGIT, and CD226, in CSF compared to NDs, while the patients with post-COVID did not. Antibody secreting B cells were elevated in the CSF of patients with viral infection (HAM/TSP, PML, and post-COVID) as well as MS patients. The elevated CD56bright NK cells in the CSF were also detected in a subset of patients with post-COVID. In addition, PD-L1 was highly expressed on CSF monocytes of patients with HAM/TSP and a subset of patients with MS, PML and post-COVID, compared to NDs. These results demonstrated that patients with post-COVID may have chronic immune dysregulation in the CSF. These results highlight the importance of immune regulation in CSF cells and the associated inflammatory milieu in subjects with virus-associated neuroinflammation and may provide new insight into immune microenvironment and rationale for targeting immunotherapy.

Poster No. 32

CSF antibody profiling of patients with HTLV-1 associated myelopathy/ tropical spastic paraparesis using VirScan

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Intrathecal antibody synthesis is a well-documented phenomenon in neurological diseases with chronic virus infection, demyelination, and neuroinflammation, as evidenced by the presence of antigen-specific antibodies, oligoclonal IgG bands and antibody secreting B cells in CSF of the patients. Since intrathecal antibody synthesis may cause chronic immune dysregulation in the CNS, it is important to understand antigen-specific antibody/B cell signatures associated with various viral exposures in predicting pathogenesis and prognosis in patients with neurologic diseases. We used a phage-immunoprecipitation-sequencing technology, i.e., VirScan, a robust platform capable of very high complexity serological screening for virus exposure across the entire human virome to determine potential viral pathogens in

patients with neurologic diseases. In this preliminary study, we examined the antibody profiling in CSF and serum of patients with human T cell lymphotropic virus 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) compared to healthy normal donors (NDs) and MS patients, using VirScan. HAM/TSP is a chronic, progressive myelopathy, in which chronically activated immune responses and infiltration of inflammatory cells into the CNS have been suggested to underlie the pathogenesis. Antibodies against HTLV-1 were highly detected in both serum and CSF of HAM/TSP patient but not in control CSF including NDs and MS patients. Unexpectedly, antibodies against human herpes virus 5 (cytomegalovirus) were also detected in the CSF of HAM/TSP patients. The preliminary results demonstrated that VirScan maybe able to identify local virus-specific antibody signitures and the associated inflammatory milieu in subjects with chronic virus infection and neuroinflammatory diseases.

Poster No. 33

Separation of EVs from Virions in Coronavirus infections

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The severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was declared a pandemic in mid-March of 2020 by the World Health Origination (WHO), laboratories around the world started research into diagnostics, therapeutics, and treatments. In recent years, the importance of extracellular vesicles (EVs) in the pathogenesis of viral infections have been found in the cases of many viral pathogens including few DNA and RNA viruses including human T-cell leukemia virus-1 (HTLV-1) and human immunodeficiency virus-1 (HIV-1). EVs from HIV-1 infected cells on uninfected macrophages induces an increase in the proinflammatory cytokines. While EVs from HTLV-1 infected cells on uninfected recipient cells promoted the localization and cellular contact by cells, this directly influences the pathogenesis of HTLV-1 as the virus mainly infects other cells by cell to cell contact. Similar to retroviruses, coronaviruses are positive strand RNA viruses, except they replicate in the cytoplasm and may regulate chromosomal DNA depending on the strain of virus. We have recently began working on beta-coronaviruses, including OC43 (BSL2 strain) and SARS-CoV-2 (BSL3 strain). Our initial experiments focus on isolation of EVs away from virions using either an iodixanol gradients or Izon sizing columns. We have successfully separated the two from one another mainly due to their density and potentially size differences. We found that EVs from multiple coronaviruses are not infectious and viral particles treated with UV irradiation are also not infectious. We also have found that coronavirus EVs caused T-cell death, which may corelate with lymphopenia observed in COVID patients. Along these lines coronavirus EVs can activate other viral genes (i.e. HIV-1 or HTLV-1) when these genes are integrated into the genome, further implying that these EVS regulate chromosomal gene expression. Finally, the mechanism(s) of how these EVs may cause such diverse effects on T-cells and other viral gene expression will be discussed.

Poster No. 34

Bictegravir-mediated lysosomal de-acidification inhibits oligodendrocyte maturation and autophagic flux

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Antiretroviral therapy (ART) has led to a reduction in the most severe forms of HIV-associated neurocognitive disorders (HAND); however, cognitive impairment continues to persist in approximately 30-50% of HIV+ patients. As the severity of HAND has shifted with the advent of ART, so has the underlying pathology, though certain deficits continue in the post-ART era. In particular, white matter (WM) alterations persist despite peripheral viral suppression by ART, suggesting that antiretrovirals (ARVs) may directly contribute to WM structural and functional deficits. Work in our laboratory has

revealed that ARVs from numerous classes, including integrase strand transfer inhibitors (INSTIs), inhibit oligodendrocyte (OL) differentiation through distinct cellular stress pathways, including oxidative stress and the integrated stress response. We sought to determine whether bictegravir (BIC), a member of the INSTI class, prevents oligodendrocyte maturation. Using our well-established cell culture system of oligodendrocyte progenitor purification and differentiation, we demonstrate that BIC inhibits oligodendrocyte maturation and reduces expression of mature myelin proteins. In order to determine the potential mechanism underlying this phenomenon, we focused on lysosomal de-acidification since our previous work has revealed that certain ARVs from other classes disrupt lysosomal pH. BIC de-acidified lysosomes in both OPCs and mature OLs, as well as significantly impaired lysosomal degradative capacity. De-acidification of lysosomes led to an inhibition of autophagic flux, a process known to be essential for proper OL function. Co-administration of the TRPML1 agonist, ML-SA1, which extrudes Ca2+ out of the lysosomal lumen and re-acidifies lysosomes, blocked the ability of BIC to inhibit OL differentiation. Future studies will examine how lysosomal dysfunction contributes to OL injury in a rodent model of HAND and how lysosomal Ca2+ contributes to the regulation of myelin sheath formation and growth. Taken together, our data reveal the critical role of proper lysosome acidification in modulating OL differentiation and maturation.

Poster No. 35

HIV-1 Tat and morphine have region-specific effects on Myrf gene regulation in CNS white matter/oligodendrocytes

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HIV-associated neurocognitive disorders (HAND) remain pervasive even with increased efficacy and use of antiretroviral therapies, and opiate use/abuse among HIV+ individuals has been documented to exacerbate CNS deficits. White matter (WM) alterations, including myelin pallor, and volume and structural alterations detected by diffusion tensor imaging (DTI), are a common observation in HIV+ individuals. A study using non-human primates infected with simian immunodeficiency virus suggests that WM may actually harbor latent virus, since antiretroviral treatment reduced levels of viral DNA in grey matter but not in WM (Perez et al, J. Neurovirology, 2018). Using a transgenic mouse that expresses the HIV-1 Tat protein, we examined in vivo effects of Tat and opiates (morphine) on WM, at times ranging from 2-6 wk co-exposure using genomic, biochemical, and morphological methods. After 6 wk chronic exposure, significant differences were observed in multiple myelin basic protein (MBP) isoforms. Outcomes were region-specific (hippocampus, striatum, corpus callosum, pre-frontal cortex), and included both individual and interactive effects. For example, HIV Tat exposure increased levels of the 18.5 kDa MBP isoform in hippocampus but not striatum; both HIV Tat and morphine affected 21.5 kDa isoform levels in striatum; morphine interaction mitigated the effects of Tat on MBP levels in hippocampus and pre-frontal cortex, but not striatum. RNA sequencing of striatal tissue revealed several candidate genes associated with oligodendrocyte precursor populations and myelin integrity that may be related to WM pathology, including transferrin, atypical oligodendrocyte markers GPR17 and NDRG1, and the oligodendrocyte transcription factor Myrf. Myrf is implicated in numerous neurological disorders, and is critical for proper oligodendrocyte differentiation and maturation. Western blot analyses identified regional differences in the effect of Tat and morphine on Myrf protein levels, some of which coincided with changes in Myrf transcriptional targets MBP and MAG. Support: DA044939.

Poster No. 36

Telaprevir as an Enterovirus D68 Antiviral to treat Acute Flaccid Myelitis in Mice

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In 2014, the US experienced an unprecedented outbreak of Enterovirus D68 (EV-D68)-induced respiratory disease. At the same time and in similar locations there was a dramatic upsurge in pediatric cases of acute flaccid myelitis (AFM), a poliomyelitis-like paralytic illness. Accumulating clinical, immunological, and epidemiological evidence has pointed to EV-D68 as the probable causative agent of biennial (2014, 2016, 2018) seasonal AFM outbreaks in the United States. There are currently no known effective antivirals against EV-D68, and current treatment is primarily supportive. Due to the urgent need for effective AFM treatment, there is significant interest in the repurposing of FDA approved antivirals for off-label use against EV-D68. Telaprevir is an FDA-approved protease inhibitor used to treat Hepatitis C infections that has been shown to inhibit the EV-D68 2A protease in vitro. We have recently demonstrated that telaprevir effectively improved paralysis outcomes in mice when administered simultaneous to EV-D68 infection. Telaprevir reduces viral titer at early disease timepoints and slows the spread of EV-D68 to the spinal cord, resulting in improved AFM outcomes in EV-D68 infected mice. Control of viral titers at early timepoints is sufficient to reduce or eliminate paralytic outcomes in Swiss Webster mouse pups, as AFM only develops in SW mice infected in the first week after birth. Our data demonstrates that Telaprevir is the first antiviral effective against EV-D68 in vivo, although it's benefit in clinical settings must still be determined.

Poster No. 37

Post-translational modifications of PINCH contribute to chemotherapeutic resistance in glioblastoma

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Gliomas are the most common adult CNS tumors and despite treatment efforts including surgery, radiation, and chemotherapy, essentially all low-grade gliomas progress to glioblastoma multiforme (GBM). Surgical resection is usually not possible and the recurrence rate of GBM is almost 100% after 9 months with a median survival of approximately 15 months due to its high capacity for invasion and resistance to therapy. Thus, improved therapeutic approaches are needed to decrease recurrence and prevent progression of astrocytoma into GBM. The PINCH protein is expressed in the mature CNS in neuropathological conditions, including Alzheimer's Disease, HIV infection, or glioma. PINCH-mediated signaling involves cell migration, spreading, and survival pathways which are all critical events in cancer progression. In fact, increased PINCH expression is related to poor prognosis in colorectal, pancreatic and breast cancer. Our new data link PINCH expression with the anti-oncogenic gene p53, suggesting that the two proteins work in concert in progression to glioblastoma (GBM). p53 is mutated in approximately 80% of gliomas leading to p53 pathway deregulation that contributes to chemotherapy resistance. Our data show that PINCH is dramatically increased in brain cancers as a function of grade of malignancy. We also observed a PINCH post-translational modification linked to p53 mutation in glioma cell lines. Since PINCH expression levels and post-translational modifications are linked to vulnerability of GBM cells to therapeutic intervention, findings from these studies will provide valuable data for potential adjuvant therapies for GBM and possibly other cancers.

Poster No. 38

Dimethyl fumarate, an approved multiple sclerosis treatment, reduces brain oxidative stress in SIV-infected rhesus macaques: potential therapeutic re-purposing for HIV neuroprotection

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Dimethyl fumarate (DMF), an FDA-approved, anti-inflammatory, antioxidant neuroprotective drug acts through rapid assimilation and antioxidative gene induction. For testing as an HIV neuroprotectant, we had shown that DMF increases antioxidant enzyme expression and reduces excitotoxin release from HIV-infected macrophages even during peak virus replication. We hypothesized that DMF treatment of SIV-infected macaques would induce brain antioxidant responses and reduce oxidative stress and neuroinflammation. Nine SIV-infected CD8+-T-Lymphocyte-depleted rhesus macaques were studied. Five received oral DMF starting 7 days prior to SIVmac251 infection and through necropsy day. In brain (11 regions), thymus, liver, and spleen were quantified by Western blot: antioxidant enzymes, astrocyte activation, cell-adhesion molecules, and synaptic and axonal proteins. Oxidative stress was determined in brain sections using immunohistochemistry (8-OHdG/DNA, 3NT/proteins) and optical redox imaging of oxidized flavoproteins containing adenine dinucleotide (Fp) and reduced nicotinamide adenine dinucleotide (NADH). Brain sections were also stained for HLA-DR and CD68. Statistical analyses were done by unpaired and paired t-test, and multivariate linear regression. Chronic daily DMF treatment associated with higher expression of the antioxidant enzymes NQO1 ($p<0.01$), GPX1 ($p<0.001$), and HO-1 ($p<0.05$) in brain, and PRDX1 ($p<0.01$) and HO-2 ($p<0.01$) in spleen, lower levels of 8-OHdG, 3NT, Fp and lower optical redox ratio. DMF treatment also associated with increased expression of cell-adhesion molecules, VCAM-1 ($p<0.01$), and ICAM-1 ($p<0.01$), and no changes in HLA-DR, CD68, GFP, NFL, or synaptic proteins. One DMF-treated animal developed a diffuse lymphosarcoma and a jejunal B cell lymphoma (lymphocryptovirus), commonly associated with SIV infection. No changes in plasma or CSF viral loads or peripheral blood hematological counts were observed. The concordantly increased brain antioxidant enzyme expression and reduced oxidative stress in DMF-treated immune-deficient SIV-infected macaques suggest that DMF could potentially induce neuroprotective responses in HIV infection. Introduction of DMF concurrently with cART may enhance neuroprotection in acute HIV infection.

Poster No. 39

Pro and anti-inflammatory factors modulate viral infection and immune responses in the brain of rhesus macaques during acute SIVmac239 infection

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Knowledge on immune activation in the brain during acute lentivirus infection is crucial for the prevention and treatment of HIV-associated neurological disorders. Therefore, we studied acute SIV infection and early immune response in three brain regions (basal ganglia, thalamus, and frontal cortex) of SIVmac239-infected rhesus macaques. Infectivity of SIVmac239 and changes in resident and infiltrating immune cells were studied in three brain regions of macaques at 7 (n=3) and 14 (n=5) days post infection (dpi), in comparison with uninfected control (n=3) and chronically infected (>180 days) macaques (n=3), using RT-qPCR, IHC, and RNAseq analysis. Although not at 7 dpi, all three brain regions had detectable virus by 14 dpi. Basal ganglia and thalamus were infected earlier than the frontal cortex and contained more virus than the latter. SIV co-localized exclusively with CD163+/CD68+ monocyte/macrophages at these early time points. An initial CD3+ T-cell depletion was observed on day 7, which recovered by 14 dpi. Proinflammatory, anti-inflammatory, and immune cells (T cells, macrophages, NK cells) signatures were upregulated earlier in PBMC (7 dpi) than the frontal cortex 14 dpi). Increased immune activation of astrocytes and significant infiltration of monocytes/macrophages in thalamus at 14 dpi coincided with peak viral load. While acute production of proinflammatory IL-6, produced partly by microglia, was sustained over time; increased anti-inflammatory cytokine TGF-beta, produced in part by monocytes/macrophages, was only transient in the brain. SIV infiltrated macaque brains during acute infection solely through

monocyte/macrophages, and precipitated CD3+ T cell depletion, suggesting delayed immune activation in the brain is most likely due to delayed viral entry. While the acute, but unsustained, upregulation of anti-inflammatory cytokines may have prevented initial damage to brain tissues, the progressive glial activation, persistent production of proinflammatory IL-6 contributed to overt neuroinflammation.

Poster No. 40

Broad-spectrum guide RNAs demonstrate efficacy in latent HIV-1-infected cells

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The latent HIV-1 reservoir constitutes one of the primary challenges antagonizing efforts toward a cure. Genomic editing with the CRISPR/Cas9 system holds promise to permanently excise or inactivate integrated provirus. To this end, a set of broad-spectrum selected molecular gRNA target (SMRT) gRNAs were identified that were predicted to cleave 100 percent of patient-derived LTR samples using an in silico prediction algorithm. Using a novel dual fluorescence system (the NL4-3 HIV-1 molecular clone which also encoded GFP and the Cas9 expression system which encoded RFP and one of the custom anti-HIV-1 SMRT gRNAs), HEK-293T cells were transfected with both gRNA/Cas9 and HIV-1 plasmids. Using these two plasmids, it was shown that when the Cas9 system was active, there could be as high as 98 percent reduction in GFP expression. gRNAs targeting the TAR region of the LTR, which is more conserved, yielded the greatest reduction in HIV-1 gene expression as measured by fluorescence microscopy and flow cytometry. In TZM-bl cells, using the highly sensitive beta-galactosidase system, at least 97 percent of LTR-driven gene expression was reduced, when multiple SMRT gRNAs were used. To more accurately model latency, the latently infected T cell line, J-Lat 10.6, was transduced with a lentiviral vector encoding Cas9 and a gRNA. SMRT1, which targets TAR, and a Tat targeting gRNA were the most effective at preventing J-Lat cells from reactivating from latency. These results have demonstrated that our custom gRNAs possess broad-spectrum cleavage activity and could contribute to HIV-1 treatment strategies or possibly even a cure at some point in the future.. Future studies will examine the any off-target effects made by these broad-spectrum gRNAs in vitro and in vivo using GUIDE-Seq.

Poster No. 41

Activation of alpha7 nicotinic acetylcholine receptor ameliorated HIV-associated neurology and neuropathology

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HIV-associated neurocognitive disorders (HAND) in the era of combination of antiretroviral therapy are primarily manifested as impaired behaviors, glial activation/neuroinflammation and compromised neuronal integrity, for which there are no effective treatments currently available. In the current study, we took advantage of doxycycline-inducible astrocyte-specific HIV Tat transgenic mice (iTat), a surrogate HAND model and determined effects of PNU-125096, a positive allosteric modulator of alpha7 nicotinic acetylcholine receptor (alpha7 nAChR) on Tat-induced behavioral impairments and neuropathologies. We showed that PNU-125096 treatment significantly improved locomotor, learning and memory deficits of iTat mice while inhibited glial activation and increased PSD-95 expression in the cortex and hippocampus of iTat mice. Using alpha7 nAChR knockout mice, we showed that alpha7 nAChR knockout eliminated the protective effects of PNU-125096 on iTat mice. In addition, we showed that inhibition of p38

phosphorylation by SB239063, a p38 MAPK-specific inhibitor exacerbated Tat neurotoxicity in iTat mice. Lastly, we used primary mouse cortical individual cultures and neuron-astrocytes co-cultures and in vivo staining of iTat mouse brain tissues and showed that glial activation was directly involved in the interplay among Tat neurotoxicity, alpha7 nAChR activation, and p38 MAPK signaling pathway. Taken together, these findings demonstrated for the first time that alpha7 nAChR activation led to protection against HAND and suggest that alpha7 nAChR and PNU-125096 hold significant promise for development of therapeutics for HAND.

Poster No. 42

Effect of the chemokine CXCL12 on PSD-95 and glutamate receptors expression and distribution in cortical neurons

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Several neurological disorders, including HIV-associated neurocognitive disorders (HAND), present with dendritic spine loss and synaptic pruning, which can lead to cognitive impairment. Importantly, these deficits may be reversible. Previous work from our lab showed that the chemokine CXCL12 increases dendritic spine density in rat cortical neurons through activation of its cognate receptor CXCR4. This was correlated with improved cognitive flexibility in an operant learning task. However, it is not clear if CXCL12 promotes spine maturation or simply stabilizes transient (silent) spines. We investigated this in primary cortical neurons by examining the expression and distribution of postsynaptic density protein 95 (PSD-95), a marker of mature dendritic spines, and the most common AMPA receptor subunit, GluA1, both at cellular and synaptic level. Our data so far show that, while CXCL12 treatments (20nM; 5 min-3hrs) do not significantly alter overall protein expression of PSD95 in cortical neurons, a consistent and significant increase in the percentage of PSD95+ thin spines is observed in these cultures (20nM; 3hrs). At this same timepoint, dendritic spine analysis also showed a significant increase in overall spine density (mainly driven by thin spines) and an increased percentage of total PSD-95+ spines. We observed a similar trend for GluA1+ spines. These findings suggest that CXCL12 may stabilize the highly dynamic thin spines promoting their maturation. Ongoing experiments will clarify if long-term CXCL12 treatment increases the expression and phosphorylation of PSD-95 and AMPA receptor subunits in neurons as well as dendritic spines, and how this correlates with dendritic spine density, morphology, and synaptic activity. In addition, we will perform live cell imaging of cortical neurons and brain slices transfected with GFP-actin to determine how the CXCL12/CXCR4 pathway affects spine maturation in real time. Overall, these studies will further our understanding of how CXCL12 regulates dendritic spines and supports cognitive flexibility.

Poster No. 43

The role of SUMOylation in CTIP2-mediated HIV-1 latency in microglial cells

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Antiretroviral therapy (ART) is highly effective at inhibiting HIV replication by targeting multiple steps in the HIV life cycle, including viral entry, integration, replication, and production. However, ART cannot eliminate the viral genome that has integrated into host-cell DNA. Microglial cells are the main HIV-1 target cells in the central nervous system and constitute an important reservoir for viral pathogenesis. In microglial cells, the co-repressor COUP-TF interacting protein 2 (CTIP2) recruits a multi-enzymatic chromatin-modifying complex and establishes a heterochromatic environment at the HIV-1 promoter, leading to HIV-1 silencing. Integrated HIV-1 gene silencing in latently infected cells constitutes a major obstacle in achieving a cure for HIV. Therefore, the mechanisms involved in HIV latency and its reversal warrant further studies to identify targets of HIV reservoir formation and clearance. SUMOylation of CTIP2 promotes recruitment of the transcriptional co-activator p300 to a CTIP2-repressed promoter with

subsequent induction of transcription. Interestingly, in other studies, prolonged treatment of native thymocytes with phorbol 12,13-dibutyrate together with the calcium ionophore A23187 also promoted the proteasomal degradation of CTIP2 through the SUMO-ubiquitin pathway, providing a mechanism for signal termination. However, the role of post-translational modifications (PTMs) in CTIP2-mediated HIV-1 latency is unknown. Here, we sought to determine whether CTIP2 is regulated by PTMs in microglial cells. Our limited understanding of the molecular mechanisms involved in the initiation and establishment of HIV-1 latency stands as a critical barrier to identifying effective preventative measures and treatments. This research addresses this gap in our understanding by examining whether and how SUMOylation, a post-translational modification that regulates many cellular processes, controls CTIP2-mediated HIV-1 latency.

Poster No. 44

Divalent metal transporter 1 is involved in morphine-mediated efflux of endolysosomal iron

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HIV-associated neurocognitive disorder (HAND) is a spectrum of neurological disorders in people with HIV that range from minor impairment to dementia in the most severe cases. Generally mild neurocognitive symptoms persist in ~50% of HIV+ patients on antiretroviral therapies, highlighting an important clinical and scientific challenge. HAND symptoms are also worsened by opioid drugs, which may be due to opioids ability to reduce dendritic spine density in brain regions involved in learning and memory. Our previous work demonstrated that opioid effects on cortical neuron's spines occur through a unique pathway involving neuronal iron metabolism. Morphine triggers iron release from neuronal endolysosomes, which upregulates the iron storage protein ferritin heavy chain (FHC). FHC then inhibits the homeostatic chemokine receptor CXCR4, which leads to reduced dendritic spine density. Here, we focused on understanding the mechanism by which endolysosomal iron is released after morphine treatment by examining divalent metal transporter 1 (DMT1), a well-known iron transporter. First, we optimized conditions to detect DMT1 and its isoforms (+/- IRE) in rat cortical neurons and found that the majority of DMT-1 was expressed in the cytoplasm. Immunofluorescent staining showed colocalization of DMT1 and the endolysosomal marker LAMP1, suggesting that both DMT1 isoforms are expressed in endolysosomes. To assess the potential contribution of DMT1 in morphine-mediated upregulation of FHC, we used a pharmacological agent ebselen, a potent inhibitor of the iron transport of DMT1. Ebselen pre-treatment prevented FHC upregulation in morphine treated cultures, suggesting that DMT1 does play a role in morphine-mediated endolysosomal iron efflux. Overall, these studies suggest that opioids worsen HAND progression by disrupting iron metabolism in the brain, and new therapeutic approaches targeting CNS iron metabolism may be relevant for HAND and other neurocognitive disorders.

Poster No. 45

HIV-1 Tat protein induced associative learning deficits in female transgenic mice

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As individuals with HIV live longer lives, owing to cART therapy, a new mild form of cognitive impairment known as HIV-1 Associated Neurocognitive Disorder (HAND) has emerged. The current series of experiments investigated the effects of HIV-1 Tat expression, in a doxycycline inducible Tat transgenic mouse model, on basic associative learning acquisition using contextual (N = 36; ~9/group) and cued (N = 30; ~8/group) fear conditioning. Mice were grouped according to sex (Male/Female) and genotype [Tat(+)/Tat(-)]. Following 6 weeks of Tat expression a mild 2 sec 0.4 mA foot shock was administered to subjects following 4 min of chamber habituation (contextual fear conditioning). In the cued fear conditioning experiment a 60 sec 2000 Hz tone at 3 min terminated with the onset of the shock. Testing

occurred 24 hrs following a single acquisition session. Results indicated no significant main effect of sex or genotype for the contextual fear condition experiment on any measure of freezing behavior (all $p > .05$); however, in the cued fear conditioning experiment there was a significant main effect of sex for freezing occurring during the CS ($p = .012$). Tukey's post hoc tests revealed that Tat(+) females ($M = .39$, $SD = .39$) demonstrated significantly lower freezing ($p = .046$) than Tat(+) males ($M = .79$, $SD = .13$) and Tat(-) males ($p = .048$; $M = .77$, $SD = .26$), but not for Tat(-) females ($p > .05$, $M = .64$, $SD = .23$). Overall these data demonstrate the effects of the Tat protein on associative learning deficits in Tat(+) female mice during cued fear conditioning while contextual learning was unaffected. These data point towards the possibility Tat expression affecting amygdalar circuitry to a greater degree than hippocampal circuitry.

Poster No. 46

Sex-specific effects of low-dose hydrocortisone on negative emotion perception in HIV

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Introduction: Sex differences in the perception of emotion and the neural circuitry underlying emotional perception have been well established. Females, particularly those with high anxiety, often show heightened identification of fearful faces. The causal role of glucocorticoids in these associations is not well understood. Here we examine these associations in persons with HIV.

Methods: In a double-blind, placebo-controlled, cross-over study, we used a single low-dose of hydrocortisone (10mg; LDH) as a mechanistic probe of the effects of elevated glucocorticoids on negative emotion perception in 65 people with HIV (31 women with HIV [WWH]). The primary outcome was accuracy in identifying emotional expressions on the Facial Emotion Perception Test (FEPT). Salivary cortisol, self-reported stress/anxiety and childhood trauma were also assessed.

Results: LDH increased salivary cortisol levels versus placebo. The effect of LDH versus placebo on FEPT accuracy depended on the combined influence of facial expression and sex ($p=0.03$). LDH influenced accuracy only for WWH ($p=0.03$), specifically for fearful faces ($d=0.44$, $p=0.04$). Women's enhanced threat detection varied with psychological burden, more pronounced among those with lower burden and trauma ($p<.05$). Exogenous glucocorticoid administration increased accuracy for fearful faces in women, but not men with HIV.

Conclusions: This result suggests a role of the HPA axis in sex differences for perception of fearful faces in WWH, potentially due to changes in GC receptor availability/activity, or improved integration of signals from facial recognition, amygdala, and emotion processing regions. The blunting of this effect in men, and in individuals with more severe trauma suggests that the relationship between optimal GC signaling and facial perception accuracy may depend less on the HPA axis and more on amygdala activity.

Poster No. 47

Machine Learning Quantification of Mouse Cortex Microglia under Experimental Conditions

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Counting cells is a cornerstone of tracking disease progression in neuroscience. A common approach for this process is having trained researchers individually select and hand count cells within an image, which

is both difficult to standardize and time-consuming. We present an automatic cell counting methodology which leverages the Trainable WEKA Segmentation (TWS) plugin freely available in the FIJI distribution of ImageJ. TWS provides machine learning tools for cell segmentation with an accessible graphical user interface. However, analyzing whole datasets effectively requires further development of methods including image processing and data analysis techniques. Automatic counts were compared to a hand counted dataset of Immunofluorescence labeled Iba1+ mouse microglia in cortex of wild-type (WT) control and HIV-gp120 transgenic mouse model of HIV-induced brain injury (HIVgp120). HIVgp120 mice recapitulate many phenomena of HIV-induced brain injury, including increased microglia numbers. 10X images were collected from sagittal brain slices of 11-14 month-old mice for each genotype. We use this data set to assess the TWS based strategy's accuracy and ability to adapt to changing cell morphology and staining that come with neurotoxic experimental conditions in vivo. The main advantage of this strategy reducing the time required to apply the automatic counting strategy compared to a manual count and the accessibility of the software in ImageJ. Using TWS reduced the time to completion by 80% as compared to manual counting, including time spent training the program. As with all ImageJ plugins, the TWS software is free to use and integrated with a host of other image analysis tools. Our study demonstrates the general applicability of this accessible technique to quickly explore large amounts of image data, leaving open the possibility to audit the results by manually counting a randomly selected subset of images. This work supported by NIH R01 MH087332, MH104131, MH105330, DA052209 (to M.K.)

Poster No. 48

Extracellular Vesicles from Infected Cells Are Released Prior to Virion Release

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Our lab has previously characterized Extracellular Vesicles (EVs) from HIV-1-, HTLV-1-, RVFV- and EBOV-infected cells. However, difference in timing between EV and virion release from infected cells remains poorly understood. Therefore, we have attempted to address the dynamics of EV and virion release from HIV-1- and HTLV-1-infected T-cells. Uninfected, HIV-1-infected, and HTLV-1-infected T-cells were synchronized in G0 phase by serum-depleted media. Later, quiescence was reversed by the release of cells in serum-rich media with inducers to activate viral transcription. The samples were harvested post-release at different timepoints and tested for EV and autophagy markers as well as for viral proteins and transcripts. Both HIV-1- and HTLV-1-infected T-cells were found to produce EV and autophagy markers differentially in comparison to uninfected controls. HIV-1-specific proteins were present at 6 hrs and their production increased overtime up to 24 hrs. HTLV-1-specific proteins peaked at 6 hrs and plateaued. Extracellular and intracellular viral RNA production correlated with viral protein expression: HIV-1 RNA production increased overtime and peaked at 24 hrs; whereas, HTLV-1 RNAs peaked at 6 hrs and plateaued. Particle concentration measurement in extracellular environment showed increase over time in both uninfected and infected samples. Finally, the HIV-1 supernatant from the 6-hrs samples was found not to be infectious; however, the virus from the 24-hrs samples was infectious and was successfully rescued from recipient uninfected cells. Interestingly, HTLV-1 was successfully rescued from both 6-hrs and 24-hrs supernatant samples, which might be attributed to the fact HTLV-1 expression was not completely suppressed by serum starvation. In summary, our data suggests that virally-infected cells secrete EVs containing viral proteins and RNAs prior to virions upon resuming normal cell cycle, thereby suggesting a potentially significant effect of EVs on naïve recipient cells prior to subsequent viral infection and spread.

Poster No. 49

Extended HIV-1 Tat and morphine exposure dynamically shifts striatal monoamine levels, Drd1 and Drd2 medium spiny neuronal function, and exploratory behaviors.

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Despite the advent of combination anti-retroviral therapy (cART), 30-50% of HIV-infected individuals exhibit neurocognitive disorders, implying that HIV still drives significant deficits in neuronal function. The striatum exhibits high viral loads and striatal medium spiny neurons (MSNs) are especially vulnerable to HIV-1. The striatal pathology can be modeled by exposing MSNs to HIV-1 protein Tat, and is exacerbated by co-exposure to opiate drugs such as morphine. Exposure of the two main populations of MSNs (dopamine Drd1 or Drd2-receptor-expressing) to Tat revealed dynamic shifts in physiology over time (in ex vivo slices) with both populations exhibiting hyperexcitability at 48-h. At 2-weeks, excitability remained elevated in Drd2-expressing but fell in Drd1-expressing MSNs, before normalizing to control firing levels at 2-months. The complex pattern of dysfunction at 2-weeks mimics parkinsonian shifts in Drd1 and Drd2-expressing MSN physiology observed with dopamine deficits. Therefore, we assayed exploratory behaviors and levels of monoamines and their metabolites after exposure to Tat and/or morphine for 2-weeks and 2-months in doxycycline-inducible GFAP-driven Tat transgenic mice. A 3-way ANOVA (genotype x treatment x time) revealed a main effect of morphine indicating decreased levels of dopamine, but enhanced levels of norepinephrine and monoamine oxidase metabolites DOPAC, HVA, and 5-HIAA in morphine treated mice. Moreover, Tat and morphine significantly interacted to alter dopamine and its metabolites DOPAC, HVA, and 3-MT across both timepoints. Behaviorally, Tat exposure for 2-weeks or 2-months increased the latency to explore a novel environment or conspecific, indicating that Tat inhibited initial environmental and social exploratory behaviors. By contrast, morphine exposure across both timepoints lengthened the time spent exploring a novel environment or food, but not a conspecific. Together, these results provide novel insight into the unique HIV-1 Tat and morphine-induced interactive effects on dopamine and monoamine metabolism that in turn may drive dichotomous effects within different motivational circuits.

Poster No. 50

Impact of morphine on a blood-brain barrier model

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The highly selective blood-brain barrier (BBB) mediates cellular and molecular passage between the central nervous system (CNS) and peripheral circulation. Densely-packed brain microvascular endothelial cells (BMECs) surrounding the capillary walls provide a semipermeable barrier between the bloodstream and brain parenchyma. Compromised BBB integrity has been linked to neurocognitive deficits that can arise from certain diseases and infections that target the CNS, including those associated with HIV-1 infection. Barrier function or regulation may also be negatively influenced by exposure to pharmaceuticals commonly used for pain management in patients suffering from CNS diseases. Morphine, a mu-opioid analgesic and metabolic product of heroin, is commonly prescribed for pain relief in a variety of conditions, including neuropathy associated with damage caused by HIV-1. Concerningly, opioid abuse occurs in nearly one third of HIV-1-infected patients and has been associated with increased severity of HIV-associated neurocognitive impairment; however, the underlying mechanism is unclear. Previous studies have

demonstrated that exposure to morphine-modulated expression of cell adhesion molecules (CAMs), has resulted in increasing BBB permeability thereby enabling transmigration of immune cells. In these studies, the cerebral microvascular endothelial cell (hCMEC/D3) line as well as a primary human co-culture of BMECs and astrocytes were used in an in vitro BBB model. Morphine exposure did not significantly alter barrier permeability, influence chemokine gradients, or induce PBMC transmigration across the BBB. These results have suggested that opiate use may not be a major contributing factor in the chronic neuro-inflammation observed in patients suffering from HIV-associated cognitive impairment.

Poster No. 51

HIV-1 expression in human medial prefrontal cortex: association with HAND

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The persistence of HIV-1 viral reservoirs in the brain, despite treatment with combination antiretroviral therapy (cART), remains a critical issue for the development of a novel cure strategy for HIV-1 infection and HAND. The hypothesis of the present study is that microglia could be the major cell type expressing HIV-1 mRNA and play a critical role on neurocognitive impairments, synaptic dysfunction, and neuroinflammation. To address the HIV mRNA distribution and potential reservoir in the brain, six HIV+ human samples from the medial prefrontal cortex region (3 female and 3 male; age between 55-74 years old), were assessed. RNAscope in situ hybridization was used to identify the distribution of HIV-1 mRNA expression in medial prefrontal cortex. Additionally, to elucidate the potential mechanism of HIV related synapse dysfunction, we performed IHC staining to clarify the synaptophysin (SYP), Tau, RhoA, PirB, NogoA, NgR3 and DCX protein levels in the brain. Our data revealed differential HIV-1 mRNA expression among the six patients, and microglia presented a predominant role for harboring the viral mRNA localization based on the morphology of positive cells. Furthermore, dual labeling of HIV-1 mRNA and Iba1 (a cell marker of microglia) indicated a co-localization of HIV-1 mRNA expression in microglia. Meanwhile, all human samples showed synaptophysin expression. However, the NogoA-NgR3-PirB-RhoA signal pathway which is related to neurocognitive dysfunction was not significantly activated. Collectively, the present study has significant implications for our understanding of HIV-1 related synaptic dysfunction and HAND.

Poster No. 52

Generating an HIV-1-specific Nanopore basecaller to enhance viral quasispecies detection

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Accounting for genetic variation is an obligate consideration during human immunodeficiency virus type 1 (HIV-1) investigation. Nanopore sequencing preserves proviral integrity by passing genomic fragments through ionic channels, allowing reads that span the entire genome of different viral quasispecies (vQs). However, this sequencing method suffers from high error rates, limiting its utility. This can be overcome through training the basecaller with gold-standard data. This was accomplished by sequencing the HIV-1 J-Lat 10.6 cell line using both Nanopore and high quality Sanger techniques. These training data were used

to minimize the difference between called reads and known sequence. Training was performed using two versions of the guppy basecaller: fast and high accuracy (HAC). Training improved both models' median Phred score across the J-Lat 10.6 genome from 6.91 to 23.78 and 10.79 to 46.87 for fast and HAC, respectively. This improved quality reduces the resolution needed to accurately detect a vQs from 136 to 50 nts/1,000 coverage for the fast basecaller and 89 to 8 nts/1,000 coverage for the HAC basecaller. Training reduces median error rates from 12.1 to 4.1% and 7.8 to 0.4% for fast and HAC basecaller, respectively. Mismatches are reduced from 32.5 to 20.2% of total errors for the fast basecaller and 32.5 to 19.9% in the HAC basecaller. Insertions are reduced from 20.0 to 17.2% and increased from 20.9 to 27.1% of total errors in the fast and HAC basecaller, respectively. Surprisingly, deletions have increased from 47.5 to 62.6% of total errors in the fast basecaller and 46.6 to 53.0% in the HAC basecaller. Future directions involve further training using U1 and J-Lat 15.4 Nanopore sequencing data to further improve performance and evaluate robustness.

Poster No. 53

Examining the effects of the chemokine CXCL12 on cortical networks with in vitro microelectrode arrays

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People with HIV often develop cognitive and behavioral deficits collectively called HIV-associated neurocognitive disorder (HAND). HAND is believed to be a consequence of subtle changes in central nervous system neuron structure and function in the absence of overt neuronal loss. Chemokines have well-known functions in the immune system, but several also promote homeostatic functions in the CNS. Our previous work in a rodent model of HAND confirmed dendritic spine deficits in the prefrontal cortex and cognitive impairment, which were both reversed by activating the CXCL12/CXCR4 chemokine signaling axis in the brain. However, it is unclear if CXCL12 promotes these outcomes by regulating cortical network function. Previous studies from others suggest that abnormal CXCR4 signaling on astrocytes triggers the release of glutamate through a TNF-alpha dependent mechanism, which can widely increase neuronal activity. Although uncontrolled increased activity can be toxic, CXCL12 protects cortical neurons from excitotoxic insults by downregulating NR2B-containing glutamate receptors and upregulating the protein Rb. As an additional protective measure, CXCL12 targets inhibitory parvalbumin-expressing axons to pyramidal cell somas, which enhances inhibitory tone and sets up an activity gate. Therefore, CXCL12 could regulate synchronous network activity through its effects on dendritic spine density, inhibitory neurons, and/or glutamate receptors. To study this, we used in vitro microelectrode arrays and followed cortical network activity over time. Control cultures significantly increased neuron spikes and synchronous network activity up to 28 days in vitro. At this timepoint, we treated cultures with CXCL12 (20 nM) and recorded mean firing rate or synchronous neuronal activity at 3-, 6-, 24-, or 48-hours post-treatment. Though still to be completed, these data suggest that CXCL12 has no net effect on cortical network function under basal conditions but rather regulates responses to toxic insults - a hypothesis that we are currently investigating both in cultured neurons and brain slices.

Poster No. 54

Abstract withdrawn

Poster No. 55

Effects of morphine on HIV neuropathogenesis

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HIV-associated neurocognitive disorders (HAND) persist in 15-40% of people living with HIV (PLWH) despite antiretroviral therapy. HIV enters the CNS, in part, by transmigration of infected CD14+CD16+ monocytes across the blood brain barrier (BBB). Substance use disorder is a major risk factor for HIV infection and some studies showed that opioids exacerbate the severity and progression of HAND. In the context of HIV infection, the mechanisms by which opioids, and specifically morphine, promote and accelerate neurocognitive dysfunction through the induction of neuroinflammation are not fully characterized. We used our model of the human BBB to study transmigration of uninfected and HIV-infected CD14+CD16+ monocytes treated with morphine, and quantified this by flow cytometry. Our preliminary results show that morphine increases CCL2 mediated transmigration of HIV-infected monocytes, but not of those that are uninfected. Once within the CNS, HIV infected monocytes can differentiate into macrophages, where they may remain as long-lived viral reservoirs that mediate CNS damage. Opioids may increase macrophage secretion of inflammatory cytokines, leading to sustained neuroinflammation. We examined the effects of morphine on CCL2, IL-8, IL-10, IP-10 and TNF-alpha production by human macrophages using a Human Cytokine/Chemokine Magnetic Milliplex kit. We showed that morphine increases all of these cytokines, and that treatment of macrophages with LPS, present in the sera of PLWH, has no additional effect on CCL2 and IL-8 increase by morphine. Our findings suggest that morphine contributes to HIV neuropathogenesis by increasing HIV infected mature monocyte entry into the CNS, contributing to viral reservoir reseeding, and production of inflammatory cytokines by macrophages.

Poster No. 56

Neurodevelopmental processes in the prefrontal cortex derailed by chronic HIV-1 viral protein exposure

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Due to the widespread access to, and implementation of, combination antiretroviral therapy (cART), individuals perinatally infected with human immunodeficiency virus type 1 (HIV-1) are living into adolescence and adulthood. Perinatally infected adolescents living with HIV-1 (pALHIV) are plagued by progressive, chronic neurocognitive impairments; the progression of the pathophysiological mechanisms underlying these deficits, however, remains understudied. A longitudinal experimental design was utilized to establish the development of pyramidal neurons, and associated dendritic spines, from layers II-III of the medial prefrontal cortex (mPFC) every thirty days from postnatal day (PD) 30 to PD 180 (Control: Male, n=8-10, Female, n=8-11; HIV-1 Transgenic (Tg): Male, n=8-10, Female, n=9-10 for each age). Three putative neuroinflammatory markers (i.e., IL-1beta, IL-6, and TNF-alpha) were evaluated early in development (i.e., PD 30) as a potential mechanism underlying synaptic dysfunction in the mPFC (n=10 for each group). Constitutive expression of HIV-1 viral proteins induced prominent neurodevelopmental alterations, independent of biological sex, in pyramidal neurons from layers II-III of the mPFC. Neurodevelopmental alterations in the HIV-1 Tg rat were characterized by prominent alterations in regressive processes, including dendritic and synaptic pruning, evidenced by a linear increase in indices of neuronal arbor complexity, dendritic branching complexity, and excitatory synapses throughout development. Examination of dendritic spine morphology revealed an age-related population shift towards dendritic spines with increased backbone length, decreased head diameter, and decreased volume in HIV-1 Tg animals relative to control animals; observations which support a progressive decrease in synaptic efficacy in the HIV-1 Tg rat. There was no compelling evidence for neuroinflammation in the mPFC of

HIV-1 Tg rats during early development. Understanding the neural mechanisms underlying chronic neurocognitive impairments in pALHIV may afford a key target for innovative therapeutics and cure strategies; an urgent need given the growing population of pALHIV. Funded by: DA013137, HD043680, MH106392, NS100624.

Poster No. 57

CSF, MRI, and cognitive outcomes in PLWH on long-duration ART

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Background: The advent of antiretroviral therapy (ART) has drastically improved the quality of life and longevity of persons living with HIV (PLWH). However, neurologic outcomes for PLWH on long-term ART remain unknown.

Methods: 157 participants on ART for >15 years (HIV+) and 102 demographically similar controls (HIV-) were included for analyses after coarsened exact matching (CEM) for both age and sex. All participants underwent a standardized neuropsychological testing battery, followed by optional research MRI and lumbar puncture. Raw cognitive test scores were used for analyses. CSF neurofilament light (NFL), total tau and cytokines were measured using the Quanterix Simoa platform. Brain volume proportions were calculated using Freesurfer (3T MRI). Box-cox transformation was used to approximate a normal distribution for CSF variables pre-regression. Weighted linear regression was used to assess the effect of HIV on outcomes.

Results: There were no significant differences in age, race, or sex between the HIV+ and HIV- groups. HIV+ participants (HIV RNA<50 copies/ml; median ART duration 21.3 (range 15.2 - 33.9) years; 79.3% male; median age 51 years) had no significant differences in raw cognitive test scores as compared with HIV- controls. The HIV+ group had an increased number of impairments on the Patients Assessment of Own Functioning Inventory (beta=1.79, p =0.004), as well as higher scores on the Beck Depression Inventory (beta=3.63, p=<0.001), and smaller proportions of subcortical gray volume (beta=-0.0013, p=0.006) on MRI. Back-transformed NFL (p = 0.019), tau (p=0.014), and TNF-a (p=<0.001) least square means were significantly elevated in the HIV+ group.

Conclusions: Using established measures of cognition, PLWH on long-term ART performed as well as controls. Some of the worse outcomes in PLWH, including depression and elevated CSF markers, may be suitable targets for future intervention studies.

Poster No. 58

Lentivirus persistence in brain: interactions between antiretroviral therapy, viral quantities and host variables

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Background: Despite antiretroviral therapy (ART), HIV-1 infection persists in different anatomical and cellular reservoirs among people with HIV (PWH). Lentiviruses infect the brain and replicate principally in microglia and trafficking macrophages. We investigated the efficacy of contemporary ART using primary human microglial and lymphocyte cultures as well as brain tissues from SIV-infected nonhuman primates (NHPs) and PWH.

Methods: ART efficacy was measured in HIV-infected human microglia and lymphocytes by released p24 quantitation. Viral RNA, integrated proviral and total viral DNA as well as host immune responses were measured in brains from SIV-infected (n=18) macaques and PWH (n=15) receiving different ART regimens. Immunodetection of lentiviral capsid proteins was performed in brain tissues.

Results: All ART compounds displayed lower EC50 values in lymphocytes than in microglia except for tenofovir, which showed 1.5-fold greater activity in microglia. SIV-encoded RNA, total and integrated proviral DNA were detected in brains from animals receiving suppressive, interrupted and no ART. HIV-1 RNA, total and integrated proviral DNA were detected in brain tissues of all PWH regardless of ART duration or plasma viral load. Both SIV and HIV-1 capsid antigens were immunodetected in brain, largely in microglia/macrophage cells regardless of treatment regimen. Factor analyses showed that host anti-viral immune response loadings represented the predominant determinants of viral burden in brain for both SIV and HIV-1 brain infections.

Conclusions: Both HIV-1 and SIV genomes and proteins persist in brain tissues despite contemporary and effective ART, which was less effective in HIV-infected microglia. ART interruption exerted minimal actions on the SIV brain reservoir without altering the neuroimmune response profile. Eradication of the HIV brain reservoir will require approaches to augment ART efficacy in the brain of PWH.

Poster No. 59

Increased EBV-DNA in CD19+-B lymphocytes in active MS patients in comparison to stable MS patients and normal donor controls

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Multiple sclerosis (MS) is an inflammatory chronic disease of the nervous system with a complex etiology. It is a leading cause of disability in young adults. MS has a multifactorial origin with genetic and the environmental components as major factors in triggering and perhaps modulating the progression of the disease. One environmental factor in particular, exposure to Epstein-Barr virus (EBV), shows the strongest association with MS. The risk of MS is higher in individuals with a history of infectious mononucleosis (IM), that it is the result of primary EBV infection during adolescence or later in life. In this study, the frequency and magnitude of detection of EBV-DNA was compared between normal donors (ND) and stable and active MS patients. Active patients were defined as patients with ≥ 1 cerebral gadolinium contrast enhancing lesion (CEL) at the sample date. Both PBMCs and sorted CD19+B cells were analyzed by digital droplet PCR for the detection of EBV viral load. As expected, higher EBV virus loads were detected in sorted CD19+ B cells than in PBMC from all cohorts. Importantly, the frequency of detection of EBV was twice as high in CD19+B cells from active MS patients (80%) than from either normal donors or stable MS patients, although the magnitude of the EBV viral load was not different between the three cohorts as determined by ANOVA test. These results support a role for EBV in the pathogenesis of MS and indicate

that the use of sorted CD19+ B cells versus total PBMCs may be a more sensitive method for EBV-DNA detection.

Poster No. 60

The effects of buprenorphine on monocyte migration and HIV neuropathogenesis

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HIV associated neurocognitive disorder (HAND) is a spectrum of neurocognitive deficits that affects 15-40% of people living with HIV (PLWH). HIV enters the brain early after peripheral infection, establishing persistent viral reservoirs in brain myeloid cells. One mechanism that contributes to HAND is transmigration of uninfected and HIV infected CD14+CD16+ monocytes across the blood brain barrier (BBB) in response to CCL2, a chemoattractant elevated in the CSF of PLWH. Studies indicate that PLWH with opioid use disorder (OUD) have exacerbated HIV neuropathogenesis that may lead to increased cognitive deficits. One treatment for OUD is opiate agonist therapy (OAT) with buprenophrine. We demonstrated that buprenorphine decreases CCL2-mediated adhesion and chemotaxis of CD14+CD16+ monocytes, early steps in monocyte entry into the brain. Our preliminary data now indicate that buprenorphine decreases CCL2 mediated transmigration of HIV infected CD14+CD16+ monocytes across the human BBB tissue culture model, which represents the final steps for monocyte entry into the CNS. HIV infected CD14+CD16+ monocytes have increased junctional proteins JAM-A and ALCAM that facilitate transmigration of these cells. We propose that buprenorphine reduces monocyte transmigration by changing expression of these proteins. Our preliminary data suggest the buprenorphine reduces ALCAM on CD14+CD16+ monocytes. To determine the ability of buprenorphine to reduce monocyte entry into the brain in vivo and thereby mitigate neurocognitive impairment, we perform studies using EcoHIV infected mice. We showed that mice treated with buprenorphine prior to EcoHIV infection do not develop cognitive impairment. This is correlated with a decrease in inflammatory monocytes in the brain. Collectively these ongoing studies will characterize buprenorphine as a potential therapy for HAND in PLWH with/without OUD.

Poster No. 61

Defining the Impact of Pericytes on Stroke Outcomes During HIV Infection

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The blood brain barrier (BBB) is a semipermeable cell layer that is essential for brain protection and homeostasis. While the role of endothelial cells in formation of the main physical barrier has been recognized, emerging evidence indicates a critical role of pericytes in the maintaining the BBB functions. BBB dysfunction is one of the hallmarks of ischemic stroke and HIV infected individuals are at a higher risk for stroke and cerebrovascular comorbidities. BBB pericytes can be a target of HIV infection and support long term replication of the virus in the CNS. Due to the position of BBB pericytes in the interplay between HIV and the CNS, we hypothesize that pericyte dysfunction can potentiate stroke and worsen post stroke outcomes. To understand the role of pericytes in stroke we employ both an in-vivo and in-vitro approach. Our in-vitro approach involves coculturing primary human pericytes and endothelial cells. Cocultures are exposed to HIV in order to study the dysfunction in paracrine signaling brought on by infection. For our in-vivo studies we use three strains of mice, one with a deletion in the platelet derived growth factor beta receptor gene (PDGFR-B +/-), one with a loss of seven tyrosine residues of the PDGFR-B receptor (F7/F7), a combination of both (F7/-), and wild-type littermate controls (+/+). Stroke is induced in mice using photothrombosis with Rose Bengal, volumes are quantified using Tetrazolium chloride (TTC) staining and evans blue injection. Brain sectioning and immunostaining is performed for CD13, fibrinogen,

and lectin which are markers for pericytes, plasma protein, and endothelial cells respectively. Excitingly, our data suggests that pericyte deficiency is sufficient to trigger BBB dysfunction, increased BBB permeability, and larger stroke volumes.

Poster No. 62

Blood-brain barrier pericytes as a target for HIV-1 infection

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Pericytes are multifunctional cells wrapped around endothelial cells via cytoplasmic processes that extend along the abluminal surface of the endothelium. The interactions between endothelial cells and pericytes of the blood-brain barrier (BBB) are necessary for proper formation, development, stabilization, and maintenance of the BBB. BBB pericytes regulate paracellular flow between cells and transendothelial fluid transport, maintain optimal chemical composition of the surrounding microenvironment, and protect endothelial cells from potential harmful substances. Thus, dysfunction or loss of BBB pericytes is an important factor in the pathogenesis of several diseases that are associated with microvascular instability. Our research indicates that BBB pericytes can be a target of HIV-1 infection able to support productive HIV-1 replication. In addition, we have evidence that BBB pericytes are prone to establish a latent infection, which can be reactivated by a mixture of histone deacetylase inhibitors in combination with TNF. HIV-1 infection of BBB pericytes has been confirmed in human post-mortem samples of HIV-1-infected brains. Overall, our study indicates that BBB pericytes can be a previously unrecognized HIV-1 target and reservoir in the brain. Supported by the NIH grants MH122235, MH072567, HL126559, DA044579, DA039576, DA040537, DA050528, and DA047157

Poster No. 63

HIV-1 Tat and morphine differentially alter behaviors related to prefrontal cortico-amygdalar circuits

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The advent of antiretroviral therapies has greatly improved the life expectancy of persons infected with HIV (PWH). However, many PWH still experience cognitive and emotional deficits that decrease their quality of life. HIV-1 trans-activator of transcription (HIV-1 Tat) can be expressed in the central nervous system (CNS) of PWH. Within prefrontal cortico-amygdalar (PFC-amygdala) circuits, HIV-Tat systematically disrupts inhibitory and excitatory synaptic function, and this can be exacerbated by opioids and may underly behavioral impairments. To explore HIV-1 Tat- and morphine-induced behavioral deficits, Tat(+) and Tat(-) male mice were exposed to HIV-1 Tat for 8 weeks and administered saline or ramping doses of morphine twice daily (s.c.) during the last 2 weeks of HIV-1 Tat exposure. Mice were behaviorally tested in a battery of PFC-amygdala-associated behaviors 4 h after morphine administration to limit opioid-induced locomotor effects. In the novelty suppressed feeding test, morphine increased eating behavior, whereas Tat-induction did not affect feeding. However, in the novelty induced hypophagia test, Tat and morphine decreased intake of the sucrose solution, indicating that Tat and morphine differentially alter anhedonia in response to palatable and non-palatable food stimuli. In home cages, Tat also decreased nesting behavior, whereas morphine decreased burrowing. These data suggest that HIV-1 Tat and morphine interfere with different behaviors of daily life that may further indicate HIV-1 Tat and morphine-induced depressive-like behavior. Although Tat(+) mice exhibited decreased acoustic startle responses, morphine increased startle reactivity indicating they independently alter sensorimotor function. HIV-1 Tat

preferentially targets inhibitory connections within the PFC. However, our preliminary data suggest that in the basolateral amygdala (BLA), but not the central nucleus of the amygdala (CEA) Tat and morphine may interact to decrease dendritic spine density. Together, our findings suggest that HIV-1 Tat and morphine differentially induce functional deficits associated with the prefrontal-amygdalar circuits.

Poster No. 64

Pathological basis of varicella zoster virus-associated adrenal dysfunction

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Latent varicella zoster virus (VZV) is present in 6% of human adrenal glands raising questions of how virus entered adrenal glands during primary infection and whether virus can cause adrenal gland damage, disrupting secretion of corticosteroids, aldosterone, epinephrine, and/or norepinephrine to produce clinical disease. Indeed, rare cases of bilateral adrenal hemorrhage and insufficiency associated with VZV reactivation have been reported. Because there is no animal model for VZV infection of adrenal glands, we obtained adrenal glands from one immunocompetent and one whole body-irradiated non-human primate (NHP) who spontaneously developed varicella from primary simian varicella virus (SVV) infection, the NHP VZV homolog. Histological and immunohistochemical analysis revealed SVV antigen and DNA in adrenal medulla and cortex of both animals. Adrenal glands had Cowdry A inclusion bodies, necrosis, multiple areas of hemorrhage, and polymorphonuclear cells; varying amounts of CD45-expressing cells were seen. No specific association of SVV antigen with beta-3 tubulin-positive nerve fibers was found; interestingly, SVV antigen was found within arterial walls, raising the possibility of hematogenous spread and explaining the diffuse hemorrhage seen throughout. We further explored the effects of VZV infection on primary human adrenal cortical cells in vitro. VZV productively infected these cells as demonstrated by increasing viral DNA from days 1, 2, and 3 post-inoculation, the presence of a cytopathic effect, and detection of VZV antigen. Analysis of conditioned supernatant from VZV- compared to mock-infected cultures showed significant increases in IL-4, Il-6, IL-8, IL-12, IL-13, and TNF-alpha. Overall, we found that varicella virus can productively infect adrenal cells, causing cell death, hemorrhage, and inflammation supporting the notion that VZV infection of adrenal glands disrupts normal hypothalamic-pituitary-adrenal axis function, leading to a constellation of symptoms including chronic fatigue, hypertension, hyperglycemia, and weakness.

Poster No. 65

Immunopathogenic TCR repertoire signature in CSF of virus-associated neurologic disease

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Objective: T-cell receptor (TCR) repertoire signatures would provide a better understanding of the pathogenesis and useful biomarker for accurate diagnosis and prognostication in immune-mediated

diseases. In this study, we examined and characterized disease specific TCR signatures in cerebrospinal fluid (CSF) of patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).

Methods: TCR-beta libraries using unique molecular identifier-based methodologies were sequenced in paired peripheral blood mononuclear cells (PBMCs) and CSF cells from HAM/TSP patients and normal healthy donors (NDs). In addition, TCR-beta repertoires were examined in HTLV-1 Tax11-19-specific CD8+ T cells from PBMCs of HAM/TSP patients with HLA-A*0201.

Results: The sequence analysis revealed that TCR-beta repertoires in CSF of HAM/TSP patients were highly expanded and contained both TCR clonotypes shared with PBMCs and uniquely enriched within the CSF. In addition, we analyzed TCR-beta repertoires of highly expanded and potentially immunopathologic HTLV-1 Tax11-19-specific CD8+ T cells from PBMCs of HLA-A*0201 positive HAM/TSP and identified a conserved motif in the CDR3 region. Importantly, TCR-beta clonotypes of expanded clones in HTLV-1 Tax specific CD8+ T cells were also expanded and enriched in the CSF of the same patient.

Conclusions: These results indicate that exploring TCR repertoires of CSF and antigen-specific T cells may provide a TCR repertoire signature in virus-associated neurologic disorders.

Poster No. 66

Morphine and HIV-1 Tat disrupt pathological biomarkers of neuroaxonal integrity in the prefrontal cortex

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Structural damage and loss of fronto-cortical white and gray matter integrity are common in HIV-infected individuals. Such pathology is hypothesized to be associated with microvessel and neuroaxonal damage. Opiate abuse is prevalent in the HIV-infected population; however, the extent to which co-exposure to HIV and opiates affect cortical white and gray matter integrity remains understudied. We evaluated (i) vascular integrity using levels of claudin-5 (cldn5) and albumin (Alb), and (ii) neuroaxonal damage using hyperphosphorylated tau load (p-tau), beta-amyloid (Abeta) level, oligodendroglia (OLG) numbers, and neurofilament light chain (NFL) level in the prefrontal cortex (PFC) of inducible HIV-1 Tat (+/-) transgenic male mice that received morphine (10-40 mg/kg, increasing by 10 mg/kg/b.i.d., s.c.) for 6 weeks. Levels of cldn5, Alb, Abeta, and NFL were evaluated by immunoblotting. Sub-anatomical Cldn5 expression, Alb leakage, OLGs, and p-tau load were determined by immunofluorescence assays in the anterior cingulate (ACC) and primary motor (M1) cortices. Tat decreased cldn5 and increased Alb levels in the PFC. Morphine exacerbated the Tat effects on cldn5 and Alb. Tat, and to a lesser extent morphine exposure, increased levels of NFL, Abeta peptide, and oligomeric forms. Enhanced NFL and Abeta pathology were observed in the comorbid brain. Immunofluorescence findings revealed dysregulation of cldn5 and enhanced Alb leakage in the ACC independently by Tat and morphine, and by the co-exposure. Cldn5 and Alb were altered only by morphine in the M1 cortex. ACC p-tau-positive cells were increased by Tat irrespective of treatment, but the opposite was observed in the M1. Morphine decreased CC1-stained OLG numbers in the ACC irrespective of genotype. No OLG reductions were observed in the M1. Neuroaxonal injury in the M1 and ACC is associated with motor and cognitive dysfunction in Alzheimer's disease. Thus, our results may explain similar neurobehavioral dysfunctions induced by HIV and/or opiate abuse.

Poster No. 67

HIV Infection and Amyloid Deposition Act In Concert to Impact Neurogenesis via Mitochondrial Dysregulation

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The number of older individuals living with HIV has increased to nearly 70% of the infected population as of 2020. This patient population is at a higher risk for developing beta-amyloid (AB) neuropathologies. One of the cell types that can be affected by AB deposition are neural progenitor cells (NPC) located in perivascular niches. This pool of NPCs can be further depleted by enhanced AB deposition, leading to delayed recovery of both motor and cognitive functions. While AB deposition is elevated during HIV infection, the role of HIV on the mechanism of AB-mediated neurodegeneration is not well characterized. We have shown that HIV increases endothelial cell secretion of extracellular vesicles (EV), which transfer AB to NPC germinal zones. Furthermore, our in vivo studies show that HIV and AB induce BBB permeability and aberrant neurogenesis. We hypothesize that cerebral HIV infection and AB deposition leads to mitochondrial dysfunction resulting in enhanced inflammatory responses and diminished neurogenesis. We will employ in vitro analysis of mitochondrial function and inflammatory markers of NPCs treated with EVs carrying AB. Excitingly, our data suggests that iPSC-derived neurons exhibit diminished growth when exposed to EVs carrying AB. Additionally, preliminary data suggests a metabolic shift in NPCs treated with AB. Understanding of neurogenesis mechanisms in the context of HIV and amyloid deposition may allow us to develop therapeutics for NPC regeneration to help modulate the severity of HIV co-morbidities.

Poster No. 68

HIV Tat and morphine-mediated activation of astrocytes involves epigenetic modification of the NLRP6 inflammasome signaling

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The Centers for Disease Control and Prevention describes HIV infection and drug abuse as intertwined epidemics, leading to compromised adherence to combined antiretroviral therapy coupled with exacerbation of HIV-associated neurocognitive disorders (HAND). Chronic low-level inflammation (mediated by viral proteins, antiretrovirals, and abused drugs) has been implicated as a significant underlying factor as well as an essential correlate of HAND pathogenesis. In this study, we hypothesized that exposure of astrocytes to HIV Tat and morphine could result in exacerbation of astrocyte activation involving: a) activation of the NLRP6 inflammasome via promoter DNA hypomethylation and, b) downregulation of miR-152, which in turn, targets NLRP6, leading to cleavage of caspase1 and release of proinflammatory cytokines, IL1beta and IL18, ultimately culminating in neuroinflammation. Whole-genome bisulfite sequencing in the frontal cortices of SIV-infected macaques demonstrated increased DNA hypomethylation of the NLRP6 promoter with a concomitant upregulation of the NLRP6 inflammasome. MiRNA array analysis of HIV Tat and morphine exposed human primary astrocytes showed decreased levels of miR-152 with a concomitant upregulation of NLRP6 inflammasome and cellular activation. Gene silencing approaches further validated HIV Tat and morphine-mediated activation of NLRP6, cleavage of caspase1 and release of IL1beta, and IL18 in human primary astrocytes. Overall, these findings underpin the epigenetic involvement of NLRP6 inflammasome signaling in astrocyte activation in the context of HIV Tat and morphine.

Poster No. 69

Human Herpesvirus 6 (HHV-6) Genome Integration in Whole Genome Sequences of Dementia Cohorts

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The infectious hypothesis of Alzheimer's disease (AD) suggests that microbes such as human herpesvirus 6 (HHV-6) play a role in pathogenesis. Contradictory evidence for the role of HHV-6 in AD highlights the need for continued research on the relationship between viruses and dementia. While HHV-6 is known to integrate into human host genomes as a means of achieving latency, the occurrence of viral integration in dementia patients has not been thoroughly investigated. Lewy body dementia (LBD) and frontotemporal dementia (FTD) are poorly understood neurodegenerative diseases leading to cognitive decline. Over 6,000 total whole genome sequences of LBD, FTD, and control cohorts from the DementiaSeq repository were screened for pathogen genomes by comparison with reference genomes from over 25,000 microbes, including HHV-6, through the PathSeq computational tool. PathSeq scores are based on the number of reads that align with the reference genome, indirectly indicating the amount of microbe genome present in the sample. Integrative Genome Viewer (IGV) was used to further identify the portions of the viral genome that were present. High PathSeq scores for HHV-6 were suggestive of chromosomally integrated HHV-6 (ciHHV-6), with ciHHV-6 in some samples being confirmed with droplet digital PCR (ddPCR). Lower PathSeq scores were consistent with integration of variably sized HHV-6 genomic segments from different regions of the viral genome. While ciHHV-6 was found in all cohorts at rates consistent with that expected based on current literature (0.6-1%), the LBD cohort had a significantly higher percentage of individuals with small segments of HHV-6 viral genome integration when compared to both control and FTD cohorts. While the effect of viral integration in these cases is unknown, possible effects include disruption of host gene expression. Further investigation of the possible role of viral genome integration in the pathogenesis of neurodegenerative disease and dementia is warranted.

Poster No. 70

HIV-1 Nef and cocaine can disrupt glutamate transporters in the nucleus accumbens

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Comorbid use of illicit drugs, such as cocaine, can contribute to the vulnerability and development of HIV-associated neurocognitive disorders (HAND). HIV infection in the brain causes neurotoxicity even though HIV does not infect neurons directly. Production of HIV neurotoxins by infected glial cells, specifically astrocytes, contributes to HAND even with cART-suppressed viral replication. When HIV replication is well-controlled during successful antiretroviral therapy, the neurotoxic HIV-1 Nef protein, the major

protein produced by infected astrocytes, is more likely to cause pathology than other HIV proteins. We have shown that expression of Nef in hippocampal astrocytes of Sprague Dawley (SD) rats causes spatial learning and recognition impairment, increased inflammation, and a compromised blood-brain barrier. Here, however, we focus on the possible intersection of HIV-1 Nef neurotoxicity and cocaine consumption. In this study, we examine the role of astrocyte Nef expression on glutamate homeostasis. Glutamate transporters maintain glutamate homeostasis in astrocytes, which are responsible for most of the synaptic glutamate clearance, and cocaine can downregulate astrocytic glutamate transporters, leading to a decreased glutamate reuptake. Therefore, we infused astrocytes transfected with Nef in the nucleus accumbens (NAc), an important structure involved in the development of cocaine abuse, in male and female SD rats. One week after surgery, the rats were injected intraperitoneally with cocaine or saline and sacrificed to collect NAc tissue. Protein and histological analysis show differences in glutamate transporter expression among groups treated with Nef and cocaine, individually and in combination. We also used a primary astrocyte and neuron co-culture to detect differences in glutamate regulation throughout each treatment. Altogether, these data suggest a convergence of HIV-1 Nef and cocaine in glutamate dysregulation and neurotoxicity. Future studies will focus on determining how the interaction between Nef and cocaine could determine neurophysiological changes that can disrupt cocaine-seeking behavior.

Poster No. 71

Analysis of CSF Extracellular Vesicle Cellular Origin Across Viral and Non-Viral Neurodegenerative Diseases

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Neurodegenerative diseases can be caused by many genetic, environmental, and antigenic (both host and non-host) factors. Some, such as Human T-Lymphotropic virus type-1 (HTLV-1) Associated Myelopathy/Tropical spastic paraparesis (HAM/TSP), have known viral causes, whereas others like Multiple Sclerosis (MS) have more uncertain etiology. Extracellular vesicles (EVs) carry cargo that pertain to their cell of derivation, and by sampling these EVs we can gain insight into the cell of origin's state at the time of EV release. We utilized multiplex analysis (MPA) using fluorescently barcoded antibody capture beads (39 markers) in combination with detection antibodies as a method for EV surface repertoire analysis in cerebrospinal fluid (CSF) from well-characterized patients diagnosed with MS (n=10), HAM/TSP (n=10), HTLV-1 asymptomatic carriers (ACs; n=5), healthy volunteers (HVs; n=10), and other viral (n=8) or non-viral (n=6) neurodegenerative diseases. Our results indicated that individuals with HAM/TSP had significantly increased levels of CSF EVs expressing CD2 ($p<0.001$) and CD8 ($p<0.001$) compared to MS patients and HVs. PCA and tSNE-1 analyses showed close grouping of HAM/TSP samples away from HVs and other disease groups. Levels of CD8 on EVs from HAM/TSP correlated positively with CD8+ T-cell numbers and total cell concentration in the CSF, but interestingly strongly negatively correlated with CD133/1 on EVs. Collectively, this pilot study indicates that CD8+ EVs in the CSF of HAM/TSP patients may be related to the viral infection and/or the pathology caused by CD8+ T-cells during the course of disease, which differs from ACs, and therefore warrant closer examination. Moreover, the potential of utilizing MPA for CSF EV analysis to implicate the cell types important in the pathology of certain neurological diseases, especially when these cell types may not be directly present at the sample site, may help point to the molecular mechanisms of disease and targeted therapies.

Poster No. 72

Transmitted/founder SHIV.D replicates in the brain, causes neuropathogenesis, and persists on ART

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An eradicative HIV cure requires safe and effective clearance of replication competent virus from all reservoirs. Memory CD4 T cells in lymphoid tissue are the best characterized persistent reservoir, but evidence suggests that myeloid reservoirs in the CNS and elsewhere comprise an important and understudied source of virus persistence. Characterization of the CNS reservoir requires physiologically relevant animal models of HIV persistence in the brain. Here, we utilized a novel transmitted/founder (TF) SHIV model based on macrophage-tropic TF SHIV.D.191859 (SHIV.D). SHIV.D encodes a clade D TF HIV-1 Env that is CCR5-tropic, efficiently replicates in CD4 T cells and macrophages. We applied our macrophage-tropic TF SHIV to characterize CNS infection, since HIV-1 replication in the brain and neuropathogenesis are closely tied with the ability to replicate in macrophage. Notably, Clade D viruses, specifically, have shown enhanced macrophage tropism and increased frequency of HIV-associated neurocognitive disorders. We studied the brains of SHIV.D-infected rhesus macaques (RM) necropsied during viremia and on suppressive ART for CNS virus replication, pathogenesis, and persistence. In RM necropsied while viremic, we see clear evidence of SHIV.D replication (RNA) and neuropathology in the brain. In SHIV.D-infected RM necropsied after >6 months of suppressive ART, we see evidence of macrophage inflammation, but infrequent cell-associated SHIV RNA by immunohistochemistry. We found SHIV cell associated-DNA in both RM across brain regions at similar levels as CD4 T cells on ART. Thus, we have clear evidence of SHIV.D replication and neuropathogenesis during viremia and persistence within the brain through suppressive ART. This model can be used to elucidate the extent, cell types, and dynamics of persistent virus infection in the CNS.

Poster No. 73

Dysregulated microglial cell activation and proliferation following repeated antigen stimulation

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Upon reactivation of quiescent neurotropic viruses, (Ag)-specific brain resident-memory CD8+ T-cells (bTRM) may respond to de novo-produced viral Ag through rapid release of IFN-gamma, which drives subsequent interferon-stimulated gene expression in surrounding microglia. Through this mechanism, a small number of adaptive bTRM may amplify responses to viral reactivation leading to an organ-wide innate protective state. Over time, this brain-wide innate immune activation likely has cumulative neurotoxic and neurocognitive consequences. We have previously shown that HIV-1 p24 antigen (Ag)-specific bTRM persist within the murine brain using a heterologous prime-CNS boost strategy. In response to Ag re-challenge, these bTRM display rapid and robust recall responses, which subsequently activate glial cells. In this study, we hypothesized that repeated recall responses to viral Ag (modeling repeated episodes of viral reactivation) culminate in prolonged reactive gliosis and exacerbated neurotoxicity. To address this question, mice were first immunized with adenovirus vectors expressing the HIV p24 capsid protein, followed by a CNS-boost using Pr55Gag/Env virus like particles (HIV-VLPs). Following establishment of the bTRM population (>30 d), prime-CNS boost animals were then subjected to in vivo recall stimulation, as well as restimulation (at 14 d post-recall stimulation), using the immunodominant HIV-1 AI9 CD8+ T-cell epitope peptide. In these studies, Ag restimulation resulted in prolonged expression of microglial activation markers and an increased proliferative response, longer than recall stimulation alone. This continued expression of MHCII and PD-L1, as well as Ki67 was observed at 7, 14 and 30 d post-AI9 restimulation. Additionally, in vivo restimulation resulted in continued and elevated production of inducible nitric oxide synthase (iNOS) among restimulated groups. Furthermore, in vivo specific Ag restimulation

produced lower levels of arginase (Arg)-1 when compared with the recall-stimulated group. Taken together, these results indicate that repeated Ag-specific stimulation of adaptive immune responses leads to cumulative dysregulated microglial cell activation

Poster No. 74

Risk assessment of Progressive Multifocal Leukoencephalopathy in ocrelizumab-treated Multiple Sclerosis patients.

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The risk of Progressive Multifocal Leukoencephalopathy (PML), a brain infection caused by John Cunningham virus (JCPyV), is the main limitation to the use of Disease Modifying Therapies for Multiple Sclerosis (MS). PML risk in course of ocrelizumab, is lower than the risk associated with natalizumab, but it is not clear if this is due to the different mechanism of action of drugs or inappropriate screening methodology. Thus, the objective of this study was to clarify the accuracy of the anti-JCV index and to quantify viral replication in ocrelizumab's patients. Thirty-five MS patients, in treatment with ocrelizumab, were enrolled. Urine and blood samples were collected at baseline (T0) and every 3 months for one year. After JCPyV-DNA extraction, a quantitative-PCR (Q-PCR) was performed. Moreover, assessment of IgM and IgG titer and anti-JCV index level were obtained. Q-PCR revealed JCPyV-DNA viruria at all selected time points, while JCPyV-DNA was never detected in plasma. IgM titer was found to decrease during ocrelizumab treatment with a statistically significant decrease in mean values ($p < 0.05$). IgG levels had a stationary trend over time ($p > 0.05$). At T0, patients who had an anti-JCV index > 1.5 were 23/35, at T2 the number decreased to 20 and, at T4, patients who had an anti JCV index > 1.5 were 18. On the contrary, patients presenting an anti-JCV index < 1.5 at T0 were 12/35, at T2 were 15/35, whereas, at T4, were 17/35. Patients with viruria were stable over time. IgM level decreased progressively whereas IgG level persisted stable. Patients with JCV index > 1.5 decreased conversely to patients with index < 1.5. Combined monitoring of the ocrelizumab's effects on JCPyV reactivation and on host immunity could offer better and complete insight toward predicting risk, leading to the recognition of predictive biomarker for PML.

Poster No. 75

ER-associated Regulation of Astrocyte Mitochondrial Function during HIV-1 Infection

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Astrocytes are key regulators of central nervous system (CNS) health and neuronal function. However, astrocyte mitochondrial dysfunction, such as induced by human immunodeficiency virus (HIV)-1, threatens astrocyte provision of essential metabolic and antioxidant support to neurons. Thus, delineating regulatory pathways that can be targeted to prevent aberrant mitochondrial homeostasis in astrocytes will be imperative for ensuring neuronal fitness/survival against CNS pathologies. Direct contact sites between the endoplasmic reticulum (ER) and mitochondria, termed mitochondria-associated membranes (MAMs), are central hubs for regulating several cellular processes required for homeostasis, including mitochondrial metabolic activity. Recent investigations have also identified unique, yet ill-defined contributions of the

three unfolded protein response (UPR) arms, beyond their classical ER stress functions, in regulating MAM tethering and/or signaling. However, these regulatory mechanisms have not yet been fully elucidated. The current investigation examines changes in astrocyte mitochondrial function, ER stress, and MAM regulation in response to HIV-1 infection. Next, we explored the role of ER-associated mechanisms in regulating astrocyte mitochondrial function. The effects of HIV-1 infection were examined using pseudotyped HIV-1 to infect primary human astrocytes. Astrocyte metabolic status was determined using Seahorse extracellular flux analyzer for a real-time assessment of cellular metabolism. Changes in protein expression of UPR and MAM mediators were determined using simple Wes assays. Finally, pharmacological inhibition of the UPR pathways was used to delineate ER-associated regulatory mechanisms mediating changes in mitochondria bioenergetics. Our studies demonstrate increased astrocyte metabolic capacity in response to HIV-1 infection, which corresponded to increased expression of UPR/MAM mediators. Moreover, pharmacological inhibition of Inositol-requiring enzyme-1alpha (IRE1alpha) impaired astrocyte mitochondrial activity. These findings illustrate the importance of ER-mitochondria communication in regulating astrocyte mitochondrial function and identify a possible mechanism to manipulate the metabolic and antioxidant coupling between astrocytes and neurons during HIV-1 pathogenesis.

Poster No. 76

Brain MR Spectroscopic Findings in Three Consecutive COVID-19 Patients: Preliminary Observations

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Introduction: There is increasing recognition of neurological disorders associated with the SARS-CoV-2 infection, including the development of diffuse white-matter (WM) abnormalities. The pathogenesis of COVID-19 WM abnormalities remains unknown although silent hypoxia has been hypothesized to have a central role in its development. Using magnetic resonance spectroscopic imaging (MRSI), we set out to evaluate the metabolic changes in the WM lesions of COVID-19 patients. Here we present examples of MRS metabolic results of COVID-19 patients and compare with other leukoencephalopathy cases and a control case.

Methods: Data were collected from three consecutive cases with COVID-19. These were compared with two previously acquired datasets of two control leukoencephalopathy cases: 1) post-hypoxic leukoencephalopathy likely related to opioids (DLP), 2) sepsis-related leukoencephalopathy (SAE) and one healthy age-matched control. Structural MRI and 3D MRSI datasets were obtained using 3T MRI scanners. MR spectroscopic datasets were processed with LCModel, and relative ratios of N-Acetyl-Aspartate (NAA), Choline (Cho), Myo-Inositol (mI), Lactate (Lac) and Glutamate + Glutamine (Glx) to Creatine (Cr) were quantified.

Results and Discussion: Our first patient (COVID-A) had multifocal necrotizing leukoencephalopathy, the second patient (COBID-B) had recent cardiac arrest, without clear leukoencephalopathy. The third patient (COVID C) was without clear leukoencephalopathy or recent severe hypoxia. Comparative analysis showed decreased NAA/Cr, indicative of neuronal loss/injury within the white matter of all 3 COVID-19 positive patients compared to the control. Compared to the control case, two of the three COVID-19 patients had significantly elevated Cho/Cr levels indicating severe demyelination. All three COVID-19 patients and the non-COVID-19 patient with DPL showed mildly elevated myo-inositol/Cr (mI/Cr) levels indicative of neuroinflammation. Lac/Cr ratios were increased in the COVID-19 patient with necrotizing leukoencephalopathy indicative of anaerobic metabolism and hypoxia. Notably, the metabolic derangements seen in the setting of COVID-19 associated multifocal necrotizing leukoencephalopathy are similar to those observed with DPL.

Poster No. 77

Cardiac fibrosis and immune activation in SIV-infected rhesus macaques

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With introduction of antiretroviral therapy (ART), human immunodeficiency virus (HIV) has transitioned to a chronic inflammatory disease, with cardiovascular comorbidities becoming more evident. PWH have a greater incidence of diastolic dysfunction compared to seronegative individuals, and in women with HIV, we have previously observed increased extracellular volume in the left ventricle that was correlated to immune dysfunction and CCR2 expression on peripheral monocytes. Here, we utilize the simian immunodeficiency virus (SIV)-infected rhesus macaque model to recapitulate left ventricle remodeling observed in PWH. We used three different cohorts for our studies: uninfected animals (SIV-), SIV-infected animals (SIV+), and SIV-infected animals receiving ART (SIV+ART). Fibrosis was assessed by mason trichrome staining for extracellular matrices in the left ventricle. Compared to SIV- and SIV+ART, SIV+ animals had a significantly greater degree of left ventricle fibrosis. To longitudinally compare immune markers of myocardial fibrosis, we observed a marked increase in growth differentiation factor 15 (GDF-15) over the course of SIV infection, which was not reduced with introduction of ART. Additionally, left ventricle fibrosis significantly correlated to surface expression of CCR2 on classical, intermediate, and non-classical monocytes. We identified osteopontin as a potential signaling factor driving fibrosis in the left ventricle, as it is integral for macrophage function, a matricellular protein of the cardiac extracellular matrix, and a cytokine for immune infiltration. There was no significant difference in the level of MMP-cleaved osteopontin between animal groups. However, we observed significant differences in the level of full-length osteopontin between the three groups, and SIV+ animals showed a significant increase in full-length osteopontin, compared to SIV- and SIV+ART. These results implicate peripheral immune activation and osteopontin as potential diving factors in cardiac fibrosis in HIV infection.

Poster No. 78

Cannabinoid receptor 2 agonist, JWH-133, downregulates HIV-induced inflammation, oxidative stress, and lysosomal exocytosis from macrophages

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Background & Objectives: HIV-associated neurocognitive disorders (HAND) affect 20-50% of HIV-positive patients, and there are no effective therapies available. HIV-infected monocyte-derived macrophages (MDM) invade the brain of these individuals, promoting neurotoxicity. We have demonstrated increased expression of cathepsin B (CATB), a lysosomal protease, in monocytes and post-mortem brain tissues of women with HAND. Increased CATB release from HIV-infected MDM leads to neurotoxicity and it is associated with inflammation, oxidative stress and lysosomal exocytosis. In search for possible therapies, we found that cannabinoid receptor 2 (CB2R) agonist, JWH-133, decreased CATB secretion and neurotoxicity from HIV-infected MDM. However, the mechanisms by which HIV infection and JWH-133 modulate CATB secretion from MDM are not completely understood. We hypothesized that HIV infection upregulates the expression of intracellular proteins that participate in mechanisms associated with CATB secretion, such as inflammation, oxidative stress, and lysosomal exocytosis, whereas JWH-133 downregulates these proteins. Methods: MDM were isolated from healthy women donors, infected with HIV-1ADA, and treated with JWH-133 every three days. At day 13 post-infection, lysates from HIV-infected MDM with increased CATB secretion (n=3) were selected and labeled with Tandem Mass Tag

(TMT), run through LC/MS/MS, and analyzed using limma software and Ingenuity Pathways Analysis. Groups' comparisons consisted of HIV(+) vehicle vs uninfected vehicle and HIV(+) JWH-133 vs HIV(+) vehicle. Proteins with a fold change ≥ |2| and p-value < 0.05 were considered as significantly deregulated. Results: HIV infection upregulated the expression of proteins associated with inflammation, oxidative stress, and lysosomal exocytosis, whereas JWH-133 downregulated the expression of these proteins. PIK3CB and PIK3R2 were the most commonly shared proteins between the pathways, and PKCalpha, PKCdelta, TFEB, and TGFbeta-1 had a known relationship with CATB. Conclusion: This study reveals alternative therapeutic targets against HAND. Future studies will validate these findings. Acknowledgements: F99NS113455, R25-GM061838, U54MD007600, SC1GM11369-01, U54MD007587

Poster No. 79

Cigarette Smoke and Nicotine Effects on Behavior in HIV Transgenic Rats

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HIV-related neurocognitive impairment can be worsened by cigarette smoking and be more severe in women. Therefore, we analyzed the effects of sex on behavioral function in HIV transgenic (Tg) rats that were exposed to either nicotine alone, to smoke from either nicotine-containing or nicotine-free cigarettes, or non-exposed. The animals were then assessed on the open field test for the total distance traveled and for the fraction of the total distance traveled and the total time spent in the center of the field, and the results then compared to WT rats subjected to the same exposures and testing. Higher total distances indicate greater locomotor activity and a higher center field measures imply a lower anxiety state. Total distances were overall higher for female and for Tg rats exposed to nicotine-free CS. Also, the total distance and both center field measures were overall higher for female rats in the control and nicotine-free CS-exposed groups. This was observed specifically for WT females as compared to WT males and, for the center field measures, for WT females as compared to Tg males. No genotype or sex-related differences were found for rats in the nicotine-free cigarette smoke (CS) and nicotine-containing CS exposed groups. Therefore, nicotine exposure did not impact genotype- and sex-related differences in motor responses and anxiety levels that were found in the control state. However, exposure to the non-nicotine components of CS resulted in locomotor activation in the presence of the HIV genes and was anxiogenic in WT and Tg male animals.

Poster No. 80

Identifying HIV Habitats in the Brain Using Diffusion Tensor and Diffusion Kurtosis Imaging with a Machine Learning Approach

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Introduction: Detection of human immunodeficiency virus (HIV) habitats in the brain is limited to terminal stages of the disease. Therefore, neuroimaging is a critical noninvasive tool for studying the neuro-pathogenesis of HIV in-vivo. However, conventional MRI analysis methods of HIV infection have resulted in contradicting outcomes in the literature, and they can only provide group-level findings without an appropriate clinical diagnosis at a single subject level. Aim: To identify habitats of active HIV infection and replication at a single subject level using diffusion tensor (DTI) and diffusion kurtosis imaging (DKI) with machine learning (ML). Methods: We collected whole-brain imaging data from 60 HIV+ patients and 60 age/gender matched HIV- controls. We processed the data for DTI/DKI fitting, and evaluated the resulting metrics (FA, MD, AD, RD, kFA, MK, AK, RK) at 30 brain anatomical regions relevant to HIV selected from the JHU-MNI-SS-type2 atlas. Our ML design used (8 metrics) x (30 regions) = 240 features

measured for 120 input subjects. We divided the 120 subjects into 96 randomly selected samples for training and 24 for testing (80/20% of the data). The ML classification was performed using non-linear support vector machine (SVM) with recursive feature elimination to select the most important features. Results: The ML classification had a 79.2% accuracy and 78.6% precision. Of the 24 test samples, 14 were HIV+ (with 11 classified correctly). The regions with the highest feature scores were the frontal lobe, thalamus, midbrain and the substantia nigra. Discussion: The regions identified by ML had the highest predictive score in discerning HIV+ from HIV- subjects. This indicates that for each of the 14 HIV+ test subjects, those regions showed signs of inflammation and neuro-degeneration signaling the presence of active HIV habitats. The accuracy and precision of the ML application can be further increased with larger sample sizes.

Poster No. 81

A Comprehensive Proteomics Analysis of the JC Virus (JCV) Large and Small Tumor Antigen Interacting Proteins: Small t Mostly Associates with Those Having Phosphatase and Chromatin-Remodeling Functions while Large T Primarily Targets the Host Protein

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The human neurotropic virus, JC virus, (JCV) is the etiologic agent of a fatal brain disease known as progressive multifocal leukoencephalopathy and encodes two oncogenic proteins from its early coding region, large (LT-Ag) and small t (Sm t-Ag) antigens. The oncogenic potential of both LT-Ag and Sm t-Ag has been demonstrated in tissue culture in vitro and various experimental animals including mouse, rats, hamsters, and monkeys in vivo. Even the contribution of such oncogenic proteins from another human polyomavirus, merkel cell polyomavirus (MCPyV) to the development of an aggressive human skin cancer, Merkel cell carcinoma, was previously established. Until recently, the known primary targets of these tumor antigens include several tumor suppressors such as pRb, p53, and PP2A. Here, we recently reported the first comprehensive proteomic list of the host proteins targeted by JCV LT-Ag and Sm t-Ag by employing a mass spectroscopy (AP/MS) assay. The proteomics data identified novel targets for both tumor antigens while confirming some of the previously reported interactions. LT-Ag was found to primarily target the protein complexes with phosphatase (PP4 and PP1), ligase (E3-ubiquitin) and ATPase (v-ATPase and Smc5/6 complex) activities. In contrast, the major targets of Sm t-Ag were identified as AIFM1, SdhA/B, PP2A, Smarca1/6, and p53. The interactions between “Sm t-Ag and Smarca5” and “LT-Ag and SdhB” and “Sm t-Ag and SDH” were further validated by biochemical assays. Interestingly, perturbations in some of the Sm t-Ag and LT-Ag targets identified in this study were previously shown to be associated with oncogenesis, suggesting novel roles for both tumor antigens in new oncogenic pathways. This comprehensive data establishes new foundations to further unravel the additional roles for JCV tumor antigens in the viral life cycle and oncogenesis. Support: NIH (R01NS090949) awarded to M. Safak

Poster No. 82

Human Neurotropic Polyomavirus, JC Virus, Agnoprotein Targets Mitochondrion and Modulates Its Functions

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JC virus (JCV), is the etiologic agent of a fatal brain disease known as Progressive Multifocal Leukoencephalopathy (PML), primarily occurring in immunocompromised individuals with AIDS, cancer, and multiple sclerosis. JCV encodes a critical regulatory protein from its late region, known as Agnoprotein which is a small and highly basic phospho-protein (71-aa). In the absence of its expression, JCV is unable to sustain its productive life cycle. Agnoprotein forms highly stable dimers and oligomers through its Ile/Leu/Phe-rich domain encompassing amino acids 23-39. The three-dimensional structure of Agnoprotein

was recently resolved by NMR (PDB: 5NHQ) revealing that it harbors two alpha-helices [minor (aa 6-13) and major (aa 23-39)] and the rest of the protein adopts an intrinsically unstructured conformation. Recent reports revealed that it is released from infected cells and perhaps taken up by the neighboring cells and the hydrophilic surface of the major alpha-helix plays a major role in the release process. We have recently reported the protein-interactome of Agnoprotein revealing that it targets various cellular networks and organelles, including mitochondria. Here, we have further characterized the functional consequences of its mitochondrial targeting and demonstrated co-localization with outer mitochondrial membrane (OMM) proteins and complexes. The mitochondrial targeting sequence (MTS, aa 1-14, MVLRQLSRKASVKV) of Agnoprotein along with the major alpha-helix domain is required for mitochondrial targeting. Data also showed alterations in various mitochondrial functions in Agnoprotein-positive cells, including a significant reduction in mitochondrial membrane potential, respiration rates and ATP production. In addition, we observed a substantial increase in ROS production and mitochondrial Ca2+ uptake. We are currently assessing the functional consequences of the increased matrix Ca+2 levels. Support: NIH (R01NS090949) awarded to M. Safak

Poster No. 83

National NeuroAIDS Tissue Consortium Resources during COVID-19 pandemic

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The National NeuroAIDS Tissue Consortium (NNTC) is an ongoing study established in 1998. The consortium aimed to establish a nationwide network of brain banks that can provide antemortem and postmortem tissues, biofluids, and respective experimental and clinical data for the neuroHIV research community. The NNTC clinical sites collect information on HIV disease severity, central and peripheral nervous system (CNS and PNS) signs and symptoms, comorbid conditions, laboratory values for a range of medical, immunological, and virological parameters, and tissue pathological diagnoses. Samples of plasma and CSF are collected from the participants followed longitudinally, and brain and other vital organs are obtained after death. In addition, the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study, with 6 clinical sites, and antemortem biofluids (e.g., plasma and CSF), skin biopsy samples, and similar data metrics, is now included in the NNTC and are available to requestors. In addition, a curated group of samples from virally suppressed individuals is available, as well as information on subjects with CSF viral escape. The NNTC has provided important biofluids, tissues and data for a variety of cellular, molecular, pathological, epidemiological, neuropsychological, and comorbidity studies (including drugs of abuse) to investigators, leading to a wealth of publications. Current efforts include enrolling additional aged subjects, brains from uninfected individuals who abused opiates, and examining the effects of the COVID pandemic on the cohort. NIH funding announcements continue to feature the NNTC as a resource for valuable samples to address investigate important aspects of HIV-related brain changes, for example in the use of single cell RNA sequencing and other technologies. The specimens and data in the NNTC continue to grow and can accelerate research into the effects of HIV on the brain, other organ systems, comorbidities, and strategies for a cure.

Poster No. 84

Therapeutic role of HIF-1alpha siRNA in attenuating Alzheimer's like pathology in HIV- associated neurological disorders

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Although cART usage has increased the lifespan of HIV+ individuals, paradoxically, its dependence is also associated with increased risk of Alzheimer's-like pathology, a comorbidity of HIV-Associated Neurocognitive Disorder (HAND). Based on our previous findings that astrocytes play a major role in HIV

Tat-mediated amyloidosis via the HIF-1alpha-BACE1-AS pathway, and since amyloids are known to be released in EVs, we sought to assess whether HIV Tat stimulated astrocyte derived EVs (Tat-ADEVs) containing the toxic amyloids could cause synapto-dendritic injury in neuronal cultures, induce neurodegeneration as well as cognitive impairments when administered in the brains of naïve mice. Rat & human hippocampal neurons exposed to Tat-ADEVs carrying amyloids resulted in reduction of dendritic spines and excitatory synapse densities. We also validated these findings ex vivo in the archival brain tissues of SIV+/ HIV+ subjects. Since HIF-1alpha is an upstream regulator of Tat-mediated amyloid production, we hypothesized that blocking HIF-1alpha could mitigate neuronal injury in vitro as well as in in vivo models. Silencing of astrocytic HIF-1alpha not only reduced the release of ADEVs and their amyloid cargoes, but also ameliorated neuronal injury. To assess neurodegeneration & behavioral impairment, we stereotactically injected Tat-stimulated ADEVs in the brains of naïve mice followed by monitoring behavioral changes, neurodegeneration, glial activation and neuroinflammation. Tat-ADEVs carrying amyloids when injected in the hippocampus of naïve mice brains resulted in reduction of excitatory synapse densities, chromatolysis of nissl granules in the neurons, increased amyloid deposition, glial cell activation as well as increased neuroinflammation. Concomitantly, these animals also showed impairment of long term memory, locomotion and increased anxiety like behavior. Tat-ADEV mediated neuropathological changes as well as cognitive impairment was restored by silencing of HIF-1alpha. This study underscores the protective role of HIF-1alpha in astrocyte & ADEV mediated Alzheimer's like pathology associated with HAND.

Poster No. 85

Role of dysregulated autophagy and NLRP3 inflammasome in HIV TAT/cocaine/cART mediated activation of microglia

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Despite the long-term use of combination antiretroviral therapy (cART), persistence of low levels of HIV proteins such as Transactivator of Transcription (TAT) in the CNS has been shown to contribute to glial activation and ensuing neuroinflammation. Accumulating evidence also implicates the role of drugs of abuse in exacerbating neurological complications associated with HIV-1. The combined effects of HIV TAT, drug abuse, and cART have however not yet been explored in depth. Herein, we sought to explore the combinatorial effects of all these three agents on dysregulated autophagy and their involvement in microglial activation. We selected a combination of three commonly used cART regimens- tenofovir (TFV), emtricitabine (FTC), and dolutegravir (DTG). Our findings demonstrated that exposure of mouse primary microglia to low-doses of HIV TAT, cocaine, and cART resulted in upregulation of autophagy markers: Beclin1, microtubule-associated proteins 1A/1B light chain 3B (LC3B-II) and p62 with impaired lysosomal functioning involving increased lysosomal pH, decreased lysosomal associated membrane protein-2 (LAMP2) and cathepsin D (CTSD),ultimately leading to dysregulated autophagy. Our data also demonstrated that HIV TAT, cocaine, and cART co-operatively activated the NLR family pyrin domain containing 3 (NLRP3) inflammasome signaling as evidenced by increased expression of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), cleaved caspase 1, and mature IL1beta. We further demonstrated that gene silencing of autophagy marker BECN1 significantly blocked the NLRP3 inflammasome signaling and autophagy-mediated activation of microglia. These in vitro findings were also validated in vivo using iTat mice that were administered cocaine and cART. This study thus suggests that HIV TAT, cocaine, and cART can co-operate to exacerbate microglial activation, involving impaired autophagy and NLRP3 inflammasome activation

Poster No. 86

Unique brain-specific antibody signatures in chronic HIV infection

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Background: The brain is an important HIV reservoir. Given its difficult accessibility to quantify viral burden, HIV-specific cerebrospinal fluid (CSF)-antibodies (rather than systemic antibodies) have been suggested as critical biomarkers of HIV-disease in the brain.

Methods: We applied a Systems Serology approach to thoroughly dissect the antibody profiles (Ig (sub)classes, Fcgamma receptors [FcgammaR] binding capacity and antibody-mediated innate immunity functions) in the plasma and CSF of 20 chronically infected (11 ART-treated and 9 untreated) HIV+ individuals.

Results: High titers of HIV-specific antibodies were detected in both plasma and CSF. However, striking brain-specific antibody signatures were identified: 1) unlike plasma antibodies that were of all Ig classes, CSF showed predominantly IgG1, IgG3, and no IgM; 2) CSF-antibodies had lower capacity to activate innate immunity functions and bind FcgammaR; 3) CSF-antibodies showed a weak binding to the neonatal FcR (FcRN), which mediates the transport of circulating antibodies to and from the brain; 4) this low FcRN affinity was not observed for antibodies targeting Flu, HSV1, HSV2, CMV and EBV, suggesting a retention of HIV-specific antibodies (but not antibodies to other viruses) within the brain; 5) ART-treatment was associated with higher polyfunctionality of plasma-antibodies compared to CSF-antibodies, pointing to a reduced effect of ART in the brain.

Conclusions: These data suggest a unique compartmentalization of subpopulations of antibodies in the CNS during chronic HIV infection, either through selective antibody transfer from the periphery across the blood-brain barrier or by local production by B cells undergoing maturation to plasma cells under brain-specific selective pressure.

Poster No. 87

Third generation PacBio sequencing of HIV-1 tat from patients of the Drexel Medicine CARES Cohort: variant occurrence, co-selection, and functional effects

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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 neuroinflammation and neurocognitive impairment. Variation within Tat has been observed to affect its function and ability to induce neurotoxicity. We recently identified and characterized predominant amino acid variants within Tat that associate with

HAND. Initial studies amplified Tat exons I and II separately from patient PBMCs from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort using PCR, and amplicons were sequenced using Illumina next-generation sequencing. Statistical analyses were able to associate variants with HAND diagnoses. To examine the co-selection of amino acid variants across both Tat exons, a PCR assay was developed to amplify both exons of the Tat gene, spanning over 4 kilobases of the HIV-1 proviral genome. This method was applied to PBMC genomic DNA from donors with HIV-1, where nearly two-thirds of tested samples produced a visible target amplicon. Samples were sequenced using PacBio third generation long-read sequencing technology. Results demonstrated high coverage at all nucleotide positions and amino acid variation across the two exons of Tat. Future studies will focus on investigating co-selection of Tat residues known to be important for protein function, variants that occur in overlapping reading frames of HIV-1, and the association of Tat variation and neurocognitive impairment phenotypes in the Drexel Medicine CARES Cohort.

Poster No. 88

Methamphetamine potentiates the SARS-CoV-2 spike protein-induced dysregulation of human brain endothelial cells

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While respiratory issues are the most publicly discussed symptoms caused by SARS-CoV-2, neurological issues also develop, and their impacts may not be fully understood for years to come. Infection of human primary brain endothelial cells (HBMEC) with SARS-CoV-2 was found to decrease levels of the tight junction proteins Claudin-5 and ZO-1. These alterations indicate changes in blood brain barrier (BBB) integrity and point towards its disruption, which may allow for the virus to pass more easily through the BBB and infect the brain parenchyma. These changes in tight junctions were also found to occur following exposure of HBMEC to only the S1 subunit of the SARS-CoV-2 spike protein. Co-exposure to methamphetamine potentiated the S1-induced changes in tight junction protein expression. Mechanistically, we are focusing on exploring the impact of dysfunctional mitochondria on these effects. This data points toward the role of increased BBB permeability in SARS-CoV-2 infection in individuals who abuse drugs, such as methamphetamine.

Poster No. 89

Role of mitochondrial SIRT3 in HIV-mediated microglial senescence

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Despite the effectiveness of combined antiretroviral therapy (cART) in suppressing HIV viral replication, chronic inflammation and immunosenescence remain cardinal features underlying the pathogenesis of HIV-associated neurocognitive disorders and premature aging. The present study was aimed at exploring the role of HIV infection in mediating mitochondrial oxidative stress, leading in turn, to microglial senescence and neuroinflammation. We tested the role of mitochondria specific sirtuin-3 (SIRT3) in HIV-mediated mitochondrial stress and microglial senescence using the chimeric EcoHIV infectious model. Our findings demonstrated that infection of mouse primary microglia (mPMs) with EcoHIV resulted in a senescence-like phenotype that was characterized by the upregulation of senescence markers p16 and p21, increased senescence-associated-beta-galactosidase (SA beta-gal) positive cells and proinflammatory cytokines. Intriguingly, infected mPMs showed downregulation of mitochondrial SIRT3, a master regulator of mitochondrial antioxidants with a concomitant increase in mitochondrial oxidative stress including increased ROS that was accompanied by decreased antioxidant defenses. Over expression of SIRT3

inhibited the EcoHIV-mediated induction of senescence-phenotype in microglial cells. These findings were also validated in the frontal cortices of EcoHIV-infected mice. In summary, this study underscores the role of mitochondrial SIRT3 and oxidative stress in mediating microglial senescence and neuroinflammation in the context of HIV infection both in vitro and in vivo. These findings thus suggest the therapeutic potential of activating SIRT3 in ameliorating HIV-mediated mitochondrial oxidative stress, microglial senescence and neuroinflammation.

Poster No. 90

HIV induces AQP4 dysfunction via A2aR leading to HAND due to glymphatic failure

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The glial-lymphatic or glymphatic fluid clearance system promotes the exchange of interstitial fluid (ISF) and cerebrospinal fluid through the arterial perivascular spaces into the brain. This process is facilitated in part by aquaporin-4 (AQP4) water channels located primarily on astrocyte end feet abutting endothelial cells of the blood brain barrier. Changes in expression levels or mislocalization of AQP4 from astrocytic end feet to the soma can lead to decreased ISF flow leading to buildup of extracellular waste products like hyperphosphorylated Tau (pTau). pTau accumulation is a neuropathological hallmark in Alzheimer's disease (AD) and in some people with human immunodeficiency virus (HIV). Approximately 50% of people with HIV (PWH) suffer from HIV-associated neurocognitive disorders (HAND), which is a spectrum disorder linked to cognitive and motor decline in PWH. Limited studies have shown that in HIV CNS infection that expression levels of AQP4 in brain homogenates from the mid-frontal gyrus of PWH with symptomatic HAND were significantly increased compared to those with asymptomatic HAND, which raises the question if AQP4 function and subcellular localization may contribute to cognitive status. In addition, common single nucleotide polymorphisms in aqp4 have been associated with more rapid cognitive decline some neurodegenerative diseases. Therefore, it is possible that common mutations in aqp4, subcellular mislocalization, dysfunction, expression levels or post-translational modifications contribute to HAND. Studies in other neuroinflammatory diseases have shown dysregulation of AQP4 through the adenosine A2aR (A2aR) signaling. A2aR activation leads to PKA/PKC-mediated inhibitory phosphorylation of AQP4 (Ser180, Ser276) that is proposed to contribute to channel internalization, mislocalization and decreased expression. In fact, Our overall hypothesis is that in PWH, changes in AQP4 may contribute, in part, to HAND by decreasing clearance of toxic aberrant proteins and HIV mechanistically alters AQP4 in part via dysregulation of A2aR.

Poster No. 91

Neuronal Cell Models of Epstein-Barr Virus Infection: Disruption of Autophagy in Neuroblastoma Cells

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Neurodegenerative diseases (ND's) affect approximately 6 million people in the US, and their incidence and severity are linked to genetic and environmental factors. Human herpesviruses such as Epstein-Barr virus (EBV), were recently implicated as potential infectious agents in the etiology of ND's. Over 90% of the world's population has EBV, which initially infects epithelial cells in the nasopharynx to then enter latency in B cells. EBV is a neurotropic virus and can infect astrocytes, neurons, and microglia. In addition, infected B and T cells travel through CNS access areas such as the Glymphatic System and the nasopharynx, where EBV could affect neuronal cell function. Processes such as autophagy and Reactive Oxygen Species (ROS) homeostasis are crucial to ND's and have already been shown to be affected by EBV. While severe neuronal consequences are rare during EBV infection, little is known about its ability to promote neuronal dysfunction that could prime neuronal cells for ND development. We hypothesize that EBV has the

potential to affect neuronal cellular processes that are relevant for the development and establishment of ND's. To study these interactions in a practical, more easily accessible cell model than primary neuronal cells, we use retinoic-acid differentiated SH-SY5Y neuroblastoma cells, which have been widely accepted as a suitable cell line for ND studies. We exposed these cells to EBV virions and can demonstrate the presence of intracellular viral genome via qPCR. Interestingly, while there is no indication that the virus establishes lytic or latent transcriptional programs in these cells, autophagy appears to be affected. Our data supports that EBV is obstructing the autophagic flux, despite the absence of viral transcripts which suggests that viral entry alone may be sufficient to trigger cellular dysfunction. Future studies will focus on ROS and mitochondrial function, which are also crucial cellular functions for ND's.

Poster No. 92

Pathway of SARS-Cov-2 invasion of the brain and implications for HIV-1 infection

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Background. Neurological complications are common in patients affected by COVID-19 due to SARS-CoV-2 infection of the central nervous system (CNS). While the mechanisms of this process are not fully understood, it has been proposed that SARS-CoV-2 can infect the cells of the neurovascular unit (NVU), which form the blood-brain barrier (BBB). Our aim was to analyze the expression pattern of the main SARS-CoV-2 receptors in naïve and HIV-1-infected NVU cells to elucidate a possible pathway of viral entry into the brain and a potential modulatory impact of HIV-1.

Methods. The gene and protein expression profile of ACE2, TMPRSS2, ADAM17, BSG, DPP4, AGTR2, ANPEP, cathepsin B, and cathepsin L was assessed in naïve or HIV-1 infected primary human brain endothelial cells, pericytes, astrocytes and immortalized human microglia cells by qPCR and immunoblotting, respectively.

Results. The receptors involved in SARS-CoV-2 infection are co-expressed in the cells of the NVU, especially in astrocytes and microglial cells. Additionally, HIV-1 infection upregulated ACE2 and TMPRSS2 expression in brain astrocytes and microglia cells.

Conclusions. These findings provide key insight into SARS-CoV-2 infection of the CNS and may help to develop possible treatments for neurological complications of COVID-19.

Poster No. 93

HIV downregulates beta-catenin in colonic epithelial cells: Implications for gut-brain axis in neuroHIV

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HIV-associated neurocognitive disorders (HAND) persist among people living with HIV (PLWH) despite the efficacy of combination antiretroviral therapy (cART) in controlling viral load. Persistent neuroinflammation is also observed in era of cART, although the mechanisms driving this inflammation in the CNS are not entirely clear. We found that plasma levels of an antagonist of the Wnt/beta-catenin pathway (DKK-1) is associated with higher prevalence of neurocognitive impairment among PLWH. To understand this observation we are studying whether disruption of Wnt/beta-catenin signaling pathway in gut epithelial cells leads to microbial product translocation into the brain where disruption of Wnt/beta-catenin signaling in astrocytes compromises neuronal health. Preliminary data from our group shows that (in primary human astrocytes) TLR 3 and 9 agonists, poly I:C and CpG, cause a 70% reduction in beta-catenin. Downregulation of Wnt/ beta -catenin in astrocytes impairs their ability to support neuronal health and leads to astrocyte senescence. Using a humanized mouse model of HIV, we show that HIV infection

leads to downregulation of beta -catenin in proximal colonic epithelial cells ($p<0.05$), loss of tight junction proteins ($p<0.05$), and functional leakage from the gut ($p<0.05$) in comparison to uninfected animals. Together, these preliminary studies suggest crosstalk between the brain and the gut through disruption of Wnt/ beta -catenin signaling that may contribute to neuroinflammation even under cART.

Poster No. 94

cART induces mitochondrial dysfunction in monocyte derived macrophages

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People living with HIV (PLWH) are affected by comorbid conditions including HIV-associated neurocognitive disorders (HAND). Combination antiretroviral therapy (cART) itself has been implicated in the development of several co-morbid conditions including neuronal toxicity. We assessed the effect of cART on monocyte-derived macrophages (MDMs) because they are a prominent cell type in neuroHIV pathogenesis. Primary human monocytes were differentiated into alternative and inflammatory phenotypes in the presence of either Atripla, consisting of: the nucleoside reverse-transcriptase inhibitors (NRTI) emtricitabine (FTC) and tenofovir disoproxil fumerate (TDF), and the non-nucleoside reverse-transcriptase inhibitor (NNRTI) efavirenz (EFV), or Triumeq consisting of: the NRTIs lamivudine (3TC) and abacavir (ABC) and the integrase inhibitor dolutegravir (DTG) at clinical doses. Triumeq but not Atripla caused cell cycle arrest in G2/M phase. Both drugs doubled the production of reactive oxygen species (ROS) and induced mitochondrial dysfunction as determined by Seahorse mito-stress test. Additionally, Triumeq increased TNFalpha, IL-6 and IL-1beta secretion by approximately 50% and doubled secreted IFNgamma. Finally, RNAseq analysis demonstrated that both drugs induced significant changes in genes involved in immune regulation, cell cycle and DNA damage. Overall, our data demonstrates that cART, independent of HIV, has significant impact on MDMs which may be linked to cART-mediated dysregulation of MDMs that contributes to persistent inflammation and comorbidities in the era of cART.

Poster No. 95

Alterations in peripheral and central nociceptive signaling circuits during HIV/SIV infection contribute to the development of HIV-associated distal sensory polyneuropathy

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With the development of combined anti-retroviral therapy (ART), human immunodeficiency virus (HIV)-associated comorbidities have remained prevalent, including HIV-associated distal sensory polyneuropathy (HIV-DSP). Our previous studies have identified that atrophy in nociceptive neurons is associated with increased inflammatory monocyte traffic to the dorsal root ganglia (DRG) during simian immunodeficiency virus (SIV) infection; however, the sensory signaling mechanism connecting this pathology to symptomology remains unclear. Our preliminary data show a significant increase ($P \leq 0.01$) in expression of the nociceptive ion channels transient receptor potential vanilloid (TRPV) and anykrin (TRPA) in the DRGs of SIV-infected ART treated rhesus macaques compared to SIV-infected ART-naïve group. Both nociceptor sensitization in the DRG and central nervous system inflammation can contribute to the development of chronic neuropathy through the release brain-derived neurotrophic factor (BDNF). Our data identifies a significant upregulation of the precursor form of BDNF, proBDNF, ($P \leq 0.01$) within the DRGs of ART treated macaques. Within the same tissue we did not observe any associated increase in the cleaved mature form, mBDNF, indicating a shift in the overall processing of BDNF in the DRGs of SIV-infected ART treated animals. Alterations in the DRG proBDNF/mBDNF ratio and increased BDNF release from peripheral sensory neurons as well as activated spinal microglia can contribute to neuropathic pain. New evidence suggests spinal microglia and resident macrophages are a viral reservoir in ART-treated macaques where SIV-associated changes are marked by neuroinflammation and glial cell activation. We

have found no significant difference in CD68 and CD3 positive immunoreactivity in the lumbar spinal cord of SIV-infected macaques when compared to SIV-infected ART treated animals. This indicates spinal neuroinflammation persists despite ART where activated glia and SIV-infected mononuclear derivatives (spinal microglia/tissue macrophages) are likely to contribute to the mechanism of chronic pain in HIV-DSP.

Poster No. 96

The impact of EcoHIV in cocaine seeking behaviors

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Background: Substance use disorders (SUDs) are characterized by high propensity to relapse and are highly comorbid with HIV infection. The underlying neurobiology of this relationship is poorly understood. Preclinical research on the neurobiobehavioral outcomes of progressive HIV infections may yield crucial information to improve SUD prognosis and reduce risk of relapse in people living with HIV.

Methods: To model progressive HIV, adult male and female wildtype C57BL6J mice were inoculated with the EcoHIV, a chimeric HIV-1 which infects murine cells by replacing envelope glycoprotein gp120 with gp80. Seven weeks after inoculation mice were trained in a cocaine CPP paradigm in which one context was associated with cocaine reward (10 mg/kg, i.p.) followed by extinction training. Reinstatement of preference was assessed following administration of yohimbine, an alpha-2 adrenergic antagonist, to determine EcoHIV effects in a model of stress-induced relapse.

Results: EcoHIV and sham-inoculated mice exhibited similar cocaine CPP and extinction. However, EcoHIV mice showed significantly increased yohimbine-induced reinstatement ($p<0.05$).

Conclusion: HIV infected mice escalated stress-induced reinstatement of cocaine seeking compared to controls mice, which suggested that people living with HIV may be at elevated risk for stress-induced relapse. Ongoing research is characterizing neural activation patterns associated with cocaine exposure in EcoHIV-inoculated mice.

Poster No. 97

Intranasal Insulin Improves Attention and Memory in Virally-Suppressed People with HIV

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Despite suppression of HIV replication with antiretroviral therapies (ART) cognitive impairment (CI) remains prevalent in virally suppressed people with HIV (VS-PWH). Although the precise mechanisms for this residual CI are not fully understood, there is considerable evidence that brain energy metabolism is progressively impaired in VS-PWH. In a randomized, double-blind, placebo-controlled study, 21 non-diabetic VS-PWH with mild-to-moderate CI were randomized to receive intranasal insulin (INI;20IU/day/nare) or placebo. Participants completed standardized neuropsychological (NP) tests at baseline, 12, and 24 weeks. A mixed effects regression of global deficit score (GDS) over time with cross-product between INI and time demonstrated a significant treatment group effect (p=.029) with improvements on GDS in the INI group at 12 and 24-weeks compared with placebo. Improvements on individual NP tests were apparent on measures of verbal memory (HVLT-R delayed free recall; p=.028) between baseline and 24-weeks, on visual memory (Rey delayed recall; p=.002) between baseline and 12 weeks, and attention (Trail Making Test-Part A; p=.006) between baseline and 24-weeks in the INI group

compared to placebo. There were no group-differences in plasma cytokines. Partial least-squares discriminate analysis modeling of plasma metabolomics showed clear a group separation between INI and placebo that was largely driven by decreases in arachidonic acid, sphingolipid and phospholipid metabolism and increases in bioenergetic and antioxidant defences in INI compared with placebo. In the INI group there were correlations between MRS and NP performance for tCho and the Rey-complex-figure (p=.049), and Glu and Hopkins-Verbal-Learning-Test (p=.025) using a mixed effect regression. The findings from this pilot study warrant further investigation of intranasal insulin as a cognitive enhancer in VS-PWH.

Poster No. 98

Targeting Positive Allosteric Modulators of CB1 Receptor as a Potential Target for HAND

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The HIV-1 transactivator of transcription (Tat) is one of the viral proteins and a neurotoxin that plays a major role in the pathogenesis of HIV-associated neurocognitive disorders (HAND). As HAND is a group of neurodegenerative cognitive disorders with an inflammatory component, the endocannabinoid (eCB) system, which regulates both cognition and immune function, presents a promising therapeutic target for treating HAND. Alternative targets, e.g. allosteric modulators, of the endocannabinoid system are explored due to the side effects associated with direct activation of cannabinoid receptors. One such CB1R positive allosteric modulator (PAM) we explored in our study was ZCZ011. Tat neurotoxicity and the effects of ZCZ011 was assessed via live cell calcium imaging and results indicated that ZCZ011 acted as a PAM and enhanced the effects of AEA. Surprisingly, ZCZ011 when tested alone was found to downregulate the intracellular calcium ([Ca2+]i) and had neuroprotective effects at higher concentrations against Tat. This can be attributed to the fact that ZCZ011 has a chiral center and therefore exists in two conformations and one of the isomers might have an agonist and PAM-like effect. We also investigated [Ca2+]i responses to microglia conditioned media (MCM) derived from microglia pretreated with ZCZ011, AEA, and/or Tat. Application of MCM Tat onto neurons caused a significant increase in [Ca2+]i and this effect was attenuated when MCM treated with ZCZ011 or AEA was applied to the neurons. However, ZCZ011 in the presence of AEA did not show any potentiation of neuroprotective effects of AEA and this may partly be due to the receptor differences on neuron and microglia where CB2 receptors are found abundantly on the later. Further experiments using MCM pretreated with CB1 and CB2 antagonists along with studies on postsynaptic density are underway to fully decipher the mechanism and effects of ZCZ011.

Poster No. 99

Nef-carrying extracellular vesicles impair mitochondrial metabolism: evidence for Nef-mediated neurotoxicity

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HIV-associated CNS dysfunction is a significant problem among people with HIV (PWH), who now live longer due to combined anti-retroviral therapy (cART). Over the course of infection, HIV generates toxic viral proteins and induces inflammatory cytokines that have toxic effects on neurons in the CNS. Among these viral proteins, HIV Nef is expressed early in progression of the infection released from infected cells

in association with extracellular vesicles (EVs). However, the impact of Nef on neuronal cell homeostasis are still elusive. Here, we show that Nef is expressed in postmortem brain specimens from PWH despite ART treatment. Our results suggest that Nef is released in extracellular vesicles isolated from HIV-1 infected or Nef expressing glial cells. Our further analysis shows that exposing primary human neurons to Nef carrying EVs lead to Nef uptake by neurons. More interestingly, a significant amount of Nef is enriched in neuronal mitochondrial fractions leading to induction of oxidative stress, increase in ROS and dysregulation of neuronal homeostasis. Altogether, our results suggest that Nef is released in extracellular vesicles from HIV-1 infected or Nef expressing glial cells that mediates the neuronal uptake of Nef, leading to neurodegeneration by inducing mitochondrial dysfunction. Overall, this study highlights the potential contribution of HIV-1 Nef protein in the development of HIV-associated CNS diseases seen in PWH.

Poster No. 100

Inhibition of Neutral Sphingomyelinase 2 disrupts the late stages of HIV biogenesis

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The binding of Gag to the inner leaflet of plasma membranes actively promotes the formation/stabilization of membrane microdomains required for the early events of HIV assembly/budding. However, the mechanisms by which Gag regulates the formation and/or stabilization of these microdomains is unknown. Here we provide the first evidence that Gag co-localizes with nSMase2; a sphingomyelin hydrolase that generates ceramide which has the biophysical property of creating membrane curvature. Pharmacological inhibition (with PDDC) or genetic deletion of nSMase2 impaired the processing of Gag and resulted in the production of non-infectious virions with apparent defects in virion maturation. This assembly/budding deficit was associated with the death of HIV infected cells by mechanisms involving perturbations of lysosomal biogenesis. PDDC treatment of HIV infected humanized NSG-mice produced a linear reduction in plasma viral loads that paralleled treatment with ARVs. Viral loads fell below detectable limits in 6 of the 8 mice treated with PDDC and 6 of 8 mice treated with ARVs within 10 weeks of drug treatment. Following drug withdrawal there was no viral rebound in PDDC treated mice who achieved viral loads below detectable limits, while 7 of 8 ARV treated mice showed viral rebound. The lack of viral rebound in PDDC treated mice was associated with a reduction in human CD14+ cells that we interpret as a selective killing of HIV infected cells by PDDC. These findings identify nSMase2 as a critical regulator of HIV biogenesis and as a potential therapeutic target.

Poster No. 101

Modeling the effects of dolutegravir on early cortical development using human forebrain organoids

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Human induced pluripotent stem cells (iPSCs) provide a renewable resource to generate cell types of interest, including neural cells, for systematic investigation. Under certain culture conditions, iPSCs self-organize to form 3D structures reminiscent of the developing fetal cortex. This provides an opportunity to

interrogate the effect of drugs and other potential pertubagens on cortical development. Currently, we have limited data on how many therapeutic drugs, including antiretroviral drugs, may impact the developing brain due to the inaccessibility of the tissue and historical constraints on the inclusion of pregnant women in clinical trials. Recent reports from an observational study in Botswana suggested that there may be slight increase in the risk for neural tube defects following dolutegravir exposure at the time of conception. To investigate the impact of dolutegravir on human neural stem cells, we measured the effects of daily drug treatment on the early stages of cortical organoid formation. We observed dose-dependent defects in organoid structure and impaired neurogenesis in dolutegravir-treated organoids, compared to vehicle-treated control organoids. Further analyses and RNA-sequencing results suggest the involvement of the integrated stress response in the dolutegravir-treated organoids and we could partially rescue the deficits with a small molecule ISR inhibitor (ISRIB). Together, these results illustrate the potential for iPSC-based strategies to reveal biological processes that may be affected by drugs during neural development and provide complementary data in relevant human cell types to augment preclinical investigations of drug safety.

Poster No. 102

Long-term HIV-1 Tat expression in the brain led to neurobehavioral, pathological, and epigenetic changes reminiscent of accelerated aging

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HIV infects the central nervous system and causes HIV/neuroAIDS, which is predominantly manifested in the form of mild cognitive and motor disorder in the era of combination antiretroviral therapy. HIV Tat protein is known to be a major pathogenic factor for HIV/neuroAIDS through a myriad of direct and indirect mechanisms. However, most, if not all of studies involve short-time exposure of recombinant Tat protein in vitro or short-term Tat expression in vivo. In this study, we took advantage of the doxycycline-inducible brain-specific HIV-1 Tat transgenic mouse model, fed the animals for 12 months, and assessed behavioral, pathological, and epigenetic changes in these mice. Long-term Tat expression led to poorer short-and long-term memory, lower locomotor activity and impaired coordination and balance ability, increased astrocyte activation and compromised neuronal integrity, and decreased global genomic DNA methylation. There were sex- and brain region-dependent differences in behaviors, pathologies, and epigenetic changes resulting from long-term Tat expression. All these changes are reminiscent of accelerated ageing, raising the possibility that HIV Tat contributes, at least in part, to HIV infection-associated accelerated ageing in HIV-infected individuals. These findings also suggest another utility of this model for HIV infection-associated accelerated ageing studies.

Poster No. 103

The astrocyte citrate carrier, SLC25A1 protects neurons from toxic peroxidated fatty acids generated by cocaine and HIV

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The brain consumes ~ 20% of the body's energy and disturbances in lipid and fatty acid (FA) metabolism in the CNS contribute to neurodegeneration and cognitive impairments. Through direct metabolic coupling interactions, astrocytes provide energetic support to neurons by delivering lactate and cholesterol and by taking up and processing peroxidated fatty acids released from neurons during stress. Disruption of lipid homeostasis in the central nervous system as observed in several neurodegenerative disorders including

HIV infection and cocaine use contributes to neurocognitive impairment, addition and may lead to accelerated cognitive decline. Oxidative metabolism is the main pathway by which the brain acquires energy, however data from numerous studies report a switch to beta-oxidation of fatty acids (FAs) in response to cocaine use and HIV infection. Tight metabolic coupling between astrocytes and neurons is required to maintain metabolic homeostasis and increased levels of ROS in the brain induces peroxidation of FA in neurons. Neurons are highly sensitive to toxic peroxidated FA (pFA) and unlike astrocytes, neuronal mitochondria are unable to efficiently consume FAs as an energy source. Astrocytes endocytose the particles for mitochondrial beta-oxidation. To assess how cocaine use by people with chronic HIV infection disrupts pathways important in astrocyte/neuron FA metabolic coupling leading to impaired lipid homeostasis in the brain, we determined mechanisms by which astrocytes processed peroxidated FA taken up from neurons and maintain their neurotrophic phenotype in the presence of cocaine and the HIV protein Tat. Importantly, our data uncovered that the astrocyte citrate carrier, SLC25A1 protects neurons from accumulation of neurotoxic peroxidated fatty acids generated by cocaine and HIV. Moreover, disruption of SLC25A1 blocks astrocytes' neurotrophic capacity in the presence of cocaine and HIV, Tat.