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P1

Evaluating multiplex gRNA treatment to inactivate LTR-mediated transcription

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HIV-1 persistence is a major hurdle to a cure. Genomic editing with the CRISPR/Cas9 system holds promise to permanently excise or inactivate integrated provirus. Broad spectrum gRNAs were designed by isolating patient PBMCs, deep sequencing their LTRs, and the most effective gRNAs were selected using a bioinformatic algorithmic pipeline. This resulted in the development of a broad-spectrum gRNA designated SMRT1. SMRT1 demonstrated knock-down of HIV-1 LTR-driven transcription in a transient transfection system. This resulted in a residual amount of LTR-driven transcription that was postulated to be due to a lack of delivery to all cells. To further elucidate the nature of the residual LTR-driven transcription, a novel dual florescence system was designed that used the NL4-3 HIV-1 molecular clone that also encoded GFP while the Cas9 expression system also encoded RFP and the anti-HIV-1 SMRT1 gRNA. Using these two plasmids it was shown that when the Cas9 system was active, there was at least a 98 percent reduction in GFP expression. Furthermore, when a VSV-G pseudotyped NL4-3 GFP was used and Cas9 (RFP) was delivered to cells, there was again an extensive reduction in GFP expression. Using a highly sensitive beta-galactosidase system, when multiple gRNAs were used at least a 97 percent reduction in LTR-driven gene expression was observed. To model a latently infected T cell, the J-Lat 10.6 cell line was used. The J-Lat 10.6 cells were transduced with a lentiviral vector that encodes for Cas9 and a gRNA from the same vector. This experiment showed that T cells which received SMRT1, or a gRNA targeting the viral protein Tat, were the most effective at stopping cells from reactivating from latency. These studies represent a step towards understanding the complex task of using CRISPR/Cas9 for HIV-1-targeted excision/inactivation therapy.

P2

Integrase Inhibitor Exposure is Linked to Neurocognitive Deficits in HIV

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Neurocognitive deficits continue to persist amongst people living with HIV (PLWH), despite availability of modern antiretroviral therapy (ART) in many developed countries. We recently observed (Gomez et al., 2018) that among the most important factors linked to low neurocognitive performance was high CNS-penetrance of ART regimens (CPE-index). CPE-scores can be problematic, e.g., newer ART medications are not included, and do not represent ART exposure over time. We tested here whether the cumulative exposure to the major classes of ARTs were predictive of neurocognitive functions in HIV, along with known factors associated with neurocognitive functions (age, education, sex, CD4 T-cell count, viral suppression, physical and mental health comorbidities). In addition, we tested the additional effects of prescribed non-ART medications with known neurocognitively adverse effects (NC-AEs, Radtke et al., 2018) on cognition in this cohort. The cohort (N=314) was recruited from the Southern Alberta HIV Clinic and most were actively prescribed ART-medications. Latent Profile Analysis (LPA) identified three neurocognitive profiles, one with neurocognitive impairment (N=45). Machine learning identified the most important factors that characterized each profile. While multiple ART classes were predictors of neurocognitive impairment, longer duration of integrase inhibitor exposure was among the most important predictors, followed by NC-AEs, even when taking age and HIV duration into account. Comparing different integrase inhibitors, individuals with past or present dolutegravir prescriptions demonstrated lower performance in attention, memory and motor functions than individuals with raltegravir and elvitegravir prescriptions. Although this epidemiological study cannot speak to causality, longer duration of ART exposure, in particular exposure to integrase inhibitors, as well as NC-AEs may negatively impact cognition in PLWH. The mechanisms through which integrase inhibitors, in particular dolutegravir, may impact CNS functions in HIV remain to tested.

P3

High rate of HIV p24 detectability in CSF with single molecule digital ELISA despite suppressive antiretroviral therapy

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Background: The central nervous system (CNS) is a reservoir of HIV persistence despite antiretroviral therapy (ART). In a previous study, we showed that HIV p24 antigen is quantifiable in CSF with digital ELISA. This previous study included mostly individuals off ART, and demonstrated that HIV p24 levels correlated with higher CSF HIV RNA and poorer neuropsychological (NP) performance. In the present study, we investigated an independent cohort of persons with HIV (PWH), the majority of whom were on suppressive ART.
Methods: 22 PWH enrolled in the study at the Emory Center for AIDS Research. This included a nine-test NP panel that generated a global deficit score (GDS). A GDS value ≤ 0.5 defined neurocognitive impairment. CSF HIV RNA levels were measured by PCR, CSF neurofilament

light chain (NFL) was measured by standard ELISA, while CSF p24 was measured with the digital ELISA platform.
Results: 22 HIV+ adults were analyzed. Thirteen (59%) were on ART for at least six months with plasma and CSF HIV RNA < 200 copies/ml, while the remainder were unsuppressed. All 13 of the suppressed participants had detectable CSF p24 concentrations that were below the lower limit of quantification. Participants with quantifiable CSF p24 were significantly more likely to have non-suppressed CSF HIV RNA ($p<0.001$) and to be impaired by GDS ($p=0.047$). The participant with the highest CSF p24 (20.6 pg/ml) had by far the highest NFL (81392 pg/ml versus median= 703 among total group) and highest GDS (3.0 versus median= 0.17 among total group).
Conclusions: This small study confirms prior findings that higher CSF p24 is associated with worse cognition. The fact that CSF p24 was detectable despite suppressive ART suggests that when measured by digital ELISA, this protein may be a novel marker of HIV CNS persistence that could be used in eradication strategies.

P4

MicroRNA regulation of Zika virus Infection

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Zika virus (ZIKV) infections have caused a wide spectrum of neurological diseases, such as Guillain-Barré syndrome, myelitis, meningoencephalitis, and congenital microcephaly. No effective therapies currently exist for treating patients infected with ZIKV. MicroRNAs (miRNAs) are a group of small RNAs involved in the regulation of a wide variety of cellular and physiological processes. To identify differentially expressed miRNAs associated with ZIKV infection, we employed nCounter© Technology (NanoString) to analyze global miRNAs expression in ZIKV-infected primary mouse cortical neurons. A total of 599 miRNAs and 770 mRNAs were examined. Our data demonstrate that ZIKV infection causes global downregulation of miRNAs with only few upregulated miRNAs. ZIKV-modulated miRNAs including miR-155, miR-203, miR-29a, and miR-124-3p are known to play critical role in flavivirus infection, anti-viral immunity and brain injury. ZIKV infection also results in downregulation of miRNA processing enzymes including Dicer-1, Drosha, DGCR8, AGO1 and AGO2 in neurons. In contrast, ZIKV infection induces dramatic upregulation of anti-viral, inflammatory and apoptotic genes. Furthermore, our data demonstrate an inverse correlation between ZIKV-modulated miRNAs and target host mRNAs induced by ZIKV. Biofunctional analysis revealed that ZIKV-modulated miRNAs and mRNAs regulate the pathways related to neurological development and neuroinflammatory responses. Moreover, functional studies targeting specific miRNA revealed a critical role of miR-155 in regulating ZIKV replication. Collectively, these data suggest that miRNAs regulate downstream gene expression, important in ZIKV disease pathogenesis, and can be targeted in the future to develop therapeutics for the management of ZIKV neurological disease.

P5

Unbiased detection of CRISPR/Cas9 cleavage to validate in silico specificity predictions for gRNAs targeting HIV-1 proviral DNA sequences

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HIV-1 persistence has been attributed to integrated proviral DNA in latent tissue reservoirs including the peripheral blood, lymphoid tissue, brain, and gut. Recent studies have removed the integrated HIV-1 provirus from individual cells and HIV-1-infected humanized mice using the CRISPR/Cas9 system. One of the challenges of a CRISPR/Cas9-based treatment has been evaluation of the specificity of the therapy across a wide range of patients. A successful excision-based therapy would require a set of gRNAs which recognize all viral quasispecies while minimizing off-target cleavage. In silico predictions indicate that gRNAs can be designed to fulfill both criteria. The current study validated these predictions. A high sensitivity DNA cleavage assay was used to measure the cleavage efficiency of gRNAs. Results showed a strong correlation between in silico predictions and in vitro performance for the set of broad-spectrum gRNAs tested. Validation of off-target efficiency predictions was performed by adapting the genome-wide unbiased identification of DSBs enabled by sequencing (GUIDE-Seq) method with next-generation sequencing. The GUIDE-Seq assay has been shown to identify the sequence locations of double-strand breaks (DSBs) in living cells including those generated by CRISPR/Cas9. This enabled high throughput quantification of off-target CRISPR/Cas9 activity with an increase in depth of coverage compared to the original GUIDE-Seq method in order to validate in silico predictions of gRNA specificity by detection of off-target cleavage events. Preliminary results indicate that the broad-spectrum gRNAs had high specificity with respect to HIV-1 with no detectable off-target hits.

P6

Morphine and Antiretroviral Therapy Impact Macrophage Functions and Macroautophagy: Implications for HIV Neuropathogenesis

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HIV enters the CNS often before an individual initiates antiretroviral therapy (ART). Infection of CNS macrophages causes release of host factors, viral proteins, and inflammatory mediators that induce neuronal damage and cognitive dysfunction termed HIV-associated neurocognitive disorders (HAND) in approximately half of people living with HIV (PLWH) despite ART. Opioids can exacerbate cognitive dysfunction in PLWH, and may contribute to HIV neuropathogenesis by affecting macrophage homeostasis. We are characterizing the impacts of morphine, an opioid, and a common ART regimen on cellular functions of primary human monocyte derived macrophages (MDM) that are dysregulated in HAND. We demonstrated that morphine and ART increase baseline and LPS-mediated phagocytosis of E. coli in uninfected MDM and may also increase latex bead uptake. We are also examining the impact of morphine and ART on secretion of cytokines relevant to HAND pathogenesis, including CCL2 and IL-1beta. Our preliminary data suggest that morphine and ART upregulate these cytokines in uninfected MDM. Dysregulation of these macrophage functions by morphine and ART may be related to

changes in macroautophagy, a quality control degradative process that regulates oxidative stress, phagocytosis, and cytokine production. Thus, we are studying the impact of ART and morphine on macroautophagy in MDM using Western bloting for LC3II, a key protein present on autophagosomes, and p62, an important cargo receptor that mediates selective autophagic processes. Our preliminary data indicate that morphine and ART together increase the rate of total autophagosome degradation. Interestingly, our data also suggest that morphine increases p62 levels by selectively inhibiting its degradation within autophagosomes. Increased p62 may contribute to changes in toll-like receptor signaling and inflammasome activation, increasing neuroinflammation and contributing to HAND. We will characterize further the molecular mechanisms underlying opioid mediated HIV neuropathogenesis with the goal of developing therapies that alter macroautophagy in CNS macrophages to treat HAND.

P7

TCF-4 is a Robust Inhibitor of HIV Transcription in CD4+ T cells: Implications for HIV latency

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TCF-4 is a downstream effector of beta-catenin, a transcriptional regulator that is highly expressed in astrocytes. Beta-catenin targets HIV for transcriptional repression by coupling with TCF-4, which binds to the HIV LTR. This beta-catenin/TCF-4 complex then associates with nuclear matrix binding protein SMAR to pull integrated HIV DNA into the nuclear matrix and away from transcriptional machinery. Astrocytes normally support a low level of productive HIV infection, however, inflammatory signals that inhibit this beta-catenin pathway result in higher levels of HIV replication, implicating an important role for the beta-catenin/TCF-4 pathway in HIV infection of the CNS. However, the relevance of this pathway is not known for CD4+ T cells, the main target of HIV-1 replication, which have lower levels of endogenous beta-catenin. In this study, we investigate the importance of TCF-4 for HIV transcriptional regulation in primary CD4+ T cells. Using an LTR reporter plasmid deficient in one or two TCF-4 binding sites in primary CD4+ T cells, we found a 4-fold increase in transcriptional activity when a single TCF-4 binding site was deleted and a 6-fold increase when both were deleted. To further investigate the impact of beta-catenin/TCF-4 transcriptional regulation on CD4+ T cell infection, we generated full-length HIV molecular clones with a mutated TCF-4 binding site. Infection of CD4+ T cells with the mutated TCF-4 binding site HIV strain resulted in 10-fold higher production of HIV transcripts gag and pol. Thus, TCF-4 binding of the HIV LTR is an important regulator of HIV transcription in CD4+ T cells despite relatively low expression of beta-catenin, suggesting that TCF-4 may partner with other transcriptional co-activators to regulate HIV transcription in CD4+ T cells. Ongoing studies are defining the transcriptional partners of TCF-4 and the relevance of this pathway in HIV latency in CD4+ T cells.

P8

Sleep disturbances in EcoHIV-infected mice

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Background: In patients living with HIV infection, the prevalence of insomnia and other sleep disturbance is nearly 2.5 times higher than healthy controls and affects nearly 70% of this population. The importance of sleep in healthy cognition has been well-established, and its disruption may contribute to neurocognitive deficits observed in infected individuals. Additionally, both HIV infection and sleep have established bidirectional relationships with neurodegenerative diseases of aging, which represent a rising affliction in these patients. This connection presents a novel opportunity for pharmacological intervention– we may ameliorate HIV-associated sleep disturbances by treating the disease itself, or improve neurocognitive function in these patients by treating the sleep disruption. In order to assay the efficacy of novel therapeutics and treatment modalities, we assessed the sleep phenotype exhibited in the EcoHIV mouse model of infection. Results: By multi-day polysomnography recordings of electroencephalography (EEG) and electromyography (EMG), we examined the uninterrupted sleep-wake patterns of EcoHIV infected mice, and uninfected control littermates. Across the entire 24-hour period, and particularly during their daytime period of deep sleep, mice infected with EcoHIV exhibited more wakefulness and less consolidated sleep than their healthy counterparts. This effect manifested in more frequent arousals, shorter sleep bouts, and decreased slow-wave power. Additionally, the amount of rapid eye movement (REM) sleep was significantly decreased. Conclusions: Similarly to people with HIV infection, the EcoHIV mouse model exhibited sleep disturbances suggestive of insomnia. These data suggest that this model carries the disease-relevant sleep phenotype, and can be used to trial possible therapeutics. Additionally, we will use established sleep-restorative drugs to determine if improved sleep can slow the progression of HIV-associated neurocognitive consequences.

P9

Inhibition of HSV-1 replication in vitro and in vivo by a gene editing strategy

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Herpes Simplex Virus Type 1 (HSV-1) is a human neurotropic virus that infects the majority of the human population worldwide. Following initial infection, HSV-1 establishes a life-long latent infection within the peripheral nerve ganglia. The mechanism determining latency and reactivation has not been fully elucidated. Various environmental factors can reactivate the virus to replicate and lead to disease progression. While our understanding of the biology of HSV-1 and its mode of regulation has been considerably enhanced during the past decade, progress in developing therapeutic strategies towards treatment of HSV-1 associated disease complication remains an unmet public health issue. Current available treatments for HSV-1 primary infection or reactivation are not specific, do not prevent establishment of latent infection or viral reactivation, and have known adverse side effects. Accordingly, we have customized the recently developed gene editing platform, CRISPR/Cas9, to specifically target the HSV-1 genome with the purpose of making indel mutations or removing large segments of the viral DNA sequences which are important for viral replication. Our preliminary results indicate that targeting essential genes of HSV-1 ICP0 and ICP27 drastically decreases their expression levels, leading to suppression of HSV-1 infection. The specificity of our gene mutation/ablation within the HSV-1 genome has been verified by genetic analysis in an in vitro cell culture model. Furthermore, expression of HSV-1 directed Cas9/gRNAs in cells protected them against HSV-1 infection. Finally, our preliminary results using an in vivo approach in a C57BL/6J mouse model suggest a possible reduction of HSV-1 related lesion in mice injected intraperitoneal and

intravenous with CRISPR/Cas9-AAV2. In conclusion, our CRISPR/Cas9 approach may be used to develop a novel, specific and efficient therapeutic platform to target the viral genome to treat HSV-1 associated complications. This work was funded by Excision Biotherapeutics.

P10

CRISPR/Cas9 system as an agent for inhibition of Polyomavirus JC infection

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease of the CNS caused by lytic infection of oligodendrocytes with human polyomavirus JC (JCV). PML lesions are areas of demyelination containing oligodendrocytes with viral nuclear inclusion bodies and bizarre astrocytes, which are also productively infected by JCV. Although JCV was isolated over forty years ago and has been extensively studied, there is still no effective therapy for PML. Recently, a novel genome editing system was developed based on clustered regularly interspaced short palindromic repeat (CRISPR) systems. The CRISPR system uses a nuclease, CRISPR-associated (Cas9), that complexes with small RNAs as guides (gRNAs) to cleave DNA in a sequence-specific manner upstream of the protospacer adaptor motif (PAM) in any genomic location. Here, we employ gene editing strategy, CRISPR/Cas9, to introduce mutations in the viral genome and by inactivating the gene encoding T-antigen, NCCR and VP-1, inhibit viral replication. Our bioinformatics screening identified several potential targets within JCV T-antigen, NCCR and VP-1 that can serve as sites for the creation of guide RNAs (gRNAs) for guiding the Cas9 on the designed area of the viral genome for editing. Results from AAV9 mediated delivery of CRISPR/Cas9 constructs in SVGA cells containing transiently transfected pBluescript Mad-1 plasmid indicate CRISPR/Cas9 specifically targets the DNA sequence corresponding to T-antigen, NCCR and VP-1, therefore suppressing viral replication. These observations provide evidence for the use of gene editing strategy as a promising tool for the eradication of the JCV genome and a possible cure for PML. This work was funded by Excision Biotherapeutics.

P11

Interplay between the DNA damage response and NF-KB signaling regulates JCV replication

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Immune dysfunction plays a critical role in the development of the lethal CNS demyelinating disease progressive multifocal leukoencephalopathy (PML), which results from infection of oligodendrocytes and astrocytes in the brain by human neurotropic Polyomavirus JC (JCV). JCV is ubiquitous in the human population worldwide and after primary infection early in life, it sustains an asymptomatic persistent infection. Later, JCV may reactivate under conditions of immunosuppression to cause PML. At present, there is no accepted effective therapy for PML, which is often fatal within a few months. Interplay between viral and host factors and the signaling pathways that regulate JCV productive replication remain poorly defined. Recently, we found that JCV infection of glial cells causes significant cellular DNA damage. This damage is associated with induction of the cellular DNA damage response (DDR), which involves activation of phospho-ATM (ataxia telangiectasia-mutated protein), gamma-H2AX phosphorylation and an increased expression level of the DNA repair protein Rad51. Furthermore, specific inhibitor of ATM inhibit JCV replication and JCV infection caused a redistribution of NEMO from cytoplasm to nucleus. Co-expression of JCV large T-antigen and Flag tagged NEMO showed the occurrence of sumoylation of NEMO, while co-expression of ATM and Flag-NEMO demonstrated physical association between ATM and NEMO. These observation indicate that Activated ATM has an important role in coordinating the molecular events involved in DNA damage signaling and repair and the activation of nuclear factor kappa-B (NF-KB) by a novel NF-KB mechanism known as nucleus to cytoplasm or "inside-out" NF-KB signaling. Importantly, we found that Rad51 complexes with NF-KB p65 subunit, which stimulates JCV transcription. Together, these findings support the hypothesis that DNA damage inflicted by JCV infection activates the DDR and induces the expression of Rad51, which acts together with NF-KB to promote productive infection.

P12

Ischemic stroke in HIV-infected brain

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HIV-associated cerebrovascular events remain highly prevalent even in the current era of antiretroviral therapy (ART). While the replication of HIV is controlled by ART, the virus is not eliminated and the patients have to continue therapy for the rest of their life. Most currently used antiretroviral drugs prevent new cells from being infected, the cells that already harbor HIV genomes can continue to produce toxic viral proteins. We hypothesize that low-level HIV replication and associated inflammation endure despite antiretroviral treatment and affect ischemic stroke severity and outcomes. Using the EcoHIV infection model and the middle cerebral artery occlusion as the ischemic stroke model in mice, we present the first experimental in vivo analysis of the relationship between HIV and stroke outcome. C57BL/6 mice were infected with 1 μg of EcoHIV p24 delivered through the left carotid artery using the ICA injection delivery method. This method increases delivery of the virus into the brain and establishes low level infection in the CNS, typical of current HIV epidemic in humans. Following infection with EcoHIV, the BBB was disrupted, placing the neurovascular unit in a proinflammatory state that may predispose the brain tissue to more severe stroke injury. Indeed, EcoHIV infection increased infract size and negatively impacted tissue and functional recovery. Ischemic stroke also resulted in increased EcoHIV presence in the affected regions, suggesting post-stroke reactivation that magnifies pro-inflammatory status. Importantly, ART with a high CNS penetration effectiveness (CPE) was more beneficial to low CPE treatment in limiting tissue injury and accelerating post-stroke recovery. These results provide potential insight for treatment of HIV-infected patients that are at risk of developing cerebrovascular disease, such as ischemic stroke.
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P13

IL-1beta stimulates the release of extracellular vesicles from astrocytes that promote the expression of neuronal receptors implicated in a morphine-addiction pathway

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The interrelationship between inflammation and drug abuse has been known since 1994, but the mechanism(s) for this interaction have been elusive. Extracellular vesicles (EVs) function as messengers that regulate communication between cells in the central nervous system. In this study,

we explored a role for EVs in mediating the cross talk between inflammation and the morphine addiction pathway. Primary rat astrocytes stimulated with IL-1beta (200 ng/ml) released EVs (ADEV-IL-1beta) containing 131 miRNAs, with 10 increased and 17 decreased >1.5 fold in abundance compared with constitutively released EVs (ADEV-CR). Using the Diana-miRPath informatic system to interrogate the 17 decreased miRNAs we found that miR-106b, miR-218a and miR-328a mapped to the morphine addiction pathway. Bioinformatic analyses predicted these three miRNAs to target multiple genes in the morphine addiction pathway including OPRM1, KCNJ3, DRD1 and GABBR2. Two of these miRNA targets were validated in HEK293T cells cotransfected with OPRM1-3'-UTR or KCNJ3-3'-UTR luciferase reporter vectors, and mimics for miR-218a and miR-106b, or a scrambled microRNA mimic (the other two genes are currently undergoing validation). In cortical neurons exposed to a dose-response of EVs (15-100 EVs/cell) we found that OPRM1, KCNJ3, DRD1 and GABBR2 mRNA and protein levels were increased in cells exposed to ADEV-IL-1beta compared with ADEV-CR. Increasing the dose of ADEV-IL-1beta delivered to neurons increased the amount of miR-106b, miR-218a and miR-328a delivered, but expression of their mRNA targets continued to increase, suggesting that the totality of miRNAs delivered were competing with each other to inhibit target genes. Together these findings suggest that ADEVs constitutively shed by astrocytes contain miRNA that function to keep key proteins of the morphine addiction pathway suppressed. Inflammation may modify the composition of ADEVs to allow the translation of these proteins to be dis-inhibited and expressed at higher levels.

P14

Short-term administration of cocaine protects gut mucosa barrier and reduces plasma level of TNF-alpha

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Cocaine affects not only the central nervous system but also systemic immunity. Cocaine increases blood-brain barrier permeability, however, its role in gut mucosal integrity and systemic inflammation is not fully understood. To study the impact of cocaine on gut permeability and systemic inflammation, we investigated mucosal integrity and permeability by IHC and qPCR, as well as plasma levels of TNF-alpha, a microbial toll-like receptor downstream cytokine using ELISA in rats after 10 days of intravenous cocaine self-administration (n = 4 and n = 10 for control and cocaine group respectively). Endpoint analyses of the gut revealed that mRNA relative expression of claudin-1 to GAPDH (1.03 ± 0.15 vs. 1.52 ± 0.20 for control and cocaine group respectively, P = 0.077, non-parametric Mann-Whitney U test) and claudin-2 to GAPDH (1.17 ± 0.32 vs. 1.78 ± 0.15 for control and cocaine group respectively, P = 0.05) was marginally or significantly increased, and plasma levels of TNF-alpha (pg/mL, 22 ± 5.6 vs. 10.9 ± 3.2 for control and cocaine group respectively, P = 0.03) were decreased in the cocaine treated group compared to the control group. In addition, histopathological analysis identified intact and non-disturbed intestinal mucosal structures following cocaine treatment. Overall, these results suggest that short-term cocaine administration exerts a protective effect on the integrity of gut mucosa and suppresses circulating inflammatory TNF-alpha.

P15

Perivascular macrophages in the neonatal macaque brain undergo massive necroptosis after simian immunodeficiency virus infection

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Children with perinatally acquired HIV infection can manifest encephalopathy defined by neurodevelopmental delay, impaired brain growth or a decline in motor and cognitive function, but these clinical symptoms rarely have clearly defined neuropathological correlates. Previously we showed that rhesus macaques neonatally infected with simian immunodeficiency virus (SIV), do not develop encephalitis and maintain low brain viral loads despite having similar plasma viral loads compared to SIV-infected adults. As perivascular macrophages (PVMs) are the primary cell type responsible for the development of HIV/SIV neuropathologies in adults, we hypothesize that differences in response to SIV infection between infant and adult PVMs contribute to the lack of encephalitic lesions in SIV-infected infants. Using multi-label immunofluorescence (IF) microscopy, we examined the frontal cortex from uninfected and SIV-infected infant and adult macaques (n=8/ea) to identify and characterize PVMs with macrophage markers CD206 and CD163, apoptotic marker cleaved caspase-3 (cCasp3) and necroptotic marker receptor-interacting protein 3 (RIP3). Infants have a unique CD206+/CD163- PVM population not found in adults. More than 60% of CD206+/CD163- cells show evidence of cCasp3-mediated apoptosis after infection and nearly 100% showed evidence of necroptosis via RIP3 activation. However, we did not observe cCasp3 or RIP3 staining in both CD206+/CD163+ and CD206-/CD163+ PVM subpopulations in infants and adults with or without infection. HIV encephalopathy in infants and children is often progressive and severe, but this clinical data is in sharp contrast to the lack of pathological evidence. The current finding of massive necroptosis of a unique subset of PVMs in infants after SIV infection provides a possible mechanism by which HIV/SIV infection causes these clinical manifestations in infants. Programmed cell death of infected PVMs would prevent classical pathologies seen in adults, but the excess neuroinflammation that may result from wide-spread necroptosis is one explanation for the clinical encephalopathy which persists in their absence.

P16

Use of Stem Cell Extracellular Vesicles as a "Holistic" Approach to CNS Repair

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Neurological diseases and disorders are leading causes of death and disability worldwide. These pathologies are often associated with high levels of neuroinflammation and irreparable tissue damage due to the dysregulation of signaling pathways that are unable to restore a homeostatic balance. Due to their multipotent properties stem cells have broad therapeutic potential and stem cell therapy has been evaluated for CNS repair. Paracrine factors, such as extracellular vesicles (EVs), mediate many of the functional effects associated with their donor stem cells. The potency of these EVs is important for CNS-related pathologies since they cross the blood-brain-barrier (1). We have isolated and recovered high yields of EVs from large scale cultures of both induced pluripotent stem cells (iPSCs) and mesenchymal stem cells (MSCs) using tangential flow filtration. Our EV characterization assays include phenotypic (size, tetraspanin expression) evaluation, protein and cytokine expression, as well as RNA analysis (2,3). Additionally, EV functionality has been assessed in vitro utilizing several cell-based assays relating to cellular growth and viability, migration, angiogenesis, and immunomodulation in recipient cells (3). Our data suggests that EVs from different sources of stem cells display unique phenotypes and exhibit differential association with various cytokines, proteins, and long non-coding RNAs (4). We propose a "dual" mode of action, whereas cellular growth is initially

slowed down due to activation of innate immune molecules by long non-coding RNAs, followed by initiation of reparative mechanisms via proteins and cytokines that promote cellular repair. Collectively, these results demonstrate the potential of stem cell EVs as a "holistic" therapeutic approach to reverse or to reduce cellular damage.

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P17

Microbial presence in brain is associated with viral burden and neuroinflammation during lentivirus infections

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Introduction: HIV-1 infection is accompanied by marked systemic inflammation that can result microbial translocation from different tissue reservoirs. Previous studies have reported detection of bacterial products in brains from humans with and without brain disease. Herein we investigated interactions between lentivirus infections, neuroinflammation and microbial presence in the brain.

Methods: Brain tissues from adult persons with and without HIV-1 infection as well as adult nonhuman primates (NHP, Indian Rhesus macaques) with and without SIVmac251 infections were investigated by RT-PCR/ddPCR, immunofluorescence and western blotting for viral, microbial and host immune responses. Further in vitro studies in which host immune responses were examined in human neural cells.

Results: In both HIV-infections and SIV-infectionss, viral burden in brain was associated with clinical and morphological brain disease. Host immune responses including glial activation and cytokine expression were also correlated with both viral burden and bacterial detection among HIV-infected humans and SIV-infected NHPs. Bacterial DNA, rRNA and mRNA were detected in all human and NHP brain samples examined although both bacterial RNA and DNA levels were significantly higher in human and NHP brains with high viral burden and inflammation. In human brain, bacterial genome load ranged from 250 to 550 copies/gm (tissue). Moreover bacterial GroEL and peptidoglycan immunoreactivity in glial cells was evident in human brain. In vitro studies revealed that exposure to cell-free supernatants from limiting-dilution bacterial cultures to brain macrophage-like cells (human microglial and THP-1 cells) induced cytokine (IL-1beta and IL-6) expression.

Conclusions: These studies indicate that bacterial genomes and proteins are present in the brains of humans and animals during lentivirus infections and were correlated with brain viral burden. Moreover, bacterial presence in brain is associated with neuroinflammation and evidence of brain disease. Thus, microbial translocation into the brain might contribute to the neuropathogenesis of lentivirus brain diseases.

P18

Associations between synthetic cathinone mephedrone, compromised blood brain barrier, and HIV-1 infection

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Synthetic cathinones are an emerging class of designer drugs consumed for psychostimulant and hallucinogenic effects. Recently in the US, the use of synthetic cathinones (bath salts) have dramatically increased, especially among adolescent and young adult populations. Several public health reports have also linked cathinone use with outbreaks of HIV-1. Here, we examined the effects that mephedrone, alone or in combination with the HIV-1 proteins gp120 and tat, may have on human brain endothelial cells and whether mephedrone affects HIV-1 replication rate. We report that mephedrone application to the endothelial cell monolayer resulted in significant decrease in transendothelial electrical resistance (-20% from baseline) indicating compromised barrier integrity. Interestingly, combination of mephedrone with tat or gp120 resulted in even more dramatic loss of barrier resistance (-30% and -60% respectively). Significant upregulation of membrane-bound ICAM-1 and VCAM-1 proteins was detected in cells exposed to mephedrone, suggesting activation of brain endothelial cells. Moreover, mephedrone treatment resulted in 2-fold increase in HIV-1 replication in in vitro culture of human monocyte-derived macrophages as measured by p24 ELISA. Our findings suggest that mephedrone exacerbates the deleterious effects of HIV-1 proteins on barrier integrity, triggers brain endothelial activation which may facilitate transmigration of HIV-1 infected immune cells across the BBB, and stimulates HIV-1 replication. Altogether, our data points to a contributing role for cathinones in HIV-1 CNS infection and neuroinflammation.

P19

Cardiovascular Disease Risk Factors and HIV Disease Markers Increase Dementia Risk and Alter White Matter Microstructure in Virally Suppressed HIV Infected Men

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Background: Cardiovascular disease (CVD) risk factors in virally-suppressed HIV+ adults may be associated with neurocognitive impairment (NCI) and white matter (WM) microstructural alterations.

Methods: 82 HIV+ (mean 55.1 years, 30% on CVD drugs; 12% with prior CVD), and 40 HIV- (mean 54.5 years, 0% with prior CVD; 20% on CVD drugs) underwent a 32 directions Diffusion Imaging Tensor (DTI) scan. Participants were split into four groups according to their HIV status and CVD status (cardiovascular risk score ,â•15% and/or history of CVD): HIV+/CVD+, HIV+/CVD-, HIV-/CVD+, HIV-/CVD-. Using IDLtrack and the ENIGMA protocol, Fractional Anisotropy (FA) and Mean Diffusivity (MD) were extracted in 11 skeleton regions of interest (SROI).

Results: High CVD risk was associated with greater dementia prevalence in the HIV+ sample (HIV+/CVD+ =14%, HIV+/CVD-=3%; p=0.08), and with mild NCI in the HIV- sample (HIV- /CVD+=45%, HIV-/CVD-=10%; p<0.01). In the HIV- sample, and relative to CVD- group, the CVD+ group had lower FA (p=0.03, d=0.97) and higher MD (p=0.003, d=1.46) in the corona radiata, and significantly higher MD in the corpus callosum (p=0.02, d=1.26) and superior fasciculi (p=0.03, d=0.97). In the HIV+ group, the CVD+ group had significantly lower FA in the superior fasciculi (p=0.04, d=0.37) and higher MD in the uncinate fasciculus (p=0.04, d=0.60) compared to HIV-/CVD- group. The HIV+/CVD+ group obtained significantly lower FA (p=0.01, d=0.65)

and higher MD (p=0.03, d=0.63) in the fornix compared to the HIV+/CVD- and HIV-/CVD- groups (FA: p=0.003 d=0.93 ; MD: p=0.008, d=0.95). Older age, taking anti-hypertensives, longer HIV duration and CRP were associated with lower FA and higher MD (p<0.0001 - p=.01). Higher blood CD4 and CD4/CD8 ratio were associated with higher FA and lower MD (p=.003 - p=.02).

Conclusions: CVD is a clinically meaningful contributor of WM microstructural alterations which may increase the presence of dementia in aging HIV+ people.

P20

Astrocyte-derived exosomes increase in the plasma of HIV patients

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HIV can infiltrate the central nervous system, triggering HIV-associated neurocognitive disorders (HAND), which prevail in nearly 50% of the patients despite antiretroviral therapy. HIV-seropositive (HIV+) patients have circulating exosomes which can spread viral proteins and other cargo. The soluble insulin receptor (sIR) is one of the proteins secreted in exosomes to the plasma and cerebrospinal fluid, higher than control individuals. Moreover, sIR+ exosomes increase with HAND. The cell type where these exosomes originate is unknown. In this study we isolated exosomes from the plasma of control (n=29) and HIV+ women (n=88), and measured two markers by flow cytometry: glial fibrillary acidic protein (GFAP) from astrocytes and L1 cell adhesion molecule (L1CAM) from neurons. Exosomes from HIV+ women express higher levels of GFAP+ exosomes (mean=54.2 MFI; p<0.0001) than controls (mean=9.07 MFI). No differences were observed in the percentage of L1CAM+ exosomes between groups. In controls, the percentage of L1CAM+ exosomes have inverse correlation with exosomes containing reactive oxygen species (ROS) (r=-0.57; p=0.0026). While in HIV+ women, exosomal GFAP levels positively correlate with the percentage of ROS+ exosomes (r=0.37; p=0.0014). Moreover, exosomal ROS levels positively correlate with exosomal sIR (r=0.28; p=0.013) and HIV Tat (r=0.34; p=0.0029) levels. In HIV+ women with normal cognition, high levels of L1CAM per exosome correlate with worse learning memory (r=-0.402; p=0.028). In HIV+ asymptomatic impaired patients, worse speed of information processing correlates with higher percentage of L1CAM+ exosomes (r=-0.738; p=0.037), and exosomal GFAP levels (r=-0.731; p=0.0396). In conclusion: (1) HIV-1 infection triggers more exosome release from astrocytes than neurons, (2) both markers inversely correlate with neurocognitive function, and (3) oxidative stress might induce a shift from L1CAM+ to GFAP+ exosomes. Further studies will determine if sIR+ exosomes are astrocyte-derived. This study could shed light onto how the CNS and the periphery communicate through exosomes in HAND patients.

P21

Targeting CCR2 and CCR5 genes using CRISPR/SaCas9/gRNA-based gene editing

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C-C Chemokine receptor type 5 (CCR5) plays a key role in HIV infection as a co-receptor for HIV entry in host cells and cell-to-cell spread. CCR5 crucial role on HIV infection came from the discovery of the delta 32 deletion mutation in the coding region of CCR5. People with homozygous mutations are almost resistant to HIV infection. CCR5Œî32/Œî32 hematopoietic stem cell transplantation was found to be a good treatment strategy in the "Berlin patient" and the "London patient". C-C Chemokine receptor type 2 (CCR2) is implicated in the transmigration of HIV-infected monocytes/macrophages through the blood brain barrier, contributing to the establishment of the central nervous system (CNS) reservoir. CCR5 is expressed on T lymphocytes, macrophages, granulocytes, dendritic cells, microglia, astrocytes, neurons, fibroblasts and also on epithelium, endothelium and vascular smooth muscle. In contrast, the expression of CCR2 is relatively limited mainly to monocytes, NK and T lymphocytes, although it may be induced in other cells under inflammatory conditions. In this study, we examined the potential role of CRISPR/Cas9 gene editing on CCR2 and CCR5. CCR2 and CCR5 have a 73% sequence homology, allowing gRNA to target both simultaneously. We designed gRNAs based on SaCas9 targeting both receptors alone and in combination. To test the ability of our construct to induce site-specific cleavage and disruption of CCR2/CCR5 genes, we used 293T embryonic kidney and U937 myeloid cells. Our results indicate the cleavage of CCR2/CCR5 genes by CRISPR/Cas9 system. To verify the specificity of our excision strategy, we performed analysis of the predicted/possible off-targets sites in the human genome. No off-targets cleavage were detected. Our experiment showed the efficiency and specificity of CRISPR/Cas9 in targeting the CCR2 and CCR5 genes and these strategies will be developed for targeting in vivo to inhibit HIV spread and traffic of cells into the brain.

P22

Interferon-alpha suppresses Polyomavirus JC replication in a STAT1-independent manner via PI3K/AKT and mTOR pathway

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The progressive multifocal leukoencephalopathy (PML) is caused by the neurotropic Polyomavirus JC (JCV), which replicates in brain oligodendrocytes and astrocytes. PML occurs occasionally under conditions of immune dysfunction and results from the reactivation of persistent virus. Our earlier studies suggest that reactivation occurs within glial cells due to the action of cytokines, e.g. tumor necrosis factor alpha (TNF-alpha), stimulating viral gene expression. In this study, we have examined the cytokines interferon alpha (IFN-alpha), which in contrast have a negative effect on JCV gene expression and replication. The role of INF-alpha-mediated activation of STAT-1 in JCV regulation was analyzed by Western blots, cell fractionation, and quantitative PCR for viral load. The PI3K inhibitor LY294002 and mTOR inhibitor Rapamycin were used to analyze the role of IFN-alpha in mediating the downstream signaling events in JCV replication. Here, we show that JCV infection is inhibited by INF-alpha in PHFA cells. INF-alpha induces expression of STAT-1 and it causes STAT1 phosphorylation and translocation to the nucleus. However, INF-alpha treatment of JCV infected cell lines, in which STAT-1 is knock down using CRISPR/Cas9 system, shows JCV suppression indicating no role of INF-alpha-activated STAT-1 on JCV replication. Treatment of JCV infected cells with inhibitors of down streaming signaling pathway PI3K and mTOR reverses inhibitory effect of INF-alpha. We conclude that STAT-1 activation by INF-alpha does not suppress the JCV replication and that INF-alpha-induced PI3K/AKT and mTOR activities are important regulator factors for anti-JCV activity.

P23

Astrocyte infection is required for retrovirus-induced spongiform neurodegeneration despite suppressed viral protein expression

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Although the major Env expressing CNS cell types have been identified for many neurovirulent retroviruses, it remains unresolved, which targets play a causal role in neuropathogenesis. Moreover, this issue is complicated by the potential for post infection virus suppression. To address these questions we explored herein whether and how cryptic neurotropism differences between ecotropic and amphotropic murine leukemia viruses (MLVs) impacted neurovirulence. RV neurotropism was first explored ex vivo using 1) acute primary glial cell cultures and 2) neural progenitor cell (NPC)- neural stem cell (NSC) neural sphere (NPH) chimeras. These experiments indicated that primary astrocytes and NPCs acutely restrict amphotropic but not ecotropic virus entry. RV CNS tropism was investigated using NSC transplant-based Cre-vector pseudotyping wherein mTmG transgenic fluorescent protein reporter mice revealed both productive and suppressed infection. Cre-pseudotyping with FrCasE, a prototypic neurovirulent ecotropic virus, identified glia and endothelia, but not neurons, as targets. Almost two-thirds (62%) of mGFP+ cells failed to show Env expression, suggesting widespread virus suppression. To circumvent RV superinfection interference confounds, targets were also identified using ecotropic packaging NSCs These experiments identified known targets: microglia, OPCs and endothelia. Additionally, one third of mGFP+ cells were identified as protoplasmic astrocytes, cells that rarely express virus in vivo. A CNS targeting comparison between isogenic ecotropic (FrCasE) and amphotropic (FrAmE) viruses showed a four-fold higher astrocyte targeting by FrCasE. Since ecotropic Env pseudotyping of amphotropic virus in the CNS dramatically exacerbates neurodegeneration, these results strongly suggest that astrocyte infection is a major disease requirement. Moreover, since viral Env protein expression is largely subdetectable in astrocytes, minimal viral protein appears sufficient for affecting neuronal physiology. More broadly, these findings raise the specter that subdetectable astrocyte expression of exogenous or endogenous RVs could play a major role in human and animal neurodegenerative diseases.

P24

Brain Metabolite Changes Following HIV Infection: Meta-analysis

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Background: Numerous studies have used magnetic resonance spectroscopy (MRS) metabolite neuroimaging to measure HIV infection effects on brain function. While many have reported changes in N-Acetylaspartate (NAA), myo-Inositol (mI), and Choline (Chol) following HIV infection, quantitative inconsistencies observed across studies have been large.
Purpose: To evaluate the consistency and temporal stability of serostatus effects on a range of brain metabolites.
Study Selection: The meta-analysis sample included cross-sectional studies between 1993 - 2019 reporting HIV infection effects on cortical and subcortical metabolites. NAA/Cr ratios (21 papers), Cho/Cr ratios (21 papers), mI/Cr ratios (17 papers) and quantitative Cr (9 papers) from basal ganglia (BG), gray matter (GM) and white matter (WM) were examined.
Data Analysis: Random effects meta-analysis using inverse variance weighting and bias corrected standardized mean differences (SMD) was used to estimate individual study Cohen's d standardized mean differences and study heterogeneity. Meta-regression was used to examine effects of study publication year and data acquisition techniques.
Data Synthesis: Meta-analysis revealed BG SMDs related to serostatus of -0.088 (NS) for NAA/Cr, 0.31 ($p<0.01$) for Cho/Cr, 0.63 ($p<0.01$) for mI/Cr and -0.29 (NS) for quantitative Cr. Nevertheless, estimates of between study heterogeneity suggested that the majority of the observed variance was between studies. In contrast, WM and GM regions showed larger changes in Chol/Cr and NAA/Cr ratios related to serostatus.
Limitations: Many studies pooled participants with varying durations of treatment, disease duration and comorbidities. Image acquisition methods changed with time.
Conclusion: While published studies of HIV effects on brain metabolism exhibit substantial variation that may result from measurement technique variations or changes in HIV treatment practice, quantitative metabolic measures showing decreased NAA/Cr, increased Cho/Cr and increased mI/Cr were consistent across studies. Brain metabolites measured with MRS can reliably detect the effects of HIV infection, serving as practical biomarkers during treatment.

P25

Efficient expression of multiplex gRNAs via tRNA transcription and maturation for guiding SaCas9 to target HIV provirus in vivo

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Gene editing via a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) platform requires both CRISPR-associated protein 9 (Cas9) and guide RNA (gRNA). While Adeno-Associated Virus (AAV) vectors are efficient for in vivo gene delivery, the packaging capacity of AAV as a vector is limited. The use of multiple gRNAs may increase the capacity of the AAV vector. Here we report that tRNAs can function as promoters to express gRNAs to replace the commonly used U6 promoter. A total 4 gRNAs in a polycistronic transcript expressed by tRNAs along with Cas9 were functional to excise fragments HIV proviral DNA in Tg26 mice harboring multiple copies of HIV-1 in their genome, after a single intravenous injection of an all-in-one vector packaged in AAV6 or AAV9. To visualize the biodistribution of SaCas9 delivered by AAVPHP.eB, a derivative of AAV9 engineered to target brain, SaCas9 was fused to a luciferase reporter NanoLuc to reveal the tempo-spatial localization of SaCas9 using in vivo and ex vivo bioluminescence imaging. In contrast to AAV9 and AAV6, which showed weak gene delivery to the brain, our results showed significant transduction efficiency of brain cells (up to 51%) with AAVPHP.eB expressing CRISPR-Cas9 and detectable HIV-1 DNA editing

P26

Morphine exacerbates HIV-1 Tat driven changes to neuroinflammatory factors in astrocytes

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Patients comorbid with HIV-1 virus and morphine drug use experience worsening neurocognitive outcomes. Nearly one-half of people infected with HIV-1 virus experience HIV-associated neurocognitive disorder (HAND) despite anti-retroviral therapy. Mechanisms underlying HAND progression alone remain unclear and are further complicated with comorbid morphine exposure. HIV-1 Tat viral protein and morphine have both been independently associated with exacerbation of neuroinflammation. Through the mechanism of Tat protein inhibition Wnt/beta-catenin, a pathway shown to be protective against HIV brain inflammation, and Tat's involvement in neuroinflammatory pathways, we explored the concurrent impact of morphine exposure in promoting dysregulation in astrocytes, cells notable for harboring latent HIV-1 virus. We hypothesized worsening HAND outcomes in increased suppression of beta-catenin

signaling and dysregulation in neuroinflammatory pathways involving neurotrophin and inflammasome signaling. To explore the impact of combined Tat and morphine exposure, we cultured and transfected primary human and U87MG astrocytes with Tat mutant plasmids informed by the neurocognitive status of the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) cohort. Luciferase reporter assay and western blot were used to quantify levels of beta-catenin. Primers to Bdnf, TrkB, and NLRP-1 were profiled via RTqPCR on Tat and morphine treated cells. Morphine potentiated the suppressive ability of Tat on beta-catenin signaling, suggesting worsening HAND in morphine use. Introducing naturally occurring Tat mutants produced variable responses in suppressing beta-catenin signaling, giving insight on potentially important functional sites involved in synergistic changes with morphine. Expression of mRNA were differentially regulated in combined Tat and morphine treatment and varied between fetal and adult astrocytes and specifically between Tat mutants, emphasizing the role of well-characterized Tat functions on neuroinflammation.

P27

Morphine and Tat Synergize to alter a unique set of microRNA in human astrocytes

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Despite effective anti-retroviral therapy, nearly half of HIV-1 infected subjects experience HIV-associated neurocognitive disorders (HAND). Substance abusing HIV-1 positive individuals experience a greater degree of impairment at a higher incidence. The precise mechanism underlying this phenomenon is unknown. The HIV 1 Tat protein and morphine have both been independently associated with exacerbation of neuroinflammation. We and others have highlighted a role of Tat in inhibiting Wnt/beta-catenin signaling. Our lab has been working to understand mechanism by which Tat mediates this effect and identified alterations in cellular miRNA expression by Tat as a possible cause. We hypothesize that morphine exposure may also alter miRNA expression and that the combined effect of these two treatments may underlie the worsening HAND seen in HIV-1 positive opioid users. To explore the impact of combined Tat and morphine exposure, we cultured and transfected primary human and U87MG astrocytes with Tat or Tat mutants without the ability to suppress miRNA expression with or without morphine. miRNA analysis indicated a possible disruption of multiple pathways related to neuroinflammation (BDNF, TrkB and NLRP-1) and these changes were confirmed by qPCR. Interestingly, morphine exposure drove changes in a different set of miRNA and the combination of combination of these two agents drove a unique miRNA profile with implications for HAND.

P28

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

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Approximately half of the HIV-infected population develops HIV Associated Neurocognitive Disorders (HAND) despite suppression of viremia with antiretroviral therapy (ART). Astrocytes, the most abundant cell type in the brain, depend on macroautophagy, a highly-regulated proteolytic process, to maintain astrocyte, and by extension, CNS homeostasis. Astrocytes are infected with HIV at a low level, as well as exposed to viral proteins, such as Nef, and to ART, all of which may impact autophagy. HIV is known to influence macroautophagy in immune cells, as are some antiretroviral drugs in certain cancer therapy models. However, only a few studies have examined a link between HIV neuropathogenesis and autophagy. We treated primary human astrocytes with HIV Nef or Tenofovir+Emtricitabine+Raltegravir (ART), and cell lysates were collected for western blot analysis of LC3-II, a macroautophagy marker. We show that HIV Nef significantly increases LC3-II flux after 24 hours of treatment, and flux remains elevated after 7 days of daily treatment. ART does not significantly change LC3-II flux after 24 hours, but increases flux after 7 days of daily treatment. These data indicate that both Nef and ART increase autophagy but with different kinetics. Astrocytes expressing dual-fluorescent LC3 and treated with Nef for 24 hours have more autophagic vesicles, both autophagosomes and autolysosomes, relative to control astrocytes, while ART-treated cells have a similar number of autophagic organelles relative to control after 24 hours, agreeing with western blot data. Our study supports a role for both HIV proteins, as well as current antiretroviral drugs, in impacting autophagy in human astrocytes, in the short-term and likely in the long-term as well. Chronic autophagic aberration resulting from Nef and ART likely leads to astrocyte toxicity and an inability to perform their many essential roles in the CNS, thereby contributing to HAND in HIV-infected people in the ART era.

P29

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 co-morbidity in people living with HIV (PLWH). Some studies show that methamphetamine increases neuroinflammation and cognitive disorders in PLWH. Methamphetamine crosses the blood brain barrier (BBB) and is associated with high risk behaviors that may contribute to HIV infection, poor ART adherence, and increased CNS viral load, resulting in neuroinflammation and neuronal damage. To characterize the impact of methamphetamine on HIV mediated neuroinflammation and seeding of viral reservoirs in the ART era, we examined its effect on mature monocyte entry into the CNS. Uninfected and HIV-infected mature monocytes were treated with methamphetamine, ART (Tenofovir and Emtricitabine), or both. Cells were added to a human BBB model, and allowed to transmigrate to CCL2 or CXCL12, chemokines elevated in the CNS of PLWH. Preliminary data indicate that methamphetamine increases transmigration of uninfected and HIV-infected cells. We also demonstrated that HIV Tat, present in the CSF despite ART, increases transmigration of mature monocytes, with higher numbers when monocytes are treated with methamphetamine. Brain microvascular endothelial cells (BMVEC), a component of the BBB, express junctional proteins that facilitate monocyte transmigration. BMVEC were treated as above, and analyzed for surface PrPc, ALCAM, ICAM-1, and JAM-A. Methamphetamine decreases PrPc, which may increase BBB permeability, while ART does not change this. ART and/or methamphetamine do not affect the other proteins. We will continue to characterize the impact of HIV and methamphetamine on monocyte and BMVEC junctional proteins. Our goal is to identify targets to limit monocyte entry into the CNS, thereby reducing inflammation and viral seeding, contributing to elimination of reservoirs and HAND.

P30

Targeting HIV-1 transcription factor binding sites using CRISPR/Cas9 to permanently deactivate latent provirus

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Even with effective control of human immunodeficiency virus type 1 (HIV-1) replication using antiretroviral therapy (ART), the therapy does not cure the chronic HIV-1 infection. Recent attempts to develop a HIV-1 cure strategy with the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system have successfully demonstrated the excision of integrated HIV-1 provirus from infected cells by introducing two CRISPR-induced double-stranded breaks (DSB) into proviral DNA. However, previous studies have shown a low frequency of complete proviral genome excision when only the long terminal repeat (LTR) is targeted. Therefore, gRNAs that target the LTR in order to permanently deactivate transcription continue to be sought. We propose to edit the core enhancer sequences of the LTR while retaining the possibility of CRISPR-induced excision events. Our computational analysis identified two gRNAs that target the NF-kappaB binding sites (gNFkB1, gNFkB2) that demonstrated high conservation across HIV-1 quasispecies. This conservation is also well preserved across HIV-1 subtypes. While colloquially there is a belief that HIV-1 NF-kappaB sites resemble endogenous cellular sites, our analysis demonstrates otherwise. When scanning all endogenous NK-kappaB sites, the predicted gRNA binding efficiency showed that none of the human NF-kappaB sites had a cleavage score over 0.19 using the CFD matrix. Conversely, HIV-1 NF-kappaB binding from 5,100 LTR sequences had an average cleavage score of 0.84. Furthermore, GUIDE-seq, which detects CRISPR-induced off-target edits in vitro, showed that the gRNA targeting NF-kappaB binding sites had high efficiency on the integrated HIV-1 in TZM-bl cells with no detectable CRISPR-induced off-target edits in the human genome. Lastly, 5' LTR-driven HIV-1 transcription reduced 45% with treatment of Cas9/gNFkB1 after post-CRISPR stimulation using PMA/I, indicating that the 5'-LTR has been deactivated by CRISPR/Cas9. These results demonstrate a working model to deactivate HIV-1 transcription with high safety by targeting critical viral transcriptional regulatory sites.

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Chromatin impact upon CRISPR treatment of latent HIV-1 infection

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Combined antiretroviral therapy (cART) is effective at reducing viral load and suppressing HIV-1 infection. However, even with effective therapy there is still no cure for HIV, in part due to cART's inability to eliminate the latent viral reservoir that harbors integrated viruses. CRISPR/Cas9 cleavage can excise or deactivate this reservoir by using gRNAs specifically targeting the transactivation response element (TAR) regions in the long terminal repeat (LTR) of the integrated provirus. However, no gRNAs have yet demonstrated 100% target cleavage. One explanation is that the TAR region is obstructed by a nucleosome (Nuc-1) in latently infected cells, which could prevent gRNAs from binding to target sequences. We hypothesize that CRISPR-Cas9 cleavage can be enhanced by loosening the chromatin architecture, making target sequences more accessible for gRNAs. We propose a "tickle-and-tweeze" strategy that utilizes sub-therapeutic doses of latency reversal agents (LRAs) as chromatin remodeling tools to relax chromatin and dissociate nucleosomes from the DNA sequences they obstruct. Previous analysis using integrative genomic data showed that DNA availability at CRISPR editing sites is significantly less than that required for endogenous gene expression. We firstly demonstrated that latency reversal using HDACis was dose-dependent in a latently infected T-cell model (J-Lat 10.6 cells). Beta-gal analysis revealed that a combination of a TAR-specific gRNA CRISPR treatment and low-dose histone deacetylase inhibitor (HDACi) significantly reduces LTR-driven transcription in TZM-bl cells. Our work supports the hypothesis that condensed chromatin can impede CRISPR-Cas9 cleavage of the HIV-1LTR; our "tickle-and-tweeze" strategy can potentially circumvent this problem. Future studies will examine the effectiveness of this strategy in J-Lat 10.6 cells using different gRNA/HDACi combinations at varying low-doses. We will also investigate whether off-target CRISPR cleavage events are generated when the chromatin environment has been relaxed.

P32

Phenotypic and functional analyses of co-occurring negative checkpoint receptors and their blockade against HTLV-induced neurologic disease

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HTLV-1 is endemic to many tropical regions with 5-10 million individuals infected globally. While most individuals infected remain asymptomatic, around 3-5% develop complications of ATLL or HAM/TSP. HTLV-1 infection causes clonal expansion of infected T cells, which after many years of chronic antigenic stimulation increase expression of several negative checkpoint receptors (NCRs) resulting in immune exhaustion. Recent work has shown that PD-1+ CD8 and TIGIT+ CD4 T cells expand significantly in individuals with HAM/TSP when compared to uninfected controls. Yet, the extent of altered combinatorial immune checkpoint receptor expression in HTLV-1-infected chronic patients remains unclear, which provide the rationale to these studies. First, an extensive bioinformatics analyses of the relevant existing data was performed. Following that, we investigated the combinatorial expression of NCRs on CD8 T cells from PBMCs of HTLV-1+ carriers HAM/TSP patients and seronegative controls from the UCSF HOST cohort. PBMCs were profiled to assess the frequencies (%) of PD-1, TIM-3, TIGIT and LAG-3 on CD8 T cells. Flow data revealed that HAM patients had significantly higher % of single Tim-3 and Lag-3 expressing CD8 T cells as compared to asymptomatic carriers (p value 0.0002, 0.0564 respectively). Furthermore, HAM/TSP patients had significantly higher triple PD-1+ TIGIT+ TIM-3+ (median: 6.2% (IQR 3.5, 7.8) (p value 0.0084)) and multiple PD-1+ TIGIT+ TIM-3+ LAG-3+ expressing global CD8 T cells (0.69% (0.40, 1.53) (0.0011)) as compared to carriers (0.13 (0.08, 0.24)). Functional

studies in correlation with proviral loads are ongoing. These data reinforce the hypothesis that multiple NCRs on CD8 T cells are an important driver of HTLV-1 pathogenesis and show that future studies that focus on combination NCR blockade may show greater efficacy in improving T cell responses and serve to improve therapeutic options to limit or control HTLV-1-associated diseases.

P33

CRISPR-Cas9 mediated disruption of ALCAM gene inhibits adhesion and trans-endothelial migration of myeloid cells

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Migration of HIV-1 infected monocytes across the endothelial barrier plays an essential role in establishing and maintenance of viral reservoir in the brain and leads to neuroinflammation, neuronal damage and subsequent HIV-induced central nervous system (CNS) dysfunction. These processes continue despite antiretroviral therapy (ART) due to limited pharmacological permeability of blood-brain barrier, the presence of residual viral replication and reactivation of latent viruses. Activated leukocytes cell adhesion molecule (ALCAM/CD166) is a junctional protein elevated on activated T cells, monocytes, dendritic cells and endothelium. Recent studies identified ALCAM to be preferentially overexpressed on HIV-1 infected, mature CD14+CD16+ monocytes from people with HIV (PWH) on suppressive ART and critical for the transmigration ability of these cells. Furthermore, high throughput CRISPR screen identified ALCAM as one of the key HIV-1 host dependency factor (critical for virus propagation but non-essential for host cells). Here, we used a pair of CRISPR guide RNAs to excise exon 1 (spanning start codon and signal peptide region) and thus create inducible ALCAM gene knockout in myeloid cells. Using lentiviral delivery, we developed several knockout clones in pro-monocytic U937, and their latently infected with HIV-1 equivalent: U1 cells. Next, verified control and knockout cell clones were tested in adhesion and transmigration assays, using monolayers of cerebral microvascular endothelial cells (hCMEC/D3). As expected, ALCAM-/- myeloid cells showed markedly reduced adhesion to and transmigration through endothelial cells. Next, using AAV6 delivery we replicated these results in primary human monocytes from three different healthy donors. In order to limit CRISPR-Cas9 editing to HIV-1 infected cells we placed Cas9 expression under the control of minimal Tat responsive HIV-1 LTR promoter (-80/+66). HIV-1BAL-GFP infection of AAV6-LTR-CRISPR-ALCAM treated CD4+ T cells and CD14+/CD16+ monocytes, resulted in induction of Cas9 expression and CRISPR mediated cleavage of exon 1 of ALCAM gene in Tat expression dependent manner.

P34

Cognitive Trajectory Phenotypes in Human Immunodeficiency Virus-Infected Patients

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The presentation of cognitive impairments (CI) in HIV-infected individuals has transformed since the introduction of antiretroviral therapies. Although the overall prevalence of CI has not changed considerably, frank dementia is now infrequent, and milder forms of cognitive impairments predominate. Mechanistic insights to the underlying causes of these residual cognitive impairments have been elusive, in part due to the heterogenous etiology of cognitive dysfunction in this population. Here, we sought to categorize longitudinal change in HIV-infected patients based on performance in specific cognitive domains. This study consisted of 193 participants from the CHARTER cohort with detailed demographic, clinical, and neuropsychological(NP) testing data obtained from 2 study visits interspersed by ,à°6 months. NP Scores were converted to domain T-Scores, and change scores were calculated for each domain between the two study visits. We followed this with a principal component analysis of change scores for each cognitive domain to produce a new smaller set of uncorrelated variables (principal components; PCs). The minimum number of PCs that explained at least 85% of the total variance in the data were retained for further analyses. K-means clustering with the Hartigan-Wong algorithm was performed on the PCs to identify cognitive domains that grouped together. Calculating the within-group sum of squares for n=1:6 clusters demonstrated that 4 clusters optimally grouped the 7 cognitive domains and the BSS:TSS ratio was 0.909, indicating that 90.9% of total variance in the variable loadings was explained by these 4 clusters. The identified phenotypes consisting of specific declines in 1) verbal fluency, 2) executive function 3) learning and recall, and 4) motor function. Each of the 4 cognitive change phenotypes identify deficits that implicate perturbations in specific neural networks. Future studies will need to validate if cognitive change phenotypes are associated with alterations in the associated neural pathways.

P35

Neurocognitive Profiles Among Virally Suppressed Women with HIV

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Neurocognitive impairment (NCI) remains frequent and heterogeneous in presentation among virally suppressed (VS) women with HIV (WWH). Herein, we identified neurocognitive profiles among 929 VS-WWH from all 13 Women's Interagency HIV Study sites at their initial neurocognitive study visit. VS-WWH completed a neuropsychological (NP) battery comprised of: Hopkins Verbal Learning Test-Revised, Trail Making Test, Symbol Digit Modalities Test, Grooved Pegboard Test, Stroop Test, Controlled Oral Word Association Test, Animal Fluency, and Letter-Number Sequencing. Using 17 performance metrics (T-scores) from the NP battery, we first used Kohonen self-organizing maps to identify patterns of high-dimensional data by mapping participants to similar nodes based on T-Scores. Nodes were clustered (via MCLUST

R package) resulting in nine clusters from an ellipsoidal multivariate mixture model with equal orientation (entropy=0.990). Four of nine identified clusters with an average T-score ≥ 45 for all metrics were combined into an "unimpaired" profile (n=311). The five impaired profiles consisted of weaknesses in: 1) sequencing (Profile-1; n=129), 2) speed (Profile-2; n=144), 3) learning (Profile-3; n=137), 4) learning and memory (Profile-4; n=86), and 5) processing speed (Profile-5; n=122). Sociodemographic, behavioral, and clinical variables were used to differentiate profile membership using Random Forest models (n=5000 trees, 5x internal cross-validation). The top ten variables distinguishing the combined impaired versus unimpaired profiles were: study site, age, education, race, illicit substance use, current and nadir CD4 count, duration of effective antiretroviral therapy (ART), and protease inhibitor use. Additional variables differentiating each impaired from the unimpaired profile included: depression, PTSD symptom burden, perceived stress, income (Profile-1); depressive symptoms, employment status (Profile 2); depressive symptoms, integrase inhibitor (II) use (Profile-3); employment, II use, income, atazanavir use, anticholinergic risk score (Profile-4); and marijuana use (Profile-5). These findings highlight the need to consider heterogeneity in NP profiles and the potential modifiable factors that may contribute to impaired profiles.

P36

Endothelial Dysfunction is Associated with Cognitive Impairment in Rakai, Uganda

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Despite antiretroviral therapy (ART), cognitive impairment (CI) in people living with HIV (PLWH) remains a frequent co-morbid condition. Biomarkers of endothelial dysfunction and neuroangiogenesis have been associated with CI in a variety of conditions, including hypertension and Alzheimer Disease, but have not been investigated in PLWH. Here, we investigated alterations in biomarkers of endothelial dysfunction and neuroangiogenesis in a cohort of ART naive PLWH (n=399) compared to HIV-uninfected individuals (n=47) in rural Uganda and their association with CI. Severity of CI was assigned using Frascati criteria relative to normative neuropsychological data collected from demographically matched HIV-uninfected individuals from the same geographical region. Serum was collected concurrent with neuropsychological testing and stored for analysis. Endothelial integrity (ICAM-1, CRP, SAA and VCAM-1), and angiogenesis (VEGF-A, VEGF-C, VEGF-D, Tie-2, Flt-1, PlGF, and bFGF), biomarkers were quantitatively determined using mesoscale multiplex assays. Endothelial activation markers (CRP, SAA, ICAM-1, VCAM-1) were elevated in PLWH compared to HIV-uninfected controls ($p \leq 0.001$). In PLWH with CI, CRP, ICAM-1, and SAA were increased compared to PLWH with normal cognition ($p \leq 0.04$). In multivariate analyses of PLWH, CRP, SAA, FLT1 and bFGF were positively associated with increasing severity of CI ($p \leq 0.03$). In univariate analyses, there was no association between any biomarker and CI in HIV-uninfected individuals. CRP and SAA were negatively associated with verbal learning, memory, and motor functions ($p \leq 0.04$), and ICAM-1 with motor functions ($p \leq 0.02$). Although, the angiogenesis markers were not significantly different in PLWH compared with HIV-uninfected individuals, FLT1 was negatively associated with verbal learning and gross motor function ($p \leq 0.046$), VEGF-A with motor functions ($p \leq 0.006$), and BFGF with verbal learning, memory, and motor functions ($p \leq 0.04$) in PLWH. These data suggest an association between endothelial dysfunction and CI in ART naïve PLWH in rural Uganda. Ongoing analyses will determine the impact of ART initiation on biomarkers of endothelial and angiogenesis.

P37

Chronic Escitalopram Treatment Promotes Synaptodendritic Recovery

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Despite the advent of combination antiretroviral therapy (cART), approximately 50% of seropositive individuals report some degree of clinical depression. Our previous research has shown significant alterations in dendritic complexity of medium spiny neurons in the nucleus accumbens of HIV-1 transgenic (Tg) rats; however, the effects of antidepressant treatment on dendritic complexity are unknown. The present study examined the effects of chronic escitalopram (SSRI antidepressant) on dendritic complexity of medium spiny neurons (MSN) in the nucleus accumbens. Adult HIV-1 Tg (male n=5, female n=5) and control F344/N (male n=12, female n=6) rats were treated with escitalopram (4mg/kg per day/40 days). Following sacrifice, coronal sections were taken through the nucleus accumbens, and the MSNs were ballistically labeled with the indocarbocyanine dye DiI. Confocal microscope images were analyzed to examine dendritic branching complexity using Neurolucida 360 (MBF Biosciences). Frequency distributions of spine length, spine volume, and spine head diameter were compiled and a Sholl analysis was used to quantify dendritic complexity. HIV-1 Tg animals demonstrated markedly less dendritic complexity when compared with F344/N controls, demonstrating clear HIV-1 Tg induced synaptodendritic damage. Treatment with escitalopram dramatically increased dendritic complexity in the HIV-1 Tg rat, with escitalopram-treated transgenic animals demonstrating significantly more dendritic complexity ($p<0.05$). Additionally, HIV-1 Tg animals treated with escitalopram exhibited greater frequency of both mushroom and stubby spine types. These results demonstrate therapeutic efficacy of escitalopram in repairing MSN dendritic damage in the HIV-1 Tg rat and suggest a role for escitalopram in treating HIV comorbid depression by a mechanism of synaptodendritic recovery.

P38

Modulation of TREM-1 activation in macrophages alters the development of acute seizures

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Temporal lobe epilepsy (TLE) is the most prevalent form of acquired epilepsy and also the most difficult to treat with the current anti-seizure drugs. Therefore, the development of new disease modifying therapies is important to treat and prevent seizures in high risk groups. Although the precise mechanisms that lead to epilepsy remain unclear, evidence from experimental and clinical work suggests that inflammation is an important contributor. Several viruses have been implicated in the development of TLE. Brain inflammation, induced by viral infection of the central nervous system (CNS), alters the excitatory and inhibitory balance among neurons and is a significant cause of acute seizures. We have developed an experimental model of virus-induced seizures/epilepsy, an animal model of TLE. In our model, C57BL/6J mice infected intra-cranially (i.c.) with Theiler's murine encephalomyelitis virus (TMEV) develop

encephalitis leading to acute seizures and epilepsy. We previously found that infiltrating macrophages play a key role in seizure development. We recently identified a population of infiltrating macrophages that express high levels of TREM-1 (triggering receptor expressed on myeloid cells 1). Activation of this receptor leads to the initiation and amplification of the inflammatory response, by increasing the production and secretion of inflammatory cytokines by these macrophages. We found that mice treated with TREM-1 inhibitor showed delayed seizure onset, decreased seizure severity and the CNS-infiltrating macrophages had significantly diminished activation towards an inflammatory reactive state. Our studies indicate that TREM-1 activation in macrophages is playing a central role in the development of acute seizures after viral infection of the CNS. We are continuing to explore the role of macrophages and how modulation of TREM-1 signaling affects inflammation and seizures.

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Human Pegivirus-1 is neurotropic and associated with neuroinflammation

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Viral infections are among the most commonly identified causes of encephalitis in humans. Human pegivirus-1 (HPgV-1) is a positive sense, single-stranded RNA virus that is a member of the Flaviviridae family. We recently reported HPgV-1 infection in the brains of two patients with fatal leukoencephalitis (Balcom, Doan et al., 2018). HPgV-1 antigen (NS5A) was detected chiefly in glial cells (astrocytes and oligodendrocytes) in cerebral white matter. To further investigate HPgV-1 infection of human glial cells, we transfected astrocytoma U251 cells with a molecular clone of HPgV-1 from which viral stocks were prepared. Primary human astrocyte cultures were then infected using these stocks and immunodetection of NS5A was performed 4, 7 and 14 days post-infection (PI). The mean viral spread (HPgV-1+ immunodetection/μm3) peaked at Day 7 PI and droplet digital PCR (ddPCR) analyses of astrocyte culture supernatants showed 7577, 10834 and 2905 viral RNA copies/mL at Days 4, 7 and 14 PI, respectively. Viral antigen detection in astrocytes was associated with cell lysis and cell death, evidenced by increased lactose dehydrogenase in the media and a decrease in DAPI intensity. To determine the in vivo neuroinflammatory effects of HPgV-1 infection, we identified an additional 11 (of 109) patients with HPgV-1 brain infection (700-11,000 HPgV-1 RNA copies/gm). The expression of proinflammatory cytokine genes (IL1B, TNFA, IL6) was markedly increased in the brains from patients with HPgV-1 co-infected with HIV-1 (n=6) compared to HPgV-1 mono-infected (n=5) patients. The present studies indicate that HPgV-1 is a neurotropic virus with the capacity to infect and spread in primary human glial cells. Moreover, HPgV-1 might act synergistically with HIV-1 to promote neuroinflammation in vivo. These findings highlight the importance of considering HPgV-1 as a potential cause of encephalitis in humans and exploring its associated neuroinflammation implicated in other neurological diseases.

P40

Zika Virus Infection in Chemosensory Cells

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Zika virus (ZIKV) is an emerging virus belonging to the genus Flavivirus. Neurological complications associated with Zika virus (ZIKV) infection suggest that the virus can invade to the central nervous system (CNS). ZIKV infects several human tissues and cells types in vitro and in vivo but their role in replication, transmission, and spread of ZIKV in humans is not known. Further, mechanisms of CNS entry in humans are to be further investigated. Taste and olfactory cells are chemosensory receptor cells with unique histological, molecular and physiological characteristics. We asked if taste and olfactory cells may support ZIKV replication. We first cultured and characterized human olfactory epithelial cells (hOECs) and human fungiform taste papillae (HBO) cells. Then we infected both cells with the PRVABC59 strain of ZIKV. Using qRT-PCR, we quantified ZIKV copies in both media and cells lysates. hOEC medium and lysates were positive for ZIKV RNA, while only few copies of ZIKV RNA copies were detected in HBO medium and lysates. We further confirmed ZIKV replication in hOECs using plaque assay analysis, western blot, and immunocytochemical staining for ZIKV specific proteins. Further, we demonstrated the presence of ZIKV particles in olfactory epithelium as well as in olfactory bulb but not in taste papillae of immunocompromised mouse (ifnar-/-) infected with PRVABC59 strain of ZIKV. Olfactory tissue and tongue were processed by immunohistochemistry for H&E staining and ZIKV proteins. Interestingly, majority of the ZIKV infected cells presented co-localization of olfactory marker protein (OMP) with viral NS1 and M proteins. These observations suggest that olfactory neuroepithelium and olfactory bulb may be an important tissue for ZIKV replication and perhaps the entry and dissemination site into the CNS.

P41

Converging Modulation of Protein Quality Control by H2O2 and Tat: Insights from Primary Rat Neuronal Culture

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Aging and HIV infection are associated with variations in protein quality control (PQC). Elevated H2O2 is associated with aging, while the viral trans-activator of transcription (Tat) protein contributes to HIV pathogenesis. However, it remains unclear how such conditions synergistically impact PQC. Results reveal that H2O2 decreases protein levels of BAG3, and induces a BAG3/BAG1-L switch. The presence of tat exacerbated such effects. Moreover, both H2O2 and tat decreased BAG3 transcription. Tat was also found to mitigate the increases in BAG3 and BAG1-L that occurred following lysosomal inhibition, suggesting the importance of BAG1-L mediated autophagy in aging and HIV conditions. Interestingly, both H2O2 and tat lead to decreased levels of those proteins integral for the formation of Stress Granules (SGs), such as G3BP1 and YB1. Taken together, present findings reveal converging effects of H2O2 and tat on neuronal PQC, and suggest the importance of PQC in facilitating neuronal dysfunction observed in aging and HIV infection.

P42

Stabilizing MEF-2: HDACIIa complex as a therapeutic strategy for ATLL

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Human T-cell lymphotropic virus type 1 (HTLV-1) affects millions of people worldwide and may lead to the development of adult T-cell leukemia/lymphoma (ATLL). While there is currently no treatment for ATLL, the mechanism of the disease and virus is being characterized. Earlier studies from our lab delineated the role of MEF-2A in increased viral gene expression and inhibition of MEF-2A lead to the reduction of in the HTLV-1viral replication and associated T-cell transformation. In recent studies, we have

identified a novel role for Myocyte Enhancer Factor-2 (MEF-2) in HTLV-1 gene expression and ATL development involving two key viral proteins Tax and HBZ. Now we wish to optimize a unique strategy to curb MEF-2 activity. MEF-2 is held transcriptionally silent via histone deacetylases (HDACs) that maintain chromatin in a condensed hypoacetylated state. A particular Class IIa inhibitor MC1568, which has a unique mode of action in stabilizing MEF-2: HDACIIa complex and enhancing their interactions. This process keeps MEF-2 tethered to the HDAC4-HDAC3 inhibitory complex and blocks its transcriptional activity. We analyzed the toxicity profile of the MC1568, via deriving the IC50 curves of various ATLL cell lines with activated T-cell as uninfected control and we noticed selective cell death only in ATLL cell lines, but no toxicity was noticed in activated T-cell controls. The rt-qPCR showed the upregulation of HDAC-9 expression in all ATLL cell-lines with the respective IC50 treatment suggesting repression via HDAC9 mediated complex.MC1568 works in an unconventional manner suppressing MEF-2 hypothetically via HDAC9 mediated inhibition and this accumulation of inert MEF-2 complexes, results in cytotoxic stress of infected cells and triggers the autophagosome. The autophagosome was confirmed by the dose-dependent accumulation of LC-3(autophagosomal marker) via protein expression and confocal microscopy and dose-dependent downmodulation of TAX and HBZ. Currently, a more in-depth mechanistic analysis of this phenomenon is under-investigation.

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Shock or Lock- Astrocyte HIV-1 reservoirs are still a threat to CNS health with altered functional and gene expression profiles

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Significant number of people living with human immunodeficiency virus-1 (HIV-1) (PLWH) suffer from HIV associated neurocognitive disorders (HAND). Central nervous system (CNS) HIV-1 reservoirs are challenging to address due to low penetration of antiretroviral drugs, lack of resident T cells, and the virus resides indefinitely in macrophages and astrocytes where the viral infection is non-cytopathic. The relevance of astrocytes to HIV-associated neuropathogenesis has been demonstrated by several previous studies. However, these studies were unable to differentiate the state of infection, i.e. active or latent, or to evaluate how this affects astrocyte biology. In this study a pseudotyped doubly labelled fluorescent reporter R/G-HIV-1 was used to identify and enrich silent and active populations of HIV+ astrocytes based on the viral promoter activity. Here we report, majority of human astrocytes were latently infected by R/G-HIV-1 early during infection and they resist reactivation by latency reactivating agents (LRAs). However, actively infected astrocytes were inducible and produce excessive amounts of neurotoxic viral proteins upon reactivation. R/G-HIV-1 infection also significantly decreased cell proliferation and glutamate clearance ability of astrocytes, which may lead to excitotoxicity. Moreover, transcriptome analyses to compare gene expression patterns of astrocyte harboring active vs silent LTRs revealed that the gene expression patterns were similar, and that the active population demonstrated more widespread and robust changes. Our data suggests that both active and latent HIV-1 infection profoundly alter astrocyte biology and strategies such as block and lock that keeps the virus latent or shock and kill therapy that reactivates the latent virus are both detrimental to astrocyte health, there by CNS homeostasis, and further heighten the burden of HAND.

P44

Reduced Dopamine and Homovanillic in Cerebrospinal Fluid in depressed People with HIV infection

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Background. Abnormalities in dopaminergic neurotransmission have been recognized recently to contribute to depression. Such abnormalities also are common in people with HIV infection (PWH), and depressive symptoms in HIV are highly prevalent and difficult to treat. We evaluated levels of dopamine (DA) and its metabolite, homovanillic acid (HVA), in cerebrospinal fluid (CSF) in PWH and HIV uninfected (HIV-) individuals in relation to depressive symptomatology.
Methods. Severity of current depression was classified using the Beck Depression Inventory-II (BDI) as minimal (BDI<14), mild (BDI 14-19) or moderate/severe (BDI>19). DA and HVA levels were measured using high performance liquid chromatography in pg/ml.
Results. Participants were 103 HIV- and 110 HIV+ individuals, mean age 42, 90% men. Lifetime MDD was present in 50% of HIV- and 65% of HIV+ ($p=0.02$). For HIV-, current depression severity was 67% minimal, 17% mild, 17% moderate/severe; and for HIV+ was 58% minimal, 17% mild, 26% moderate/severe. HIV interacted with BDI severity such that DA ($p<0.01$) and HVA levels ($p<0.05$) were lower in PWH with moderate/severe depressive symptoms (Cohen's ds>0.47), but not in HIV- (Cohen's ds<0.40).
Conclusions. Results suggest that the pathophysiology of depression in PWH differs from that in HIV- individuals. With the rise in consideration of dopamine agonists for the treatment of depression, these results suggest that PWH may show greater response to these agents than HIV-.

P45

Cross reactivity of varicella zoster virus monoclonal antibodies with simian varicella virus antigens provide novel reagents for studies of varicella pathogenesis

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Similar to varicella zoster virus (VZV) infection in humans, simian varicella virus (SVV) causes varicella (chickenpox), establishes a latent infection in ganglionic neurons and reactivates to produce zoster (shingles). While SVV pathogenesis, latency and reactivation has been widely studied in non-human primates (NHP), it has been hampered by the lack of availability of virus-specific reagents. VZV and SVV genomes share extensive similarity and code for antigens that have a high degree of homology. Recently, a panel of VZV-specific monoclonal antibodies have been generated and some of these antibodies cross react with SVV antigens in Western blot analysis. Herein, we tested one of the SVV-reactive monoclonal antibodies specific for VZV open reading frame (ORF) 5, which codes for glycoprotein K (gK), in flow cytometry by intracellular staining of SVV infected cells in culture. SVV gK shares 60% homology to its VZV homologue. In SVV-infected Vero cells, approximately 16% of cells were found to express ORF 5 antigen, with a background levels of 2%, in uninfected cells. We are currently examining blood mononuclear cells (MNCs) from NHP infected in culture as well as during varicella. Our findings show for the first time that SVV-specific immune responses can potentially be studied after primary infection and reactivation using VZV monoclonals in this unique animal model of VZV infection.

P46

The differential apoptotic capacity of closely related paralytic and non-paralytic strains of Enterovirus D68.

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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 points to EV-D68 as a major causative agent of recent biannual (2014, 2016, 2018) seasonal AFM outbreaks in the US. Studies in our lab and others have demonstrated that, in contrast to the original prototype strains of EV-D68 (Fermon, Rhyne), many EV-D68 strains isolated from patients in 2014 cause paralytic disease in mice, suggesting that the neuroinvasive phenotype is a recently evolved trait of EV-D68. Two clade B2 EV-D68 strains: IL/14-18952 (IL-52) and CA/14-4231 (CA-31) share 98% genome sequence homology and contain only 11 coding differences between them, yet have drastically different phenotypes in infected mice. The IL-52 strain of EV-D68 effectively replicates in muscle and spinal cord tissue of C57BL6/N neonatal mice, causes severe paralysis associated with death of motor neurons, and induces activation of the apoptotic marker, caspase-3. In contrast, the CA-31 strain replicates in muscle but does not spread to spinal cord or induce paralysis in infected mice. Furthermore, infection with CA-31, in contrast to that of IL-52, does not result in activation of caspase-3. These differences in dissemination and apoptotic activation between closely related EV-D68 strains provide important insights into our understanding of the novel neuroinvasive phenotype of contemporary EV-D68 strains.

P47

Repurposing Agents Results in Selective Death Induction of HIV-1 Infected Myeloid Reservoirs

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Background: Existing antiretroviral therapy (ART) cannot efficiently eliminate HIV-1 within the CNS. HIV-1 persistence in myeloid sanctuaries represents a major barrier to eradication, and drives HIV-1 associated neurocognitive dysfunction (HAND), which occurs in up to half of HIV-infected individuals even with well-controlled viremia. Safe, specific agents that selectively eliminate key cells harboring the myeloid reservoir are urgently needed. Our group has identified two safe, FDA approved agents rufinamide and bergenin (non-HIV-1 indication) that demonstrate selectivity for killing of only HIV-infected macrophages and microglia.
Methods: Cell isolation: Primary human monocytes were isolated from healthy donors; macrophages differentiated with GM-CSF; microglia were differentiated with GM-CSF/IL-34-based cocktail. Infections: Macrophages or microglia were infected with HIV-189.6 (MOI 0.5) for 72 hr in the presence of 0.01-10 ¬μM rufinamide or bergenin, HIV-1 alone, or HIV+VPX (positive control). Effect on HIV-1 infection and cell death was quantified by FACS (p24+/live/dead cells). HIV-1 acceleration was quantified with RT-PCR for 2-LTR circles and HIV RNA. Effect on SAMHD1/pSAMHD1 was quantified (western blot).
Results: Rufinamide and bergenin do not kill uninfected macrophages or microglia. Both agents demonstrate selectivity for killing HIV-1-infected macrophages/microglia, and significantly accelerate HIV-1 replication; agents do not alter SAMHD1/pSAMHD1 levels.
Conclusions: Rufinamide and bergenin demonstrate selectivity for killing only HIV-1 infected macrophages and microglia, and are not toxic to uninfected cells. Agents accelerate HIV-1 replication in macrophages/microglia, implying acceleration results in selective cell death of infected myeloid cells. Acceleration of replication is SAMHD1-independent. Bergenin and rufinamide demonstrate selectivity towards killing of only HIV-1-infected macrophages and microglia, warranting further mechanistic studies, and eventual studies in humans, to evaluate the use of these agents towards elimination of myeloid derived viral sanctuaries systemically and within the CNS.

P48

Plasma Extracellular Vesicles (EVs) Carrying CYP Enzymes and Their Implications in CNS Toxicity

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Cytochrome P450 (CYP) enzymes metabolize the majority of xenobiotics to their soluble forms. Although primarily found in hepatocytes, CYP enzymes are also expressed in extrahepatic tissues, e.g. brain cells and peripheral macrophages. Recently, we have shown that several CYP enzymes, including CYPs 1B1, 2A6, 2E1, and 3A4, are packaged in extracellular vesicles (EVs) derived from the plasma of healthy subjects; moreover, our study revealed that CYP2E1 is packaged more abundantly than the other isoforms tested. We also demonstrated that EV CYP2E1 is metabolically active. EVs are nanosized (<200nm), membrane-bound carriers of biological material (e.g. proteins, nucleic acids, etc.) that are involved in intracellular communication and are studied as potential therapeutic targets. CYP2E1 is involved in alcohol and acetaminophen metabolism; CYPs 2A6 and 1B1 metabolize tobacco constituents. These CYP-mediated pathways produce toxic intermediates and ROS, which contribute to oxidative stress and cytotoxicity. Our recent study found that plasma EVs synergistically increase alcohol and acetaminophen-induced toxicity upon exposure to naïve cells, via a CYP2E1 pathway. Further, unpublished data suggests that circulating plasma EVs can cross the blood-brain-barrier (BBB), deliver CYP2E1 to macrophages and microglia, and exacerbate alcohol-induced toxicity across the BBB. Since alcohol and tobacco-induced oxidative stress can impact CNS cells, and CYP-mediated oxidative stress contributes to enhanced HIV replication in vitro, it is reasonable to suggest that EVs carrying CYPs are released from the liver, circulate via plasma, and exacerbate toxicity and HIV-1 pathogenesis in CNS cells. We are currently investigating the contribution of alcohol-induced EV CYP2E1 across the BBB to alcohol-mediated HIV-1 disease progression and HIV-associated neurocognitive diseases (HAND), which could help develop new treatment strategies for people living with HIV who drink alcohol.

P49

Protein Quality Control-mediated Nuclear Homeostasis in Primary Cardiomyocytes

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Genotoxic insults causing DNA damage lead to activation of DNA damage response (DDR) which either by promoting repair of damaged DNA or enhancing turnover of components involved in DNA repair ameliorate cell survival. However, when genotoxicity is beyond repair, DDR directs cells toward programmed cell death to inhibit damaged cell expansion. Accumulation of unrepaired DNA as a result of DDR dysfunction has been implicated in the pathogenesis of failing hearts. In this regard, the role of protein quality control (PQC) machinery as one of the major tools

for cellular homeostasis in controlling trafficking of damaged DNA as well as DNA repair pathways remains largely unclear. Among various molecular players involved in PQC, Bcl2-associated athanogene 3 (BAG3) by association with heat shock protein 70 (HSP70) has been demonstrated to target accumulated aggregates for further degradation and removal through autophagy process especially in the myocardium. Mutations of BAG3 have been implicated in pathogenesis of various cardiovascular complications including dilated cardiomyopathy. However, the importance of BAG3 in maintaining homeostasis of nuclear components and key underlying molecular players remain to be demonstrated. In this study, we have investigated the impact of BAG3-mediated PQC and the potential underlying signaling cascade in homeostasis of nuclear components under normal and proteotoxic stress condition in primary cardiomyocytes. Data indicated that BAG3 suppression led to significant accumulation of ubiquitinated aggregated proteins including damaged DNA in cardiomyocytes and under proteotoxic stress condition, damaged DNA were transported from nucleus to cytoplasm and removed via BAG3-mediated PQC. Results from this study suggest that fine-tuning the BAG3-mediated PQC might be a promising tool to modulate cardiac response under pathological condition.

P50

Role of Bcl2-associated Athanogene 3 in Turnover of Gap Junction Protein, Connexin 43, and Electrophysiological Activity of Primary Culture of Neonatal Cardiomyocytes

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Connexin 43 (Cx43) is the most expressed gap junction protein in ventricular cardiomyocytes and plays a critical role in myocardial cell-to-cell coupling and electrical conduction. Pathological stress which dysregulate Cx43 expression, turnover and post-translational modification can adversely impact myocardial coupling, thus contributing toward development of cardiac arrhythmias and heart failure. Among various molecular regulators of cardiac protein quality control, Bcl2-associated athanogene 3 (BAG3) is a stress-induced pleiotropic protein which targets aggregated proteins and damaged organelles for degradation and removal via autophagy-lysosome pathway. BAG3-mediated protein quality control is essential for cardiac homeostasis; such that mutations of BAG3 have been implicated in the pathogenesis of various cardiovascular disorders including dilated cardiomyopathy. In this study we have investigated turnover of Cx43 in primary neonatal rat ventricular cardiomyocytes (NRVCs) and found that impairment of autophagy-lysosome pathway, either by inhibiting lysosomal activity or suppressing the level of BAG3, dysregulates turnover of Cx43. Results show that pharmacological inhibition of lysosomal activity leads to accumulation of Cx43 aggregates and BAG3 knock-down results in significant downregulation of Cx43 turnover and protein stability. Overall, these observations ascribe a novel function for BAG3 indicating that BAG3 through interactions with cytoskeleton protein, tubulin, regulates intracellular trafficking and lysosomal-mediated turnover of Cx43 and potentially impacts communication of cardiac muscle cells through gap junctions. We further investigated the impact of BAG3 on electrophysiological activity of NRVCs by employing microelectrode array (MEA) technology. Data indicated that BAG3 suppression led to impaired electrical signal generation as well as signal propagation throughout the in vitro culture of cardiomyoctres. Overall, by providing more in-depth analyses and characterization of electrophysiological parameters, this study reveals that BAG3 is an essential regulator of electrical activity of neonatal cardiomyocytes.

P51

Apigenin modulates dendritic cell activities and restraint inflammation via RelB inhibition in the context of neuroinflammatory diseases

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Natural flavonoids are a class of polyphenolic compounds, ubiquitously present in plants, fruits, and vegetables, have a range of biological activities including antioxidant, anti-carcinogenic, and anti-inflammatory effects. Apigenin, one such flavonoid abundantly present in celery, parsley, and chamomile has been used for centuries to treat Parkinson's, neuralgia, shingles, and other diseases. Due to its lower intrinsic toxicity, Apigenin has gained lots of interest as a potential therapeutic agent to treat various neurological disorders such as multiple sclerosis (MS). As an autoimmune disorder, multiple immune cell types contribute to the pro-inflammatory environment by the secretion of cytokines. Dendritic cells (DC)s mediate T cell polarization and activation into different subsets, specifically Th1 and Th17 cells, the main effectors of this disease. In order to investigate the effect of Apigenin in regulating immune response, we tested and found that, Apigenin possibly exerts its effects through shifting the DC modulated T-cell responses from Th1 and Th17 type towards Th2 and Treg directed responses evident through the decrease in T-bet, IFN-gamma (Th1), IL-17 (Th17) and increase in IL-4 (Th2), IL-10, TGF-beta, and FoxP3 (Treg) expression. RelB, an NF-kappaB pathway protein, is central to DC maturation, its antigen presentation capabilities, and DC-mediated T cell activation. Apigenin reduced cytoplasmic levels of RelB and also RNA and protein levels of its downstream targets TNF-alpha, CD40, and IL-23 in these DCs. These results provide key information that Apigenin by regulating DC activity controls the molecular events and marking its potential therapy for the neuroinflammatory disease.

P52

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

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To date, the lack of a suitable small animal model has hindered our understanding of Human T-cell lymphotropic virus (HTLV)-1 chronic infection and associated neuropathogenesis defined as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The host immune response plays a critical role in the outcome of HTLV-1 infection, which could be better tested in the context of humanized (hu) mice as tested in the present study. we employed Balb/c-Rag1-/-gammac-/- or Rag1 and Bone Marrow-Liver-Thymic (BLT) mouse models for engraftment of human CD34+ hematopoietic stem cells. Flow cytometry and histological analyses confirmed reconstitution of Rag1 and BLT mice with human immune cells. Following HTLV-1 infection, proviral load (PVL) was detected in the blood of Rag-1 and BLT hu-mice as early as 2 weeks post-infection (wpi) with sustained elevation in the subsequent weeks followed by Tax expression. Additionally, infection was compared between adult and neonatal Rag1 mice with both PVL and Tax expression considerably higher in the adult Rag1 mice as compared to the neonates. Establishment of peripheral infection led to lymphocytic infiltration with concomitant Tax expression and resulting myelin disruption within the central nervous system of infected mice. In addition, up-regulation in the expression of several immune checkpoint mediators such as programmed cell death-1 (PD-1), T-cell Ig and ITIM domain (TIGIT), and T cell Ig and mucin domain-3 protein (Tim-3) were observed on CD8+ T cells in

various organs including the CNS of infected hu-mice. Collectively, these studies represent the first attempt to establish HTLV-1 neuropathogenesis in the context of Rag-1 and BLT hu-mice as potential novel tools for understanding HTLV-1 neuropathogenesis and testing of novel therapies such as immune checkpoint blockade in the amelioration of chronic HTLV-1 infection.

P53

Novel elvitegravir nanoformulation for drug delivery across the blood-brain barrier to achieve HIV-1 suppression in the CNS

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Over the last two decades, the use of antiretroviral therapy (ART) has remarkably decreased the morbidity associated with HIV-1 infection. However, the prevalence of HIV-1-associated neurocognitive disorders (HAND) is still increasing. The blood-brain barrier (BBB) is the major impediment for penetration of antiretroviral drugs, causing therapeutics to reach only suboptimal level after systemic administration. To solve this problem, we developed an innovative nanoparticle-based delivery strategy which can improve the virus suppression in the CNS reservoir macrophages and microglia. Poly(lactic-co-glycolic acid) (PLGA)-based elvitegravir nanoparticles (PLGA-EVG NPs) were prepared by nano-precipitation technique. The penetration of EVG native drug /nanoformulation was determined using an in vitro BBB model and validated in mice. The efficacy of HIV-1 suppression in the CNS was assessed by exposing EVG native drug/nanoformulation in HIV-1-infected human monocyte-derived macrophages/microglia in the in vitro BBB model. The PLGA-EVG NPs showed particle size of ~47 nm from transmission electron microscopy and zeta potential of ~ -6.74 mV from dynamic light scattering. The results showed ~25% of PLGA-EVG NPs can cross the in vitro BBB after 24 hours, and also showed a significant increase in penetration of PLGA-EVG NPs compared to EVG native drug at the dose of 5 μg/mL and 10 μg/mL. In the mouse model, brain EVG concentration was found to be ~3-fold higher in PLGA-EVG NPs compared with the native drug. Most importantly, the PLGA-EVG NPs were able to show an enhanced HIV-1 suppression in HIV-1-infected human monocyte-derived macrophages and monocyte-derived microglia after crossing the BBB without altering the BBB integrity. Compared with EVG native drug, our EVG nanoformulation demonstrated an improved BBB penetration and HIV-1 suppression both in vitro and in vivo systems. Overall, this is an innovative and optimized treatment strategy that has a potential for therapeutic interventions in reducing HAND.

P54

HIV anti-retrovirals skew monocyte-derived macrophage phenotype and function

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The introduction of combination antiretroviral therapy (cART) has transformed HIV-1 diagnosis from a death sentence to a manageable chronic disease. People living with HIV (PLWH) are now living longer but their quality of life is significantly impacted by co-morbidities. One such being HIV-induced neurocognitive disorders (HAND). HAND symptoms include impairments in learning, concentration and motor function that can impact daily life. Although these symptoms are largely attributed to HIV-induced neuroinflammation, emerging evidence highlights drug-induced toxicities as a contributor of HAND. In the central nervous system (CNS), macrophages and microglia play an important role in maintaining homeostasis; namely through phagocytosis of dead cells or debris, repair of damaged tissue, and regulation of the neuroimmune response to danger signals. Macrophage phenotype is fine-tuned by signals in their micro-environment, resulting in a myriad of macrophage subtypes ranging from inflammatory to anti-inflammatory. We assessed here the impact of antiretrovirals on macrophage phenotype and functions. Primary human monocyte-derived macrophages (MDMs) were treated with the two most commonly prescribed cART regimens worldwide, Atripla and Triumeq. Both drugs induced the frequency of CD4+ CCR5+ and CD4+ CXCR4+ expressing undifferentiated (M-MDMs) and alternatively-differentiated (M2a-MDMs) by 2-fold; they also reduced phagocytic capacity and induced reactive oxygen species (ROS) by 10%-20% and 1.5-fold, respectively, in M2a-MDMs. In addition, Triumeq induced apoptosis in M-MDMs and M2a-MDMs by 2%-4%, reduced TNF-alpha secretion from M-MDMs by a third, increased VEGF 5-fold, and increased IP-10 secretion from M2a-MDMs 2-fold relative to vehicle treated MDMs. These studies demonstrate that ARVs, independent of HIV, impact MDMs survivability, function and phenotype.

P55

Role of bTRM in controlling viral reactivation

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Background: MCMV brain infection elicits neuroimmune responses that terminate acute infection. We have previously demonstrated that a subset of neurons harbor latent virus and reactivated virus can be grown out of explant cultures. While CD8+ T-cells are critical in controlling acute infection, immunological factors which regulate viral reactivation remain to be elucidated. Here, we investigated the role of brain resident-memory T-cells (bTRM) in controlling recrudescence within brain using murine-model of latent MCMV infection.
Methods: Using RNAscope, we probed IE1 nucleic acid within brains of MCMV-infected animals at D5 and D30 p.i. Using RT-PCR, we assessed expression of IE1, E1, and gB transcripts. Using flow cytometry, we assessed depletion of CD8+ and CD103+ T-cell following intracerebroventricular injection of either alpha-CD8 Ab (65 μg) or alpha-CD103-sap (2 μg) into latently-infected brain. Using luciferase-expressing transgenic mice [FVB.129S6(B6)-Gt(ROSA)26Sortm1(Luc)Kael/J], we longitudinally assessed reactivation of cre-MCMV after CD8+ or CD103+ T-cell depletion using IVIS100.
Results: MCMV caused acute brain infection, as demonstrated by X-gal staining of beta-gal-expressing virus, and IE1 staining using RNAscope at 5 d p.i. After 30 d p.i., MCMV established latent infection, as indicated by positive RNAscope staining for viral nucleic acid despite an absence of viral transcripts (IE1, E1, and gB) detectable using RT-PCR. After establishment of latency, we injected either depleting alpha-CD8 Ab or alpha-CD103-sap into the brain and showed a 90-95% T-cell depletion. Further, we employed FVB mice that express luciferase under the ROSA26 promoter, but have a stop signal flanked by LoxP sites. We infected these animals with cre-MCMV that express cre-recombinase under control of IE1 promoter. In these studies, we observed enhanced imaging signals indicative of viral reactivation in animals that were depleted of CD8+ or CD103+ T-cells.
Conclusion: MCMV persists in a latent state and undergoes intermittent viral reactivation that is quickly quelled by ongoing TRM responses.

P56

Utilizing broad-spectrum guide RNAs to purge latent HIV-1-infected cells

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The latent viral reservoir of HIV-1 provides a major challenge for efforts toward a cure. Genomic editing with the CRISPR/Cas9 system holds promise to permanently excise or inactivate integrated provirus. To this end, broad spectrum gRNAs were designed by isolating patient PBMCs and deep sequencing their LTRs. This resulted in the development of broad-spectrum gRNAs named SMRT1 to 10. Based on an in silico prediction algorithm, it was demonstrated that our broad-spectrum gRNAs were predicted to cleave 100 percent of patient-derived LTR samples. In TZM-bl cells, P4R5 cells, and the molecular clone pLAI, SMRT gRNAs were shown through beta-gal expression to be effective individually and in combination, even more so than previously established gRNAs, while keeping cell viability high. We have previously performed a number of approaches to measure gRNA effectiveness that include flow cytometry, beta galactosidase, and fluorescent microscopy. These results have demonstrated that the gRNAs possess broad-spectrum cleavage activity and could contribute to HIV-1 treatment strategies or possibly even a cure at some in the future. Our next steps will be to continue testing our SMRT gRNAs in increasingly physiologically relevant cells, individually and in combination, and to identify what factors may augment or inhibit the effectiveness of our HIV-1 treatment strategy. Additionally, to increase the rigor of our techniques, we will utilize more quantitative techniques such as qRT-PCR to measure intracellular and extracellular HIV-1 mRNA levels during the course of therapeutic intervention using CRISPR technologies.

P57

Role of extracellular vesicles upon exposure to cigarette smoke condensate in HIV replication and CNS toxicity

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Smoking is known to exacerbate HIV pathogenesis and neurotoxicity, especially in monocytes and macrophages, likely through the cytochrome p450 (CYP)-mediated oxidative stress pathway. Extracellular vesicles (EVs) have been shown to alter HIV pathogenesis through inter-cellular communication. However, the exact role of EVs in smoking-mediated HIV pathogenesis and CNS toxicity is unknown. In this study, we investigated the effect of cigarette smoke condensate (CSC) on the characteristics of monocyte and macrophage-derived EVs and their influence on HIV replication and neurotoxicity. First, we characterized the EVs in terms of physical properties such as size and zeta potential, marker proteins and transmission electron microscopy. Next, we demonstrated that CSC reduced total protein and antioxidant enzyme (AOE) levels in EVs from HIV-infected and uninfected macrophages. Interestingly, the EVs from CSC-treated uninfected cells exert protection from cytotoxicity and viral replication in HIV-infected macrophages. However, EVs from HIV-infected cells lost their protective capacity. To observe the effect of smoking on CNS toxicity by EVs, we exposed CSC treated HIV-uninfected macrophage-derived EVs to microglia cells. We found that CSC-exposed EVs have significantly higher toxicity compared to CSC treatment. However, the toxicity decreased when EVs and CSC were treated together. In future, we will determine the role of HIV-infected macrophage-derived EVs on microglia. The results so far suggest that EV-mediated defense is more pronounced during the early stages of HIV-infection, which diminishes at a latter phase. Furthermore, EVs from uninfected cells demonstrated a CSC-mediated upregulation of catalase, with a decrease in the levels of catalase and PRDX6 in EVs derived from HIV-infected cells. These results suggest a potential role of AOEs, which are differentially packaged into CSC-exposed HIV-infected and uninfected cell-derived exosomes, on HIV replication in recipient cells. Overall, our study suggests a novel role of EVs in tobacco-mediated HIV pathogenesis and CNS toxicity.

P58

Beta-catenin inhibits ZIKV early infection of Human Fetal Astrocytes

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In the last decade there were several large outbreaks of Zika Virus (ZIKV) infection in the Americas. In newborns ZIKV is associated with significant neurologic abnormalities including microcephaly. ZIKV infects neuronal progenitor cells as well as human astrocytes. We assessed here the impact of beta-catenin on ZIKV infection of human fetal astrocytes (HFAs). Beta-catenin is robustly expressed in HFAs and functions as a transcriptional co-activator and/or is associated with adherens junctions to support cell-to-cell communication. HFAs were infected at 0.5 MOI with three different strains of ZIKV, the Puerto Rican strain 2015 PRVABC59; the Columbian strain 2015 FLR; and the Ugandan strain 1947 MR766. Induction of beta-catenin was established through treating HFAs with a pharmacological reagent BIO or transfection with a constitutively active beta-catenin construct. ZIKV level was determine by RT-PCR and/or RNA scope. Activation of beta-catenin in HFAs reduced ZIKV viral entry by 50% at five hours post infection, as determined by RT-PCR and reduced number of infected cells by 5-fold as determined by RNAscope Further, activation of beta-catenin reduced the expression of key proteins for ZIKV entry into astrocytes, Tyro3 and AXL, by 50%. These data indicate that beta-catenin inhibits early steps in ZIKV infection likely through downregulation of the attachment receptors Tyro3 and AXL and suggest that disruption of beta-catenin may facilitate higher level of ZIKV infection of HFAs.

P59

Chronic dengue virus encephalitis in a patient with progressive dementia with extrapyramidal features

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Dengue virus is the most common arbovirus worldwide and represents a significant public health concern. Infections with dengue virus are usually self-limiting and chronic dengue infections have not been previously reported. While investigating the etiology of central nervous system disease in a patient with progressive dementia with extrapyramidal features, we elucidated a chronic dengue infection within the central nervous system. Viral immune responses in both serum and cerebrospinal fluid were profiled by VirScan, a phage-display assay, and enrichment of dengue viral antibodies were detected in cerebrospinal fluid as compared to serum. While no virus was detected in serum or cerebrospinal fluid, post-mortem analysis confirmed dengue virus in the brain by quantitative polymerase chain reaction, immunohistochemistry, in situ hybridization by RNAscope and viral genome sequencing. Dengue virus was also detectable by polymerase chain reaction and sequencing from brain biopsy tissue collected 33 months ante-mortem, confirming a chronic infection despite a robust immune response directed against the virus. Immunoprofiling and whole exome sequencing of the patient did not reveal any immunodeficiency and sequencing of the virus demonstrated wild-type dengue virus in the central nervous system. Our findings suggest that dengue virus infections may persist in the central nervous system and should be considered in patients with progressive dementia with extrapyramidal features in endemic regions or with relevant travel history. Further, this work highlights the utility of comprehensive antibody profiling assays to aid in the diagnosis of encephalitis of unknown etiologies.

P60

Antisense Oligonucleotides target HIV mRNA to inhibit expression of viral transcripts

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HIV remains a chronic infection with potential long-term deleterious consequences that include chronic inflammation and neurocognitive impairment. Evidence suggests that ongoing low-level viral replication and/or production of specific viral proteins in the presence of antiretroviral therapy (ART) play a role. There is a driving need in HIV research to control sources of residual productive or partial viral replication and to provide alternative therapies that fill the gaps left by currently available treatments. The HIV Trans-Activator of Transcription (Tat) is one of the earliest viral proteins made during HIV expression. Tat is a neurotoxic and pro-inflammatory viral protein that is also essential for productive viral replication, establishment of the infection, and viral reactivation. Tat is not directly targeted by current therapies and may be produced even in the presence of ART. We sought to inhibit Tat production at the mRNA level by using antisense oligonucleotides (ASO) that were complementary to the viral tat (ASO-1) and env (ASO-2) transcripts. ASO-1 and ASO-2 significantly decreased tat mRNA levels and total viral RNA (vRNA) in HEK293T cells. Intracellular p24 and gp120 proteins were reduced to nearly undetectable levels in ASO-transfected HEK293T at 48 hours. Next, we evaluated the effect of these ASO on HIV replication in the context of an acute infection. Anti-HIV ASO were readily taken up by peripheral blood mononuclear cells (PBMCs) and persisted intracellularly for >30 days. A single 100 nM dose of either ASO-1 or ASO-2 inhibited expression of tat and total vRNA in infected PBMCs by >70% at 10 days post-treatment, and this effect was further enhanced up to 98% by dual administration of both ASO-1 and ASO-2. These studies indicate that targeting HIV replication at the translational level may be a beneficial addition to existing therapies which may not prevent production of viral regulatory proteins such as Tat.

P61

Involvement of the Cortical Cholinergic Receptor System in Symptoms of Cognitive Aging with HIV

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As of the year 2016, an estimated 50% of the United States' population living with the Human Immunodeficiency Virus (HIV-1) is at least 50 years or older, due to the success of Anti-Retroviral Therapy (ART) steadily increasing the life-expectancy of those living with the disease. Long-term survival with HIV interacts with numerous treatment and lifestyle factors over the lifespan which may increase the risk of developing HIV-Associated Neurocognitive Disorders (HAND), a collection of cognitive impairments involving memory, attention, executive functioning deficits, often in spite of successful ART keeping peripheral viral markers controlled or undetectable. This pattern of cognitive symptoms in HAND closely overlaps those seen in the process of Cognitive Aging in seronegative healthy older adults, which is associated with the decrease in integrity of the Neuronal Acetylcholinergic Receptor System. To understand the degree to which these separate processes interact, we investigated the relative contribution of acetylcholinergic system tone to the cognitive phenotype of HAND, using a comprehensive cognitive test battery after administration of a double-blinded, placebo-controlled anti-cholinergic drug challenge. We found that both the nicotinic and muscarinic agonists showed heightened cognitive effects on the HIV-positive participants, relative to the HIV-negative participants, particularly on measures of speed, attention, and memory functioning. The differential responsivity to cholinergic antagonists between serostatus groups indicates a relative deficit of cortical cholinergic system functioning in the HIV-positive brain, and for these cognitive domains, is consistent with an accentuated pattern of cognitive aging, subsequent to HIV-diagnosis.

P62

Two-pore channels and Tat endolysosome escape

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Tat is essential for HIV-1 replication and plays an important role in the HIV-1 associated neurological complications. Secreted Tat enters cells via receptor-mediated endocytosis. Upon endocytosis, Tat is internalized into endolysosomes, from which it must be released into the cytoplasm before entering the nucleus to facilitate HIV-1 viral replication. However, the underlying mechanism whereby Tat escapes endolysosome remains unclear. Accordingly, we investigate the underlying mechanisms whereby Tat escapes endolysosomes and subsequent HIV-1 LTR transactivation. In U87MG cells stably integrated with LTR luciferase reporter, we demonstrated that Tat-mediated LTR transactivation requires the lysosomotropic agent chloroquine. Using endolysosome leakage assay, we demonstrated that chloroquine exerts endolysosome penetrating effects. Using a split-GFP Tat endolysosome escape assay, we demonstrated that chloroquine enhanced Tat endolysosome escape. Furthermore, we demonstrated that chelating endolysosome calcium with high affinity rhodamine-dextran or chelating cytosolic calcium with BAPTA-AM attenuated Tat endolysosome escape and LTR transactivation. Significantly, we demonstrated that pharmacological blocking and knocking down the endolysosome resident two-pore channels (TPCs) attenuated Tat

endolysosome escape and LTR transactivation, and this effect is selective as knocking down TRPML1 was without effect. Our findings suggest that calcium released from TPCs are involved in Tat endolysosome escape and subsequent LTR transactivation. TPCs represent a novel therapeutics target against HIV-1 latency and HIV-1 induced CNS dysfunction. (Supported by R01MH119000, R01MH100972, and R01MH105329)

P63

Endolysosome iron restricts Tat-mediated HIV-1 LTR transactivation by increasing HIV-1 Tat oligomerization and beta-catenin expression

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HIV-1 transactivator of transcription (Tat) protein is required for HIV-1 replication and has been implicated in the pathogenesis of HIV-1 associated neurocognitive disorder (HAND). HIV-1 Tat enters cells via receptor-mediated endocytosis and following its internalization into endolysosomes HIV-1 Tat can reside inside endolysosomes or following its escape from endolysosomes it can enter the nucleus where it activates the HIV-1 LTR promoter. HIV-1 replication is affected by the iron status of people living with HIV-1. However, very little is known about how iron affects Tat activation of the HIV-1 LTR promoter. Here we determined the extent to which endolysosome iron affects Tat-induced HIV-1 LTR transactivation in part because HIV-1 proteins can de-acidify endolysosomes and endolysosome de-acidification can affect levels of iron in endolysosomes, cytoplasm and mitochondria. We found that ferric (Fe3+) and ferrous (Fe2+) iron restricted Tat-mediated HIV-1 LTR transactivation. In comparison, chelation of endolysosome iron with deferoxamine (DFO) or 2-2 bipyridyl, but not cytosolic iron with deferiprone or deferasirox, enhanced Tat-mediated HIV-1 LTR transactivation. Tat oligomerized in the presence of iron and DFO prevented the oligomerization. DFO also reduced protein expression levels of the HIV-1 restriction agent beta-catenin in the cytosol and nucleus. These findings suggest that DFO increases HIV-1 LTR transactivation by increasing monomeric Tat, increasing secretion of monomeric Tat, and/or reducing beta-catenin protein expression levels. Thus, intracellular iron might play a significant role in regulating HIV-1 replication and thereby alter the effectiveness of therapeutic strategies. (Supported by MH100972, MH105329, MH119000, DA032444)

P64

Extracellular Vesicles and Viruses: Who Comes Out First?

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In recent years, we have been able to separate Extracellular Vesicles (EVs) from viruses using HIV-1, HTLV-1, Rift and Ebola infections (1-4). It is not clear to date whether there is a timing difference between EV and virus release from infected cells. We recently attempted to address the kinetics of EV and virus release from multiple infected cells using serum starvation experiments from infected (100%) cells. Both supernatants and cell pellets were collected at 0, 3, 6, 12, and 24 hours and processed for presence of EVs and viral proteins as well as RNA gene expression. Results from supernatants of uninfected cells showed a peak of tetraspanin proteins CD63, CD81, and CD9 at 6 hours and a gradual drop of all EV associated proteins by 24 hours. However, the EVs from infected cells showed all three tetraspanins present at 3 hours and expression gradually increased up to 24 hours. When compared to HTLV-1 infected cells, the three tetraspanin proteins were present at 6 hours and expression continued to increase up to 24 hours. HTLV-1 infected cells also showed a unique pattern of CD81 expression. Viral protein expression for both HIV-1 and HTLV-1 (p24 and p19, respectively) were observed at later hours. RNA gene expression analysis was performed and this data correlated with viral protein expression. Finally, experiments of infectivity from 6 and 24 hour supernatants were performed in cells. Collectively, our data indicates that EV release may occur prior to viral release in infected cells, thereby implicating a potentially significant effect of EVs on uninfected recipient cells prior to subsequent viral infection.
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P65

Application of the novel "iDISCO" full-brain tissue clearing method

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The iDISCO (immunolabeling-enabled three-dimensional imaging of solvent-cleared organs) method is a quick, inexpensive, and easily adaptable tissue staining and clearing procedure that allows neuroscientists to study a protein of interest in a whole, unaltered tissue sample. Traditional immunolabeling procedures of the brain are often restricted to thinly sliced samples, which limits the ability to label and observe intact structures, axonal projections, or vasculature. However, when brain tissue can be left whole during processing, entire pathways and structures are maintained. Previous clearing methods take considerable time to fully clear the tissue, and can often denature more sensitive antibodies. The iDISCO method rapidly and fully clears tissue, only requires standard laboratory equipment, and is compatible with many antibodies. The procedure consists of five main steps: sample collection, methanol pretreatment, immunolabeling, tissue clearing, and imaging. iDISCO utilizes Alexa Fluor dyes, which create samples that can be imaged several times while remaining stable. While the iDISCO method was initially tested and validated for mice embryos and brains, the protocol was adapted by our laboratory to image intact hemispheres of the HIV-1 transgenic (Tg) rat. Antibodies for tyrosine hydroxylase and Iba-1 were validated in this transgenic model using iDISCO. Utilizing this method, the full dopaminergic circuit has been imaged and can be studied in three dimensions in the HIV-1 Tg rat. Activated microglia were also visualized, allowing for a more thorough, full-brain view of inflammation. The brain functions as an interwoven network, with circuits working together, rather than structures functioning independently of one another. Viewing the brain as a whole system that allows for clear circuit-level analysis, rather than as a series of individual anatomical structures, is the biggest advantage of the whole-brain tissue clearing iDISCO method.

P66

TREM2 and CSF1R as markers of chronic CNS inflammation in an SIV pigtailed macaque model of HIV CNS disease

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CNS macrophages play a key role in HIV-related CNS pathology. Chronic activation of CNS macrophages is thought to drive this disease phenotype although this low-level pro-inflammatory state is poorly characterized and understood. TREM2 and CSF1R are both receptors constitutively expressed on cells of the myeloid lineage, and in the brain they are exclusively expressed on CNS macrophages, including microglia. These receptors share downstream signaling elements and a common transcription factor, PU.1. It has previously been reported that TREM2 and CSF1R may be important in neurodegenerative diseases such as

Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and HIV CNS disease. Here, we characterized TREM2 and its relationship to CSF1R in an SIV pigtailed macaque model of HIV CNS disease. By in situ hybridization (ISH) and qRT-PCR, we show that TREM2 mRNA increases with SIV infection ($P = 0.01$), and is significantly correlated with CSF1R mRNA expression ($R = 0.79$, $P < 0.001$). With suppressive cART, TREM2 and CSF1R mRNA return to baseline. In agreement with these results, we also show that CD163+ CNS macrophages exclusively express PU.1, and PU.1 protein levels increase with SIV infection by IHC ($P = 0.03$) and return to baseline with suppressive cART. At the protein level, TREM2 and CSF1R are also elevated with SIV infection. However, with suppressive cART, TREM2 protein returns to baseline while CSF1R protein levels remain elevated, suggesting a differential protein regulatory mechanism for TREM2 and CSF1R. Consequently, TREM2 and CSF1R, although sharing many of the same downstream effects, may have different roles in HIV CNS pathology.

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Proteomic and cytokine profiling of plasma extracellular vesicles derived from HIV-infected alcohol drinkers and cigarette smokers.

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Background: Abuse of alcohol and tobacco can exacerbate HIV pathogenesis by transferring materials through extracellular vesicles (EVs). EVs present a stable and accessible source of biological information from one cell to various types of cells. Therefore, we aimed to study the specific EVs proteins, cytokines and chemokines which are altered in both HIV and drug abusers to identify a physiological marker to indicate the immune status and neuronal damage of HIV-positive drug abusers.
Methods: EVs were isolated from plasma of the following subjects by double isolation method to improve their purity: a) Healthy b) HIV c) Alcohol drinkers d) Smokers e) HIV+alcohol drinkers f) HIV+smokers. Quantitative proteomic and cytokine profiling of EVs were performed using commercially available kits.
Results: The EVs were characterized according to the ISEV guidelines. A total of 343 proteins were detected in EVs of all the study groups. Comparison of proteins among all the study groups revealed that hemopexin was significantly altered in HIV+drinkers compared to drinkers and HIV subjects. Further, our study is the first to show properdin expression in plasma EVs, which was decreased in HIV+smokers and HIV+drinkers compared to HIV patients. Plasma EVs package cytokines and their levels are altered in HIV-positive drug abusers. The percentages of EVs IL-1ra, and RANTES were significantly reduced in HIV subjects compared to healthy subjects. The IL-1ra level was higher in EVs of non-HIV-infected alcohol drinkers compared to those of HIV+drinkers. The MCP-1 levels in the plasma of HIV+smokers was higher than in either HIV-positive non-drug abusers or HIV-negative smokers.
Conclusion: The present findings suggest that hemopexin, and properdin show potential as markers for HIV-drug abuse interactions. Further, altered cytokine levels in plasma as well as EVs of HIV-positive drug abusers, suggest a novel mechanism of neuropathogenesis in cases of drug abuse and HIV comorbidity.

P68

Global analysis of changes in RNA-protein interactions and RNA secondary structure following excitotoxic neuronal injury in Rattus norvegicus

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HIV-associated neurocognitive disorders (HAND) affect ~50% of HIV patients, despite antiretroviral treatment (ART) use. Pathologic features of HAND include synaptic loss and neuroinflammation mediated by excitotoxins and viral proteins, amongst others. To characterize neuronal dysfunction mechanisms in HAND, several genomic, transcriptomic, and proteomic studies have been conducted. However, proper interpretation of these data sets requires additional information about post-transcriptional regulatory processes, like RNA processing, stability, and translation. Such mechanisms are particularly important in neuronal function and, accordingly, defects in RNA-binding proteins (RBPs) and RNA regulation have been linked to other CNS diseases. We set out to identify global changes in RNA-protein interactions and RNA secondary structure in an excitotoxicity paradigm in primary cortical neuroglial cells (14 days in vitro (DIV)). We have used the novel protein interaction profile sequencing (PIP-seq) method to identify sites within an RNA molecule that are bound by an RBP, termed protein protected sites (PPS). Our data show dramatic shifts in RNA secondary structure between untreated- and NMDA-treated cells near the start and stop codons of protein coding genes. RNA secondary structure has been implicated in regulating mRNA translation and stability, suggesting that these shifts might reflect differential regulation of these processes in NMDA-treated cells. We also can identify sequence motifs enriched in NMDA-specific PPSs and use them to identify NMDA-dependent RBPs, which might shed light on RBPs post-transcriptional role in excitotoxic stress. Furthermore, while most PPSs are found within protein coding genes, there is a distinct population of PPSs identified in long intergenic non-coding RNAs (lincRNAs), which is overrepresented in untreated cells. In total, we have globally examined RNA secondary structure and the RBP-ome of primary cortical neuroglial cells in a simple excitotoxic paradigm, providing insights into the potential role RBPs and secondary structure changes in diseases associated with excitotoxic stress, such as HAND.

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CIRCULATING PLASMA EXTRACELLULAR VESICLES IN ALCOHOL-INDUCED HIV-1 PATHOGENESIS AND NEURONAL DAMAGE

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Alcohol use, which is highly prevalent in people living with HIV/AIDS (PLWHA), contributes to increased HIV pathogenesis and neuronal damage, eventually leading to HIV-associated neurocognitive disorders (HAND). Recently, we established the role of a cytochrome P450 2E1 (CYP2E1)-mediated oxidative stress pathway in alcohol-induced HIV pathogenesis. Since the expression of alcohol-inducible CYP2E1 is low in the brain, it is not sufficient to induce alcohol-mediated HIV pathogenesis in the brain. We hypothesize that, upon exposure to alcohol, extracellular vesicles (EVs)/exosomes produced primarily from hepatocytes contain a substantial level of CYP2E1. These EVs circulate via plasma and infiltrate into the CNS and deliver CYP2E1 to HIV-infected brain cells to induce alcohol-mediated HIV neuropathogenesis. In this study,

we identified a substantial level of functional CYP2E1 in human plasma EVs. These EVs crossed the blood-brain-barrier (BBB), delivered EVs to brain cells, and induced alcohol-mediated toxicity. Briefly, we isolated and characterized EVs from plasma obtained from de-identified healthy individuals. We observed that the relative level of CYP2E1 in EVs is higher than in hepatic cells, which are the powerhouses of CYP2E1. Further, using in vitro BBB and in vivo animal models, we demonstrated that plasma EVs containing CYP2E1 can cross the BBB and deliver it to macrophages and microglia across the BBB. We further showed that the plasma EV CYP2E1 cargo is capable to induce ethanol-mediated toxicity in macrophage across the BBB. To validate the in vitro findings, we showed an increased level of plasma EV CYP2E1 in alcohol-drinking mice, and their effect on alcohol-induced toxicity. This is the first evidence of the substantial expression and circulation of CYP2E1 in plasma EVs and their crucial role in mediating alcohol-induced toxicity in brain cells. Establishing the role of EV pathway via CYP2E1 in alcohol-induced HIV-1 neuropathogenesis would impact the treatment of PLWHA who drink alcohol.

P70

Modulation of neuronal dendritic spine morphology and function by astrocyte derived extracellular vesicles following morphine withdrawal

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Interactions of HIV or its viral proteins with morphine are known to induce neuronal damage. Previously, we reported that constitutively released astrocyte-derived extracellular vesicles (ADEV-CR) provide trophic support to neurons by delivering protein and miRNA cargo that promote survival. In this study, we found that morphine (500nM) slowed the release of ADEVs to 55.68% of ADEV-CR in a 24h, whereas withdrawal of morphine resulted in a rebound of ADEV release to 131% of ADEV-CR. ADEVs shed in response to morphine withdrawal (ADEV-MW) were enriched with the cytokines IL-1beta, TNF-alpha, IFN-gamma, IL-10, IL-13, IL-4 and IL-5 ($p<0.05$;ANOVA) compared with ADEV-CR. Exposure of primary hippocampal neurons to ADEVs shed during morphine withdrawal reduced the overall number of dendritic spines by 65.6%, with prominent decreases in stubby (57.56%), and mushroom (55.76%)-shaped spines compared to neurons exposed to ADEV-CR (all comparisons $p<0.01$;ANOVA). Reductions in mature dendritic spines were associated with a 40% reduction in spontaneous neuronal spike and a 30% reduction in burst activity ($p<0.001$;ANOVA), but no change in neural network connectivity. We next treated EcoNDK infected mice with escalating dose of morphine for 5 days (10-100 mg/kg x2 inj./day), followed by 48h of morphine withdrawal. The total number of plasma EVs were reduced by 28.93% after morphine treatment compared with vehicle administered EcoNDK mice ($p<0.05$;ANOVA), and rebounded to concentrations not different from vehicle treated EcoNDK mice during drug withdrawal. Plasma viral load increased during morphine withdrawal ($p<0.01$). Reductions in cortical PSD95 in EcoNDK-infected mice were accentuated by morphine exposure and did not return to baseline during drug withdrawal. The rebound in plasma EVs and the reduction in PSD95 during drug withdrawal were prevented by treating mice with PDDC, an inhibitor of neutral sphingomyelinase that blocks EV release. These data suggest that morphine intoxication and drug-withdrawal modulate the release and molecular cargo of ADEVs.

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Biomarkers for repeated brain injury: implications for long term outcome

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Each year in the United States alone, over 1.7 million individuals sustain a traumatic brain injury (TBI) with 1.4 million (80%) of these injuries classified as concussion. Historically, clinicians have relied upon self-reported symptoms by the patient to diagnose a concussion, which can lead to misdiagnosis. To meet the need for an objective diagnostic marker, extensive research into biomarkers for concussion is underway. Biomarkers most often assessed for diagnosis and prognosis of TBI overlap largely with those utilized in other neurodegenerative diseases including HIV-associated neurocognitive disorders (HAND), Alzheimer's disease (AD). Among the most widely used blood biomarkers are glial fibrillary acidic protein (GFAP), S10beta, neurofilament light (NFL), tau and ubiquitin carboxyl-terminal hydrolase L1 (UCHL-1). Other fluid markers under investigation include proteins or RNA present in saliva, cerebrospinal fluid, or enriched in exosomes. The ease of collection and assessment are significant considerations when collecting samples acutely and longitudinally over the course of recovery from concussion or during disease progression in HAND or AD. Importantly, understanding the cellular origin, normal and pathological functions, and possible reasons for changes in blood levels must be considered to interpret findings. In this context, we collected blood and exosome-enriched fractions and assessed numerous clinical parameters (near point convergence, balance, sport concussion assessment tool, etc.) from athletes in rugby, American football and BMX dirt jumpers at baseline and before and after the game or competition. During the sport event, participants wore accelerometers to detect number of hits to the head, magnitude of the impact and directionally (linear (g) or rotational (rot/s2). Our data show that some biomarkers and clinical measures are directly associated with head impacts, whereas, other are not. The data indicate the utility of some measures and illustrate the need for further studies into TBI detection, concussion diagnosis and predictive outcome measures.

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Extended exposure to HIV-1 Tat or acute morphine dynamically shifts firing rates in dopamine D1 and D2 receptor-expressing medium spiny neurons (MSNs) in the dorsal striatum

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Despite the advent of combination anti-retroviral therapies (cART), 30-50% of HIV-infected individuals still exhibit neurocognitive disorders, implying that HIV still drives significant deficits in neuronal function. The striatum appears to be especially vulnerable to HIV-1, harboring high viral loads and exhibiting neuronal injury,Äîpresumably leading to the motor effects seen in patients with advanced HIV. These neurotoxic effects can be modeled with exposure to HIV-1 Tat and can be exacerbated by co-exposure to opiate drugs. Opioid and HIV-1 interactions are especially relevant because HIV-infected individuals are more likely to receive opioids for pain and/or to have opioid use disorder. To discern the effects of HIV-1 Tat and acute opiate exposure on the physiology of the two populations of MSNs (Drd1 or Drd2 expressing), we crossed Drd1-

tdTomato (D1) or Drd2-eGFP (D2) receptor-expressing lines with doxycycline-inducible, GFAP-driven HIV-1 Tat transgenic mice. Using these lines, we looked selectively at D1 or D2 MSNs and explored the progression of physiological changes caused by HIV-1 Tat exposure over time using whole-cell patch clamp recording of MSNs in ex vivo striatal slices. Our data indicate that 48 h or 2-weeks of Tat exposure significantly increased D2 MSN firing rates. By contrast, D1 MSNs showed initial increases at 48 h, followed by a transient decrease in firing rates at 2 weeks in response to Tat. Together these results seem to indicate that the initial excitotoxity due to Tat progresses into a more complex pattern of dysfunction which mimics parkinsonian shifts in D1 and D2 physiology observed after dopaminergic neuron loss. Interestingly, acute morphine exposure consistently increased D1 MSN firing irrespective of exposure to Tat and variably affected the D2 MSN firing. Given that few MSNs express the ¬μ-opioid receptor our results suggest critical differences in the networks of neurons and/or glia which influence each MSN subtype.

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A JCPyV-PML Capsid Mutation in MuPyV Mediates Humoral Immune Evasion and Preferential CNS Replication

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Progressive Multifocal Leukoencephalopathy (PML) is a fatal demyelinating brain disease caused by JC polyomavirus (JCPyV). Virtually all humans are asymptomatically infected lifelong with JCPyV. Individuals with immunological deficits, however, are at risk of developing PML. PML pathogenesis is closely linked with emergence of JCPyV variants bearing mutations in regions of the VP1 major capsid protein that bind host cell receptors. The impact of these mutations in altering cell tropism/neurovirulence and/or immune escape in vivo is unclear. Humans are the only host reservoir for JCPyV. Using mouse polyomavirus (MuPyV) as a natural pathogen-host model, we engineered a MuPyV mutant containing a V296F substitution in VP1 that maps to a frequent VP1 mutation, S269F, in JCPyV. The V296F mutant virus showed decreased replication in the kidney, the major site of polyomavirus persistence, but comparable replication in the brain. Strikingly, the V296F mutation also conferred complete resistance to a MuPyV VP1-specific monoclonal antibody (mAb), whose specificity largely overlapped with the endogenous anti-VP1 response in mice. CryoEM reconstructions of the mAb in complex with the wild type virus revealed that the V296F mutation blocked attachment of the mAb via steric hindrance. Additional VP1 mAb escape mutants isolated in vitro displayed alternate mechanisms of disrupting mAb recognition and virus neutralization. Together, these data support a model of antibody-driven selection of VP1 for escape mutations, which impair infection and persistence in the kidney but retain tropism for the CNS.

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Impact of morphine on an in vitro 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 and 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.

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Continued Viral Seeding of the CNS by Latent and Transcriptionally and Translationally Active HIV+ Monocytes from PLWH on Long Term Suppressive ART.

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The CNS is infected early after peripheral infection, leading to establishment of CNS viral reservoirs that persist despite successful ART. These reservoirs release factors that mediate neuroinflammation and neuronal damage. Establishment and reseeding of viral reservoirs is, in part, due to the transmigration of monocytes across the blood-brain barrier (BBB) into the CNS to CCL2, a chemokine elevated in the CSF of people living with HIV (PLWH) on ART. This is mediated by CCR2, the only known CCL2 receptor on monocytes. We demonstrated that monocytes, cultured, infected, and treated with ART in vitro, preferentially transmigrate across a human BBB model to CCL2, and that ART-treated monocytes harboring virus (HIV+) have a selective advantage in this process compared to ART-treated uninfected but HIV-exposed (HIVexp) monocytes. This is facilitated by increased junctional proteins JAM-A and ALCAM. We demonstrated that, Cenicriviroc, a CCR2/CCR5 dual inhibitor, and anti-JAM-A and anti-ALCAM antibodies, reduce/block the preferential transmigration across the BBB of HIV+ monocytes even with ART. Using DNA/RNAScope analysis on PBMC from PLWH prescribed ART for over 10 years, we now show that HIV-DNA+CD14+ monocytes may have a selective advantage to transmigrate across the BBB, as compared to HIV-DNA-CD14+ monocytes. These HIV-DNA+CD14+ monocytes appear to have an even greater selective advantage to transmigrate than HIV-DNA+CD3+T-cells. We also demonstrate that monocytes with transcriptionally and translationally active virus, as shown by the detection of HIV-nef mRNA or HIV-24 protein, have increased selective and

preferential transmigration across the BBB, while CD3+ T-cells do not. We propose that the ongoing entry of HIV+ monocytes into the CNS, and not CD3+ T-cells, is critical for maintenance of viral reservoirs even in the presence of ART. This underscores the importance of developing therapies to block the entry of HIV-infected mature monocytes and stop viral reseeding into the CNS.

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Antiretroviral therapies affect the metabolic changes in white adipose tissue in mice

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Metabolic abnormalities are common in people living with HIV (PLWH) on ART and include impaired glucose tolerance, dyslipidemia, changes in the ability to store and mobilize energy from adipose tissues. Understanding the potential side-effects of ART is important for PLWH, and for individuals using ART for Pre-Exposure Prophylaxis. Adipocytes are central regulators of energy homeostasis, and changes in their function can have profound effects on energy metabolism. In this study we determined if ART produced metabolic changes in adipose. Three-month-old male C57BL/6 mice were injected with vehicle or a combination of indinavir, lamivudine and zidovudine (45 mg/kg each) once a day for 28 days. Triple TOF mass spectrometry was used to detect and quantify ~170 metabolites in white fat, brown fat, plasma and liver. We found that ART was associated with robust changes in the metabolic profile of visceral white adipose tissue, where 39% of the detected metabolites were significantly different compared with vehicle injected controls ($p<0.05$, Wilcoxon rank-sum test). Most (41 of 48) of these metabolites were increased and included amino acids, nucleic acids and their derivatives. The remaining 7 decreased metabolites were fatty acids with the exception of one carbohydrate. None of the detected metabolites were significantly changed in in brown fat or plasma. In liver 15% of the metabolites were significantly different compared with vehicle-injected mice ($p<0.05$, Wilcoxon rank-sum test). Half (13 of 26) of the metabolites were increased including amino acids, bile acids, and some nucleic acids. The remaining 13 lipids, fatty acids, vitamins and derivates were decreased. These metabolic changes suggest that ART dysregulates liver and white adipose tissue energy metabolism, and that these metabolic abnormalities may not be detected in blood.

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EcoHIV in rats: infection in primary microglia

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The EcoHIV infection model in mice has been well-described and has proven to be of significant utility in experimental neuroAIDS. Extension of this infection model to rats, often used in studies of drug abuse and neurocognitive disorders, would be advantageous in the study of neuroAIDs. In the present study, we created a rat model of HIV infection by first using primary glia cells cultured from the F344/N rat. To restrict the virus replication to rodents, the coding region of gp120 in HIV-1/NL4-3 was replaced with gp80 from murine leukemia retrovirus. The chimeric virus construct, chimeric ecotropic HIV (EcoHIV) was synthesized in 293FT cells for 48 hours in vitro culture. Next, the primary glia cells were isolated from wild type F344/N rat and cultured up to 90% confluency. Then the conditioned medium (EcoHIV) from 293FT cell culture was added to the primary glia cell cultures to validate the infection efficiency. We found detectable EcoHIV in the primary rat glia cells within 24 hours after infection. In addition, immunostaining for the microglial cell marker, Iba1, was performed. We found that Iba1 positive cells, i.e. microglia, were the major cell type for rat EcoHIV infection. Collectively, EcoHIV infection in rat brain extends the model of HIV-1 infection for valuable investigation of HIV-1 associated neurocognitive disorders and drug abuse.

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HERV-K Np9 induces neurotoxicity by activating c-myc signal pathway

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About 8% of the human genome is composed of retrovirus-like sequences called human endogenous retrovirus (HERVs), yet their function is poorly understood. Recent studies have shown that HERVs may play critical roles in both pathological and physiological conditions. Our group found that HERV type K (HERV-K) subtype HML-2 was activated in the brain of patients with amyotrophic lateral sclerosis (ALS). HERV-K encodes a regulatory protein called cORF or Rec, which is the equivalent of Rev in HIV-1. However, class 1 HERV-K has a deletion of 292 bp in the N-terminal region of envelope gene, which changes the alternative splicing to make a protein called Np9. Rec has the ability to promote nuclear export of full-length HERV-K viral RNA, while the function of Np9 is not clear. In this study, we found that the expression of Rec and Np9 was increased in the brain of ALS patients. Both Rec and Np9 were translocated into the nucleus. They can activate the expression of HERV-K when co-transfected with a HERV-K plasmid. Both Rec and Np9 caused toxicity in induced pleuripotent stem cell derived human neurons. However, only Np9 can interact with transcription factor PLZF, and activate c-myc signal pathway. In conclusion, HERV-K Np9 may induce neurotoxicity by activating c-myc signal pathway.

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Astrocyte-derived Extracellular Vesicles mediate HIV-1 Tat and opioid-induced pericyte loss and blood-brain-barrier breach

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It is well-established that despite combination antiretroviral therapy accumulation of early viral protein HIV Tat, implicated in cytotoxicity and neuroinflammation remains unabated, leading in turn, to the pathogenesis of HIV-associated neurocognitive disorders. Adding further complexity to this is the inherent comorbidity of drug abuse in approximately one-third of all HIV infected individuals. It is well-recognized opioids abuse can result in breach of the blood-brain barrier (BBB), ultimately enhancing monocyte transmigration and inflammation in the CNS. Pericytes, essential constituents of the BBB, play a key role in maintaining the integrity of the BBB. The role of pericytes in Tat/morphine-mediated neuroinflammation, however, remains less understood. Our current study aimed at examining the effect of miRNAs released from Tat/Morphine-stimulated astrocyte-derived extracellular vesicle (ADEV) cargo, in mediating pericyte loss at the BBB, in turn, leading to increased influx of peripheral monocytes. Stimulated ADEVs were taken up by pericytes inducing migration (Boyden chamber & wound healing assays) of these cells. Additionally, further validation of Tat/morphine-ADEV-mediated migration of pericytes was also done using an in vitro 3D model of pericytes and human endothelial cells. Our findings suggested that stimulation of astrocytes with Tat/morphine resulted in upregulation of ADEV miRs-423 and -23a respectively, both of which were taken up by pericytes, increasing migration of these cells away from the endothelial cells. Upregulation of miR-423-3p in Tat-ADEVs targeted p21 which, in turn, was critical for promoting cell migration. Interestingly, morphine-

mediated upregulation of ADEV miR-23a, also resulted in cell migration via targeting of the phosphatase and tensin homolog. In conclusion, our findings indicate Tat/ Mor-mediated dysregulation of miRNA expression in the astrocytes involves cellular crosstalk with the pericytes, via the EV cargo, leading ultimately to loss of pericyte coverage at the brain endothelium, breach of the BBB and increased influx of peripheral monocytes, resulting in neuroinflammation.

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Maximizing the silencing potential of anti-HIV-1 SpCas9 gRNAs using deep learning

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Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 has shown potential to be an effective HIV-1 cure strategy. It works by causing a double-stranded break in a region with sufficient complementarity to a predesigned guide RNA (gRNA) sequence, which is then repaired through non-homologous end joining. The resulting indels can potentially disrupt proviral DNA function and prevent viral activation, serving as a functional cure. A popular target for anti-HIV-1 gRNAs has been the 5' long terminal repeat (LTR), a 634-nucleotide region which functions as the HIV-1 promoter. An indel within the LTR could prevent proviral transcription, effectively silencing HIV and preventing it from infecting other cells. However, to maximize gRNA silencing potential, we need to understand how sequence variation within the LTR influences its transcriptional ability. Although predicting transcriptional activity has been challenging, recent advances in deep learning have allowed researchers to isolate the promoter-specific contribution to transcription from DNA sequence alone. To predict how LTR genetic variation influences transcriptional activity, a deep convolutional neural network (CNN) was constructed using publicly available promoter and transcriptomic data from CD4+ T cells. After 60 iterations of training, the r2 value of the predicted activity versus actual activity was 0.74. To ensure that the information learned from human promoters can be applied to predicting LTR activity, another CNN was trained to distinguish human promoters from non-promoters. 734 LTR sequences were then passed through this network, where the results show that 78.8% of the LTRs were predicted to be human promoters. These results have suggested that the information learned about human promoters can be applied to LTR transcriptional prediction. Future directions involve further improving network structure, optimizing hyperparameters, and incorporating a cell type proxy to simulate an LTR's behavior in different cell types and activation states.

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CRISPR induced disruption of MOGS gene inhibits HIV progeny virion infectivity.

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The envelope protein of HIV-1 is heavily glycosylated with a total of 24 N-linked glycosylation sites covering much of its surface. It has been shown that these glycosylations are important for the proper intracellular proteolytic maturation of the envelope precursor glycoprotein gp160, into gp41 and gp120 and for post-CD4 binding functions of mature gp120. Inhibition of mannosyl-oligosaccharide glucosidase (MOGS, alpha-glucosidase 1), a key enzyme in processing N-linked glycan, leads to incomplete processing of viral envelope resulting in a reduction of virion infectivity and cell-to-cell spread of virus infection by suppression of cell fusion events. Recently it was reported that cells derived from patients with a rare genetic disease, type II congenital disorders of glycosylation (CDG-IIb), caused by mutations in the MOGS gene have a reduced ability to support a productive infection by various enveloped viruses including HIV-1. Here using CRISPR-Cas9 we tested the effects of MOGS gene knockout on HIV-1 infection. First, we developed and characterized single-cell MOGS gene knockout clones in TZM-bl and Jurkat cells. Next cells were challenged by infection with HIV-1 NL4-3-GFP reporter virus. No significant differences were detected in the level of primary infection in MOGS -/- cells comparing to WT cells, as measured by GFP reporter positivity and Gag p24 levels in supernatants from infected cells. However, virions released from infected MOGS negative cells showed markedly reduced of infectivity, which was examined by secondary infections of highly susceptible to HIV-1, HutR5 T-lymphoid cell line. Western blot analysis confirmed a lack of proper glycan trimming of gp160 precursor protein in HIV-1 infected MOGS -/- cells. Presented CRISPR based disruption of the MOGS gene provides a new, resistance-refractory antiviral strategy to block HIV-1 infection.

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HTLV-1 3' LTR modulates Myocyte enhancer factor-2(MEF-2) activity in pathogenesis of Adult T-cell leukemia/lymphoma

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HTLV-1 is a complex human retrovirus, an etiologic agent in causing malignant and intractable T-cell neoplasia. About 5% of the infected population would progress to acquiring a more aggressive form of non-Hodgkin's lymphoma (NHL), termed as Adult T-cell leukemia/ Lymphoma (ATLL). MEF-2 transcription factors are a family of genes that are involved in distinct functions in various tissues and isoforms of MEF-2 are 2A, 2B, 2C, 2D in mammals whose mutations are implicated in various cancers. Earlier studies from our lab delineated the role of MEF-2A in increased viral gene expression and inhibition of MEF-2A lead to the reduction in HTLV-1 viral replication and associated T-cell transformation. The objective of these studies is to establish predominate MEF-2 isoform in ATLL utilizing different cell systems representing productive viral infection and/or ATLL. We have adopted novel cyto-analytical techniques to quantitate gene transcripts and protein expression patterns at a single cell level for all four MEF-2 isoforms (A-D). We also investigated differential LTR (5' and 3') recruitment and interaction of these isoforms with Tax and HBZ. Finally, we conducted a series of knockdown and small molecule inhibitor studies to understand effects of selected isoform on viral replication and cancerous T-cell growth. These studies revealed post-translationally modified, overexpression of MEF-2C and downregulated expression of MEF-2B which are known to be oncogene and tumor suppressor, respectively. In addition, differential interaction of MEF-2 isoforms with HTLV-1 promoter as well as with Tax and HBZ was also seen. Knockout studies further solidified the involvement of MEF-2C in ATLL, which resulted in the downmodulation of HBZ protein dose-dependently. The enrichment of MEF-2C in the 3'

LTR and the downregulation of HBZ which transcribes from the antisense region of the viral genome, provides more leads towards transcriptional activity of the lesser investigated 3'LTR of the virus.

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The role of extracellular vesicles (EVs) and EV-cargo in virally-mediated CNS diseases

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Background: Extracellular vesicles (EVs) have gained increased attention as novel orchestrators of intercellular communication and key mediators of pathogen-host dynamics in viral infections. In HTLV-associated myelopathy (HAM), HTLV-1 specific T cells are identified in the cerebrospinal fluid (CSF) of patients in the absence of cell-free virus. In the rapidly fatal disease Progressive Multifocal Leukoencephalopathy (PML), caused by JC virus, infected cells lack the viral receptors for JCV, however post-mortem autopsy samples of PML patients demonstrate JCV within these same cells. We propose that some of these observed paradoxes are explained by this novel route of EV-mediated viral transmission. We hypothesize that a subset of neuroinvasive viruses exploit EVs in order to promote viral spread and chronic inflammation.
Methods: This is a proof-of-principle pilot study to determine the feasibility of isolating and characterizing EVs from study participants in our Neuroimmunology clinic. Study participant CSF and serum were analyzed. Exosome isolation was performed with qEV SEC columns or MagCapture Exosome Isolation Kit PS. EV concentration and size distribution was determined by Nanoparticle Tracking Analysis. HTLV-1 proteins (gp61, gp46, Ub-Tax, Tax), JCV proteins (T ag, VP1), exosome markers (CD63, CD9) and actin were detected by Western Blot. JCV viral DNA was detected by ddPCR.
Results: EVs were successfully isolated from the CSF of PML (n=1) and healthy volunteers (n=2) and the serum of multiple sclerosis (n=2) and HAM (n=2) patients. qEV SEC isolation demonstrated EV heterogeneity in the size and type (CD63+, CD9+, CD81+) of EVs in healthy serum, healthy CSF and MS and HAM serum.
Conclusions: Our preliminary results demonstrate the feasibility of isolating various EV subpopulations from the serum and CSF of healthy and diseased study participants. Ongoing studies are aimed at validating these findings in a larger samples and further characterizing the role of EVs in neuroinvasive CNS diseases.

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Recombinant simian varicella virus expressing cas9 and virus-specific guide RNA provides a novel vaccine model to inactivate latent virus DNA

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Varicella-zoster virus (VZV) causes varicella (chickenpox), becomes latent in ganglia and reactivates to cause zoster (shingles) and other serious neurological disorders, primarily in the elderly. There are one million new cases of zoster annually in the USA. While the VZV vaccine (vOka) given to prevent varicella in children is attenuated, vaccine virus does become latent like wild-type VZV and can reactivate. Although latency cannot be prevented, development of a VZV mutant that does not reactivate would prevent zoster and associated serious neurological and ocular complications. Simian varicella virus (SVV), a primate counterpart of human VZV causes a similar disease in monkeys and has served to be an excellent animal model. A genetic approach using the clustered regulatory interspaced short palindromic repeat (CRISPR)-assisted system (Cas) and a short complementary single-stranded RNA (gRNA), which specifically targets genomes and precisely excises a segment has been used for editing genomes of pathogens. Using bacterial artificial chromosome clones containing the complete SVV genome, we have constructed a recombinant SVV that expresses the 125 kda Cas9 protein and an option to insert gRNA specific for SVV genes that are required for its replication. We will insert gRNA specific for SVV ORF (open reading frame) 63 that is required for virus replication. Our novel approach will provide a foundation to develop preclinical and clinical studies toward prevention of varicella reactivation.

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Detection of virus in the brain with acute SIV infection in rhesus macaque

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HIV enters the central nervous system(CNS) during acute infection, but early time points are difficult to study in humans. Experimental CNS infections in nonhuman primate models have generally used swarm SIV stocks, passaged neurotropic viruses, or with concomitant CD8 depletion, which often resulted in overwhelming encephalitis1-4. A recent study of SHIV infection analyzed virus and lymphocytes presences in the brain of rhesus macaques after 12 weeks of infection5. In this study, we aimed to determine if SIV was detectable in the CNS within 7 and 14 days after infection. 9 rhesus macaques were infected rectally with SIVmac239X. 4 monkeys were sacrificed on day-7 post-infection and 5 were on day-14. We performed RNAscope to detect SIV positive cells in the frontal cortex, basal ganglia and thalamus and immunohistochemistry to identify CD3, CD68 and CD163 positive cells in the brain. We also quantified viral DNA and RNA quantities in gross brain tissues. Quantitative PCR consistently detected viral DNA/RNA copies in brain tissues as early as 14 days post-infection and in one animal at 7 days post-infection. RNAscope determination of SIV resulted in detection of virus at both 7 and 14 day post-infection, with the highest number of SIV-infected cells in basal ganglia followed by thalamus. There were no significant differences in the numbers of SIV -infected cells between days-7 and -14 of SIV infection. Co-labelling of SIV RNA probes and cellular markers for immune cells revealed that CD163 positive macrophages were involved in viral invasion of the brain. CD68 positive macrophages and CD3 T-cells were mostly detected along the blood vessels but occasionally also in the brain parenchyma. In an acute model of SIV infection, we showed that SIV can be detected in the brain as early as 7 days post-infection, and is carried by CD163 positive peripheral macrophages.

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Removal of SIV proviral DNA fragments by CRISPR from blood and lymphoid cells of living ART treated non-human primates.

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Our research group successfully utilized CRISPR/Cas9 gene editing to excise HIV-1 genome from latently infected human cells. To test our approach in a preclinical setting, we developed a similar strategy for SIV. We utilized a bioinformatics tool to identify a pair of nucleotide sequences in order to originate a pair of gRNAs with the highest predicted on-target and lowest scores for off-target cleavage within the 5' LTR and Gag gene of SIVmac239. Then, we employed AAV-9 as a vector to deliver CRISPR/Cas9 designed to target sequences spanning the LTR and Gag genes and permanently inactivating proviral DNA. In our study, 8 adult Chinese rhesus macaques male were intravenously (i.v.) infected with SIVmac239. At 8 weeks post infection, animals were treated daily with a drug regimen of tenofovir disoproxil fumarate, emtricitabine and dolutegravir. Then, ex vivo gene editing was performed in PBMCs by AAV9-CRISPR/Cas9 transduction, PCR amplification and Sanger sequencing of the amplicons to assess the potency and precision of viral DNA elimination. Moreover, we performed an in vivo proof of concept study on 4 animals, 3 were given an i.v. infusion of AAV-9-CRISPR/Cas9 and after 3 weeks, animals were necropsied and blood and tissues were harvested for virological and gene excision evaluations. In all SIV-infected animals, ex vivo excision of viral DNA was confirmed by the detection of distinct DNA fragments of 465bp and 358bp resulting from the removal of intervening DNA sequences between 5'LTR to Gag and Gag to 3'LTR, respectively. Delivery was confirmed by the presence of Cas9 and expression of both gRNAs and results from Sanger sequencing confirmed the breakpoint of the viral DNA. In vivo, both excisions were confirmed in blood and tissues of animals that received AAV-9-CRISPR/Cas9 infusion.

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Host and virus chromatin interactions during lytic infection of HFL cells with VZV.

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Varicella zoster virus is a ubiquitous alphaherpesvirus that causes varicella (chickenpox) on primary infection, establishes latency in the peripheral and enteric nervous system, and can reactivate to cause zoster (shingles) frequently associated with serious neurological complications. During latency most VZV genes are transcriptionally silent with transcription from the episomal VZV DNA limited to the Varicella Latency Transcript. Previous data suggests that gene silencing during latency is through epigenetic modification of the latent VZV genome, but this may also include higher order DNA structures. We hypothesize that higher order chromatin structures of both the virus and host are involved in transcriptional profiles during infection and latency. We used paired-end RNA-seq and CTCF ChIP-seq to identify changes in host transcription profile and insulator occupancy induced by VZV infection of human lung fibroblast cells. As a proof-of-principle, we also interrogated four distinct viewpoints along the host genome for VZV DNA interactions to investigate if intramolecular chromatin associations between the virus and host could be identified during lytic infection. Our work shows the feasibility of using multiple approaches to determine the effect of virus infection on host chromosomal structure as well as highlighting unique experimental and bioinformatic hurdles involved in these studies.

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Effect of HIV-1 Tat-induced senescence on astrocytes and BMECs important for blood-brain barrier

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In spite of the medical advances made, human immunodeficiency virus type 1 (HIV-1)-infected patients still face serious complications, including neurocognitive impairment. Although anti-retroviral therapy (ART) has decreased the severity of neurocognitive impairment, more than 50% of HIV-1-infected individuals still suffer from some form of impairment. The driving force behind this observation is still unclear. Notably, the severity of impairment has been shown to be correlated to the levels of a number of chemokines, inflammation, and activated cells, rather than with the number of HIV-1-infected cells in the brain. Factors involved in the onset of neurodegenerative diseases have been linked to neuroinflammation and altered blood-brain barrier (BBB) integrity and may be mediated by HIV-1 accessory proteins. The HIV-1 protein Tat has been shown to cause neuropathologic changes, inflammation, increased BBB permeability, and inhibition of apoptosis in the CNS, all of which are also features of cell senescence. Senescence is a cell mechanism usually involved in aging that is driven by telomere shortening and oxidative stress and results in a permanent exit of the cell cycle with resistance to apoptosis. We have shown that brain microvascular endothelial cells (BMECs) and astrocytes treated with Tat had increased SA-beta gal-positive staining, a biomarker of senescence, as well as decreased ZO-1 staining, a junction protein, indicating that Tat exposure to cells of the BBB can result in senescence and decreased BBB integrity. Future studies will determine how Tat exposure affects other biomarkers of senescence such as p16 and p21. When senescence occurs prematurely, it has been shown to be associated with neurodegenerative disorders such as Alzheimer's disease, and it is likely that Tat-induced premature senescence of cells of the BBB could also be linked to neurocognitive impairment.

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Effects of morphine on hiv neuropathogenesis

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HIV-associated neurocognitive disorders (HAND) persist in greater than 50% of people living with HIV (PLWH) despite antiretroviral therapy (ART). HIV enters the CNS by transmigration of infected CD14+ CD16+ monocytes across the blood brain barrier (BBB) in response to chemokines including CCL2. This subset of monocytes is increased in the peripheral blood of HIV-infected individuals and is even higher in HIV-infected people with substance use disorder. Opioids are among the most common drugs of abuse, and some studies showed that they 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. Mature monocytes were allowed to transmigrate to CCL2, and were quantified by flow cytometry. Our preliminary results show that morphine increases CCL2 mediated transmigration of HIV-infected monocytes when compared to the transmigration of infected, untreated cells. Once within the CNS, HIV infected monocytes can differentiate into macrophages, where they may remain as long-lived viral reservoirs. Opioids may enhance macrophage secretion of inflammatory cytokines, leading to sustained neuroinflammation. We examined the effects of morphine on CCL2 and IL-8 production by macrophages during a four-day period. Additionally, we treated macrophages with LPS, which is present in the sera of PLWH, to study its effects with morphine. We showed that morphine increases CCL2 and IL-8, and that this effect is not changed when LPS is present. Our findings suggest that morphine contributes to HIV neuropathogenesis by increasing HIV infected mature monocyte entry into the CNS and by stimulating the production of inflammatory cytokines by macrophages.

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Mouse Model of Chronic West Nile Neuroinvasive Disease: Inflammation and Behavior

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Viruses that can invade the central nervous system (CNS) are increasing in incidence around the world. They can cause severe, potentially lifelong sequelae that can greatly reduce quality of life in those affected. Although there has been some progress in determining the causes of the long-term neurological dysfunctions, little is known about what causes these deficits in survivors. West Nile virus (WNV) is a mosquito-borne virus with a worldwide distribution that causes severe neurological disease, called West Nile neuroinvasive disease (WNND). Over half of those surviving WNND develop long-term neurological deficits including depression, memory loss, and motor incoordination. Several other neurotropic arthropod-borne viruses, particularly alphaviruses and flaviviruses, cause diseases with similarly disabling outcomes. It is difficult to perform research on these sequelae using animal models due to the stringent requirements of biocontainment needed for many of the pathogens of concern. We have established a behavioral unit in biosafety level-3 (BSL-3) settings for use in screening mice for behavioral changes post-infection. Using WNV as the pilot virus, we have screened for behavioral deficits up to two months post-infection. Up to one-third of infected mice show dysfunction in memory and motor learning. Persistent WNV RNA was detected in multiple regions of the brain in mice both with and without detectable neurobehavioral dysfunction. Inflammatory nodules were also found in multiple regions of the brains of mice more than one month post-infection. Pathological changes in mice with and without neurobehavioral changes are being further evaluated through immunohistochemistry and quantification of inflammatory cytokines in regions of the brain related to memory function. We aim to gain a better knowledge of how WNV affects the CNS in the chronic stages, and to develop a model for future research into neuroinvasive virus sequelae and for evaluation of therapies treat neurological deficits following WNND.

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Association of GRIN2A GT(n) VNTR with cognitive impairments in people living with HIV

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Severity of HIV-associated neurocognitive disorders (HAND) is highly variable in people with HIV (PWH). Glutamate excitoxicity is one of the several mechanisms by which HIV causes damage to neurons. Although there are likely several key players that induce glutamate excitotoxicity, alterations in ionotropic receptor N-methyl-D-Aspartate (NMDA) signaling is recognized as a major source. Data show that glutamate excitotoxicity is primarily dependent on calcium influx, whereby glutamate attaches to the two NMDA receptors (NR2A and B) on neurons. Research shows that carriers of a long GT(n) variable tandem repeat (VTR) in the promoter region of the NR2A gene (GRIN2A; the gene that codes for the NR2A subunit within the NMDA channel) were more likely to have smaller amygdala and hippocampal regions pointing to the likelihood for specific cognitive domain dysfunctions (memory and learning) in these individuals. NMDA receptors are important modulators for synaptic plasticity for learning and memory within these brain regions. We hypothesize that this may be due in part to the GT(n) VTR in the promoter region of GRIN2A, thereby, altering levels of GRIN2A transcription, translation and downstream regulation of glutamate/calcium levels. It is estimated that approximately 30% of the general population have the homozygous long GT(n) VTR in the GRIN2A promoter. In health, there is apparently no biological consequence of having this repeat; however, we predict that in diseases (HIV infection) or injuries (traumatic brain injury) that impact the CNS, the long terminal repeats lead to worse outcome. The GT(n) VTR within the promoter region of GRIN2A has been associated with a number of neurodegenerative and neurocognitive diseases, but no studies have been conducted to assess the potential association of GT(n) VTR in GRIN2A and HAND. Our data address the potential contribution of this genotype to neuronal dysfunction and HAND.

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Association between (GT)n promoter polymorphism and recovery from concussion

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Variability in recovery between concussed athletes can be attributed to several risk factors. One risk factor not definitively explored is genetic variation. Genetic variations such as variable number tandem repeats (VNTR) in the promotor region are normal in the population, and can lead to disparities in the amount of protein produced, which could be associated with neuronal recovery. Little research has been conducted to investigate promoter VNTRs within genes responsible for recovery following a concussion. The authors implemented a prospective cohort design using a standardized concussion protocol to diagnose and followed 93 athletes to full recovery at 3 different sites to determine the association between promotor GT(n) VNTR polymorphisms and recovery time within concussed athletes. The GT(n) VNTR within the promoter region of GRIN2A, KCNH2, GRIK1, NEFL were genotyped using capillary electrophoresis. GT(n) VNTR promotor polymorphisms were dichotomized

into long (L) and short (s) alleles. Using unadjusted negative binomial regression models we found athletes carrying the LL GRIN2A GT(n) or the ss NEFL GT(n) VNTR within the promoter region were more likely to experience a prolonged concussion recovery, where they were not able to return to play for approximately 60 days. This could be presumably attributed to altered proteins or protein levels that disrupts neuronal recovery. This pilot study suggests that these VNTRs are associated with prolonged concussion recovery. In future studies we plan to measure the extent to which the L or s alleles alter the level and the activity of the GluNR2a and NEFL proteins that GRIN2A and NEFL produce, respectively.

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Sustained morphine exposure and withdrawal differentially affect HIV-1 Tat-induced dendritic spine losses within CA1 pyramidal neurons

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Opiate abuse can exacerbate human immunodeficiency virus-1 (HIV)-associated neurocognitive deficits. Hippocampal area CA1 pyramidal neurons are a potential point of convergence between neurotoxic HIV proteins (e.g., Tat, gp120) and opioids. Previously, we observed reductions in key interneuronal populations within the CA1 microcircuitry of the hippocampus in Tat transgenic mice, coinciding with significant structural and electrophysiological alterations in pyramidal neurons. These disruptions in GABAergic interneurons could impact pyramidal neuron function downstream; 20 min pretreatment with the GABA(A) agonist muscimol (100 nM) reduced Tat (100 nM) and morphine (500 nM)-induced alterations in neuronal intracellular calcium concentration, while bicuculline (20 μM) prolonged Tat-induced elevation of intracellular calcium in the presence of morphine ($p<0.001$). In vivo, doxycycline-inducible expression of Tat in adult mice reduced apical dendritic spine density on dentate gyrus granule cells ($p=0.008$), CA1 pyramidal neurons ($p<0.001$), and CA3 pyramidal neurons ($p<0.001$). Simultaneous morphine exposure (escalating dosage, 10-40 mg/kg) did not have a significant effect. By contrast, higher dose (25 mg time-release pellet, s.c., 5 d) morphine altered the proportion of dendritic spine subtypes in CA1 pyramidal neurons, indicative of maladaptive synaptic plasticity. These changes were dependent on genotype (Tat-/Tat+) and dendritic layer. Morphine treatment decreased the number of thin/filopodial spines in the stratum radiatum (SR, $p=0.0117$). Acute morphine withdrawal reduced mature mushroom spines in SR ($p=0.0098$). Tat exposure reduced stubby spines in morphine-treated stratum oriens (SO, $p=0.0118$) and SR ($p=0.0175$) dendritic layers. Morphine withdrawal increased stubby spine density within the stratum lacunosum-moleculare (SLM) regardless of Tat genotype ($p=0.0406$) while reducing thin/filopodial spines in Tat+ mice alone ($p=0.029$), suggestive of reduced synaptic plasticity. These morphine- and Tat-induced alterations in synaptic spine density and morphologic subtypes coincide with neurophysiological and behavioral deficits associated with neuroHIV and opioid abuse. This work was supported by NIH grants R01 DA018633 (KFH) and NIH F32 DA047193 (VDM).

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Prepulse Inhibition: A Diagnostic Biomarker for HIV-1 Associated Neurocognitive Disorders (HAND)

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HAND, characterized by alterations in the core components of cognitive function and age-related disease progression, persist in the post-cART era. However, currently available diagnostic tools lack the sensitivity and specificity needed for an accurate diagnosis for HAND. Scientists and clinicians have been on a quest for an innovative diagnostic biomarker for HAND in the post-cART era. To address this knowledge gap, the potential utility of preattentive processes as a diagnostic biomarker for HAND, measured using prepulse inhibition (PPI), was examined using complementary statistical techniques in intact male and female F344/N HIV-1 transgenic (Tg; N=20 litters) and control rats (N=17 litters). First, discriminant function analyses and receiver operator characteristic curves were utilized to assess the utility of PPI as a diagnostic biomarker. In the HIV-1 Tg rat, PPI accurately diagnoses the presence of the HIV-1 transgene, relative to control animals, with both high sensitivity (i.e., 89.3%-100%) and high specificity (i.e., 79.5%-94.1%). These results generalize across experimental paradigms, the functional lifespan, sensory modalities, and biological sex. Second, statistical mediation was assessed to empirically evaluate whether preattentive processes serve as a neurobehavioral mechanism underlying HAND. Three longitudinal, lag-1 autoregressive mediation models were utilized to assess whether deficits in preattentive processes mediate the effect of the HIV-1 Tg on alterations in learning, sustained attention and/or long-term episodic memory over time. Findings support preattentive processes as a partial mediator of genotype effects in all of the examined relationships across the functional lifespan, explaining between 44% to 58% of the HIV-1 transgene effect; results which confirm the role of PPI as an intervening variable in causal pathways between HIV-1 and several neurocognitive markers. Evidence from multiple, complementary statistical techniques, therefore, heralds an opportunity for the development of a brief, non-invasive biomarker for HAND in the post-cART era. Funded by NIH grants DA013137, HD043680, MH106392, NS100624.

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Selective Estrogen Receptor beta Agonists: A Therapeutic Approach for HIV-1 Associated Neurocognitive Disorders

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The persistence of HIV-1 associated neurocognitive disorders (HAND) in the post-cART era, afflicting between 40-70% of HIV-1 seropositive individuals, supports a critical need for the development of adjunctive therapeutic treatments. Selective estrogen receptor beta agonists, including S-Equol (SE), have been implicated as potential therapeutic targets for the treatment of neurocognitive disorders. In the present study, the therapeutic efficacy of 0.2 mg SE for the treatment of HAND was assessed to address three key questions in the HIV-1 transgenic (Tg) rat. First, does SE exhibit greater therapeutic efficacy when treatment is initiated relatively early (i.e., between 2 and 3 months of age) in the course of viral protein exposure? Second, does the therapeutic utility of SE generalize across multiple neurocognitive domains? Neurocognitive assessments tapping preattentive processes, learning, sustained attention, and selective attention were conducted. In each neurocognitive domain, at least 60% of HIV-1 Tg animals treated with SE exhibited enhanced neurocognitive function, approximating controls. Third, are preattentive processes sensitive to the initiation of SE treatment in HIV-1 Tg animals? In HIV-1 Tg animals, preattentive processes, measured via visual prepulse inhibition, diagnosed treatment with SE with 87.5% accuracy, 66.7% sensitivity, and 100% specificity. Thus, the therapeutic efficacy of SE is greater when treatment is initiated relatively early in the course of viral protein exposure and generalizes across neurocognitive domains supporting an adjunctive therapeutic for HAND in the post-cART era. Furthermore, preattentive processes may herald an innovative diagnostic biomarker for milder forms of neurocognitive impairment. Funded by NIH grants DA013137, HD043680, MH106392, NS100624.

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Stabilization of HIV-associated macrophage inflammatory activity by a p75 neurotrophin receptor ligand

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When macrophages are stimulated with the mature neurotrophin, nerve growth factor (NGF), the natural ligand for the neurotrophin receptor, TrkA, they respond by down regulating the secretion of neurotoxic factors. In contrast, proNGF enhances the toxic activity of macrophages through interactions with the p75 neurotrophin receptor (p75). This functional dichotomy parallels the actions of pro- and mature neurotrophins on neurons during aging and in neurodegenerative diseases and suggests that modulation of p75 may have therapeutic anti-inflammatory benefits. To explore this possibility, we cultured monocyte derived macrophages (MDM) and treated them with the p75 ligand, LM11A-31. LM11A-31 shifted the macrophages toward a ruffled, less toxic phenotype with a maximum effective concentration of 30 nM. Incubation with 10 nM LM11A-31 reduced the toxic activity of MDM conditioned medium and induced a unique secretory phenotype which dominated over effects of HIV virions. Secretome analysis with antibody arrays revealed a unique secretory profile that was rich in growth factors but failed to fit traditional M1 or M2 profiles. Results from a specific assessment of known activation markers were most consistent with anti-inflammatory, phagocytic and tissue remodeling functions. None of the protein changes could clearly be linked to the neurotoxic or protective activity although the robust secretion of proteins such as growth and differentiation factors suggest that the secretion of protective agents may be just as important as suppressing the release of toxic factors. Although much work is still needed to reveal the functions of many proteins in the secretome, such as growth and differentiation factors, the data clearly indicate that the ligand LM11A-31 has excellent therapeutic potential due to its ability to induce a more protective phenotype that restricts activation by HIV.

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Full length Tat 101 is the most frequently sequenced length variant in HIV-1-infected patient samples within the Drexel Medicine CARES cohort and LANL

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Human immunodeficiency virus type 1 (HIV-1) encodes for Tat, a multifunctional regulatory protein that is involved in transcriptional enhancement and in causing neurotoxicity and central nervous system (CNS) dysfunction. This study examines Sanger sequencing of HIV-1 subtype B Tat from 2006 to 2015 within the Drexel University College of Medicine CNS AIDS Research and Eradication Study (CARES) cohort to define the spectrum of Tat length in patients. The Los Alamos National Laboratory (LANL) database was used for comparison purposes. Miscoded stop codons were present in the CARES cohort and LANL sequence databases and protein variability was highly similar. The majority (approximately 85%) of Tat sequences in CARES and LANL are 101 residues in length. Unique Tat lengths found in CARES and not LANL were 31, 36, and 39 residues in length. When CARES patients were longitudinally examined, sequence lengths of 101 had a low probability of reducing to below 48, and sequences had a high probability of increasing to above 86 during their next visit, when below 48 residues in length. These results have suggested that Tat length has been conserved to retain the majority of the protein function highlighting its importance in viral replication. Interestingly, meta-analysis of recent literature has determined that the truncated Tat 86 and not the full-length Tat 101 is the most frequently utilized experimentally. Association between Tat length and clinical parameters from the sequences could not be accurately performed due to the relative distribution of Tat lengths, therefore no correlation between Tat lengths and any clinical parameters were observed. When examined in vitro, the truncated Tat constructs initially appeared to transactivate the LTR, but further testing suggested that read-through was occurring, resulting in leaky expression of the full-length construct.

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Neuronal BAG3 Suppression Induced by HIV-1 Tat Disrupts the Synaptic Vesicles Homeostasis

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HIV-1 proviral components continue to express toxic proteins despite the ART treatment. HIV-1 Tat, a major neurotoxic product of HIV-1, is secreted from infected cells and modulates numerous pathways in the surrounding tissues. Synaptic vesicles homeostasis is crucial for the neuronal developmental processes and neuronal signal transmission and its impairment has been linked to numerous neurological disorders. BAG3 is a co-chaperone known for its affinity to form chaperone complexes, thus regulating the heat shock proteins activity and PQC processes. In this study we demonstrated how BAG3 mRNA and protein levels are downregulated as a result of HIV-1 Tat expression in primary neurons. We also showed the negative impact of BAG3 downregulation on the neuronal lysosomal autophagy through the ATG5 suppression and reduction in LC3-I to LC3-II conversion flux. We also investigated the effect of BAG3 in the homeostasis of synaptic vesicles represented by synapsins (Syn) and synaptotamin 1 (Syt1) as important pre-synaptic proteins which are widely expressed in neurons, mediating the release of neurotransmitters to the synaptic cleft. We observed that as a result of BAG3 knockdown synapsins and synaptotagmin 1 accumulate in the cell body and along the axons as opposed to their distributed localization at the axon-terminals in normal neurons. Our protein assays also showed that synapsins stability and more importantly their normal turnover via the lysosomal autophagy are significantly impaired upon the BAG3 suppression. These observations have much implication in the context of HIV-1 induced neurological impairments, as our complementary studies confirmed the effect of Tat on the formation and stability of synapsins networks similar to that of the BAG3 knockdown. In the light of these studies, it can be concluded that HIV-1 Tat modulates the neurotransmitter release partly due to its impact on BAG3, synapsins and synaptotagmin 1, contributing to the neuropathophysiology of HAND.

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RNA Deep Sequencing Reveals the Alteration of Neuronal Cholesterol Biosynthesis by HIV-1 Tat

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HIV-1 Tat protein by egressing from the permissive infected cells and permeating into non-permissive neuronal cells perturbs homeostasis of the naïve neurons, hence contributes to the neuropathogenesis of the viral infection in HIV-1 positive patients. Thorough bioinformatics Interrogation of the results from mRNA deep sequencing of rat hippocampal neurons after exposure to Tat identified dysregulation of several genes that are collectively involved in lipid and cholesterol metabolism. Results from lipid metabolism array validate upregulation of sterol o-acyltransferase 1/acetyl-coenzyme A acyltransferase 1 (SOAT1/ACAT1), sortilin-related receptor L (SORL1), and low-density lipoprotein receptor-related protein 12 (lrp12), all of which are Implicated in biogenesis of beta-amyloid plaques. Further studies in neuronal cells, confirmed elevated Levels of SOAT1/ACAT1, total cholesterol, free cholesterol and particularly cholesteryl esters (CE) as a result of HIV-1 Tat treatment. Treatments of the cells with cocaine revealed a modest counteracting effect on the interference of Tat-cholesterol metabolic pathway. These Results offer a new insight into molecular events involved in neurodegenerative activity of HIV-1 and its potential overlap, at least in part, with pathogenic events associated with the development of AD-related dementia.

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Elevated plasma eicosanoids are associated HIV-disease status and working memory in people living with HIV

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Despite effective suppression of Human Immunodeficiency Virus (HIV) replication with antiretroviral therapies approximately 50% of people living with HIV (PLWH) develop cognitive impairment (CI). While we do not fully understand why some PLWH develop CI while others do not, persistent low-level inflammation appears to contribute to CNS damage and CI. Eicosanoids are a family of signaling molecules produced through the oxidation of 20-carbon fatty acids. Eicosanoids derived from the omega-3 fatty acids docosahexaenoic acid (DHA), or eicosapentaenoic acid (EPA) tend to be protective, while those from omega-6 fatty acids arachidonic acid(AA), or linoleic acid(LA) metabolism are more often pro-inflammatory. Here we measured 42 eicosanoids in plasma from 95 PLWH from the CHARTER cohort and 25 HIV-uninfected participants using liquid chromatography tandem mass spectrometry (LC-MS/MS). All participants completed a neuropsychological test battery assessing seven cognitive domains (verbal, executive function, SIP, learning, recall, working memory, and motor). We compared eicosanoid levels between PLWH and HIV-uninfected using Pearson correlations to examine associations between eicosanoid levels, HIV clinical markers, and cognition. At baseline, 8 EPA metabolites, 16 AA metabolites, 10 DHA metabolites, and 3 LA metabolites were significantly elevated ($p<0.05$; ANOVA with Tukey post-hoc) in PLWH compared to HIV-uninfected individuals. We found that the majority of plasma eicosanoids were negatively associated with current (37) and nadir CD4 (28), suggesting an association of these eicosanoids with disease status. In contrast, we found a positive correlation of eicosanoids (17) with viral loads. Additionally, 37 eicosanoid metabolites were negatively associated with plasma bilirubin suggesting a potential association of eicosanoid production with liver function. Moreover, higher eicosanoid levels were associated with lower t working memory (34 of 42 eicosanoids). These findings suggest that HIV-disease status and liver function may regulate the production of inflammatory eicosanoids (largely products of AA and LA metabolism) and lower working memory performance.

P101

Tryptophan in position 375 enhances sensitivity to soluble CD4 neutralization but doesn't universally associate with macrophage-tropism in Transmitted/Founder (T/F), acute and late state viruses of different subtypes

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Background: HIV-1 vaccines need to elicit antibodies that target conserved envelope (Env) epitopes to protect against diverse HIV-1 clades. To achieve this, it is necessary to know how different amino acids affect Env trimer structure. The CD4 binding site (CD4bs) on the Env glycoprotein trimer is major target for cross-reacting neutralizing antibodies (nabs). Previously, we used EMPIRIC (Exceedingly Meticulous and Parallel Investigation of Randomized Individual Codons) saturation mutagenesis to identify Env residues that open the CD4bs. Several substitutions in CD4 binding loop residue 375 resulted in a more exposed CD4bs without modifying the trimer apex (Duenas-Decamp, M et al., PLOS Pathogens, 2016)
Methods: Here, we introduced a tryptophan at 375 position in different Transmitted/Founder (T/F), acute stage and late state macrophage and non-macrophage viruses of different subtypes. We evaluated changes in 375W mutant structure by testing the sensitivity of Env+ pseudovirions to neutralization by sCD4 and monoclonal antibodies (mAbs) b6, b12, VCR01, 3BNC117, and 17b. These mutants were also used to evaluate macrophage infection and thus determine their macrophage-tropism (mac-tropism).
Results: We observed an increase in sensitivity against sCD4 in all the mutants tested. None of the wild type and mutants were neutralized by b6 or 17b mAbs. The 375W mutation did not affect the b12 epitope. VRC01 and 3BNC117 neutralization was dependent on the virus tested. We found that a tryptophan in position 375 conferred mac-tropism in Z1792M, a non-macrophage tropic Clade C virus. Interestingly, macrophage tropic viruses such as B33, B59 and JRFL that carry 375W lost their capacity to infect macrophages.
Conclusions: Overall Env trimer structure influences the mac-tropism because a single mutation can increase or decrease mac-tropism depending on the virus studied. A tryptophan at position 375 in Env exposes the CD4bs in T/F, acute stage and late state macrophage and non-macrophage viruses of different subtypes.

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Immunogenicity of novel MHC Class I epitopes for a therapeutic vaccine against HTLV-1

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Human T-cell leukemia virus type 1 (HTLV-1) infects approximately 20 million people worldwide. While 90% are asymptomatic, 5% develop severe diseases including adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Patients with chronic HTLV-1 infection have high frequencies of HTLV-1-activated CD8+ T cells, and the two main HLA alleles (A2, A24) are present in 88% of infected individuals. There is no effective vaccine against either of disease state, ATL or HAM thus we launched an epitope discovery program to identify neo antigens for a therapeutic vaccine. We utilized an immunoproteomics approach to characterize MHC-I restricted epitopes presented by HLA-A2+, A24+ MT-2 and SLB-1 cell lines. Unlike traditional motif prediction algorithms, this approach identifies epitopes associated with cytotoxic T-cell responses in their naturally processed forms, minimizing differences in antigen processing and protein expression levels. Out of nine identified peptides, we confirmed six novel MHC-I restricted epitopes that were capable of binding HLA-A2 and HLA-A24 alleles and used in vitro and in vivo methods to generate CD8+ T cells specific for each of these peptides. MagPix MILLIPLEX data showed that in vitro generated epitope-specific CD8+ T cells secreted IFN-gamma, granzyme B, MIP-1alpha, TNF-alpha, perforin and IL-10 when cultured in the presence of MT-2 that is a HTLV-1 infected cell line. Degranulation assay confirmed cytotoxic response through surface expression of CD107 on CD8+ T cells when cultured with MT-2 cells. A CD8+ T-cell efficacy assay indicated significant antiviral activity of CD8+ T cells specific against all identified peptides. These epitopes are thus candidates for a therapeutic peptide-based vaccine against HTLV-1, and our results provide preclinical data for the advancement of such a vaccine.

P103

The Effects of buprenorphine on Mechanisms of HIV Neuropathogenesis

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HIV associated neurocognitive disorder (HAND) is a spectrum of neurocognitive deficits that affects approximately half of people living with HIV (PLWH). HIV enters the brain early after peripheral infection and establishes viral reservoirs that are difficult to eliminate. One mechanism that contributes to HAND is the transmigration of uninfected and infected CD14+CD16+ monocytes across the blood brain barrier (BBB) in response to CCL2, a chemoattractant elevated in the CSF of PLWH. Some 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 buprenorphine, an opiate agonist therapy (OAT). We previously demonstrated that buprenorphine decreases CCL2-mediated adhesion to brain microvascular endothelial cells (BMVEC) and chemotaxis of CD14+CD16+ monocytes to CCL2. We are now characterizing the effects of buprenorphine on transmigration of uninfected and HIV infected human primary CD14+CD16+ monocytes across the BBB. Our preliminary data indicate that buprenorphine decreases CCL2 mediated transmigration of HIV infected CD14+CD16+ monocytes across a tissue culture human BBB model. HIV infected CD14+CD16+ monocytes have increased expression of junctional proteins JAM-A and ALCAM that is correlated with increased transmigration of these infected cells. One way buprenorphine may reduce this transmigration is through changes in expression of these junctional proteins. We are therefore characterizing the impact of buprenorphine on expression of JAM-A and ALCAM on CD14+ CD16+ monocytes. We will also examine the transmigration of mature monocytes from PBMC obtained from PLWH taking buprenorphine and compare to those not taking this OAT. Ultimately these data will characterize buprenorphine as a potential therapy for HAND regardless of opioid use.

P104

Cytokine production within the cortico-basal ganglia-thalamocortical circuity and anhedonia are altered by HIV-1 Tat and morphine

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Many HIV-1-infected individuals experience depression and decreased motivation, despite antiretroviral therapies. HIV-1 trans-activator of transcription (HIV-1 Tat) is expressed in the central nervous system (CNS) of infected individuals and may cause inflammation within the cortico-basal ganglia-thalamocortical circuit, underlying these emotional impairments. Additionally, opiates can further exacerbate HIV-1 Tat-induced inflammation and neuropsychological deficits. Accordingly, we investigated the effects of 48 h to 8 weeks of HIV-1 Tat induction on cytokine production within the PFC, striatum, and thalamus, and the effects of HIV-1 Tat and morphine on anhedonia behaviors in adult male transgenic mice. In the PFC, Tat increased expression of chemokines CCL4, CXCL1, and G-CSF, and anti-inflammatory cytokines IL-4 and IL-10; whereas chemokines CCL5 and eotaxin and pro-inflammatory cytokine IL-1beta expression was increased in the striatum. Interactive effects indicated that Tat increased expression of IL-17 and IL-6 in the PFC and striatum, respectively, after 8 weeks of induction. In contrast, chemokine CCL5 and proinflammatory cytokines IL-1alpha and IL-13 were decreased in the thalamus. To assess the effects of HIV-1 Tat and morphine on anhedonia, Tat(+) and Tat(-) 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 4 h after drug administration to assess morphine-induced locomotor effects. In the novelty suppressed feeding test morphine increased eating behavior; whereas Tat-induction did not affect feeding. Interestingly, in the novelty induced hypophagia test, HIV-1 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. Together, our findings suggest that HIV-1 Tat uniquely and systematically induces cytokine production throughout the cortico-basal ganglia-thalamocortical circuit, and cytokine production may trigger HIV-1 Tat and morphine-induced anhedonia and depression.

P105

Transcellular transfer of mitochondria from hypermetabolic astrocytes to neurons during cocaine and HIV-1 exposure

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Exposure of primary astrocytes to the HIV-1 protein Tat and to cocaine induce a metabolic switch to a highly metabolically active phenotype that limits the astrocytes' neurotrophic ability. These changes are accompanied by increased mitochondrial fission with the fragmented mitochondria packaged into extracellular vesicles assembled from the mitochondrial membrane. Astrocyte-derived extracellular vesicles (AEVs) are then released and taken up by neurons. Cell type-specific mitochondrial labeling revealed distinct astrocytic and neuronal mitochondrial populations within neurons. Neuronal uptake of AEVs from astrocytes exposed to

cocaine and Tat significantly decreased both neuronal fitness and mitochondrial function. Conversely, inhibition of astrocytic mitochondrial fragmentation by expressing GTPase inactive Drp1 or dominant negative Orai1 variants significantly decreased neuronal uptake of astrocytic mitochondria and preserved neuronal fitness. Our data identified astrocyte-derived mitochondria as neurotoxic factors in the AEVs released from astrocytes exposed to Tat or cocaine.

P106

Inflammation induced-PINCH causes mitochondrial mislocalization and bioenergetic deficit in neurons

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Diseases and disorders with a chronic neuroinflammatory component are often linked with changes in brain metabolism. Among neurodegenerative disorders, people living with HIV and Alzheimer's disease are particularly vulnerable to metabolic disturbances, but mechanistic connections among inflammation, neurodegeneration and bioenergetic deficits in the central nervous system are less well defined. The particularly interesting new cystine histidine-rich protein called PINCH is nearly undetectable in healthy mature neurons, but is robustly expressed in tauopathy-associated neurodegenerative diseases including human immunodeficiency virus (HIV) infection and Alzheimer's disease. The molecular mechanisms and cellular consequences of PINCH expression were previously unknown. In this context, we have identified the transcription factor responsible for PINCH induction in chronic neuroinflammatory conditions and the effects of PINCH expression in neurons. Our data show that Tat/TNFalpha activation of MEF2A via increased cellular calcium induces PINCH transcription leading to PINCH's interaction with the actin cytoskeleton, altered mitochondrial distribution and impaired neuronal metabolism. Blocking Tat/TNFalpha-induced PINCH preserves mitochondrial localization and maintains metabolic functioning.

P107

Z-DNA-binding protein 1 (ZBP1) is critical for controlling virus replication and survival of West Nile virus encephalitis

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West Nile virus (WNV), a neurotropic flavivirus, is the leading cause of viral encephalitis in the United States. Recently, ZIKV infections have caused serious neurological diseases and birth defects, specifically Guillain-Barrè syndrome and microcephaly. Z-DNA binding protein 1 (ZBP1) is a cytoplasmic sensor that that has been shown to play a significant role in initiating a robust immune response. We previously reported that WNV and ZIKV infections induce dramatic up-regulation of ZBP1 in mouse brain as well as in infected primary mouse cells. Herein, we show the critical role of ZBP1 in restricting the pathogenesis of WNV and ZIKV infection. Deletion of ZBP1 resulted in significantly higher morbidity and mortality after infection with a pathogenic WNV NY99 strain in mice. No mortality was observed in wild-type (WT) mice infected with the non-pathogenic WNV Eg101 strain. Interestingly, infection of ZBP1-/- mice with WNV Eg101 was lethal resulting in 100% mortality, suggesting that ZBP1 is required for survival after WNV infection. Viremia and brain viral load were significantly higher in ZBP1-/- mice compared to WT mice. In addition, protein levels of interferon (IFN)-alpha, and inflammatory cytokines and chemokines were significantly higher in the serum and brains of ZBP1-/- mice compared to the WT mice. Primary mouse cortical neurons and mouse embryonic fibroblasts (MEFs) derived from ZBP1-/- mice produced higher virus titers compared to WT cells after infection with WNV NY99 and WNV Eg101. Similarly, neurons and MEFs lacking ZBP1 exhibited significantly enhanced replication of PRVABC59 (Asian) and MR766 (African) strains of ZIKV compared to WT cells. The knockout of ZBP1 function in MEFs inhibited ZBP1-dependent virus-induced cell death. In conclusion, these data reveal that ZBP1 restricts WNV and ZIKV production in mouse cells and is required for survival of a peripheral WNV infection in mice.

P108

Dopamine increases HIV entry into macrophages by increasing calcium release via an alternative signaling pathway

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Despite combined anti-retroviral therapy, many individuals with HIV still experience neurocognitive decline and neuropathology, particularly in dopaminergic regions of the CNS. Further, drug abuse and age-related psychiatric comorbidities, both of which involve the use of dopaminergic drugs, affect a significant portion of the HIV+ population. This suggests dopamine plays a role in the development of NeuroHIV and demonstrates a critical need to understand how dopamine may alter HIV neuropathogenesis. We previously showed that dopamine increases HIV entry into primary human macrophages (hMDM), a major target for HIV in the CNS. This is mediated by activation of both D1-like and D2-like dopamine receptors, which canonically signal through opposing effects on cAMP. This indicates that dopamine receptors may be acting through a common mechanism to enhance the entry process. One such mechanism could be the non-canonical dopamine signaling pathway in which activation of both subtypes of dopamine receptor lead to PLC beta-mediated PKC activation and IP3-mediated calcium release via Gq (D1-like) or Gby (D2-like). Our data support the involvement of this pathway, showing dopamine stimulates calcium release and PKC phosphorylation in hMDM in a Gq-dependent manner, indicating a role for D1-like receptors. Inhibition of calcium flux prevents the dopamine-mediated increase in HIV entry, suggesting activation of this pathway mediates the impact of dopamine on viral entry. The interaction of the viral envelope protein gp120 with the co-receptor CCR5 activates the same Gq-PLC-beta signaling cascade. This suggests that dopamine may increase viral entry by potentiating the gp120-CCR5 signaling pathway. To further assess the role of this signaling pathway on dopamine-mediated entry, we will utilize a novel entry assay to assess the requirements for Gq and PLC-beta. These studies will further define the mechanisms by which dopamine alters viral entry, providing insight into the signaling mechanisms critical for viral entry.

P109

Characterization of primary varicella zoster virus infection in guinea pigs

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Varicella zoster virus (VZV) produces varicella on primary infection, establishes latency then reactivates with aging or immunosuppression to produce zoster. During primary infection and reactivation, VZV can also produce neurological disease, including vasculopathy (presenting as stroke and cognitive impairment), myelopathy and cranial nerve palsies. A critical barrier in dissecting mechanisms of VZV-associated disease is the lack of a reproducible, well-characterized small animal model for VZV-infection. Prior studies demonstrated that the guinea pig (Cavia porcellus) is a candidate model that recapitulates human VZV primary

infection, latency and reactivation. We reproduced and optimized these studies for a comprehensive characterization of primary VZV infection in the guinea pig. Using outbred, hairless guinea pigs with jugular vein catheters to facilitate inoculations and blood draws, peripheral blood mononuclear cells (PBMCs) were isolated and infected using a monolayer of VZV-infected guinea pig fibroblasts. The VZV-infected PBMCs were able to transmit infection to other fibroblasts in vitro; when VZV-infected PBMCs were re-injected through via the catheter, a transient viremia was seen within 5 days post-infection (DPI). From 15-17 DPI, 3/3 VZV-infected guinea pigs developed clusters of raised, erythematous skin lesions in the cervical dermatomes, corresponding to inoculation site. Furthermore, the rash in one of the animals continued to spread to the right ophthalmic distribution of the trigeminal nerve. Preliminary experiments looking at synaptic function of guinea pig hippocampus showed robust long term potentiation. Our findings demonstrate a well-defined protocol for establishing VZV infection in a small animal model that holds promise for studying the pathogenesis of primary infection in vivo, as well as for testing virus contributions to synaptic dysfunction and contributions to neurodegenerative diseases.

P110

AIDS, but not HIV Disease, Reduces Gray Matter Volume

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Background: Age, illicit drug use, HIV disease, as well as CNS opportunistic infections, can affect brain structure, with the striatum being particularly vulnerable. Nevertheless, the impact of peripheral AIDS defining illness (ADI) on brain structure has been less broadly investigated. We examined peripheral ADI effects on brain structure, using a hierarchical neuroanatomical approach motivated by known serostatus effects related to sensorimotor and episodic memory functions.
Methods: In a cross-sectional study of HIV infection, including 95 virally suppressed seropositive and 84 seronegative, demographically matched participants ages 30-70 years, we examined ADI effects on brain structure. Volumes of gray matter (GM), white matter (WM), cerebrospinal fluid (CSF) tissue compartments, as well as selected cortical and subcortical ROIs were estimated from high resolution, T1-weighted MRI data using computational neuroanatomy techniques (SPM12/CAT12). In the participants with HIV disease, linear regression was used to model global and regional effects of ADI,, incorporating age, sex, CD4 nadir, drug use and total intracranial volume as covariates.
Results: Older age was associated with standardized parameter estimates showing reduced GM (β = -0.28;$p<0.05$), WM (β = -0.15; $p<0.05$), and increased CSF (β = 0.35;$p<0.05$) volumes. ADIs, but not HIV disease, were associated with reduced total GM (β = -0.19;$p<0.05$) volume. Nevertheless, neither ADI nor HIV infection affected WM, thalamic, hippocampal, or precuneus volumes.
Conclusions: While previous work demonstrated that HIV infection has a relatively localized effect on striatal structure [1], the current findings demonstrate that ADIs are the most powerful predictors of global GM volume. The lack of interaction between either HIV infection or ADI with age suggests that there is an acute impact of ADI on brain structure, but no associated alteration in the rate of change with age.
1. O'Connor, E.E., et al., HIV infection and age effects on striatal structure are additive. J Neurovirol, 2019.

P111

Lipocalin-2 deficiency ameliorates neuronal damage and behavioral deficits in a transgenic model of HIV-induced brain injury

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Up to 50% of individuals infected with human immunodeficiency virus (HIV)-1 develop some form of neurological and neurocognitive complication categorized as HIV-associated neurocognitive disorders (HAND). The pathological mechanisms leading to HAND remain incompletely understood. We have recently shown that the acute phase protein lipocalin-2 (LCN2) is one of the most upregulated genes in a transgenic (tg) mouse model of HIV-induced brain injury. Moreover, LCN2 is necessary to indirectly protect neurons with maraviroc, an inhibitor of the chemokine receptor CCR5, against damage initiated by a CXCR4-preferring HIVgp120. To explore the in vivo role of LCN2, we cross-bred transgenic mice expressing HIV envelope protein gp120 in the central nervous system (HIVgp120tg), which display features observed in NeuroHIV patients, with a genetic knockout of LCN2 (LCN2ko). LCN2 deficiency prevented behavioral deficits, and largely abrogated synaptic and dendritic damage, minimized the increase in microglial cells and reduced p38 MAPK activity caused by the viral protein gp120. However, neuronal protection was dependent on the presence of CCR5. We found that LCN2ko animals have an increased in expression levels of CCR5-natural ligands, and HIVgp120tg mice lacking LCN2 and CCR5 showed restoration of neuronal damage as well as microglial activation accompanied by a significant increase in p38 MAPK activity. Furthermore, we observed that LCN2 is significantly upregulated in brains of HIV+ patients when compared to non-infected controls. Additionally, higher levels of LCN2 were present in HIV+ patients with brain injury than in HIV+ patients without brain injury suggesting a role for LCN2 in HIV-associated brain injury. Altogether, our findings point to a previously unknown interaction between LCN2, CCR5, and p38 MAPK in the context of HIV-associated brain injury and neurocognitive impairment. Supported by NIH, MH087332, MH104131, MH105330 and DA026306 (P5) to M.K.

P112

HIV-1 Tat Promotes Age-Related Cognitive, Anxiety-like, and Antinociceptive Impairments in Mice that are Moderated by Aging Endocrine Status

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Dysfunction of the hypothalamic-pituitary-gonadal axis is a common co-morbidity among HIV-1-infected individuals (~1/5 reporting hypogonadism). This incidence is greater among those over the age of 50, who now comprise more than 60% of the U.S. HIV+ population. The underlying mechanisms are unknown, but both combined antiretroviral therapeutics or HIV-1 proteins, such as the trans-activator of transcription (Tat), are associated with dysregulation of lipid storage/synthesis or mitochondrial function, rate-limiting processes for steroidogenesis. Evidence for Tat-mediated behavioral deficits in aged animal models is sparse and findings are not typically stratified by endocrine status. We anticipated that conditional Tat expression in aged (~1.5 y/o), male and female transgenic mice (Tat+ mice) would impair cognitive performance and increase anxiety-like behavior, mechanical allodynia or thermal hyperalgesia, and the incidence of age-related co-morbidities (splenomegaly, cardiomegaly, tumorigenesis) compared to age-matched controls (Tat- mice). We further expected aged females that maintained their reproductive status (pre-estropausal) to be more resilient to Tat/age-related co-morbidities than those that had transitioned to reproductive senescence

(post-estropausal). We found the latency to escape a radial arm water maze and the incidence of memory errors to be significantly greater among Tat+ males and post-estropausal females compared to respective Tat- controls. Either Tat-exposure or being post-estropause significantly increased anxiety-like behavior as measured by the number of entries in the center of an open field or the proportion of time spent on the open arms of an elevated plus maze. Tat increased mechanical allodynia among aged females, but not aged males, with no effect on thermal hyperalgesia. Tat expression was also associated with splenomegaly and cardiomegaly in females (not males), and a greater incidence of liver tumors among post- vs pre-estropausal females. Steroid replacement improved symptomology in males, but has not yet been assessed in females. Thus, endocrine status may be an important predictor in Tat/age-related decline.

P113

Novel Role of Extracellular Vesicles in HIV/SIV Neuropathology

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Combined Antiretroviral Therapy (cART) effectively achieves systemic virological suppression in HIV-infected patients; however, HIV Associated Neurocognitive Disorders (HAND) still progress in aviremic patients. HIV Nef and Gag proteins are detectable in PBMCs from aviremic patients supporting low level constant transcriptional activity in latently infected cells. We confirmed HIV/SIV Nef is carried within extracellular vesicles (EV) and transported between cells. This novel mechanism of viral protein transfer allows for viral proteins to enter uninfected cells and cells lacking the CD4 entry receptor, including astrocytes. Extracellular communication via EVs carrying viral proteins could explain long-term chronic activation and inflammation within the CNS associated with HIV in aviremic patients. To evaluate astrocyte activation by EVs carrying viral proteins, EVs were isolated from mixed glial cultures from SIV-infected rhesus macaques (RM) and cocultured with naïve primary astrocytes. Astrocyte activation was assessed via immunocytochemical staining of TLR2 and vimentin and ProcartaPlex immunoassay. Co-culture with EVs showed significant activation of astrocytes. To determine immune response and systemic trafficking of EVs, in vivo intravenous infusion of two rhesus macaques with fluorescently labeled EVs isolated from control or SIV-infected RM was performed. Heterologous infusion of EVs did not cause adverse reaction in either macaque. Flow cytometry identified infused EVs on the surface or within monocytes circulating within the blood at 4hrs post-infusion. EVs were identified within tissues at 48hrs post-infusion. This is the first in vivo EV infusion in a nonhuman primate model in the context of HIV. This infusion model provides a means for future studies to evaluate the pathogenesis associated with EVs carrying HIV viral proteins as well as explore EVs as therapeutic carriers. This work was supported by grants from the National Institutes of Health including MH113517, NS104016, PS1-OD11104, U42-OD024282, U42-OD10568, DA040394 and a Ruth L. Kirschstein National Research Service Award (5T32OD011124-13).

P114

HIV-Tat autocleaves and forms novel amyloid fibrils which are highly neurotoxic

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HIV-Tat protein is released from HIV-infected cells even under antiretroviral therapy and causes activation of lymphocytes, glial cells and neurotoxicity. Using atomic force microscopy (AFM) and mass spectrometry we found that HIV-Tat can spontaneously cleave into shorter peptides, some of which are consistent with the protein's predicted inteins. Unlike other fragments, the HIV-Tat 32-62 peptide is neurotoxic by itself. Starting with nM concentrations, using AFM we visualized micron-long branching fibrils with ~80 nm persistence lengths and layered structure. At higher concentrations, the fibrils thickened, and their branching angles decreased. With further increase in concentration, besides fibers, large irregularly shaped aggregates formed in solution and, at interfaces, extended sheet-like aggregates and tens-of-microns-long fibers appeared. Birefringence microscopy, FTIR and polarized Raman microspectroscopies of the aggregates suggest that the peptide has highly oriented side chains and backbone and contains 45% beta-sheet, consistent with the amyloid fiber structure. This is different than the full-length HIV-Tat, which is alpha-helical in bound state and in aggregates. HIV-Tat 32-62 forms fibers much faster and is more potently neurotoxic than amyloid beta. Raman measurements showed that the free cysteines in aggregates form in time S-S bonds, which make the aggregates insoluble. The replacement of Cys34, Phe38 and Ile45 with Ala in the peptide chain led to reduced aggregation, no fibril formation and lower neurotoxicity. We identified microns-long Tat 32-62 fibers in cells expressing the peptide or the full-length Tat. Our model of the fibril involves an antiparallel beta sheet backbone of the protofibril to which other protofibrils attach in a stepwise manner and are eventually connected through S-S bonds. These results indicate that HIV-Tat autocleavage makes possible the aggregation of the 32-62 fragment which leads to neurotoxicity through an amyloid type of mechanism.

P115

Recall responses from brain-resident memory CD8+ T-cells (bTRM) induce reactive gliosis

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HIV-associated neurocognitive disorders (HAND) persist even during combination antiretroviral therapy (cART) when viral loads in cerebrospinal fluid (CSF), as well as plasma, are below detectable limits. While the cause of HAND is unknown, recent studies link chronic immune activation, neuroinflammation, and CSF viral escape in cART-treated patients to increased disease progression. In this study, we tested the hypothesis that specific, recall immune responses from brain-resident memory T-cells (bTRM) activate glial cells and induce production of neurotoxic mediators. To address this question, we used a heterologous prime-boost strategy in which mice were first immunized with recombinant adenovirus vectors expressing the HIV-1 p24 capsid protein, followed by a CNS-boost using Pr55Gag/Env virus like particles (HIV-VLPs). We observed that the murine brain became populated with long-lived CD8+ bTRM cells specific for immunodominant HIV-1 Gag epitopes.

Increased expression of the memory cell markers: CD127, CD69, CD103, and CD49a were identified on these bTRM cells. Furthermore, bTRM cells produced high levels of IFN-gamma and IL-2 upon ex vivo Ag restimulation. When prime-CNS boost animals were subjected to in vivo recall stimulation using the immunodominant HIV-1 AI9 peptide, HIV-specific CD8 TRM cells rapidly initiated tissue-wide inflammation. Higher levels of HIV-specific bTRM cells were induced upon cognate antigen rechallenge. Reactivation of bTRM cells resulted in microglial activation and displayed elevated levels of MHC II and PD-L1 (B7-H1); markers of cellular activation, demonstrating tissue-wide reactive gliosis. IHC staining further confirmed glial cell activation following restimulation. In addition, elevated levels of MHC II, Iba (ionized calcium binding adaptor molecule)-1, IFN-gamma, iNOS (inducible nitric oxide synthase), CXCL10 and CXCL9 mRNAs were observed following ex vivo AI9 restimulation of brain mononuclear cell: primary glial cell co-cultures. Taken together, these results indicate that specific, adaptive recall responses from bTRM cells induce reactive gliosis and drive production of neurotoxic mediators.

P116

Maraviroc Treatment Delays SIV Rebound from the CNS Latent Reservoir

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The SIV/macaque model of HIV allows in-depth study of latent viral reservoirs including comparisons of reactivation of virus from both peripheral and CNS latent reservoirs following treatment interruption. Using the SIV/pigtailed macaque model, we treated 16 SIV-infected pigtailed macaques with CNS penetrant ART consisting of dolutegravir, PMPA, and FTC beginning 12 days post-inoculation. 10 of the 16 SIV-infected ART treated macaques also received maraviroc (MVC). Virus replication was suppressed in plasma and CSF within 50 days of ART. After >100 days of suppression, ART (DTG, PMPA, FTC) was stopped in all 16 macaques. Of the 10 animals receiving ART and MVC, MVC treatment was stopped in 6 animals at the same time as ART withdrawal; MVC monotherapy was continued after stopping ART in the other 4 animals. Modeling of rebound virus in plasma showed similar growth kinetics once virus became detectable in all three treatment groups, however the timing of initial virus detection was delayed by an average of 4 days in animals that received ART+MVC. All animals, regardless of treatment, rebounded to a stable plateau of 10^5 copies/mL, ~2 logs lower than their pre-treatment plasma set points. A similar pattern was seen in CSF; a delay in time of initial detection with MVC treatment followed by similar rebound kinetics once SIV was detectable. However, CSF SIV rebound in the animals who remained on MVC after stopping ART was delayed by 2 weeks versus the other ART and ART+MVC withdraw groups. These findings demonstrate that CCR5 inhibition delays viral rebound in both plasma and CSF compartments consistent with a reduction in the latent viral reservoir in periphery and CNS. Furthermore, continuing MVC after stopping ART markedly delays CSF rebound but not plasma rebound, illustrating that continuing CCR5 inhibition post-treatment interruption selectively targets rebound from CNS reservoirs.

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Active apoptosis and regeneration of peripheral nociceptive neurons during SIV infection.

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Human immunodeficiency virus (HIV)-associated sensory neuropathy (HIV-SN) is a common neurological comorbidity of HIV infection and prevails in the post-antiretroviral therapy (ART) era. HIV infection drives pathology in the dorsal root ganglia (DRG) through mechanisms including inflammation, altered metabolism, and neuronal dysfunction. Importantly, DRG neurons are long-lived neurons with high axonal regenerative capacity and resilience to environmental changes; however, death of DRG neurons is permanent. Here, we characterized damage to and regeneration of specific neuronal populations in a simian immunodeficiency virus (SIV)-infected macaque model with or without ART. DRG neuronal populations were identified by neurofilament H-chain 200 (NF200), I-B4 isolectin (IB4), or tropomyosin receptor kinase A (TrkA) expression and assessed for population size, apoptotic markers, and regeneration signaling. IB4+ non-peptidergic and TrkA+ peptidergic neurons showed a decreased population size (cell death) in the DRG of SIV-infected animals compared to uninfected animals. ART mitigated the loss of IB4+ non-peptidergic neurons but not TrkA+ peptidergic neurons. We observed both pro-regenerative, stress-induced signaling (ATF3) and apoptotic signaling (cleaved caspase 3) in DRG neurons during SIV infection. Neurons in the DRG showed accumulation of cytosolic cleaved caspase 3 and nuclear-localized activating transcription factor 3 (ATF3), which was significantly lower in uninfected animals and SIV-infected animals receiving ART. Non-peptidergic and peptidergic neurons colocalized with cytosolic cleaved caspase 3 and nuclear-accumulated ATF3, showing apoptosis and active regeneration in sensory neurons. These data suggest that non-peptidergic and peptidergic neurons are susceptible to pathological changes from SIV infection, and although intervention with ART reduced damage to the DRG, peptidergic neurons remained susceptible to damage even with reduced viral load.

P118

Modeling shock and kill therapy for SIV brain infection

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Antiretroviral therapy (ART) has greatly increased worldwide life expectancy for human immunodeficiency virus-1 (HIV-1) infected patients. Even with the remarkable success of ART, the virus persists in many different cells and tissues. The formation of viral reservoirs represents a major obstacle to HIV-1 eradication. Viral reservoirs contain latently infected long-lived cells. The brain has minimal ART penetration and is a natural anatomical reservoir for HIV-1 infection. The "Shock and Kill" therapeutic initiative aims to reactivate latently infected cells by latency reversing agents (LRAs) and kill these reactivated cells by strategies involving the host immune system. The effectiveness and safety of LRAs in the brain needs to be carefully investigated and understood. By developing a three-compartment mathematical model, a predictive model of brain viral infection is generated from empiric simian immunodeficiency virus (SIV) data. SIV nonhuman primate (NHP) studies provide brain tissue specimens where the duration of infection and the timeframe of any treatment strategies are known. Empiric SIV data from two different NHP SIV infection models will be used. One SIV model provides pigtailed macaque data for short term disease progression (approximately 7-95 days post-infection) and the other SIV model provides Indian Rhesus macaque data for longer term disease progression (140-211 days post-infection). This mathematical model was used to quantify the dynamics of latently and productively infected cells in the brain during SIV infection. The transmission rate at which susceptible brain macrophages become productively infected was estimated to be in the range 6.41x10^-6 to 1.44x10^-5 per year for SIV infection among untreated pigtailed macaques and was estimated to be in the range 6.51x10^-6 to 1.71x10^-5 per year for SIV infection among untreated rhesus macaques. This mathematical model was used to simulate the effects of LRAs and the "Shock and Kill" therapy in the brain during SIV viral infection.

P119

Multiplexed digital gene expression profiling of West Nile neurovirulence in C57BL/6J and C57BL/6N mouse models

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West Nile virus (WNV), a neurotropic flavivirus, is the leading cause of viral encephalitis in the United States. Host genetic factors are potential regulators of human WNV disease susceptibility, and in the mouse model, genetic background has been linked to WNV pathogenicity. The inbred C57BL/6J mouse is frequently used to generate knockout or knockin strains and is a well-established model of WNV disease. A closely related substrain, C57BL/6NJ, is the strain used for all knockout mice generated under the International Knockout Mouse Consortium. It is not clear whether C57BL/6J and C57BL/6NJ differ in their susceptibility to WNV disease. Moreover, the host genetic factors of WNV disease susceptibility in these mouse strains have not been documented. In order to explore these effects and recapitulate the immune genetic differences among mouse strains, we studied changes in the gene expression following WNV infection in C57BL/6J and C57BL/6NJ mouse models. We used the nanostring nsolver platform to quantify gene expression in the brain tissues isolated from WNV-infected C57BL/6J and C57BL/6NJ mice. Our data show that C57BL/6J and C57BL/6NJ differ in their susceptibility to WNV disease. WNV infection in C57BL/6NJ mice results in high brain viral load, increased immune cell infiltration, and higher levels of inflammatory cytokines and chemokines in infected brain tissues compared to the C57BL/6J mice. In addition, mRNA levels of interferon regulatory transcription factors were significantly higher in the C57BL/6NJ mice compared to the C57BL/6J mice. In summary, this work establishes a clear difference in WNV pathogenicity mediated by host inflammatory processes in the closely related C57BL/6J and C57BL/6NJ mouse strains. Our study uncovered distinct differences between C57BL/6J and C57BL/6NJ mouse after infection with WNV, which may be exploited in future studies to identify host factors and/or specific genetic elements that regulate host-dependent inflammatory mechanisms involved in WNV pathogenicity.

P120

Effects of Sex on Cigarette Smoke- and Nicotine-Induced Motor and Behavioral Abnormalities in HIV-1 Transgenic Rats

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Background: Cigarette smoke (CS) exposure may be associated with an increased risk of neurocognitive impairment (NCI) in HIV-1 infection and worse in women than men. Therefore, we studied the impact of sex/gender on these effects using the HIV-1 transgenic rat, which contains 7 of 9 HIV-1 genes.
Methods: Rotarod test (RRT), novel object recognition test (NORT), and open field test (OFT) data from groups of Tg and wild-type Fisher F344 (WT) rats that were either non-exposed (CON) or exposed to either regular CS (CIG), nicotine-free CS (NF) or to nicotine alone (NIC) were analyzed by three-way analysis of variance. The factors included in the analysis were genotype, sex and exposure.
Results: There were main effects for genotype on the NORT and OFT, for sex on the RRT and OFT and for the exposures on all three tests. Females rats showed overall better performance on the RRT, and WT genotype and female sex was associated with increased exploratory activity on the OFT. Regular CS exposure was linked with the overall enhanced performance on the RRT and OFT, whereas nicotine decreased overall exploratory behavior on the OFT. Analysis of two-way interactions showed that, in the presence of the HIV transgene, responses were overall increased in male and decreased in female animals. On the RRT, male and female rats were similarly response to CS, but females were more responsive to nicotine, and, on the OFT, CS increased exploratory behavior more for males than females. The three-way interaction between the factors was associated with increased exploratory behavior.
Conclusions: CS and nicotine showed opposing effects on motor activity and anxiety in WT versus Tg and in male versus female rats that could underlie observations regarding these exposures in men and women, particularly those at risk for the development of HIV-related NCI.

P121

Enterovirus D68 particles exist in naked and membrane bound forms

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Enterovirus D68 (EV-D68) is an emerging pathogen that causes bi-annual respiratory disease and is associated with rapid-onset muscle-weakness and limb paralysis in children (termed acute flaccid myelitis, AFM). Recent, but not historic, viral isolates from the United States are capable of infecting spinal cord motor neurons in vivo and neuronal cells in vitro, however the biological basis of the increased virulence and recently acquired ability of contemporary EV-D68 to induce CNS disease is unknown. The presence or absence of an envelope surrounding the capsid of an animal virus influences its stability, transmission, and immune recognition within the CNS. Picornaviruses (including EV-D68) are considered to be "non-enveloped" viruses that package their genomes in icosahedral protein capsids that are released during cell lysis. It has recently been shown that several picornaviruses can be released from cells in a non-lytic fashion via extracellular vesicles. We examined EV-D68 viral structure using ultracentrifugation through density gradients and found that, in addition to the expected naked viral capsid (density of 1.21-1.23g/cm3), there was a second viral peak consistent with an enveloped form (density near 1.1g/cm3). This second peak was completely eliminated following treatment with a non-ionic detergent. In addition, electron microscopy revealed that this second viral peak contained a "membrane-bound" conglomerate of viral particles approximately 140nm in diameter. Taken together, our data suggests the existence of a previously undiscovered membrane-bound form of EV-D68.

P122

Cigarette smoke extract exposure alters extracellular vesicle release from several CNS cell types

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Introduction: HIV-associated neurocognitive disorders (HAND) affect many people living with HIV. Peripherally circulating brain-derived extracellular vesicles (EVs) and their encapsulated RNAs may serve as biomarkers for HAND, however cigarette smoking can also modulate expression of these markers. Smoking rates are also higher in HIV+ populations. To better understand how cigarette smoke extract might modulate EV release and cargo, we examined several CNS-derived cell lines, including astrocytes (U87MG), microglia (SV40), and immortalized human oligodendrocytes (HOG). Methods: CSE was prepared by bubbling through culture medium using a standardized method. All cell types were exposed to either 0% or 50% CSE for 24 hours. Cell viability was assessed by MuseTM Cell Analyzer, and EVs were isolated from culture conditioned media (CCM) by size exclusion chromatography (SEC). The void (fractions 1-6), EV (7-10), and protein (11-14) were pooled and concentrated. EVs were characterized by transmission electron microscopy (TEM), Spectradyne particle counting, and Western blotting. Results: For all cell types, viability was only slightly reduced in response to 50% CSE exposure (~90%). Vesicles present in the EV

fractions displayed the "cup-shaped" artifact associated with TEM fixation, as well as the absence of EVs in both the void and protein fractions. Spectradyne particle counts indicated CSE exposure substantially increased the CCM particle count in the EV fraction when compared to control. Western blotting revealed the presence of the expected EV markers, including CD63, CD81, and TSG101 in the EV fractions, while their absence was observed in the void and protein fractions. Conclusion: Despite a remarkable resistance to CSE-induced cell death, CNS cells display physiologic responses to CSE that include vesiculation pathways. We are now studying the RNA cargo of brain-derived EVs to identify markers of HAND that are affected or not by tobacco smoke.

P123

Neuroinflammatory and integrated stress response signatures persist in a human iPSC tri- culture model of HIV infection despite antiretroviral treatment

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HIV-Associated Neurocognitive Disorders (HAND) affect over half of HIV-infected individuals worldwide. While antiretroviral therapy (ART) has reduced the severity of HAND, the prevalence has increased due to increased life expectancy. Therapeutically targetable mechanisms underlying HAND remain elusive due to a lack of tools to study the direct interactions among HIV-infected microglia, neurons, and astrocytes. We developed a human-induced pluripotent stem cell (HiPSC) based model; whereby, we independently differentiate HiPSCs into neurons, astrocytes, and microglia and systematically combine to generate a tri-culture with or without HIV-infection and ART. scRNAseq analysis on tri-cultures including HIV infected iMg revealed inflammatory signatures and integrated stress response, specifically EIF2 signaling, in all three cell types; although, the microglia were most affected. ART mostly resolved these signatures but did not completely quell inflammation in the microglia and neurons. Remarkably, ART alone induced a similar response to infection. scRNA analysis also revealed activation of Fcgamma receptor mediated phagocytosis pathway in infected iMg. However, there was a reduction in synaptic phagocytosis by infected microglia, suggesting that enhanced phagocytosis by HIV-infected microglia may not account for the synaptodendritic damage observed in HIV-infected patients. Pathway analysis also revealed increased RhoGDI and CD40 signaling in the HIV-infected microglia exposed to ART. This activation was associated with a persistent increase in TNFalpha expression. This work establishes an all human tri-culture that recapitulates key features of HIV infection in the CNS and provides a new model to examine the effects of HIV infection and its treatment with antiretroviral compounds in a multicellular context.

P124

Comparisons of Brain DTI and DKI Metrics in Individuals with Clade C HIV Infection

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Introduction: HIV enters the brain leading to low-level inflammation and alterations to the tissue structural integrity of the brain, which are associated with mild-to-moderate neurocognitive impairments. Prior diffusion tensor imaging (DTI) studies have shown altered brain structural integrity due to HIV infection. Yet this finding is not consistent across the literature as some studies reported near-normal structural integrity in HIV+ individuals. Our goal is to determine if diffusion kurtosis imaging (DKI) can provide a more reliable measurement of the brain structural integrity.
Methods: DKI data were acquired using a 3T scanner from 64 HIV+ and 56 HIV- subjects. Sixty dual-shell DW-images (b = 1000/2000 s/mm2) and 9 b0 images acquired with 30 gradient directions were used for DTI/DKI analysis. The data were pre-processed for motion and eddy-current distortions, then fitted to generate DTI (FA, MD, AD, RD) and DKI (kFA, MK, AK, RK) parametric maps. We evaluated these metrics at regions known to harbor high HIV viral load: frontal and temporal lobes, cerebellum, caudate, putamen, globus pallidus, thalamus, and substantia nigra. We applied an F-test ($p<0.05$ corrected for multiple comparisons) to find significant between-group differences and also calculated Cohen's d.
Results: With DTI metrics, group differences are only significant in the caudate and thalamus with decreasing FA, increasing MD, AD, and RD for the HIV+ group. DKI metrics show significant differences in two additional regions, susbtantia nigra and caudate, with the trend showing a decrease in kFA, MK, AK, and RK for the HIV+ group. Cohen's d was <0.5 for all DTI metrics and while some DKI metrics showed higher differences (>0.5).
Conclusion: Both the DTI and DKI metrics show alterations to the structural integrity of brain tissue in HIV+ subjects. However, the DKI metrics exhibited relatively 1) higher number of regions with significant differences, 2) higher effect sizes.

P125

A comprehensive proteomic analysis of JC virus (JCV) agnoprotein-associated proteins: Agnoprotein primarily targets the host-cell proteins with coiled-coil motifs

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JC virus (JCV), the etiologic agent of progressive multifocal leukoencephalopathy (PML) which primarily occurs in a subpopulation of the individuals with an underlying immunocompromised conditions including those with cancer, multiple sclerosis and AIDS, encodes a small, highly basic phospho-protein, named agnoprotein (Agno). Although its functions are not completely understood, in the absence of its expression, JCV cannot sustain its productive life cycle. It plays regulatory roles in viral replication, transcription, and virion biogenesis. It forms stable dimers/oligomers and is released from the infected cells. The physical and functional interaction of Agno with various host proteins was previously reported. However, understanding its detailed regulatory roles during the viral life cycle is hampered by the lack of a complete map of such interactions. Here, we report the first comprehensive interactome of Agno with its host cellular targets utilizing a powerful "Two-Strep-Tag" affinity purification system coupled with mass spectroscopy. Enrichment analysis of the proteomic data revealed that Agno primarily targets the cellular proteins with "coiled-coil" motifs. Agno-host interactions occurs at the organelle level, including mitochondria, nucleus/nucleolus and ER-Golgi network and at the cellular processes level, including protein synthesis and degradation, and cellular transport. Among its newly discovered cellular partners, we further investigated the Agno-CRM1 (Exportin) interaction by mutagenesis and protein-protein interaction studies. Agno interacts with CRM-1 through its nuclear export signal (NES) located within its major alpha helix. Agno NES sequence shows high similarity to that of HIV Rev. HIV Rev-M10-like double mutation within Agno NES (L33D-E34L) nearly abolished the Agno-CRM-1 interaction and consequently significantly down-regulated the efficiency of the viral replication, suggesting a role for Agno in CRM-1-mediated

functions during the viral replication cycle. This comprehensive proteomic data provides new opportunities to further unravel the critical regulatory roles of agnoprotein during the JCV life cycle.

P126

JC virus late coding region encodes a novel nuclear protein, ORF4, which targets PML-NBs and modulates their expression and reorganization

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JC virus (JCV) is the etiologic agent of the progressive multifocal leukoencephalopathy (PML) and contains a small, double-stranded circular DNA genome. The JCV early and late coding regions generate a number of splice products, some of which encode novel proteins. We have recently reported the discovery and characterization of two such open reading frames from the JCV late coding region, generating two small proteins, ORF1 and ORF2, (Saribas et al., J. Cell Physiol., 233:4137-4155, 2018). Here, we report the discovery of an additional ORF from the same coding region, designated as ORF4, which encodes a 173 aa-long protein. ORF4 protein is identical to the C-terminus of JCV VP1 (aa 182-354). Immunocytochemistry studies revealed that ORF4 protein localizes to the nucleus with a punctate distribution pattern and specifically targets one of the remarkable protein assemblies, termed promyelocytic leukemia nuclear bodies (PML-NBs), where it enhances their expression and alters their organization. The PML-NBs are known to play critical roles in both intrinsic and innate immune responses against viral infections. Reorganization of PML-NBs by other polyomaviruses, including BK virus and merkel cell polyomavirus was previously reported but the identity of any viral protein associated with such alterations was unknown to date. We now identified the ORF4 protein as the only JCV protein responsible for the enhanced expression and reorganization of the PML-NBs. Additional studies revealed that besides PML protein, ORF4 also induces the expression of other two permanent members of the PML-NB complexes, ATRX and hDaxx, but attenuates that of Sp100. Further studies revealed that ORF4 contains a novel nuclear localization signal (NLS) of its own, which is also functionally shared by the JCV VP1. We are currently further investigating the regulatory roles of ORF4 in modulation of the intrinsic and innate immune responses induced by PML-NBs towards JCV infections.

P127

Suppression of Zika virus infection in brain by the antiretroviral drug, rilpivirine

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Zika virus (ZIKV) infection is associated with microcephaly in neonates and Guillain-Barré syndrome in adults. ZIKV produces a class of non-structural (NS) regulatory proteins that play a critical role in viral transcription and replication including NS5, which possesses RNA-dependent RNA polymerase (RdRp) activity. Here, we demonstrate that rilpivirine (RPV), a non-nucleoside reverse transcriptase inhibitor (NNRTI), used in the treatment of HIV-1 infection, inhibits the enzymatic activity of NS5, and suppresses ZIKV infection and replication in primary human astrocytes. Similarly, other members of the NNRTI family, including etravirine and efavirenz showed inhibitory effects on viral infection of brain cells. Site directed mutagenesis identified 14 amino acid residues, within the NS5 RdRp domain (AA265-903), which are important for the RPV interaction and the inhibition of NS5 polymerase activity. Administration of RPV to ZIKV infected interferon-alpha/beta receptor (IFN-A/R) knock out mice improved the clinical outcome and prevented ZIKV-induced mortality. Histopathological examination of the brains from infected animals revealed that RPV reduced ZIKV RNA levels in the hippocampus, frontal cortex, thalamus and cerebellum. Repurposing of NNRTIs, such as RPV, for inhibition of ZIKV replication offers a possible therapeutic strategy for the prevention and treatment of ZIKV associated disease.

P128

HIV-1 Nef disrupts oligodendrocyte morphology and myelin integrity in the central nervous system.

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HIV-associated neurocognitive disorder (HAND) is a spectrum of cognitive impairments that remain a common consequence of HIV infection. While the advent of combined antiretroviral therapy (cART) has substantially reduced the most severe forms of HAND, milder forms continue to affect 30-50% of HIV-positive individuals. Clinical and experimental studies have implicated preferential white matter damage in HAND pathogenesis, but the mechanisms underlying HIV-associated demyelination remain unknown. Our lab has previously shown that the HIV-1 accessory protein Nef is released from cells in extracellular vesicles (EVs) and impairs cholesterol efflux from macrophages in the periphery by down-regulating and inactivating a critical cholesterol transporter, ATP-binding cassette A1 (ABCA1). Since oligodendrocytes require a tremendous amount of cholesterol for the synthesis, formation, and potentially the maintenance of myelin sheaths in the central nervous system (CNS), the current study examined the effects of Nef EVs on oligodendrocyte morphology and myelin structure in the CNS. EVs carrying recombinant Nef were produced by transfected HEK293T cells and applied to mouse cerebellar slice cultures ex vivo, mouse spinal cords in vivo, and mixed mouse cortical cultures in vitro. EVs produced by cells transfected with an empty vector served as control. Immunohistochemical analysis of Nef-treated cerebellar slice cultures showed decreased myelin along cerebellar axons, indicated by decreased ratio of myelin basic protein (MBP)/MBP+ neurofilament medium protein (NFM) immunoreactivity. Spinal cord white matter of adult mice injected with Nef also displayed decreased MBP immunoreactivity consistent with myelin lesions that were not observed in controls. Furthermore, treatment with Nef altered complex processes in mature oligodendrocytes in vitro. Together, these data suggest that Nef perturbs myelin integrity in the CNS by impairing oligodendrocytes. Further work will examine the role of ABCA1 and reverse cholesterol transport in Nef-mediated myelin impairment.

P129

Impairment of dopamine- and cAMP-regulated neuronal phosphoprotein-32 (DARPP-32) in SIV-infected rhesus macaques

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In the era of combination retroviral therapy (cART), about 50% of people with HIV (PWH) still have neurocognitive impairment. Molecular pathways required to release neurotransmitters and the downstream signals

are impaired in HIV, leading to inefficient neuronal activities. Dopamine plays an important role in reward, emotion and movement pathways in the brain. DARPP-32 is a target of dopamine receptor D1, by which it conveys downstream responses of the receptor activation to multiple targets. DARPP-32 endogenously inhibits the activity of protein phosphatases such as PP1, PP2A and PP2B by a complex round of phosphorylations at tyrosine 34 (T34) by PKA. Conversely, phosphorylation at tyrosine 75 (T75) by CDK5, converts DARPP-32 to an inhibitor of PKA affecting DARPP-32 inhibitory activities on the protein phosphatases. This interplay leads to the alterations in the phosphorylation levels of protein phosphatase targets. Here, we investigated the expression, phosphorylation and cellular distribution of DARPP-32 in 5 uninfected rhesus macaques and 15 SIV-infected, 6 of which were also treated with a conventional ART regimen. We determined a significantly higher overall expression and phosphorylation of DARRP-32 in the frontal cortex and hippocampal of SIV-infected and SIV-infected ART animals when compared with uninfected animals. In addition, synaptosome fractionations also demonstrated that DARPP-32 is highly enriched at the synapse in both SIV-infected and SIV-infected ART animals. Immunohistochemistry experiments of the frontal cortex and hippocampus revealed an enriched presence of T34 and T75 phosphorylated forms of DARPP-32 along neuronal processes indicating the altered distribution of DARPP-32. Furthermore, we used rat hippocampal and striatal neuronal cultures to verify our observations. We found that HIV Tat protein induces the expression of DARPP-32 in cultured primary neurons. We postulate that HIV Tat induces the higher expression and alters the phosphorylation of DARPP-32 in multiple sites in neurons impairing the brain dopaminergic pathways in PWH.

P130

IFNAR1 Plays a Critical Role in Neuronal Injury Induced by HIV-1

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Infection with HIV-1 causes brain injury and HIV-associated neurocognitive disorders (HAND). Nearly, half of the HIV positive population develops some form of HAND. Our laboratory uses a transgenic mouse (tg) model of HIV-associated brain injury expressing the viral gp120 envelope protein under the control of a modified GFAP promoter in the astrocytes. These HIVgp120tg mice display key neuropathological features of human HIV brains, including neuronal damage, differential gene expression and behavioral deficits. HIV-1 infection and transgenic expression of gp120 both activate the innate immune system, including the production of the type I interferons (IFN) alpha and -beta, which signal via IFNalpha receptor (IFNAR) 1 and -2. We recently showed that treatment with IFNbeta could completely abrogate in vitro and in vivo neuronal damage triggered by HIV envelope protein gp120. Neuroprotection by IFNbeta was dependent on IFNAR1. In this study, we cross-bred IFNAR1 knockout (IFNAR1KO) with HIVgp120tg mice in order to investigate the role of IFNAR1 in HIV induced brain injury. We found that the genetic deletion of IFNAR1 resulted in partial protection from neuronal damage and behavioral deficits in HIVgp120tg mice. The IFNAR1KO prevented the damage of neuronal dendrites in females and males of HIVgp120tg mice but ameliorated the loss of presynaptic terminals only partially and primarily in females. IFNAR1 deficiency also diminished, in a sex-independent fashion, microglial activation, whereas astrocytic GFAP expression remained elevated. IFNAR1-deficiency rescued the spatial learning and recognition memory of HIVgp120tg females but not males. Altogether, our study suggests that in the absence of protective IFNbeta concentrations IFNAR1 contributes in a sex-dependent manner to neuronal injury triggered by HIV-1.

P131

Defining HIV-1 Tat genetic co-linear variation within patients of the Drexel CARES Cohort using the third-generation PacBio sequencing platform

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HIV-1 mortality has decreased with the prolonged use of suppressive antiretroviral therapy (ART) while the incidence of HIV-1-associated neurocognitive disorder (HAND) has increased. The HIV-1 Tat protein has been shown to cause neurotoxicity and be associated with neuroinflammatory and neurodegenerative CNS disease. Variation within Tat has been observed to affect its function and ability to induce neurotoxicity. We have recently performed studies to identify and characterize predominant amino acid variants within Tat that associate with HAND. Initial studies amplified Tat exon I and II separately from patient PBMCs from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort using PCR, and subsequently sequenced the amplicons using Illumina next-generation sequencing. Multiple positional hotspots of high variation in Tat were identified within both the first and second exons. Statistical analyses were applied to the amino acid positions and variants were each associated with a HAND diagnosis. To be able to examine the co-selection of amino acid variants that exist in both Tat exons, PCR primers encompassing both exons were generated. The amplification strategy utilized primers encompassing both exons of the Tat gene, spanning over 3 kilobases of the HIV-1 proviral genome. This method was applied to an initial set of 8 samples, which were sequenced using PacBio next-generation long-read sequencing technology. Results demonstrated variation in the number of dominant sequences, where some patient amplicons had a single dominant sequence and others had several minor variants. Following this preliminary analysis, the study was expanded to include an additional 57 patient samples, which were PCR amplified using the same strategy, but utilized primers that extended from Tat exon I into the 3' long terminal repeat (LTR) region. Future studies will focus on the association and impact of Tat amino acid variation and co-selection on Tat-mediated neurotoxicity and HAND.

P132

Genetic deletion of the p75 neurotrophin receptor attenuates the loss of dendritic spines and alleviates behavioral impairments in gp120 transgenic mice.

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Human immunodeficiency virus type 1 (HIV-1) positive individuals exhibit an array of neurocognitive symptoms, termed HIV-associated neurocognitive disorders (HAND). These symptoms persist despite combined antiretroviral therapies. HAND may be partly explained by a combination of neuronal simplification, loss of synaptic connections, and reduction in dendritic spine volume. Activation of the p75 neurotrophin receptor (p75NTR) by a precursor form of brain-derived neurotrophic factor (proBDNF) drives neuronal simplification and aberrant dendritic spine morphology. ProBDNF is upregulated in several brain areas in HAND subjects and in animal models of HAND which express the neurotoxic envelope protein gp120. To establish a relationship between p75NTR activation and gp120-mediated neurodegeneration, we crossbred gp120 transgenic (tg) and p75NTR-/- mouse colonies to obtain wild type (wt), gp120tg, p75+/-gp120tg, and p75-/-gp120tg mice. Golgi-Cox staining of hippocampal and striatal neurons in gp120tg mice demonstrated sharply reduced spine density and selective loss of mature spines compared to other genotypes. The gp120tg experimental group also demonstrated a decrease in the expression of the postsynaptic marker PSD95, which was prevented in p75+/-gp120tg and p75-/-gp120tg mice. Finally, we tested all four groups of mice to assays of spatial navigation and motor learning to reveal whether the removal of p75NTR restores both the cognitive and motor impairments observed in gp120tg mice. The deletion of the p75NTR gene prevented the cognitive impairment observed in gp120tg mice, suggesting normalization of hippocampal spines. Moreover, the removal of p75 attenuated motor learning deficits in gp120tg mice, demonstrating a striatal-specific impairment. We propose that targeting p75NTR may be a viable strategy for adjunct therapies in HAND individuals.

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Zika envelope protein causes toxicity to human neural stem cells and neuronal cultures via GSK3beta

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Zika virus transmission erupted into two recent outbreaks in 2013 and 2016. The viral infection can have devastating neurological manifestations which includes developmental abnormalities such as microcephaly. In adults it can cause encephalitis, myelitis, or Guillain-Barré syndrome. Unfortunately, no vaccines or therapeutic interventions currently exist for clinical use. Previous drug screens focused largely on targeting viral replication to modulate cytopathic effects in host cells following exposure to full-length Zika virus. Here we show that infection of human neurons with Zika virus leads to loss of spontaneous electrical activity followed by cytopathic effects. In human neuronal cultures, the envelope protein alone was sufficient to induce neurotoxicity. The protein was highly toxic to neurons and neural stem cells, but not astrocytes. Ultrastructural studies showed prominent mitochondrial swelling. The toxicity was viral strain dependent. We developed a human neuron cell-based throughput assay to screen selected compounds for potential neuroprotective activity. The screen suggests that the toxicity is mediated via the GSK3beta signaling pathway resulting in oxidative stress. As such, these compounds may provide neuroprotective efficacy as adjunctive therapy to treat Zika virus infections.

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Capitalizing upon Voluntary Wheel Running as a Neurorestorative Strategy

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Long-term benefits of physical activity on neurocognitive outcomes among HIV-uninfected older adults are well established. Cross-sectional and longitudinal (2.6 yrs) data suggest physical activity is also positively associated with maintenance of cognitive function of HIV-infected persons. Using the HIV-1 transgenic (Tg) rat, we explored the neurorestorative potential of 11-hour access to voluntary wheel running. Following baseline measurements of adult animals, the influence of voluntary wheel access on maintaining cognitive function was examined in a repeated measures design with assessments conducted every two weeks for a total of six weeks. We predicted that baseline measurements would confirm alterations in neurocognitive function in the HIV-1 Tg rats, but that access to voluntary wheel running would attenuate the progressive loss of function. Specifically, baseline measurements were taken on a spontaneous alternation Y-maze task, auditory and visual pre-pulse inhibition, as well as gap inhibition. Each task was specifically chosen to test working memory and temporal processing deficits that allow multiple trials to utilize a progressive study design. Upon completion of the pretests, animals were placed in residential cages with voluntary access to running wheels for 11 hours nocturnally; food and water were available ad libitum. Microstructural analysis of wheel running incorporates latency to start, the number and length of running bouts as well as inter-bout interval. Analyzing the baseline data, visual PPI revealed an effect on both genotype, $F(1,12)= 5.166$ $p\leq0.05$ and sex, $F(1,12)= 21.8$ $p\leq0.05$. Auditory PPI yielded similar significant results of genotype, $F(1,12)=6.1$ $p\leq0.05$.and sex, $F(1,12)=23.6$ $p\leq0.05$. Gap inhibition revealed only a significant effect of sex, $F(1,12)=5.9$ $p\leq0.05$. Preliminary data for spontaneous alternation task also suggest a deficit in the HIV-1 Tg animals. The focus on the maintenance of neurocognitive function in conjunction with the postmortem exanimation of dendritic spine complexity and morphology will allow a more complete assessment of neurotherapeutic potential.

P135

Congenital Zika Syndrome in Guinea Pigs

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Zika virus (ZIKV) infection during pregnancy may cause diverse and serious congenital defects in the developing fetus. In this study, we utilized pregnant guinea pigs to study congenital Zika syndrome. Female guinea pigs early in pregnancy (weeks 3-4 of gestation) were inoculated with Asian ZIKV strain (PRVABC59) or PBS (mock) via subcutaneous route. Dams were weighed daily, and blood was collected at regular intervals to assess the presence of virus. Weight loss was observed in ZIKV-infected dams during the first week of infection. ZIKV-infected animals seroconverted and significant viral secretion in serum was detected. During the period between infection and delivery of the pups, significant viral RNA and NS1 protein were detected in all animals from 2 to 5 days after infection, with peak viral replication at day 3. Dams developed remarkably robust ZIKV-specific neutralizing antibody response, and anti-ZIKV antibodies were also detected in pups. Notably, ZIKV was efficiently transmitted from infected guinea pigs to their naïve co-caged mates. ZIKV infection of pregnant guinea pigs caused fetal damage. Sixty percent of ZIKV-infected dams showed abnormal pregnancies in that they all delivered at least one or more abnormal pup. Pups from ZIKV-infected animals exhibited significant intrauterine growth retardations. ZIKV was detected in the brain of pups from ZIKV-infected animals. ZIKV RNA and anti ZIKV-antibody levels in the dams reliably predicted abnormalities in pups. ZIKV detection in the brain tissues correlated with the pup abnormalities. In conclusion, the pregnant guinea pig model provides quantifiable congenital abnormality readouts to assess fetal outcome

and may serve as a good model to test therapeutics, and to study the mechanisms of ZIKV congenital pathogenesis.

P136

HCV cure in HIV coinfection dampens inflammation and improves cognition through multiple mechanisms

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Background: Chronic inflammation in HIV/HCV coinfection increases cognitive impairment. With the new direct-acting antiviral (DAA) therapy for HCV, our objective was to determine if chronic inflammation would be decreased and cognition improved with HCV sustained viral response (SVR) in coinfection.
Methods: We studied 41 participants before and after DAA treatment for HCV alone or with viral controlled HIV. We measured monocyte activation, plasma levels of inflammation and cognitive impairment. Monocyte-derived exosomes were studied with RNA sequencing before treatment and targeted miRNAs after treatment.
Results: All HCV-coinfected subjects achieved SVR. Plasma sCD163 and neopterin were decreased in HCV mono and coinfected persons. Blood CD16+ monocytes were decreased in coinfection after HCV treatment. Overall cognition improved 25% in coinfection. HCV SVR decreased monocyte interferon genes MX1, IFI27 and CD169 in coinfection and MX1, LGALS3BP, HLADQA1 and TNFAIP6 in HCV monoinfection. Monocyte exosomes from coinfected persons after treatment were significantly increased in miR-19a, miR-221 and marginally miR-223, all associated with decreasing inflammation and NFŒ∫B activation.
Conclusions: HCV SVR in coinfection brings monocyte activation markers to levels of HIV alone. While both chronic viral infections stimulate interferon genes and proteins as well as inflammatory markers, they do so in a differential manner. Cognitive impairment in coinfection is significantly reduced strongly suggesting that previous reports on the percent of impairment in HIV may have been greatly influenced by HCV coinfection.

P137

CSF Markers of AD-Related Pathology Relate Specifically to Memory Impairment in Older People with HIV

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Background: Aging with HIV presents new complications including the risk for Alzheimer's disease (AD) and its precursor, amnestic mild cognitive impairment (aMCI). Identifying aMCI among people with HIV (PWH) is challenging because memory impairment, the defining feature of aMCI, is also common in HIV-associated neurocognitive disorders (HAND). The neuropathological hallmarks of aMCI/AD are amyloid-beta42 plaque and phospho-Tau accumulation. We assessed whether cerebrospinal fluid (CSF) markers of AD-related pathology (lower amyloid-beta42; higher phospho-Tau and phospho-Tau/amyloid-beta42 ratio levels) could help identify PHW that may be on the aMCI/AD trajectory by determining their relationship to memory performance versus other cognitive domains commonly-impaired in HAND (executive function, attention/working memory) in older PWH.
Methods: Participants included 31 PWH aged 50,àí68 years (84% male, 58% White) from the National NeuroAIDS Tissue Consortium (NNTC) who had CSF and a neurocognitive assessment within one year. Forty-five percent were diagnosed with HAND. CSF amyloid-beta42 and phospho-Tau were measured by commercial immunoassay. The neurocognitive assessment included tests measuring learning and delayed recall (Hopkins Verbal Learning Test-Revised and Brief Visuospatial Memory Test-Revised), executive function (Trail Making Test-Part B), and attention/working memory (Letter Number Sequencing). Linear regressions examined the relationship between neurocognitive test scores and levels of CSF phospho-Tau, amyloid-beta42, and phospho-Tau/amyloid-beta42 ratio. Analyses were adjusted for demographics, apolipoprotein-E ε4 allele carrier status, clinical comorbidities, and HIV disease and treatment characteristics.
Results: Phospho-Tau levels did not relate to neurocognitive test scores. Lower CSF amyloid-beta42 levels related to poorer BVMT-R Learning ($p=.006$). Higher phospho-Tau/amyloid-beta42 ratio related to poorer BVMT-R Learning and BVMT-R and HVLT-R Delayed Recall ($ps<0.05$). No CSF markers related to executive function or attention/working memory.
Conclusions: Among CSF markers of AD pathology, the phospho-Tau/amyloid-beta42 ratio was a more reliable marker of memory-specific cognitive deficits, and, thus, may have utility in identifying older PWH at-risk for aMCI/AD.

P138

Microglia contribute to HIV-associated synaptic loss in the spinal pain neural circuits

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HIV patients with chronic pain develop synaptic degeneration in the spinal cord dorsal horn, but the patients without the pain disorder do not show this neuropathology, indicating a pathogenic contribution of the synaptic degeneration to the development of HIV-associated pain. However, the mechanism underlying the synaptic degeneration is unclear. We report here that HIV-1 gp120, a neurotoxic protein that is specifically associated with the manifestation of pain in HIV patients, induces synapse loss via microglia. Further studies elucidate that gp120 activates microglia by stimulating Wnt/beta-catenin-regulated fractalkine in neuron. The results suggest a critical role of microglia in the pathogenesis of HIV-associated synaptic degeneration in the spinal pain neural circuit.

P139

Combinatorial effects of HIV Tat and cocaine on mitochondrial damage, defective mitophagy and activation of microglia: Implications for NeuroHIV

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Epidemiological studies have shown that cocaine abuse is associated with a higher incidence of HIV infection and accelerates the progression of NeuroHIV by potentiating neuroinflammation. While emerging evidence demonstrates the individual effects of HIV Tat (Trans-activator of transcription) and cocaine on mitochondrial damage, defective mitophagy, glial activation,and neuroinflammation, the combinatorial effects of the two and the molecular mechanism(s) underlying these effects remain less understood. In the present study, we sought to examine the cooperative effects of Tat and cocaine on mitochondrial damage, impaired mitophagy and its role in microglial activation and neuroinflammation. Microglial cells were exposed to varying combinatorial doses of HIV Tat and cocaine and assessed for changes in mitochondrial impairments. Our findings demonstrated that exposure of microglia to HIV Tat and cocaine resulted in altered mitochondrial membrane potential compared to cells exposed to either

agent alone. Additionally, in the presence of HIV Tat and cocaine, there was up-regulated expression of mitophagy markers such as Pink1,Parkin,and Drp-1, which, in turn, was accompanied by increased expression of mediators of autophagy. This was further validated by confocal microscopy demonstrating increased accumulation of mitophagosomes in the presence of HIV Tat&cocaine. Taken together, these findings suggested that HIV Tat&cocaine inhibited the mitophagy flux leading to accumulation of mitophagosomes in microglia. Our results also demonstrated that HIV Tat&cocaine-mediated defective mitophagy resulted in significant activation of microglia and increased expression of proinflammatory cytokines. Collectively, these findings demonstrate that both HIV Tat&cocaine could, via their co-operative actions, exacerbate mitochondrial damage, defective mitophagy and microglial activation, thereby compounding the severity of neuroinflammation in the context of HIV and cocaine abuse. Future directions include exploring therapeutic strategies aimed at mitigating mitochondrial damage & defective mitophagy as a means to alleviate microglial activation in the context of comorbidity of chronic viral infection and cocaine abuse. (Supported by DA043138; DA047156)

P140

ApoE isoforms elicit cell-type specific responses in the CNS that likely contribute to age-related HAND

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Age-related neurodegenerative diseases like HIV and Alzheimer's disease (AD) are accompanied by increased neuroinflammation, exacerbated bioenergetic deficits and alterations in astrocyte-neuron signaling and communication. The ApoE protein regulates lipid homeostasis by transporting lipids needed for brain cell fitness in health and for repair after injury. Our previous studies by Cotto et al., in Glia, 2017 showed that the HIV protein Tat, alone or in combination with cocaine, disrupted ApoE-mediated transfer of cholesterol to neurons thereby, resulting in bioenergetic deficit and neuronal damage. There are three ApoE isoforms: E2, E3 and E4, therefore, three homozygous (E2/2, E3/3, E4/4) and three heterozygous (E3/2, E4/3, E4/2) genotypes occur in humans, with E2/2 accounting for approximately 8.4% of the population, E3/3, 77.9%, and E4/4, 13.7%, with the frequency of the E4 allele increased to 40% in AD patients. In addition, extensive data suggest that ApoE4 contributes to HIV associated cognitive impairment (HAND), neuropathology and infectivity. Herein, we hypothesize that ApoE genotype influences astrocyte-neuronal interactions contributing to increased cell signaling disruption that likely plays a role in HAND. To address this hypothesis, we utilized several cell models to assess changes in crosstalk related to ApoE/cholesterol transfer, bioenergetic changes and altered astrocyte-neuronal signaling. Specifically, induced pluripotent stem cells (iPSC)-derived astrocytes and neurons from individuals with the E4 and E3 genotype were used to assess these changes. We also confirmed these findings in human primary astrocytes with different ApoE genetic isoforms and in transgenic mice expressing human ApoE3 and ApoE4. These findings suggest that different ApoE isoforms elicit cell-type specific responses in the CNS that likely contribute to age-related HAND.

P141

The lncRNA LOC102549805 (U1) modulates neurotoxicity of HIV-1 Tat protein

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Due to its neurotoxic activity, HIV-1 Tat has offered a convenient probe to investigate neuronal cell homeostasis pathway. In this study we discovered an interplay between Tat and an uncharacterized long non-coding RNA, LOC102549805, that impact on several cell survival pathways including mitochondria function, bio-energetic pathway, Ca2+ influx and cell death pathways in the presents of Tat. The current experiments reveal the capacity of Tat to potentially contribute to HIV pathenogenesis within the CNS through alteration in a host cell's non-coding RNAs. Results from primary rat neurons exposed to, or expressing, the Tat protein revealed differential expression of multiple lncRNAs, including a significant up-regulation of LOC102549805. Interestingly, LOC102549805 was shown to have a cis-regulatory effect on its nearby gene, neuropeptide B/W receptor (NPBWR1), resulting in its significantly increased transcription and translation. Moreover, LOC102549805 demonstrated a crucial role in energy homeostasis pathways, as it was shown to increase cytoplasmic Ca2+ and Ca2+ retention in mitochondria, while mitigating mitochondrial O2 reduction and subsequent ATP production. In addition, LOC102549805 was shown to alter pathways implicated in neuronal survival, as it increased the level of lysosomal autophagy marker (LC3-II) as well as apoptosis indicator (cleaved caspase-3) but decreased the anti-apoptotic protein (BCL-2) as well as a lysosomal degradation marker (p62). Present findings suggest crucial roles for the Tat-mediated over-expression of LOC102549805, a previously uncharacterized lncRNA, suggesting it might be a novel target to control the progression of HIV pathogenesis within the CNS.

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HIV-1 Tat protein perturb lipid homeostasis in neurons through lncRNA U1 (LOC102549805) over-expression

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Lipodystrophy is associated with HIV infection but little shown about the underlying mechanism and the role of viral proteins in HIV-mediated lipid disturbance. In the current study, we demonstrated the regulatory effect of HIV-1 Tat protein on lipid homeostasis in neurons via alteration the expression level of lncRNA LOC102549805 (U1). In this study, we imitated the Tat-mediated over-expression of lncRNA-U1 by transducing primary neurons with U1 virus. Our results revealed a mitigated level of ApoE-4 (apolipoprotein 4) in both Tat/U1 transduced primary neurons. ApoE-4 plays a major role in regulating lipid metabolism as well as redistributing cholesterol and phospholipids in CNS. Accordingly, our result showed reduction in the level of LDL receptor and Soat1 proteins, the downstream pathway of activated apolipoprotein signaling. On the other hand, the level of phosphorylated tau has declined, which is the downstream product of ApoE4 aggregation. These findings help to explain the neurocognitive disorders associated with HIV infection, as ApoE-4 alteration shown to be associated with neuropathology, including stroke and AD, as well as other brain injuries.

P143

Wnt7a regulates MDM phenotye and function: relevance to HIV induced neuroinflammation

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Monocyte-derived macrophages (MDM) display a myriad of phenotype and functions, and in the central nervous system contribute to neuroprotection or neurodegeneration. We described a unique MDM phenotype

generated in response to Wnt7a; a member of the Wnt ligand family which orchestrate cell development, phenotype and function. We previously demonstrated that Wnt7a skews monocytes to differentiate into MDMs with a cytokine profile distinct from inflammatory and alternative MDM controls (M1 and M2a MDMs respectively), including elevated IL-6 secretion and reduced IL-10 and IL-12 secretion. Wnt7a-MDMs also display reduced CD11b expression, and reduced phagocytic capacity when compared to M1 and M2a MDM controls. We demonstrate here that conditioned media (CM) from Wnt7a-MDMs diminish astrocyte proinflammatory (A1) phenotype. Specifically, Wnt7a-MDMs reduced transcription of complement proteins C3 by fivefold and C4a and C4b by two folds relative to M2a-MDMs. Wnt7a-MDMs also reduced astrocyte expression of lipocalin-2 by approximately 300 folds relative to M1-MDMs. Wnt7a-MDMs secreted less C1q, a driver of A1 phenotype, than both MDM controls, and promoted more astrocyte proliferation relative to M1 MDMs. Lastly, in the context of HIV-Associated Neurocognitive Disorders (HAND), Wnt7a expression was consistently lower in the brain of HIV associated dementia (HAD) patients relative to seronegative and neurocognitively normal patients. Overall these data demonstrate that Wnt7a-MDMs reduces astrocyte inflammation and suggest that levels of Wnt7a in the CNS may be neuroprotective.

P144

Altered expression of nociceptive ion channels, TRPV and TRPA, in a SIV-infected rhesus macaque model of HIV-associated distal sensory polyneuropathy

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With development of combined anti-retroviral therapy (cART), human immunodeficiency virus (HIV)-associated comorbidities, including HIV-associated distal sensory polyneuropathy (HIV-DSP), have remained prevalent. Pathophysiological hallmarks of HIV-DSP include dorsal root ganglia (DRG) damage, degeneration of peripheral intraepidermal nerve fibers, and increased immune cell traffic to the DRG. Our previous studies have identified a decrease in both peptidergic and non-peptidergic nociceptor neurons as well as increased inflammatory monocyte traffic to the DRG during simian immunodeficiency virus (SIV) infection; however, the sensory signaling mechanism connecting pathology to symptomology remains unclear. Changes in neuronal nociceptive ion channels at both the transcriptional and functional levels have been shown in chronic inflammation, suggesting that HIV-associated chronic inflammation may also alter sensory neuron ion channels. Here, we used 23 adult male rhesus macaques, which were subdivided into 3 groups; uninfected (N=8), SIV-infected (N=9), and SIV-infected treated with a cART regimen of Tenofovir, Emtricitabine, and Raltegavir (N=6). We found altered transcription of the nociceptive ion channels; transient receptor potential vanilloid (TRPV) and ankyrin (TRPA). Using quantitative RNAscope, we detected a significant difference in TRPV expression among the three groups (ANOVA, P=0.003). There was a significantly greater expression in TRPV in the SIV-infected/cART treated group when compared with our SIV-infected untreated group (Dunn's, P=0.004). Similarly, we detected a significant difference in TRPA expression among groups (ANOVA, P=0.01), where the SIV-infected/cART treated group had the highest proportion of TRPA-positive neurons. These findings suggest that changes in nociceptive ion channels occur in SIV infection, even with a reduction of viral load by cART. Further studies are needed to connect expression, function, and modification of nociceptor ion channels to symptoms of HIV-DSP.

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HIV-associated neurocognitive disorders are associated with persistent CNS immune dysfunction

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Background: Despite effective viral suppression with antiretroviral therapy, approximately 50% of people living with HIV (PLWH) are affected by HIV-associated neurocognitive disorders (HAND). These disorders have severe impacts on the ability of PLWH to function independently by affecting memory, learning and motor skills. Prolonged immune activation and inflammation caused by persistent HIV infection and/or gut dysbiosis is thought to indirectly contribute to the pathogenesis of HAND. However, the precise inflammatory profile of the CNS of individuals with HAND remains ill-defined. Here we investigated the inflammatory nature of the CNS of PLWH with neuro-asymptomatic or symptomatic HAND.

Methods: Formalin-fixed paraffin embedded frontal cortex tissue from HIV-uninfected (n=8), and HIV-infected (n=30) individuals with varying degrees of HAND [neurologically normal (n=9), asymptomatic cognitive impairment (n=8), symptomatic cognitive impairment (n=13)] were stained by multiplex immunohistochemistry to identify CNS resident immune cells (CD68+Tmem119-: macrophage, Tmem119+: microglia, GFAP: astrocytes). Matched frozen tissue was assessed for gene expression of inflammatory mediators by qPCR analyses.

Results: Gene expression of IL-18, IL-6, CXCL-10 and TNF were significantly altered in PLWH in comparison to HIV-uninfected individuals ($p<0.05$ for all). These alterations also persisted in tissue from PLWH with neurocognitive impairment relative to neurocognitively normal HIV-uninfected controls. Astrocyte activation was significantly greater in tissue from PLWH with symptomatic HAND compared neurocognitive normal individuals ($p<0.05$). Tissue from individuals with symptomatic HAND also showed an increased percentage of IFNgamma producing myeloid cells when compared to tissue from HIV-infected neurologically normal or HIV-uninfected individuals ($p<0.05$).

Conclusion: Cognitive impairment in PLWH is associated with altered immune cell responses, potentially indicative of cellular dysfunction and senescence. Identifying the mechanisms driving immune cell dysfunction in HAND may inform therapeutic strategies to restore CNS immunity and improve cognitive function in PLWH.

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Associations between antiretroviral drugs on depressive symptomatology in homogenous subgroups of women with HIV

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Antiretroviral therapy (ART) is inconsistently associated with depression. These associations may depend on factors such as biological sex, age, and health status. Identifying such factors may help optimize treatment of HIV and depression. We implemented a novel approach to examine interindividual variability in the association between ART agents and depressive symptoms. 3,434 women living with HIV (WLWH) from the Women's Interagency HIV Study (WIHS) were computationally divided into subgroups based on sociodemographic (e.g., age) and longitudinal (from 1995 to 2016) behavioral and clinical profiles (e.g., substance use, HIV RNA, CD4 counts). Five comparable subgroups (n's ranged from 482 to 802) were identified and characterized as those with: controlled HIV/vascular comorbidities; profound HIV legacy effects; younger women [<45 years of age] with hepatitis C; primarily 35-55 year olds; and poorly controlled HIV/substance use. Within each subgroup, we examined associations between ART agents used over the past 6 months and item-level depressive symptoms on the Center for Epidemiologic Studies Depression Scale. Tenofovir (4 of 5 subgroups) followed by efavirenz, emtricitabine, stavudine, lopinavir, etravirine, nelfinavir, ritonavir, and maraviroc were the most common agents associated with depressive symptoms, although the pattern and directionality varied by subgroup. For example, lopinavir was associated with fewer symptoms among the subgroup with a legacy HIV effect but more symptoms among the subgroup with well-controlled HIV/vascular comorbidities. Unexpectedly, dolutegravir and raltegravir were not associated with depressive symptoms among any subgroup. Findings underscore marked interindividual variability in ART agents on depression in WLWH. Sociodemographic, clinical, and behavioral factors are important determinants of the relationship between ART agents and depressive symptoms in WLWH.

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Differential Localization of Antiretroviral Therapy in Brain: Mapping Prodrug and Pharmacologically Active Metabolites

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Introduction: Antiretroviral therapy (ART) suppresses HIV replication to undetectable levels. However, ART does not quell virus in the brain to the same extent as the periphery. As a result, there are limitations in dosing of the brain that perpetuate a spectrum of cognitive disorders termed HIV Associated Neurocognitive Disorders (HAND). HAND is a major consequence of HIV infection that affects between 30-70% of infected individuals and greatly impacts the survival and quality of life of those who are affected. There is a paucity of data regarding the mechanisms by which substance abuse contributes to HAND.

Methods: To determine the localization of ART, brain was obtained from rhesus macaques infected with simian immunodeficiency virus and treated with ART. Following perfusion, the brain was cryosectioned and matrix assisted laser desorption ionization (MALDI) imaging mass spectrometry performed.

Results: Two first line ART drugs, tenofovir (TFV) and emtricitabine (FTC), were imaged in five brain regions. Additionally, the pharmacologically active triphosphate metabolites capable of inhibiting HIV/SIV were also determined. TFV and FTC were present in brain to a significantly lower extent than the periphery. However, FTC was more abundant in brain than TFV. Metabolism into the pharmacologically active metabolites occurred in a heterogenous manner that was restricted to "hot spots", where relatively high concentrations of ART were found.

Conclusions: There is a limited penetrance of the pharmacologically active ART metabolites into brain, particularly in regions involved in the dopaminergic reward pathway. This suggests that the brain is undertreated during HIV infection which will perpetuate HAND.

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Soluble insulin receptor levels in plasma, exosomes and urine: novel biomarkers for HIV-associated neurocognitive disorders

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HIV can infiltrate the central nervous system, triggering neurological complications in these patients. HIV-associated neurocognitive disorders (HAND) range from asymptomatic (ANI), mild (MND) or dementia (HAD), in few cases. The soluble insulin receptor (sIR) is secreted to the plasma and cerebrospinal fluid either free or in exosomes, higher in HIV-seropositive (HIV+) patients than control individuals. We have demonstrated that there is an association between plasma and CSF sIR levels and HAND in our cohort of HIV+ women. However, measuring proteins in urine represents a less invasive and more accessible diagnostic tool. The objective of this study was to investigate if sIR was present in the urine of seropositive women and its association with HAND and renal function. We measured the full-length sIR in the urine of control (n=29) and HIV+ (n=76) women by ELISA. Glomerular filtration rate (GFR), an indicator of kidney function, was similar in controls and HIV+ women (p=0.48). Urine sIR positively correlates with GFR (r=0.60; p=0.031) in controls, but not in HIV+ patients. Plasma sIR positively correlates with the levels of exosomal sIR (r=0.46; p=0.0089) and exosomal HIV-Tat (r=0.448; p=0.01), confirming our previous findings. In all HIV+ women, urine sIR negatively correlates with the levels of plasma sIR (r=-0.34; p=0.0036). In symptomatic impaired women (MND and HAD), urine sIR has a negative correlation with exosomal sIR (r=-0.37; p=0.048) and with the therapy CNS penetration efficacy (CPE) scores (r=-0.47; p=0.019), and positively correlates with speed of information processing z-scores (r=0.48; p=0.008). Our findings suggest that the increased levels of sIR secreted to exosomes and plasma are retained and not excreted in the urine, correlating with HAND in HIV+ patients. The combination of inverse plasma/urine levels of sIR could represent a novel biomarker for HAND.

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Mechanisms of PSGL-1 restricts HIV-1 infectivity

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PSGL-1 (P-selectin glycoprotein ligand-1) is a dimeric, mucin-like, 120-kDa glycoprotein that binds to P-, E-, and L-selectins. PSGL-1 is primarily expressed on the surface of lymphoid and myeloid cells and is upregulated during inflammation to mediate leukocyte tethering and rolling on the surface of the endothelium for migration into inflamed tissues. In addition, PSGL-1 expression has been shown to be induced in monocytes in HIV-infected patients. Recently, PSGL-1 has also been identified as an INF-gamma-regulated anti-HIV-1 restriction factor that inactivates virion infectivity. However, the mechanisms of PSGL-1-mediated anti-HIV activity remain to be elucidated. Here, we report that the expression of PSGL-1 in virus-producing cells inhibits virion infectivity by inhibiting virion attachment to target cells. Mapping studies show that the extracellular, N-terminal domain of PSGL-1 is necessary for its anti-HIV-1 activity, which also does not require PSGL-1 dimerization. Mechanistically, PSGL-1 may inhibit virion binding to target cells through structural hindrance of virus particle interaction with target cells. HIV-1 infection, and expression of both Vpu and Nef, downregulate PSGL-1 from the cell surface, enabling the virus to partially escape PSGL-1-mediated restriction. These findings demonstrate that PSGL-1 is a new host factor with a novel mechanism of action.

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Extracellular vesicles carrying HIV Nef may contribute to the development of HIV CNS dysfunction.

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HIV-associated CNS dysfunction is a significant problem among individuals who live longer due to the combined anti-retroviral therapy (cART). HIV utilizes viral proteins and subsequent cytokine induction to unleash its toxicity on neurons. Among these viral proteins, HIV Nef is found in neurons of postmortem brain specimens from PWH. However, the source of neuronal Nef, mechanism of its uptake by neurons, and its impact on neuronal cell homeostasis are still elusive. Our studies using an SIV model of neuroHIV showed high levels of Nef expression in frontal cortex, hippocampus and cerebellum. Interestingly, brain sections from the same regions of a group of SIV-infected macaques treated with ART revealed that frequent numbers of Nef positive cells were still present in the cerebellum and hippocampus. In addition, our data using RNAscope for SIV RNA followed by IHC for the detection of Nef protein revealed Nef protein positive cells were SIV RNA negative suggesting that Nef protein detection is not limited to the cells infected with the virus in the brain. To gain more insight into the cell types with Nef expression, we performed a series of co-immunostaining studies with cell type specific markers and Nef protein. Interestingly, in addition to microglia, macrophages and astrocytes, many neurons with NeuN staining were also positive for Nef protein. Since neurons are not prone to the HIV-1 infection, Nef is most likely taken up by these cells. Indeed, our data suggest that Nef protein is released in extracellular vesicles (Nef-EVs) from HIV-infected glia. Further analysis of the possible impact of these Nef-EVs on neurons in primary culture models revealed that Nef-carrying EVs were readily taken up by neurons, a significant amount of Nef was enriched in mitochondrial fractions, induced oxidative stress and dysregulated neuronal electrophysiology. These results suggest that Nef-EVs may contribute to HIV CNS dysfunction

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A structurally novel inhibitor of neutral sphingomyelinase 2 selectively kills HIV-infected cells

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Existing antiretroviral (ARV) regimens suppress replication of the Human Immunodeficiency Virus (HIV), but do not eliminate HIV-infected cells, or viral-reservoirs. Withdrawal of ARV therapy results in viral rebound in majority of people living with HIV, suggesting new approaches to intensify current ARV regimens are necessary to eliminate persistent viral infection. We found that the sphingomyelin hydrolase, neutral sphingomyelinase2 (nSMase2) is required for HIV to complete its lifecycle. Inhibition of nSMase2 with a novel small molecule inhibitor (PDDC) dose-dependently suppressed HIV-replication with a minimum effective dose of 300 nM, and was selectively cytotoxic to cells with actively replicating HIV (H9, U1, and primary CD4+ cells). Similar results were obtained by molecular knock-down of nSMase2. The mechanism by which PDDC kills infected cells appears to involve endolysosomal stress and caspase-3 dependent apoptosis. Viral entry, HIV gene transcription or translation are not modified by PDDC suggesting that inhibition of viral replication occurs at a post-translational step. We found that nSMase2 binds to Gag and is incorporated into virions, suggesting a vital role for this enzyme in the HIV lifecycle. Daily treatment of humanized NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice with PDDC (10 mg/kg, I.P.) produced a linear decrease in plasma viral loads, with 5 of 7 mice below detection limits within 8-10 weeks. None of the 5 mice with undetectable viral loads showed viral rebound during 8-weeks of drug-withdrawal. In contrast, 6 of 7 NSG-mice treated with ARVs achieved undetectable viral loads, but 5 of these 6 mice rebounded within the first 2-weeks of drug withdrawal. Adoptive transfer of splenocytes isolated from PDDC-treated mice with undetectable viral loads into uninfected NSG-mice did not result in plasma viremia, confirming absence of replication competent virus. These findings demonstrate that inhibition of nSMase2 selectively kills HIV-infected cells and could be a useful adjunctive therapy for successful "cure" efforts.

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Cysteinyl Leukotrienes Mediate In Vivo HIV-1 gp120 Associated Brain Injury

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As of 2018, UNAIDS estimates that the number of people living with human immunodeficiency virus (HIV) has grown to 36.9 million with 21.7 million receiving treatment with combination antiretroviral therapy (cART). HIV-associated neurocognitive disorder (HAND) is estimated to affect not only survival and quality of life but can also impact everyday functioning. Current estimations show upwards to 50% of the maturing HIV-positive population exhibiting some degree of cognitive impairment. Studies in our laboratory indicate that p38 MAPK is essential for the neurotoxic phenotype of HIV gp120-stimulated macrophages and microglia, as well as the induction of neuronal apoptosis triggered by macrophage-derived toxins and proinflammatory molecules. Microarray analyses of human macrophages stimulated with HIV envelope protein gp120 and of brains of transgenic mice expressing the viral gp120 under the control of a modified GFAP promotor in astrocytes (gp120tg mice) have suggested the involvement of cysteinyl leukotrienes (cysLTs) in HIV neurotoxicity. Leukotrienes (LTA4, LTB4) and cysteinyl

leukotrienes (LTC4, LTD4, and LTE4) are the products of the 5-lipoxygenase (5-LOX) metabolism of arachidonic acid. CysLTs (LTC4, LTD4, and LTE4) are formed by the addition of cysteine derivatives. Antagonism of the cysteinyl leukotriene receptor 1 (CysLTR1) or inhibition of CysLT-producing enzymes has been implicated in neuroprotection. Our lab utilizes gp120tg mice which exhibit neuropathology similar to that observed in human HIV patients including decreased synaptic and dendritic density, microgliosis, astrocytosis, and behavioral impairment. In these mice, we have observed a neuroprotective outcome resulting from genetic ablation of the CysLTR1. Interestingly, the knockout of CysLTR1 did not prevent gp120-induced increases in astrocyte or microglial activation. In addition, deficiency of the upstream factor LTC4 synthase (LTC4S) in gp120tg mice also confers neuroprotection, further supporting a crucial role for CysLTs in HIV-associated brain injury.

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Using human iPSC-based forebrain cortical organoids to model in utero exposure to dolutegravir

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Prevention of mother-to-child-transmission of HIV remains a prominent global health priority but determining the safety and efficacy of antiretroviral drugs during pregnancy remains a challenge. Recently published results from an observational study of birth outcomes in Botswana reported a potential association between dolutegravir exposure at the time of conception and an increased prevalence in the development of neural tube defects. Given this clinical observation, we sought to investigate the effects of dolutegravir on neural development using human induced pluripotent stem cells in a 3D organoid model of early brain development. Forebrain cortical organoids recapitulate the structural organization, composition of diverse cell types, as well as some of the molecular dynamics of early stages of cortical neurogenesis. These forebrain organoids are composed of numerous neural rosettes in which neural progenitor cells are organized in a distinctive radial orientation. To model early prenatal exposure to the drug, we treated these organoids with dolutegravir once a day in the culture media and observed a robust phenotype after two weeks of exposure. Forebrain organoids exposed to dolutegravir exhibited a dose-dependent response resulting in a disruption of the neural rosette structures, suggesting deficits in the function and organization of early neural progenitor cells. Dolutegravir-exposed organoids also appear smaller in overall size, with fewer and thinner neural rosettes, as compared to either vehicle-treated control organoids, or organoids exposed to raltegravir, another antiretroviral integrase inhibitor. Ongoing studies are focused on identifying molecular and cellular mechanisms underlying this phenotype by characterizing specific pathways that are altered upon dolutegravir exposure. Together, these findings add to a growing body of work demonstrating the utility of human forebrain organoid models as a platform for toxicity screening and studying the neurodevelopmental effects of prenatal drug exposure.

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Depressive-like behaviors in EcoHIV mouse and potential therapeutics

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Despite the wide-spread use and success of antiretroviral therapy (ART), more than 30% of people living with Human Immunodeficiency Virus infection (PLWH) in the US suffer from depression. This is a prevalence rate that is two times higher compared with the general population. Nonetheless, a lack of pathophysiological mechanisms underlying the mechanisms for depression in PLWH hampers advances in its prevention and treatment. Understanding the pathological progression of HIV comorbidities in humans is difficult, partly due to analyses being largely limited to the availability of post mortem tissue samples. Efforts to overcome this challenge have been made by establishing models of lentivirus induced brain disease in animals, including the EcoHIV infected mice. The EcoHIV mouse model is useful to replicate the milder disease course seen in virally-controlled PLWH, including cognitive impairments. Importantly, to our knowledge, the depressive-like behavioral phenotypes of the EcoHIV mouse model remains uninvestigated. By performing the 3-chamber social interaction test, we observed that EcoHIV infection leads to social interaction deficit in mice. Treating mice with our newly developed glutamine antagonist JHU-083 partially normalized the impaired social behaviors in the EcoHIV mouse. Based on these results, we propose that EcoHIV model is a useful model to mimic HIV-induced depressive-like behaviors and test the effects of compounds that may have potential antidepressant properties.

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