J Neuroimmune Pharmacol (2017) 12(Suppl 1):S3–S77
DOI 10.1007/s11481-017-9731-z

BRIEF REPORT

The 23rd Scientific Conference of the Society on Neuroimmune Pharmacology: Program and Abstracts

Published online: 1 March 2017

Program and Abstracts of the 23rd SNIP Scientific Conference

Program

Wednesday March 29, 2017

Registration Opens 2 pm
Satellite Symposium on HIV in the CNS 2 – 5 pm
Speakers TBA
In collaboration with the City-Wide NeuroAIDS Group, aponsored by the Comprehensive NeuroAIDS Center (CNAC) at Temple University

Trainee Poster Session 5 – 8 pm
Sponsored by the Early Career Investigator Committee

1st Annual DISC Networking Hour8 – 10 pm
Sponsored by the Diversity and Inclusion SNIP Committee
Speaker: **Dr. Ana Nuñez**, M.D., Associate Dean for Diversity, Equity and Inclusion, Drexel University College of Medicine, PA, USA
Diversity, Equity and Inclusion in Neuroimmune Pharmacology – A Mentoring Challenge

ThursdayMarch 30, 2017

Breakfast 7 – 8 am

Presidents' Welcome 8 – 8:05 am
Dr. Michal Toborek, M.D., Ph. D., Leonard M. Miller Professor of Biochemistry and Molecular Biology, Vice-Chair for Research for the Department of Biochemistry and Molecular Biology, University of Miami, FL, USA

Presidential Symposium 8:05 – 9:50 am
Dopamine Neurotransmission in HIV-1 Infection
Chairs: **Dr. Vishnudutt Purohit**, Ph.D., NIDA, NIH, MD, USA
Dr. Habibeh Khoshbouei, Pharm.D., Ph.D., University of Florida, FL, USA

Dr. Peter J. Gaskill, Ph.D., Drexel University College of Medicine, PA, USA
Role of Dopamine Receptor Activation in HIV Entry

Dr. Franca Marino, Ph.D., University of Insubria, Varese, Italy
Dopamine and immunity: from basic evidence to therapeutic opportunities

Dr. Jun Zhu, Ph.D., University of South Carolina, SC, USA
Molecular mechanism of HIV-1 Tat interacting with human dopamine Transporter

Dr. Rosemarie Booze, Ph.D., University of South Carolina, SC, USA
HIV-1 disrupts motivational processes via dopamine transporter dysregulation

Dr. T. Celeste Napier, Ph.D., Rush University, IL, USA
Pathophysiology of the nucleus accumbens in HIV-1 transgenic rats is potentiated by cocaine self-administration

Dr. Jay McLaughlin, Ph.D., University of Florida, Gainesville, FL, USA
Brain Exposure to HIV-1 Tat Protein Potentiates the Psychostimulant Effects of Morphine, Modulates Consumption and Reinstates Extinguished Reward-Seeking

Break 9:50 – 10:10 am

Presidential Lecture 10:10 – 11 am
Introduction: **Dr. Michal Toborek**, M.D., Ph. D., University of Miami, FL, USA
Dr. David Sulzer, Ph.D., Columbia University Medical Center, NY, USA
Antigen display by dopamine neurons

Symposium 2 11 – 12:30 pm
Novel mechanisms of CNS infection by emerging neurotropic viruses
Chair: **Dr. Eliseo A. Eugenin,** Ph.D., Rutgers, USA
Dr. Mark Challberg, Ph.D. NIAID, NIH, MD, USA

Speakers:
Dr. Mark Challberg, Ph.D., NIAID, NIH, MD, USA
Introduction to Flaviviruses

Dr. Eliseo Eugenin, Ph.D., Rutgers University, NJ, USA
Flaviviruses, a critical threat: Focus in CNS

Dr. Laura Yockey, Ph.D., Yale University, CT, USA
Zika and CNS development

Ms. Courtney Veilleux, Rutgers University, NJ, USA
Productive Zika infection in the human brain

Dr. Catherine Blish, Stanford University, CA, USA
Zika virus infection induces cranial neural crest cells to produce cytokines at levels detrimental for neurogenesis

Meet the Mentors Luncheon 12:30 – 2 pm
Sponsored by the Early Career Investigator Committee

Plenary Lecture 2 – 2:45 pm
Introduction: *TBD*

Dr. Paul Kenney, Ph.D., Mount Sinai School of Medicine, NY, USA
Immune signaling in the habenula and nicotine addiction

Symposium 3 2:45 – 4:15 pm
Role of microbiome in health and disease: Implications in HIV disease progression and drug abuse
Chair: **Dr. Sabita Roy,** Ph.D., University of Miami, FL, USA

Dr. Shilpa Buch, Ph.D., University of Nebraska, NE, USA
Opening statement

Dr. Dan Knights, Ph.D., University of Minnesota, MN, USA
Plenary speaker, Title of talk: TBD

Dr. Jason Brenchley, Ph.D., NIAID, MD, USA
Dysbiosis does not influence disease progression in SIV-infected Asian macaques

Dr. Charles Mactutus, Ph.D., University of South Carolina, SC, USA
Integrity of the microbiome of the HIV-1 transgenic rat:
Efficacy of the gut-brain-axis for treatment of neurocognitive impairment

Dr. Sabita Roy, Ph.D., University of Miami, FL, USA
Title of talk: TBD

Dr. Santanu Banerjee, Ph.D., University of Miami, FL, USA
Title of talk: TBD

Dr. Ivan Vujkovic-Cvijin, Ph.D., NIH, MD, USA
Title of talk: TBD

Break 4:15 – 5 pm

Poster Session 5 – 8 pm

JNIP Editorial Board Meeting 7 – 9 pm

Friday March 31, 2017

Breakfast 7 – 8 am

Local Symposium 8 – 10:30 am
Neuroinflammatory Bases of Cognitive Impairment and Other CNS Disorders
Chair: **Dr. Olimpia Meucci**, M.D., Ph.D., Drexel University, Philadelphia, PA, USA
Dr. Dennis Kolson, M.D., Ph.D., Univ. of Pensylvania, Philadelphia, PA, USA

Dr. Norman Haughey, Ph. D., Johns Hopkins, MD, USA
Neuroinflammation in Multiple Disease States

Dr. Paola Bezzi, Ph.D., University of Lausanne, Lausanne, Switzerland
Neuroglia interactions and neurotransmission: new focus on monoaminergic pathways

Dr. Angelo Lepore, Ph.D., Thomas Jefferson University, Philadelphia, USA
Neuroinflammatory mechanisms in axon regeneration and neuropathic pain following SCI:
role of astrocyte communication with microglia and macrophages

15 minute break

Dr. Davide Ragozzino, Ph.D., University La Sapienza, Rome, Italy
Synaptic pruning and microglia

Dr. Joan W. Berman, Ph.D., Albert Einstein College of Medicine, NY, USA
CCL2-driven mchanisms of neuroinflammation & drug abuse

Dr. Robert Nagele, Ph.D., Rowan University, NJ, USA
Autoantibodies as biomarkers in neurodegenerative diseases

1st Annual DISC Lecture 10:30 – 11:25 am
Introduction: **Dr. Kelly Jordan-Scuitto**, Ph.D., University of Pennsylvania
Dr. Astrid Cardona, Ph.D., University of Texas at San Antonio, TX, USA
Absence of fractalkine/CX3CR1 induces perivascular clustering of microglia and fibrinogen leakage during systemic inflammation in mouse models of diabetic retinopathy

Symposium 5 11:30 – 12:30 pm
Mechanisms of mitochondrial dysfunction associated with HIV-induced neurotoxicity
Chairs: **Dr. Adam Fields**, University of California at San Diego, CA, USA
Dr. Vasudev Rao, Ph.D., NIMH, MD, USA

Dr. Josue Perez Santiago, Ph.D., University of California San Diego, CA, USA
Mitochondrial Injury and Aging in HIV Infection

Dr. Avdoshina, V, M.D., Ph.D., Georgetown Univ. Med. Center, Washington, DC, USA
Neurotoxic effect of Human Immunodeficiency Virus: cytoskeleton, mitochondria, and neuronal apoptosis

Dr. Todd Hulgan, M.D., M.P.H., Vanderbilt University, TN, USA
Mitochondrial DNA variation and HIV-associated neurotoxicity

Dr. Kelly Stauch, Ph.D., University of Nebraska, Medical Center, Omaha, NE, USA
Title: *TBD*

Lunch 12:30 - 2 pm

NIH Workshop on Grant Writing for Trainees 12:30 - 2 pm
(Boxed lunch provided)

Participants
Dr. Roger Sorenson, Ph.D., NIDA, NIH, MD USA
Dr. Vishnudutt Purohit, Ph.D., NIDA, NIH, MD USA
Dr. Rao Rapaka, Ph.D., NIDA, NIH, MD USA
Dr. Changhai Cui, Ph.D., NIAAA, NIH, MD USA
Dr. Jag Khalsa, Ph.D., NIDA, NIH, MD USA
Dr. Jeymohan Joseph, Ph.D., NIMH, NIH, MD USA
Dr. Vasudev Rao, Ph.D., NIMH, NIH, MD USA

Symposium 6 2 – 3:30 pm
Alcohol and neuroimmune pharmacology
Chairs: **Dr. Abraham Bautista**, Ph.D., Dir. OEA, NIAAA, NIH
Dr. Santosh Kumar, Ph. D., Univ. of Tennessee Health Sci. Center, TN, USA

Dr. Ilker K Sariyer, DVM, Ph.D., Assistant Professor, Department of Neuroscience and Center for Neurovirology, School of Medicine, Temple University, Philadelphia, PA
Pre-mRNA missplicing of MCL-1 is involved in ethanol induced neurotoxicity

Dr. Dayne Mayfield, Ph.D., Professor, Waggoner Center for Alcohol and Addiction Research, University of Texas at Austin, Austin, TX
Alcohol effects on microRNA, neuroimmune gene network, neuroinflammation

Dr. Marisa Roberto, Ph.D., Professor, The Neurobiology of Addictive Disorders, California Campus, The Scripps Research Institute, San Diego, CA
Neuroimmune factors in alcohol-induced neuroadaptation in the central amygdala

Dr. Michael Lewis, Ph.D., Professor, Department of Psychology, Hunter College CUNY & Doctoral Program, CUNY Graduate Center Alcohol Neuropharmacology Alcohol neuropharmacology:
Interaction with food intake and reward mechanisms

Dr. Hee-Yong Kim, Ph.D., Senior Investigator, Laboratory of Molecular Signaling, NIAAA, Bethesda, MD 20892
Docosahexaenoic acid in the nervous system: Modulation by ethanol

Dr. Narasimha Midde, Ph.D., Research Associate, Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN
Role of cytochrome P450 in alcohol-mediated effects in brain cells: Strategy to target novel CYP pathway.

Break 3:30 – 4 pm

Workshop 4 – 5:30 pm
Exploration of Alternate Career Options for the Young Investigators in Neuroimmune Pharmacology
Co-Chairs**: Dr. Sanjay B. Maggirwar**, Ph.D., University of Rochester, NY, USA
Kathleen Borgmann, University of North Texas, TX, USA

Sanjay B. Maggirwar, Ph.D., M.B.A.,
Need to Reform Biomedical Workforce Training

Additional speakers: *TBD*

Saturday April 1, 2017

Breakfast 7:00 – 8:30 am

Symposium 7 8:30 – 10 am
Cannabinoids in the Immune System
Chairs: **Dr. Roger Sorenson**, Ph. D., NIDA, NIH MD, USA
Dr. Toby K. Eisenstein, Ph.D., Temple University, PA, USA

Dr. Pal Pacher, MD, Ph.D., NIAAA, NIH, MD, USA
Advances in the Role of CB2 Activation in the Attenuation of Inflammation

Dr. Yuri Persidsky, M.D., Ph.D,. Temple University, PA, USA
Cannabinoid type 2 receptor activation in neuro-immune modulation at blood brain barrier: Role in neuroprotection

Dr. Yumin Zhang, M.D., Ph.D., Uniformed Services University of Health Sciences, MD, USA
Inhibition of the 2-AG hydrolytic enzyme ABHD6 in neuroinflammatory diseases

Dr. Toby K. Eisenstein, Ph.D., Temple University, PA, USA
CB2 Agonists as Immunosuppressive Molecules: Mechanisms of Action

Break 10 – 10:25 am

Bill Narayan Lecture 10:25 – 11:30 am
Introduction: **Dr. Howard E. Gendelman**, M.D., Margaret R. Larson Professor of Internal Medicine and Infectious Diseases, Chair, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA

Dr. Serena Spudich, M.D., Professor of Neurology; Division Chief, Neurological Infections & Global Neurology, Yale University, CT, USA
Establishment of CNS HIV-1 reservoirs and brain injury: Is typical antiretroviral therapy too little, too late?

Early Career Investigator Symposium 11:30 – 2:30 pm
(Boxed lunch provided)
Chairs: **Dr. Marisela Agudelo**, Ph.D., Florida International University, FL, USA
Dr. Howard E. Gendelman, M.D., Univ. of Nebraska Med. Ctr, Omaha, NE, USA

Speakers TBA – Selected ECITA Awardees, both pre- and post-doctoral, will give 5 minute presentations

Open Business Meeting 2:30 – 3:30 pm

Break 3:30 – 4 pm

Symposium 8 4 – 5:30 pm
Computational and Systems Biology applied to the Brain
Chair: **Dr. Maria Cecilia Marcondes,** Ph.D., The Scripps Research Institute, CA, USA

Dr. Celia M. Schunter, Ph.D., King Abdullah University of Science and Technology, Thuwal, 23955-6900, SA
The role of the brain in the adaptation to climate change

Dr. Chris Gaiteri, Ph.D., AD Center, Rush University, Chicago, IL, 60612, USA
Neuroimaging the molecular state of the brain in cognitive decline and Alzheimer's disease

Dr. Michelli Faria de Oliveira, Ph.D., University of California San Diego, CA, USA
HIV compartmentalization in the CNS

Dr. Maria Cecilia G. Marcondes, Ph.D. The Scripps Research Institute, CA, USA
Astrocyte-specific signatures in response to methamphetamine exposure *in vitro*

Banquet 7 – 10 pm
Hosted by the new SNIP president, **Dr. Jonathan Geiger**, Ph.D., Chester Fritz Distinguished Professor, School of Medicine and Health Sciences, University of North Dakota

Speaker: **Dr. Paul McGonigle**, Director, Division of Interdisciplinary and Career-Oriented Programs; Professor of Pharmacology & Physiology, Drexel University, College of Medicine, Philadelpia, PA, USA
Academic Drug Discovery

Announcements, Awards and Adjournment of Conference

Meeting adjourned.

Abstracts for the 23rd SNIP Scientific Conference

Abstracts are ordered in three major groups: 1) speakers (35 abstracts), 2) poster presenters Wednesday (W1-71) and 3) poster presenters Thursday (T1-77). Within each group the abstracts are ordered alphabetically by the last name of the first/presenting author.

1) Speakers (speaker abstracts available on 2/10/2017 are included)

Neurotoxic effect of Human Immunodeficiency Virus: cytoskeleton, mitochondria, and neuronal apoptosis

Avdoshina, V, MD, Ph.D.[1], Wenzel, ED, BS[2], Caragher, SP, BS[1], Fields, JA, Ph.D.[3], Castellano, P, Ph.D.[4], Taraballi , F, Ph.D.[5], Eugenin, E, Ph.D.[4], Masliah, E, MD[3], Mocchetti, I, Ph.D.[1]; [1]Department of Neuroscience, Georgetown University Medical Center, Washington, DC, 20057 United States. [2]Department of Pharmacology, Georgetown University Medical Center, Washington, DC, 20057 United States. [3]Department of Pathology and Neuroscience, University of California San Diego, La Jolla, CA, 92093 United States. [4]Department of Microbiology, Biochemistry and Molecular Genetics, International Center for Public Health Rutgers, The State University of New Jersey, Newark, NJ, 07103 United States. [5]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, 77030 United States.

Despite of combined antiretroviral therapy success, neurological impairments leading to HIV-associated neurocognitive disorders (HAND) persist. Neurite pruning and loss similar to those observed in HAND is seen in neurons exposed to the HIV envelope protein gp120. The present study was undertaken to discover the role of cytoskeleton in gp120 neurotoxicity. We tested the hypothesis that gp120 interacts specifically with neuronal microtubules. Using dot blot analysis and surface plasmon resonance we established that gp120 binds with high affinity to neuronal specific β-3 tubulin (TUBB3), but not to tubulin isoforms expressed by other cell types. Gp120 also inhibits tubulin polymerization in vitro and decreases its acetylation in rat cortical neurons and HIV+ human postmortem brain samples. We have identified the binding site of gp120 to TUBB3 and designed a small peptide (Helix-A) that prevents gp120-TUBB3 interaction. Helix-A has been cross-linked to mesoporous silica nanoparticles (Helix-A nano) to enhance the intracellular delivery of the peptide. We then tested the neuroprotective property of Helix-A nano against three strains of gp120 in rat cortical neurons. Helix-A nano prevented gp120-mediated neurite simplification as well as neuronal loss. We also examined mitochondria movements as a tool to ascertain whether gp120 affects antero- and retrograde transport. Time-lapse imaging of mitochondria showed that gp120 significantly reduces mitochondrial trafficking. Overall our data suggest the key role of gp120-neuronal microtubule interaction in HIV-mediated neurotoxicity.

Supported by NS079172, NS074916, P30AI087714, MH096625, NS083426, AG043384, MH062962, MH5974, MH83506

CCL2-mediated mechanisms of neuroinflammation in the context of HIV infection and drug abuse

Berman, J.W., Ph.D.[1]; [1]Departments of Pathology, and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, 10461 United States.

Transmigration of monocytes across the BBB contributes to CNS infection, inflammation, and neuronal damage that mediate HIV-associated neurocognitive disorders (HAND) despite ART. The number of mature CD14CD16 monocytes is increased in HIV+ people, is even higher in HIV+ drug abusers, and is critical to HIV CNS disease. CD14CD16 monocytes transmigrate preferentially across the BBB to the chemokine CCL2. HIV infected, CD14CD16 monocytes transmigrate in even greater numbers due to increased junctional proteins JAM-A and ALCAM, and increased CCR2, the CCL2 receptor. Dopamine, elevated in the brain in response to drug use, enhances this transmigration. Buprenorphine, a therapeutic for heroin addiction, reduces CD14CD16 cell adhesion to brain endothelial cells and their chemotaxis to CCL2. It also reduces monocyte entry into the CNS and prevents cognitive impairment in the ECOHIV mouse model. CD14CD16 monocytes in HIV+ people consist of cells infected with HIV (HIV+), and cells exposed to viral/host factors but not infected (HIVexp). HIV+CD14CD16 monocytes preferentially transmigrate across the BBB in comparison to HIVexpCD14CD16 cells. Cenicriviroc, a CCR2/CCR5 inhibitor, as well as JAM-A antibody prevent this preferential transmigration. CCR2 and JAM-A may be therapeutic targets for HAND. CCR2 is increased on CD14CD16 cells from HIV+ people with HAND, and not on those from unimpaired or impaired people due to non-HIV causes. Neuroimaging showed that CCR2 on CD14CD16 monocytes correlates with decreased neuronal metabolites in the basal ganglia. CCR2 may be a biomarker for HAND.

Supported by NIMH

Zika virus infection induces cranial neural crest cells to produce cytokines at levels detrimental for neurogenesis

Blish, C. Ph.D.[1], [1]Stanford Immunology, Stanford School of Medicine, Stanford University, Stanford, CA 94305, USA

Zika virus (ZIKV) infection during pregnancy is linked to microcephaly, which is attributed to infection of developing brain structures. ZIKV infects neural progenitor cells in vitro, though its effects on other developmentally relevant stem cell populations, including cranial neural crest cells (CNCCs), have not been assessed. CNCCs give rise to most cranial bones and exert paracrine effects on the developing brain. Here, we report that CNCCs are productively infected by ZIKV, but not by the related dengue virus. ZIKV-infected CNCCs undergo limited apoptosis but secrete cytokines that promote death and drive aberrant differentiation of neural progenitor cultures. Addition of two such cytokines, LIF or VEGF, at levels comparable to those secreted by ZIKV-infected CNCCs is sufficient to recapitulate premature neuronal differentiation and apoptotic death of neural progenitors. Thus, our results suggest that CNCC infection by ZIKV may contribute to associated embryopathies through signaling crosstalk between developing face and brain structures.

HIV-1 disrupts motivational processes via dopamine circuitry dysfunction

Booze, RM, Ph.D.[1], Bertrand, SJ, Ph.D.[1], Harrod, SB, Ph.D.[1], Mactutus, CF, Ph.D.[1]; [1]Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States.

Motivational alterations/apathy in HIV-1+ individuals are associated with decreased performance on tasks involving frontal-subcortical circuitry and the nucleus accumbens. The HIV-1 transgenic rat was used to assess long-term HIV-1 protein exposure on motivated behavior using cocaine (0.01-1.0 mg/kg/infusion) and sucrose (1-30%, w/v) maintained responding with fixed-ratio (FR) and progressive-ratio (PR) schedules of reinforcement. Cocaine-maintained responding was profoundly attenuated in HIV-1 Tg rats (FR1 0.33 mg/kg/infusion and PR 1.0 mg/kg/infusion). For sucrose-reinforced responding, HIV-1 Tg rats displayed no change in EC50, relative to controls, across concentration-response curves under either FR or PR schedules of reinforcement suggesting no change in sensitivity to, or reinforcing efficacy of, sucrose reinforcement. However, the HIV-1 Tg rats reached (FR1) criterion more slowly than controls suggesting an overall decrease in response vigor. When choosing between cocaine and sucrose, control rats initially chose sucrose but with time shifted to a cocaine preference. In contrast, HIV-1 disrupted choice behaviors. DAT function was altered in the striatum of HIV-1 Tg rats; however, prior cocaine self-administration produced a unique effect on dopamine homeostasis in the HIV-1 Tg striatum. Synaptic dysfunction may be fundamental in determining the expression of dopaminergic goal-directed behaviors in the HIV-1 Tg rat. These findings help foster therapeutic strategies to treat motivational alterations (such as apathy) in vulnerable HIV-1 positive populations.

Supported by NIH DA013137, HD043680, MH106392

Absence of fractalkine/CX3CR1 induces perivascular clustering of microglia and fibrinogen leakage during systemic inflammation in mouse models of diabetic retinopathy

Cardona, AE. Ph.D. [1], Andrew S. Mendiola[1], Rolando Garza[1], Sandra M. Cardona[1], Shannon A. Mythen[1], Sergio A. Lira[2], Katerina Akassoglou[3,4], [1]Department of Biology and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, San Antonio, TX, USA; [2]Immunology Institute Icahn School of Medicine at Mount Sinai, New York, NY, USA; [3]Gladstone Institute of Neurological Disease, University of California, San Francisco, San Francisco, CA, USA; [4]Department of Neurology, University of California, San Francisco, San Francisco, CA, USA.

The emergence of widespread combination antiretroviral therapy (cART) use has altered the clinical character of neurologic disease observed in HIV-1 and has raised a host of questions related to the role of the central nervous system (CNS) as a site of HIV-1 persistence and a potential barrier to HIV-1 cure. This presentation will review what is known regarding establishment of HIV-1 in the CNS during early infection, and whether early intervention with cART or other treatments may alter the character of a potential CNS 'reservoir' for HIV-1. Based on studies of acute HIV-1 in humans and acute SIV in macaque models, it has become evident that CNS HIV-1 infection during acute and early infection triggers a unique sequence of pathogenic and immunoregulatory events. Early treatment with cART reduces levels of CSF HIV-1 RNA and signs of neuronal injury and immune activation that are present in individuals on cART started during chronic infection, however cART alone may not resolve all of these abnormalities. This talk will present studies that investigate the persistence of HIV during cART, including detection of low level CSF HIV-1, characterization of CSF HIV-1 escape, and monitoring of HIV-1 resurgence in the CNS after cART interruption. Furthermore, new investigations employing immunological approaches to reduction of CNS reservoirs will be described. Conclusions: Persistent perturbation of the CNS the setting of apparently successful cART may in part reflect a reservoir for HIV-1 within this tissue compartment. Early cART reduces the magnitude of systemic HIV-1 reservoirs and may also provide benefit to the brain, but adjunctive interventions may be needed to fully ameliorate HIV-1 related immune activation and viral persistence in the CNS.

CB2 Agonists as Immunosuppressive Molecules: Mechanisms of Action

Eisenstein, T. K., Ph.D.[1], Robinson, R. H., Ph.D.[1], Joseph, J. M., BS[1], Adler, M. W., Ph.D.[1]; [1]Center for Substance Abuse

Research, Lewis Katz School of Medicine of Temple University, Philadelphia, PA, 19140 United States.

There is currently great interest in evaluating therapeutic effects of cannabinoids, particularly for treatment of pain and for a variety of autoimmune conditions that have an inflammatory component. Δ9 -THC has been shown by many investigators to be immunosuppressive. It activates both CB1 and CB2 receptors. CB1 is expressed mainly on neurons and CB2 is most abundant on cells of the immune system. Synthetic cannabinoids that are selective for the CB2 receptor have been shown to induce immunosuppression in a number of animal models of autoimmune diseases. Our laboratory has compared Δ9 -THC, WIN55212-2 (a synthetic cannabinoid with activity at both CB1 and CB2), and synthetic CB2 agonists for activity in a mouse model of graft rejection and in an inflammatory pain assay. Using CB1 and CB2 selective inhibitors, CB2 k/o mice, and arrays of immune mediators, we have examined which receptor mediates these selected biological effects, and immunomodulatory mechanisms. Using the mixed lymphocyte reaction (MLR), an in vitro correlate of graft rejection, we have found that compounds active at the CB2 receptor inhibit responses of T-cells to foreign tissue. The immune response is down-regulated via production of IL-10, an anti-inflammatory cytokine, and induction of T-regulatory (Treg) cells. In a pain model using formalin injection of the mouse paw, both CB1 and CB2 receptors mediated analgesia. Since engagement of CB2 receptors on leukocytes does not elicit psychoactive activity, CB2 selective compounds provide a potential new class of cannabinoid drugs with medicinal capacity.

Supported by Grant from the Pennsylvania Department of Health and NIDA grants DA013429 and T32-DA07237

Flaviviruses, an expanding threat in public health

Eugenin EA.[1], [1]Public Health Research Institute, Newark, NJ, USA

The flaviviruses dengue, West Nile, and Japanese encephalitis represent three major mosquito-borne viruses worldwide. These pathogens impact the lives of millions of individuals and potentially could affect non-endemic areas already colonized by mosquito vectors. Unintentional transport of infected vectors (Aedes and Culex spp.), traveling within endemic areas, rapid adaptation of the insects into new geographic locations, climate change, and lack of medical surveillance have greatly contributed to the increase in flaviviral infections worldwide. The mechanisms by which flaviviruses alter the immune and the central nervous system have only recently been examined despite the alarming number of infections, related deaths, and increasing global distribution. In this review, we will discuss the expansion of the geographic areas affected by flaviviruses, the potential threats to previously unaffected countries, the mechanisms of pathogenesis, and the potential therapeutic interventions to limit the devastating consequences of these viruses.

Neuroimaging the molecular state of the brain in cognitive decline and Alzheimer's disease

Gaiteri, CG, Ph.D.[1]; [1]Rush University Alzheimer's Disease Center, Rush University, Chicago, IL, 60612 United States.

Many neuroimaging studies have shown that variation in neuroanatomical structures is associated with disease or disease risk. Utilizing these findings in molecular biology research is challenging, because the molecular basis for variation in a given brain structure is generally unknown. By utilizing gene expression and methylation, we show that person-to-person variation in many regions of the brain is associated with the expression of hundreds of genes and DNA methylation of thousands of loci. These structurally-correlated genes are concentrated in several expression and methyation molecular systems. This combination of the spatial resolution of neuroimaging with the drug development potential of omics provides a novel coherent framework for Alzheimer's disease.

Dopamine increases macrophage susceptibility to HIV infection

Gaskill, PJ, Ph.D.[1]; [1]Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, 19012 United States.

Within the CNS, cells of the myeloid lineage, such as macrophages, are the principle targets for HIV infection. In substance abusers and those using therapeutics such as selegiline, Ritalin or L-DOPA, increased numbers of CNS macrophages are exposed to dopamine. Dopamine mediates the addictive and reinforcing effects of all drugs of abuse, and is also increased by a number of prescription medications. Our research demonstrates that exposure to concentrations of dopamine greater than 10-8 M significantly increase the entry of HIV into primary human macrophages. This increase is due to the activation of either D1-like or D2-like dopamine receptors, and also requires interaction with the HIV co-receptor CCR5. This suggests a common pathway by which dopamine acts on HIV entry, as we have shown that blocking calcium release abrogates the impact of dopamine on entry. The interaction between the dopamine receptors and CCR5 may involve either heteromerization or downstream crosstalk. Our data indicate that CCR5 and dopamine receptors can heterodimerize in primary human macrophages, but also suggest that dopamine receptors can potentiate calcium flux

through actions on PLC. These data indicate that illicit drugs and other substances which act on the dopaminergic system may activate a common pathway mediating increased susceptibility of macrophages to HIV infection. Thus, HIV-infected individuals who abuse drugs or use therapeutics which increase CNS dopamine may enhance the development of neuroinflammation and HAND through the impact of dopamine on CNS macrophages.

Supported by NIDA

Protective, Instructive and Destructive Components of the Neuroinflammatory Reponse

Haughey, NJ, Ph.D.[1]; [1]Department of Neurology, Division of Neuroimmunology, The Johns Hopkins University School of Medicine, Baltimore, MD, 21287 United States.

Neuroinflammation has been implicated as a protective, and pathogenic response to infection, trauma, degenerative, and psychiatric disease. Although each of these conditions have distinct etiologies, there are a number of commonalities in the cytokine/chemokine and innate immune response. The acute stage of a neuroinflammatory response is fundamentally an attempt to protect the brain. The central nervous system mediators for these acute responses are primarily brain resident glia that generate and release cytokines/chemokines, and exosomes to regulate the host response to damage or infection. The brains inflammatory and immune response is tightly regulated by a number of molecular balances that are intended to limit the duration and intensity of the inflammatory/immune response. When these regulatory systems fail in the setting of disease, or do not activate when the inflammatory environment is sub-threshold to initiate these controls, a chronic low-level inflammation and immune activation can result. Chronic inflammation and immune activation can compromise the integrity of the blood brain barrier, and contribute to neuronal damage. Attempts to regulate the neuroinflammatory environment have produced mixed results that range from cognitive worsening to improvement. In this seminar we will compare commonalties and differences in the temporal course of neuroinflammatory and immune responses across multiple neurological diseases, the historical and current approaches to therapy.

Supported by NIMH, NIDA

Mitochondrial DNA variation and HIV-associated neurotoxicity

Hulgan, T, MD[1]; [1]Division of Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN, 37220 United States.

Neurocognitive impairment (NCI) remains an important complication in HIV-infected persons. HIV-associated NCI and neuroinflammation represent diverse and complex phenotypes and endophenotypes, respectively. It is likely that host genetic variation contributes to both phenotype diversity and the complex relationships between endophenotypes and phenotypes. The critical and complex roles of mitochondria in energy production, oxidative stress and inflammation, and apoptotic regulation make them vulnerable targets and key mediators of cellular damage in response to environmental stresses. Given the high energy demands of the central nervous system, mitochondria are critically important in brain neuronal function and neurodegeneration. Ancestry-related mitochondrial DNA (mtDNA) variation is associated with differential mitochondrial function in model systems and humans, and has been associated with outcomes of HIV infection and its treatment. The potential importance of mtDNA variation in neurotoxicity and NCI is supported by the prominence of neurologic phenotypes in inherited mtDNA diseases and associations between mtDNA variation and chronic neurodegenerative diseases. Our group hypothesized that mtDNA variation is associated with neurotoxic effects of chronic HIV infection- including neuroinflammation and NCI. This presentation will summarize key background information on mitochondrial genetics, results from recent mtDNA-focused analyses in the CHARTER Study, and future directions to better elucidate mechanisms of effects of mtDNA variation and neurotoxicity in HIV.

Supported by NIMH R01 MH095621 (PIs Hulgan and Kallianpur)

Immune signaling in the habenula and nicotine addiction

Kenney, P., Ph.D.[1]; [1] Mount Sinai School of Medicine, NY, USA.

Medial habenula (MHb) neurons that project to the interpeduncular nucleus (IPN) regulate the set-point for nicotine aversion. Genetic or pharmacological manipulations that attenuate the stimulatory effects of nicotine on the MHb-IPn circuit increase nicotine intake in human smokers and laboratory smokers. Hence, adaptive responses in the MHb-IPN circuit in response to nicotine intake is likely to play a critical role in the development of tobacco dependence in human smokers. Recently we have found that a history of nicotine consumption in human smokers or nicotine exposure in the laboratory animals decreases the size of the MHb, as measured using magnetic resonance imaging. Using whole-brain tissue clearing in laboratory animals, we find that this decrease in MHb volume is the result of nicotine induced death of habenular cholinergic neurons. These findings suggest that cholinergic neurons are uniquely sensitive to neurotoxic actions of nicotine. The MHb is unique from a neuroimmune perspective, as neurons in this

site express interleukin-18 (IL-18), a cytokines heavily implicated in neurodegenerative processes. In this presentation I will discuss new data implicating interleukin-18 in regulating the stimulatory effects of nicotine on MHb neurons. I will also show data suggesting that IL-18 deficiency increases the sensitivity of MHb neurons to the neurotoxic actions of nicotine and provide insights into underlying mechanisms..

Docosahexaenoic acid-derived effects on neurodevelopment and neuroprotection: modulation by ethanol

Kim, H-Y, Ph.D.[1]; [1]Laboratory of Molecular Signaling, NIAAA/NIH, Rockville, MD, 20852 United States.

Docosahexaenoic acid (DHA, 22:6n-3) is highly enriched in neural tissues mainly as membrane phospholipids. Our laboratory has demonstrated neurotrophic and neuroprotective roles of DHA including its capacity to promote neuronal development and synaptic function and to improve functional outcome after traumatic brain injury (TBI). DHA promotes neuronal survival primarily due to its unique ability to alter neuronal membrane properties, thereby facilitating activation of key kinases required for cell survival. In contrast, ethanol exerts opposite effects by influencing DHA metabolism and interacting with DHA-dependent signaling events. More recently, we discovered that N-docosahexenoyethanolamine (synaptamide), an endogenous metabolite of DHA, potently induces neuronal differentiation of neural stem cells (NSCs), neurite outgrowth and synaptogenesis while suppressing neuroinflammation by specifically binding to orphan G-protein coupled receptor 110 (GPR110, ADGRF1). GPR110 knockout mice show significant deficits in synapse number and learning and memory function. Considering that DHA is the precursor of synaptamide, the GPR110 ligand, and also positively affects the recovery after brain injury, synaptamide/GPR110 signaling provides a novel target for controlling not only neurodevelopment but also neuroprotection. Molecular and signaling mechanisms underlying DHA-mediated neurotrophic and neuroprotective effects and their modulation by ethanol will be presented along with potential preventive/therapeutic strategies.

Supported by NIAAA intramural program

Astrocyte progenitor transplantation promotes axon regeneration and recovery of respiratory function following spinal cord injury by modulating the inflammatory macrophage response

Lepore, AC, Ph.D.[1]; [1]Department of Neuroscience, Vickie and Jack Farber Institute for Neuroscience, Sydney Kimmel Medical College at Thomas Jefferson University, Philadelphia, PA, 19107 United States.

Stem cell transplantation-based replacement of astrocytes is a novel and potentially powerful therapeutic strategy for treating CNS diseases such as traumatic spinal cord injury (SCI). Astrocytes play a number of critical roles in the intact CNS, while astrocyte dysfunction contributes to pathogenesis of a host of nervous system disorders. Astrocytes normally form borders to restrict immune cell entry into CNS parenchyma in intact CNS and at glial scar sites after injury. Astrocytes can also dampen the inflammatory phenotype after CNS perturbation via mechanisms such as the release of immunomodulatory factors. The pro-inflammatory macrophage response after SCI plays an important role in preventing regeneration of injured axons, thereby limiting recovery of function. We find that astrocyte progenitor transplantation significantly diminishes the infiltration of resident microglia-derived and peripheral monocyte-derived macrophages into the lesion site in our rodent model of cervical contusion-type SCI. Furthermore, astrocyte lineage transplants promote robust regeneration of bulbospinal respiratory axons that are centrally involved in controlling inspiratory breathing, as well as significant recovery of diaphragm function. Interestingly, axon regrowth occurs specifically through lesion areas with robust transplant integration and reduced macrophage response. Collectively, our findings demonstrate that astrocyte transplantation can promote axon regeneration and consequent recovery of respiratory function following cervical SCI by modulating the intraspinal macrophage response.

Supported by NIH 1R01NS079702, Paralyzed Veterans of America Research Foundation #346986

Alcohol neuropharmacology: Interaction with food intake and reward mechanisms

Lewis, MJ, Ph.D.[1]; [1]Department of Psychology, Hunter College CUNY, New York, NY, 10065 United States.

Research from several approaches suggests the interaction of mechanisms that mediate alcohol abuse and dependence with those that control food intake and nutrition. Alcohol is a calorically rich food as well as a drug of abuse. Food deprivation increases alcohol intake and preference and alcohol intake can increase food choice and intake. The mechanisms underlying these increases are not well understood. Voluntary alcohol intake is altered by various manipulations of basic nutrients and the neurobiological systems that mediate them, suggesting a complex interaction between alcohol and food intake. We have found that that the motivation for alcohol intake is closely related to mechanism that signal and control fat intake. The orexigenic neuropeptide galanin increase alcohol preference and intake as well as fat intake. In addition CD36, a vital signaling mechanism in triglycerides uptake and fat metabolism, alters alcohol effects centrally and peripherally. Both

alcohol and fatty foods increase dopamine (DA) activity in the nucleus accumbens; however, decreased DA functioning with chronic intake of both follows this effect. In addition both chronic administration of alcohol and a high fat diet alter endocannabinoid concentrations in this brain area. Understanding the interrelationship of alcohol intake and reinforcement with the intake and regulation of fat may provide valuable insight into alcohol dependence, food intake, eating disorders and related neuroimmune diseases.

Supported by NIHRC2 AA019413 of the NIAAA, NIH and from the National Institute on Minority Health and Health Disparities of the National Inst

Integrity of the microbiome of the HIV-1 transgenic rat: Efficacy of the gut-brain-axis for treatment of neurocognitive impairment

Mactutus, CF, Ph.D.[1], Booze, RM, Ph.D.[1]; [1]Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States.

The emerging view that HIV is a disease of the gut was explored in the HIV-1 transgenic (Tg) rat, a useful model of neurocognitive deficits that result from long-term HIV-1 viral exposure, as seen in HAND and pediatric HIV/AIDS. S-equol, a metabolite produced via the gut microbiome following ingestion of soy isoflavone daidzein, has neurorestorative effects. A 2 x 2 factorial design was employed with 42 adult female ovariectomized HIV-1 Tg and F344/N control rats (background strain) randomly assigned to one of two treatment groups: Sucrose vs. S-equol (ns=10-11). All animals were ovariectomized and fed a minimal phytoestrogen diet (≤20 ppm of phytoestrogen) to remove endogenous sources of estradiol. With a clinically relevant dosage level, S-equol (four 0.5 mg pellets) or four sucrose control pellets per animal were administered daily for 70 days. 16S rRNA gene sequence surveys were used to characterize baseline and the S-equol response of the gut microbiome. Evidence for baseline differences between HIV-1 vs. control stool samples was suggested by alpha diversity analysis (p

Supported by Supported by NIH DA013137, HD043680, MH106392.

Astrocyte-specific signatures in response to methamphetamine exposure *in vitro*

Marcondes, MC, Ph.D.[1], Bortell, N[1], Basova, L, Ph.D.[1], Semenova, S, Ph.D.[2], Fox, HS, MD, Ph.D.[3], Ravasi, T, Ph.D.[4], Marcondes, MC, Ph.D.[4]; [1]Department of Molecular and Cellular Neurosciences, The Scripps Research Institute, La Jolla, CA, 92037 United States. [2]Department of Psychiatry, University of California School of Medicine, San Diego, CA, 92037 United States. [3]3Department of Experimental Pharmacology, University of Nebraska Medical Center, Omaha, NE, 68182 United States. [4]4KAUST Environmental Epigenetic Program , King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia, Thuwal, Thuwal, 23955 Saudi Arabia.

Astrocyte activation is one of the earliest findings in the brain of methamphetamine (Meth) abusers. Our goal in this study was to identify the characteristics of the astrocytic acute response to the drug, which may be critical in pathogenic outcomes secondary to the use. We developed an integrated analysis of gene expression data to study the acute gene changes caused by the direct exposure to Meth treatment of astrocytes in vitro, and to better understand how astrocytes respond, what are the early molecular markers associated with this response. We examined the literature in search of similar changes in gene signatures that are found in Central Nervous system disorders. We identified overexpressed gene networks represented by genes of an inflammatory and immune nature and that are implicated in neuroactive ligand-receptor interactions. The overexpressed networks are linked to molecules that were highly upregulated in astrocytes by all doses of methamphetamine tested, and that could play a role in the Central Nervous System homeostasis. The strongest signatures were the upregulation of MAP2K5, GPR65, and CXCL5, and the gene networks individually associated with these molecules. Pathway analysis revealed that these networks are involved both in neuroprotection and in neuropathology. We have validated several targets associated to these genes. Thus, gene signatures for the astrocytic response to Meth were identified among the upregulated gene pool, using an in vitro system. The identified markers may participate in CNS dysfunction or protection to acute drug exposure.

Supported by NIH/NIDA

Dopamine and immunity: from basic evidence to therapeutic opportunities

Marino, F, BS[1], Cosentino, M, MD, Ph.D.[1]; [1]Center for Research in Medical Pharmacology, University of Insubria, Varese, varese, 21100 Italy.

Dopamine (DA) is a neurotransmitter involved in crucial central nervous system (CNS) functions including motivation, cognition, movement and reward. DA is however also synthesized and utilized by immune cells. DA acts on 5 different dopaminergic receptors grouped into two families: the D1-like and the D2-like and dopaminergic receptors (DR) are present on the surface of immune cells. Increasing evidences suggests a pivotal role of DA in the neuroimmune network,

contributing to the CNS-immune system interplay and in the communication among immune cells. DA affects possibly all human immune cells. Results from animal models and clinical studies support the involvement of dopaminergic pathways in immune cells in several diseases affecting primarily the CNS, like multiple sclerosis or Parkinson's disease, as well as in peripheral diseases eventually affecting in second instance the CNS, such as cancer and HIV infection. Emerging evidence indicates the occurrence of dopaminergic immune mechanisms also in metabolic disease and in the regulation of hematopoiesis. Pharmacological modulation of dopaminergic pathways can be obtained by use of indirectly acting agents targeting DA synthesis, storage and release, uptake and metabolism. Many directly and indirectly acting dopaminergic drugs are in use for non-immune indications (e.g. cardiovascular, neurologic, neuropsychiatric), and many of them might be easily and conveniently repurposed in the neuroimmune network, as they have a usually favourable safety profile and a low price, thereby benefiting both patients and the healthcare systems.

Supported by =

Alcohol effects on microRNA, neuroimmune gene networks, and neuroinflammation

Mayfield, R.D., Ph.D.[1]; [1]Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX, 78712 United States.

Long-term alcohol use can result in lasting changes in brain function ultimately leading to alcohol dependence. These functional alterations are associated with dysregulation of complex gene networks that span multiple brain regions, and growing evidence implicates microRNAs as key regulators of these networks. We have examined network changes in microRNA and mRNA target expression in response to alcohol in both human postmortem brain and in rodent brain after treatment with various alcohol exposure paradigms. These analyses have identified a number of neuroimmune-related pathways including ERK1/2 (mouse equivalent Mapk3, Mapk1), Bcl2 (in amygdala networks) and Srf (in prefrontal cortex networks). These identified hub genes demonstrate robust microRNA-mRNA network alterations in response to alcohol exposure. In addition, temporal analyses identified NfkB- and Smad3-centered networks in nucleus accumbens. These results demonstrate that alcohol exposure results in complex temporal changes in microRNA-mRNA gene network structure and that manipulation microRNAs may rescue the aberrant synaptic plasticity associated alcohol consumption.

Supported by This work was supported by NIAAA grants AA020926, AA020683, AA012404

Brain exposure to HIV-1 Tat protein potentiates the psychostimulant effects of morphine, modulates consumption and reinstates extinguished reward-seeking

McLaughlin, JP, Ph.D.[1], Cirino, TJ[1], Eans, SO, MS[1], Medina, JM[1], Hymel, KA, Ph.D.[1], Paris, JJ, Ph.D.[2], Kaufman, MJ, Ph.D.[3]; [1]Dept. of Pharmacodynamics, University of Florida, Gainesville, FL, 32610 United States. [2]Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23298 United States. [3]McLean Imaging Center, McLean Hospital, Harvard Medical School, Belmont, MA, 02478 United States.

We hypothesized that HIV-1 Tat expression in brain would modulate the rewarding effects of opioids, specifically morphine. Using the GT-tg bigenic mouse model, where brain-selective Tat expression is controlled by activation of a doxycycline (Dox) promotor, we tested the effects of Tat protein on morphine-conditioned place preference (CPP) and voluntary morphine consumption in a two-bottle choice (TBC) assay. Western blot analysis confirmed the expression of Tat protein in GT-tg bigenic mice. In behavioral testing, saline-treated GT-tg bigenic mice, or Dox-treated G-tg mice (lacking the Tat gene) all demonstrated a morphine-CPP responses similar to that of saline- or Dox-treated C57BL/6J mice. In contrast, Tat expression significantly increased morphine-CPP 3-fold only in GT-tg bigenic mice treated with Dox. Consistent with this observation, exposure to Tat protein increased the levels of dopamine released in response to heroin in GT-tg mice. The potentiation of morphine-CPP was prevented by pretreatment with an inhibitor of Tat protein, didehydro-Cortistatin A, and dependent on the magnitude of exposure to Tat protein. Likewise, expression of Tat protein produced an exposure-dependent reinstatement of an extinguished morphine place preference response in previously uninduced mice. Overall, these data suggest that expression of HIV-1 Tat protein potentiated the rewarding effects of morphine in an exposure-dependent manner, and suggests a biological means by which HIV infection may increase the vulnerability to substance abuse and relapse in abstinent subjects.

Supported by R01-MH085607 (from NIMH) and R01-DA039044 (from NIDA)

Role of cytochrome P450 in alcohol-mediated effects in brain cells: Strategy to target novel CYP pathway

Midde, NM, Ph.D.[1], Rahman, MA, MS[1], Kumar, S, Ph.D.[1]; [1]Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN, 38163 United States.

Although alcohol dehydrogenase is the first to metabolize alcohol in the liver, CYP2E1 is the major pathway to metabolize alcohol in chronic and binge drinkers, which subsequently produces reactive oxygen species (ROS) and reactive acetaldehyde causing liver damage. CYP2E1 has also been implicated in alcohol metabolism in brain cells including monocytes (lineage of brain macrophages and microglia) and astrocytes. Recently, we showed that CYP2E1 is significantly expressed in monocytic and astrocytic cells. Next, we showed that alcohol induces CYP2E1 and causes cellular toxicity in brain cells. Further, we showed that selective CYP2E1 inhibitor, diallyl sulfide (DAS) and CYP2E1 siRNA, are capable of rescuing cells from alcohol-mediated toxicity. The oxidative stress produced by CYP2E1 subsequently induces CYP2E1 through PKC/Nrf-2 pathway leading to a vicious cycle of producing perhaps an uncontrolled level of oxidative stress in monocytes and astrocytes. Thus targeting CYP2E1 is imperative to minimize alcohol-induced toxicity in the CNS cells. Although DAS is a specific CYP2E1 inhibitor, it is also a CYP2E1 substrate, which upon metabolism leads to toxic metabolites. We are currently developing novel DAS analog that is relatively stronger inhibitor but weaker substrate of CYP2E1 than DAS. Our initial results showed that 4 of the 8 analogs have better cytotoxicity profiles than DAS, and they also rescue alcohol-induced toxicity in hepatic, monocytic, and astrocytic cells. Studies are underway to further characterize selected analogs using both in vitro and in vivo approaches.

Supported by NIAAA/NIH AA-022063

Autoantibodies as biomarkers of neurodegenerative diseases

Nagele, R, Ph.D.[1], DeMarshall, C, Ph.D.[1]; [1]Dept. of Cell Biology and NJ Institute for Successful Aging, Rowan University, Stratford, NJ, 08084 United States.

In neurodegenerative diseases such as Alzheimer's and Parkinson's disease (AD and PD), pathological changes are initiated many years prior to emergence of symptoms and generate brain-specific tissue debris that spills over into the blood. This triggers the production of disease-associated autoantibodies, whose normal function is to clear this debris from the blood. We have found that detection of disease-associated changes in autoantibody profiles is useful for the diagnosis of these diseases. For example, using human protein microarrays to screen blood autoantibody profiles, we identified small panels of autoantibodies that are useful as diagnostic biomarkers to detect early-stage AD at the mild cognitive impairment (MCI) stage as well as early-stage PD with exceptionally high overall accuracies (100% and 92%, respectively) and distinguish discrete stages of AD and PD progression. Lastly, these biomarker panels were also capable of distinguishing MCI and early-stage PD patients from those with other neurological and non-neurological diseases with high overall accuracy, thus confirming disease specificity. The development of accurate, blood-based diagnostic tests for patients at early stages of AD, PD and other neurodegenerative diseases will enable early treatment, allow earlier enrollment into clinical trials, and facilitate monitoring of disease progression while under treatment by their physicians or while participating as subjects in clinical trials for new potential therapeutics. It also opens the door to the possibility of pre-clinical detection.

Supported by Osteopathic Heritage Foundation, Michael J Fox Foundation

Pathophysiology of the nucleus accumbens in HIV-1 transgenic rats is potentiated by cocaine self-administration

Napier, TC, Ph.D.[1], Chen, L, Ph.D.[2], Wayman, WN, Ph.D.[2], Hu, X-T, MD, Ph.D.[2]; [1]Department of Psychiatry, Rush Univ Sch of Medicine, Chicago, IL, 60612 United States. [2]Department of Pharmacology, Rush Univ Sch of Medicine, Chicago, IL, 60612 United States.

The nucleus accumbens (NAc) is involved in the pathogenesis of cocaine addiction and is exposed to significant viral loads in human immunodeficiency virus (HIV)-infected individuals. Understanding the pathophysiology that affects the NAc in the co-morbid state is necessary for developing effective therapeutic interventions. To ascertain if the HIV exacerbates cocaine-induced pathophysiology in the NAc core (NAcc), we assessed NAcc neuronal excitability in adult male HIV-1 transgenic (Tg) and non-Tg rats that self-administered cocaine (COC-SA) vs. saline-yoked controls (SY). To do so, primary NAcc neurons (medium spiny neurons; MSN) were recorded in ex vivo forebrain slices harvested 14 days after the last SA operant session using whole-cell patch-clamp electrophysiology. We found that: (i) COC-SA significantly decreased evoked firing of MSNs in non-Tg rats, while this effect was potentiated in Tg rats. (ii) Changes in membrane properties (e.g., resting membrane potential, input resistance, and rheobase) support the findings that MSN excitability was reduced by COC-intake for non-Tg rats, but was increased in Tg rats after COC-SA. (iii) L-type Ca2+ channels were upregulated in NAcc of Tg rats when compared to non-Tg rats. This represents the first demonstration of NAcc neuropathophysiology in Tg rats and findings reveal a functional dysregulation after chronic exposure to COC and HIV-1 proteins in vivo. Study outcomes suggest that HIV proteins render NAcc reward circuits more vulnerable to excitatory stimuli, especially when co-exposed to chronic cocaine.

Supported by NIH DA033882 and DA033206

Diversity, Equity and Inclusion in Neuroimmune Pharmacology – A Mentoring Challenge

Dr. Ana Nuñez, M.D.[1], [1] Drexel University, College of Medicine, PA, USA

The field of Neuroimmune Pharmacology has a myriad of complexities to learn in order to be successful. Similar to lab techniques, the techniques about mentoring and networking are equally important as the next interesting idea. And yet, the 'how to' for success can appear elusive. This session will build on mentoring experiences at the conference and is sponsored by the Diversity and Inclusion Committee (DISC). It will be an engaging session for participants to envision their future success in their communities of science.

Mitochondrial DNA, Inflammation and Neurocognitive Dysfunction in HIV infection

Pérez-Santiago, J, Ph.D.[1], Schrier, RD, Ph.D.[1], Oliveira, MF, Ph.D.[1], Var, SR, BS[1], Day, TRC, BS[1], Massanella, M, Ph.D.[1], Cherner, M, Ph.D.[1], Woods, SP, Ph.D.[1], Letendre, SL, MD[1], Gianella, S, MD[1], Mehta, SR, MD[1]; [1]Department of Medicine, University of California San Diego, San Diego, CA, 92103 United States.

Even with the advent of antiretroviral therapy, more than 50% of HIV infected individuals experience a degree of neurocognitive impairment (NCI). This is driven in part by persistent inflammation. Mitochondrial DNA (mtDNA), when released by cells, can cause potent inflammation. We evaluated the relationship between mtDNA levels in cerebrospinal fluid (CSF) to measures of inflammation and neurocognition in HIV+ adults enrolled in 2 independent cohorts at the HIV Neurobehavioral Research Program: 1) with HIV suppression in blood and CSF (n=37) and 2) with full HIV suppression in CSF and blood (n=37). We quantified mtDNA levels in CSF using droplet digital PCR and measured inflammatory markers sCD14, sCD163, IP-10, MCP-1, IL-6, IL-8, TNF-α, neopterin, and neurofilament-light (NFL) by immunoassay in CSF supernatant or blood plasma. Higher levels of cell-free mtDNA in CSF were strongly associated with severity of NCI ($r=0.77$, $p<0.01$) in subjects with partially suppressed VL. Also, higher mtDNA levels were associated with higher levels of IP-10 in CSF ($r=0.70$, $p<0.01$) and MCP-1 in blood ($r=0.66$, $p=0.01$) in subjects with NCI. However, during viral suppression, higher mtDNA levels were associated with better neurocognitive performance ($r=0.43$, $p=0.02$), less neuronal damage measured by NFL, and more inflammation measured by MCP-1 ($r=0.56$, $p<0.01$) in CSF, and TNF-α ($r=0.43$, $p<0.01$) and IL-8 ($r=0.44$, $p<0.01$) in blood. While higher mtDNA levels were associated with more inflammation, inflammatory mechanisms in the presence of viral replication may impact neurocognition differently.

Supported by MH80663, MH081482, MH073419, AI036214, AG044325, AI093163, AI100665, AI007384, MH062512, MH101012, DA026306, 1S10RR031646-01, PST5TP2, MH097673,CNPq-Brazil

Cannabinoid type 2 receptor (CB2) activation in neuroimmune modulation at blood brain barrier

Persidsky, Y, MD, M.Ph.[1]; [1]Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

Previous studies have shown that the receptor-mediated cannabinoid system during neuroinflammation can produce potent neuroprotective and anti-inflammatory effects. Little is known about how selective activation of CB2 affects the activated state of the brain endothelium and blood brain barrier (BBB) function during neuroinflammation. Using human brain tissues and primary human brain microvascular endothelial cells (BMVEC), we demonstrate that the CB2 is highly upregulated during HIV infection and inflammatory insults. In vitro CB2 agonists increased barrier tightness and increased the amount of tight junction proteins in BMVEC, decreased adhesion/migration of monocytes across BBB models and expression of adhesion molecules in BMVEC treated with proinflammatory mediators. These results were further confirmed in vivo where CB2 agonists atteñated adhesion to and migration of leukocytes across the BBB (assessed by intravital microscopy), diminished expression of adhesion molecule and attenuated BBB 'leakiness' in mouse model of LPS or TNF-induced neuroinflammation. We recently identified novel CB2 agonists which tightened BBB, diminished monocyte adhesion/migration across BBB models in vitro and protected BBB in animal models after oral administration. We also demonstrated that selective CB2 activation in human leukocytes diminished their ability to engage the brain endothelium and migrate across BBB in vitro and in vivo preventing its injury. Therefore, CB2 ligands offer a new strategy for BBB protection during neuroinflammation.

Supported by U01AA023552, R37 AA015913

Microglia shape presynaptic properties at glutamatergic CA1 synapses

Ragozzino, D, Ph.D.[1], Basilico, B, BS[1], Pagani, F, Ph.D.[3], Maggi, L, Ph.D.[1], Di Angelantonio, S, Ph.D.[1], Gross, C[2], Limatola, C[1]; [1]Dept. Physiology and Pharmacology, University Sapienza of Rome, Rome, ROMA, I00185 Italy. [2]Mouse Biology Unit, EMBL, Monterotondo, Monterotondo, ROMA, 00015 Italy. [3]CLNS@Sapienza, Istituto Italiano di Tecnologia, Rome, ROMA, 00185 Italy.

During postnatal life microglial cells directly contact neurons, actively participating in synapse elimination and maturation. Hence, altered neuron-microglia interactions during development may lead to defective maturation of neuronal connectivity. CX3CL1/CX3CR1 axis represents a unique pathway of communication between neurons and microglial cells. We report that in mice model lacking CX3CR1 and defective in neuron-to-microglia signaling, hippocampal CA3-CA1 synapses develop with specific and less efficient properties. Indeed, Cx3cr1 KO synapses are characterized by an immature AMPA/NMDA ratio persisting in adulthood and displayed reduced functional connectivity, as revealed by the input-output curve of AMPA-mediated currents. Detailed analysis of evoked glutamatergic currents of Cx3cr1 KO and WT mice revealed a strong difference restricted to the AMPA component of EPSC. In particular, Cx3cr1 KO hippocampal synapses have higher paired pulse potentiation and a higher number of failures but normal potency compared to WT, suggesting that these synapses are characterized by a lower glutamate release probability. Consistently, when recordings were performed in condition of high release probability, Cx3cr1 KO synapses showed typical synaptic multiplicity and AMPA/NMDA ratio, pointing to a presynaptic defect. Altogether, these data show that the absence of microglial CX3CR1 profoundly affects glutamatergic synaptic function in the hippocampus, leading to unreliable synaptic contacts characterized by a low release probability.

Neuroimmune factors in alcohol-induced neuroadaptation in the central amygdala

Roberto, Marisa[1], Bajo, Michal, Ph.D.[1], Varodayan, F. P, Ph.D.[1], Khom, S, Ph.D.[1], Steinman, M. Q, Ph.D.[1], Patel, D., Ph.D.[1], Hedges, P., Ph.D.[1], Montgomery, S.E., BS[1], Roberts, A. J, Ph.D.[2]; [1]1Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA, 92037 United States. [2]Department of Molecular and Cellular Neurosciences, The Scripps Research Institute, La Jolla, CA, 92037 United States.

Neuroimmune signaling is critical in the transition to alcohol dependence, as ethanol activates the neuroimmune system and cytokines modulate alcohol-related behaviors. The brain IL-1 system is hypothesized to alter neuronal communication in addiction-related brain regions, such as the in the central nucleus of the amygdala (CeA) and medial prefrontal cortex (mPFC). Here, we used a 2 bottle choice-chronic interemittent ethanol vapor expsoure paradigm to induce ethanol dependence in mice and tested the effects of IL-1β on GABA transmission in CeA and prelimbic cortex (PrL) using whole cell-patch clamp recordings. We found that superfusion of IL-1β (50 ng/ml) increased the frequency of spontaneous Inhibitory Postsynaptic Currents (sIPSCs) in the CeA of ethanol naive, non-dependent and dependent mice, indicating that IL-1β induces GABA release in this region. In all groups, IL-1β produced variable effects on CeA sIPSC amplitudes and kinetics, suggesting that IL-1β can also alter postsynaptic GABAA receptor function. Notably, IL-1β (50 ng/mL) decreased GABA release on PrL layer II/III pyramidal neurons in naive and non-dependent mice, but increased GABA release in ethanol-dependent mice. Interactions between the IL-1 system and acute ethanol are currently being assessed. Overall, our results suggest that chronic ethanol induces limited modulation of the IL-1 system and its effects on GABA transmission in the CeA, but induced profound adaptation in the mPFC, which could have important implications for the role of the immune system in the development of alcohol dependence.

Supported by Supported by NIH/NIAAA AA013498, AA015566, AA013517, AA020893

Pre-mRNA missplicing of Mcl-1 is involved in ethanol induced neurotoxicity.

Sariyer, IK, Ph.D.[1], Sariyer, R, BS[1], De Simone, FI, Ph.D.[1], Donadoni, M, BS[1], Chang, S, Ph.D.[2]; [1]Department of Neuroscience, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States. [2]2. Institute of NeuroImmune Pharmacology, Seton Hall University, South Orange, NJ, 07079 United States.

Heavy and chronic ethanol exposure can cause significant structural and functional damage to the adult brain. The developing nervous system is even more vulnerable to ethanol exposure. Prenatal exposure of ethanol during pregnancy can lead to fetal alcohol spectrum disorders (FASDs), characterized by malformation of the nervous system and mental retardation. The most devastating consequence of ethanol exposure is the neurotoxicity associated with the depletion of neurons. It is crucial to elucidate mechanisms of neuro-apoptosis in order to develop effective therapeutic approaches to overcome ethanol-induced neuropathologies. Regulation of splice variants in the brain can modulate protein functions, which may ultimately affect behaviors associated with alcohol dependence and ethanol-mediated neurotoxicity. Limited number of studies has shown that pre-mRNA splicing patterns of genes are potentially altered and involved in behavior changes associated with alcoholism. Since alcohol consumption is associated with neurotoxicity, it is possible that altered splicing of survival and pro-survival factors during the development of alcoholism may contribute to the neurotoxicity. Our results suggest that ethanol exposure can lead to pre-mRNA missplicing of Mcl-1, a pro-survival member of the Bcl-2 family, by downregulating the expression levels of serine/arginine rich splicing factor 1 (SRSF1). The pre-mRNA of Mcl-1 can be alternatively spliced to remove exon 2, which

produces shortened form of Mcl-1, named Mcl-1S. While the longer gene product Mcl-1L enhances cell survival, the alternatively spliced shorter gene product Mcl-1S promotes apoptosis. Our results have indicated that ethanol exposure to neurons leads to a decrease in the ratio of Mcl-1L/Mcl-1S by favoring pro-apoptotic Mcl-1S splicing over anti-apoptotic Mcl-1L isoform suggesting that Mcl-1S may play a crucial role in neurotoxicity associated with alcohol consumption.

The role of the brain in the adaptation to climate change

Schunter, C, Ph.D. [1], Ravasi, T, Ph.D. [1]; [1]Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology, Thuwal, 23955-6900.

The increase of CO2 in the oceans, termed ocean acidification, is projected to have detrimental effects on marine organisms. The magnitude of the impact on the marine ecosystems will depend on species capacity to adapt. Recent studies show that the behaviour of reef fishes is impaired at projected CO2 levels; however, individual variation exists that might promote adaptation. Offspring of CO2-tolerant and CO2-sensitive parents were reared at nearfuture CO2 (754 uatm) or present-day control levels (414 uatm) and exposed to higher levels of CO2 at different life stages. We study the transcriptomes and proteomes in the brain of Acanthochromis polyacanthus (spiny damselfish), to evaluate short-term, long-term (one generation) and transgenerational molecular responses to more acidified oceans. Withingeneration CO2 exposure lead to an increased expression of genes involved in GABAergic neurotransmission as well as the potassium-chloride cotransporter 2 (kcc2). The reversal of the transmembrane gradient for HCO3- and Cl- with elevated levels of extracellular bicarbonate is the likely cause for the excitement of the neuronal transmission which in turn causes the behavioural impairment in fish at near-future high CO2 levels. We find a clear signature of the parental sensitivity to CO2 in the molecular phenotype of the offspring, mainly driven by circadian rhythm genes. Furthermore, expression patterns with prior parental CO2 acclimation largely differ to short-term or long-term exposures emphasizing the importance of transgenerational acclimation in exposure experiments.

Establishment of CNS HIV-1 reservoirs and brain injury: Is typical antiretroviral therapy too little, too late?

Spudich, S., M.D.[1]; [1]Dep. Neurology, Yale University School of Medicine, CT, USA

The emergence of widespread combination antiretroviral therapy (cART) use has altered the clinical character of neurologic disease observed in HIV-1 and has raised a host of questions related to the role of the central nervous system (CNS) as a site of HIV-1 persistence and a potential barrier to HIV-1 cure. This presentation will review what is known regarding establishment of HIV-1 in the CNS during early infection, and whether early intervention with cART or other treatments may alter the character of a potential CNS 'reservoir' for HIV-1. Based on studies of acute HIV-1 in humans and acute SIV in macaque models, it has become evident that CNS HIV-1 infection during acute and early infection triggers a unique sequence of pathogenic and immunoregulatory events. Early treatment with cART reduces levels of CSF HIV-1 RNA and signs of neuronal injury and immune activation that are present in individuals on cART started during chronic infection, however cART alone may not resolve all of these abnormalities. This talk will present studies that investigate the persistence of HIV during cART, including detection of low level CSF HIV-1, characterization of CSF HIV-1 escape, and monitoring of HIV-1 resurgence in the CNS after cART interruption. Furthermore, new investigations employing immunological approaches to reduction of CNS reservoirs will be described. Conclusions: Persistent perturbation of the CNS the setting of apparently successful cART may in part reflect a reservoir for HIV-1 within this tissue compartment. Early cART reduces the magnitude of systemic HIV-1 reservoirs and may also provide benefit to the brain, but adjunctive interventions may be needed to fully ameliorate HIV-1 related immune activation and viral persistence in the CNS.

Autoimmune aspects of Parkinson's disease

Sulzer, D, Ph.D.[1]; [1]Psychiatry, Columbia University, New York, NY, 10032 United States.

While adult human neurons are not typically antigen presenting cells, many substantia nigra dopamine neurons express MHC-I. The death of these neurons causes the motor disorders of Parkinson's, and in mouse substantia nigra neurons, the appropriate combination of neuronally presented antigen and T cell causes cell death. In blood of ~40% of Parkinson's patients and few age matched controls, CD4+ and CD8+ T cells are present that respond to two regions in alpha-synuclein, a protein misprocessed in the disorder. As degradation of alpha-synuclein and other proteins by lysosomes changes with disease and age, it is possible that autoimmune response to neoepitopes play roles in neurodegenerative and other aging related disorders.

Productive Zika infection in the human brain

Veilleux, CA.[1], [1]Rutgers University, New Brunswick, NJ.

The pathogenesis of Zika infection in the brain remains largely unknown. Incidence of microcephaly and Guillain-barre

Syndrome are heightened in multiple localities that have autochthonous Zika transmission. Of major importance is determining the mechanisms of Zika virus access to the brain. Our laboratory has demonstrated that other neurotropic viruses facilitate entry into the brain across the blood brain barrier and are sustained within the brain via infected astrocytes. Our laboratory has also previously identified viral reservoirs within the brain. Thus, our research aims to examine Zika virus access in the brain and the potential for human brain cell populations to act as viral reservoirs for Zika. Our results indicate that during Zika infection of human brain cells, astrocytes are productively infected. Furthermore, Zika infection of astrocytes contributes to high viral concentrations within the brain and may represent key reservoirs and mediators of apoptosis in the central nervous system during viral infection. The objective of our research is to characterize the mechanism of viral infection and replication in human fetal astrocytes. We hypothesize that infection of human fetal astrocytes by Zika virus causes apoptosis that contributes to the devastating CNS consequences documented by this virus. Our results illustrate a distinct mechanism of Zika infection in human fetal astrocytes. Understanding infection in primary CNS resident cells is critical in understanding the pathogenesis of Zika infection in the developing brain.

Vaginal Exposure to Zika Virus during Pregnancy Leads to Fetal Brain Infection

Yockey, L.[1], [1]Yale University, New Haven, CT.

Zika virus (ZIKV) can be transmitted sexually between humans. However, it is unknown whether ZIKV replicates in the vagina and impacts the unborn fetus. Here, we establish a mouse model of vaginal ZIKV infection and demonstrate that, unlike other routes, ZIKV replicates within the genital mucosa even in wild-type (WT) mice. Mice lacking RNA sensors or transcription factors IRF3 and IRF7 resulted in higher levels of local viral replication. Furthermore, mice lacking the type I interferon (IFN) receptor (IFNAR) became viremic and died of infection after a high-dose vaginal ZIKV challenge. Notably, vaginal infection of pregnant dams during early pregnancy led to fetal growth restriction and infection of the fetal brain in WT mice. This was exacerbated in mice deficient in IFN pathways, leading to abortion. Our study highlights the vaginal tract as a highly susceptible site of Our study highlights the vaginal tract as a highly susceptible site of ZIKV replication and illustrates the dire disease consequences during pregnancy.

Inhibition of the 2-AG hydrolytic enzyme ABHD6 in the treatment of EAE

Zhang, Y, MD, Ph.D.[1]; [1]Dept. of Anatomy, Physiology and Genetics, Uniformed Services University, Bethesda, MD, 20814 United States.

Alpha/beta-hydrolase domain 6 (ABHD6) is a novel 2-arachidonoylglycerol (2-AG) hydrolytic enzyme, that can fine-tune the endocannabinoid signaling in the central nervous system. Unlike inhibition of the major 2-AG hydrolytic enzyme monoacylglycerol lipase (MAGL) that can cause cannabinoid receptor desensitization and behavioral tolerance, inhibition of ABHD6 increases 2-AG to a therapeutic window without producing side effects. In this study, we investigated the role of targeting ABHD6 in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Post-symptom treatment with an ABHD6 inhibitor WWL70 increased the brain levels of 2-AG and ameliorated the clinical signs of EAE, T cells infiltration, microglia activation and the expression of activated leukocyte cell adhesion molecules. The production of iNOS, COX-2, TNF-α and IL-1β was significantly reduced by WWL70 treatment. The neuroprotective effect of WWL70 was demonstrated by increased survival of mature oligodendrocytes, reduced demyelination and axonal loss in WWL70 treated EAE mouse spinal cord. The therapeutic effect of WWL70 on EAE was absent by co-administration of CB2 receptor antagonist, but not CB1 receptor antagonist. Consistently, WWL70 did not afford any protection in CB2 receptor knockout mice after EAE induction. Furthermore, we found that WWL70 can inhibit prostaglandin E2 (PGE2) production in lipopolysaccharide activated microglia in vitro and in the EAE mouse spinal cord. These results suggest that inhibition of ABHD6 can be used as an ideal strategy for MS treatment.

Supported by National Multiple Sclerosis Society, DMRDP, DoD

Molecular mechanism of HIV-1 Tat interacting with human dopamine transporter

Zhu, J, MD, Ph.D.[1]; [1]Drug Discovery and Biomedical Sciences, University of South Carolina, Columbia, SC, 29208 United States.

Abnormal dopaminergic transmission has been implicated as a risk determinant of HIV-1-associated neurocognitive disorders (HAND). Cocaine has been shown to exacerbate the severity of HAND. HIV-1 Tat protein and cocaine augment synaptic dopamine (DA) levels and viral protein release by inhibiting DA transporter (DAT) activity. By producing oxidative stress-induced damage to dopaminergic neurons, prolonged exposure to Tat eventually causes DAT-mediated dysregulation of DA to accelerate the progression of HAND. This talk will focus on molecular mechanism by which how cocaine and Tat synergistically work to create binders that derail neuronal communication in the brain. Our published work demonstrates that Tat directly binds to DAT and displays an allosteric modulatory effect on DAT function. Through

integrated computational modeling prediction and experimental validation, we have identified key residues in human DAT with which Tat interacts, which are critical for Tat-induced inhibition of DAT and transporter conformational transitions. We will introduce the functional influences of the different mutations of these human DAT residues in basal DA uptake by DAT and Tat-DAT interaction. These findings provide mechanistic insights into identifying residues on DAT for Tat binding, which allows the exploration of the molecular targets on DAT for therapeutic interventions, stabilizing physiological DA neurotransmission and improving neurocognitive function of HAND in concurrent cocaine abusers.

Supported by NIH/NIDA/DA035714, DA041932

2) Posters – Wednesday

W1.Resveratrol Analog TIMBD Inhibits HIV1-gp120 Associated Inflammation in Human Astrocytes

ABDALLA, F, MS[1], Nookala, A, BS[1], Kumar, A, Ph.D.[1], Bhat, H, Ph.D.[1]; [1]Pharmacology and Toxicology, University of Missouri Kansas City, Kansas City, MO, 64108 United States.

Human Immunodeficiency Virus (HIV)-associated neurocognitive disorder (HAND) is one of the undermining disorders that affect majority of HIV infected patients. Patients with HAND suffer from both cognitive and motor dysfunction which is characterized by memory loss and changes in personality. Astrocytes are considered the major reservoirs of the HIV in the brain. Resveratrol (Res) is one of the known phytoestrogens with potential anti-inflammatory activity. However studies suggests resveratrol's poor specificity and bioavailability as a reason for its poor efficacy. To overcome these problems associated with Res, our research group has developed novel Res analog (TIMBD). The inflammatory cytokines and chemokines associated with HIV-gp120 in astrocytes plays a critical role in HAND development in HIV patients. Patients with HIV are reported with high levels of IL6, IL8 and CCL5 which have been shown to be critical in the damage process. In this study we demonstrated that TIMBD has the potential to inhibit HIV-gp120 induced neuro-inflammation. Briefly, SVG astrocytes were cultured in growth media. Cells were transfected with mock/or HIV-1 gp120 plasmid and treated with TIMBD for up to 48 h. The mRNA and protein levels of IL6, IL8 and CCL5 were quantified. Protein levels of transcription factors AP1, pSTAT3 and NF-κB were measured using western blotting. We demonstrated that TIMBD but not Res was able to decrease the expression of those pro-inflammatory mediators. We concluded that TIMBD is able to modify the inflammatory mediators in HIV-gp120-transfected astrocytes.

W2.CRISPR/Cas9-mediated excision of HIV-1 proviral genome sequences

Allen, A[1], Sullivan, N[1], Nonnemacher, M[1], Dampier, W[1], Homan, G[1], Desimone, M[2], Pirrone, V[1], Kercher, K[1], Passic, S[1], Williams, J[1], Wigdahl, B[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]School of Biomedical Engineering and Health Systems, Drexel University, Philadelphia, PA, 19104 United States.

HIV-1 persistence during long-term antiretroviral therapy is a major hurdle to a cure. Genomic editing techniques, like the CRISPR/Cas9 system, hold promise to permanently excise integrated virus from a host cell. However, due to the rapid mutation rate intrinsic to HIV-1 replication and numerous intra- and extracellular selective pressures, the virus in patients exists as a collection of distinct genomic variants, termed quasispecies. Presented here is a methodology for designing gRNA sequences to cleave a spectrum of HIV-1 quasispecies in the absence of in silico off-target impact. PBMC genomic DNA was isolated from patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort as well as from brain and spleen tissue from the National NeuroAIDS Tissue Consortium (NNTC) and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using Next Generation Sequencing (NGS). A package of 4 or 10 gRNAs were selected based on the training data set, which in silico cleaved the entire detectable quasispecies within the remaining CARES Cohort patient DNA samples and the majority of the NNTC patient samples. The package was further tested in silico against all subtype B North American LTRs from the Los Alamos National Laboratory (LANL) Database and was shown to cleave all LANL HIV-1 LTR sequences. Currently, we have cloned these packages of gRNAs and have initiated functional studies to confirm the in silico studies performed to date. These studies represent a step towards understanding the complex task of using excision therapy to target HIV-1.

Supported by This work is supported by NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32

W3.LP-BM5 virus Exacerbates Murine AIDS-associated symptoms in gp120 transgenic mice

Arabatzis, TJ, BS[2], Wakley , AA, Ph.D.[1], McLane, VD, Ph.D.[3], Cao, L, MD, Ph.D.[1]; [1]Department of Biomedical Sciences: College of Osteopathic Medicine , University of New England, Biddeford, ME, 04005 United States. [2]College of Arts and Sciences, University of New England, Biddeford, ME, 04005 United States. [3]Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME, 04005 United States.

Of the 36.7 million people globally with HIV, 25-69% of them suffer from HIV-associated neurocognitive disorders (HAND) (UNAIDS 2016). The LP-BM5 virus is a well-characterized and often used murine AIDS (MAIDS) model for investigating HIV/AIDS and HAND. LP-BM5 causes immunodeficiency in susceptible mouse strains, as HIV does in humans. However, LP-BM5 virus, a murine leukemia virus, does not express HIV proteins (such as gp120 and tat) known to contribute to HAND pathology; limiting its broader use as a HIV/AIDS model. On the contrary, HIV protein transgenic (tg) mice expressing soluble viral proteins, such as gp120, have been established as HIV/AIDS models. However, tg mice only develop CNS pathology, and not immunodeficiency. In this current study, we examined the progression of LP-BM5 infection in gp120tg mice to test whether we could generate a more clinically relevant murine HAND model. Gp120tg(+) mice showed an exacerbation of the disease following infection when compared to their gp120(-) littermate controls and wild type (WT) C57BL/6 controls. At 12 weeks post-infection, LP-BM5 infected gp120tg(+) mice had higher IgG2a serum levels and an increased viral burden in hippocampi compared to the gp120(-) littermate and WT controls. In addition, gp120tg LP-BM5 mice made more errors in the T-maze alternation test suggesting an association between a higher viral load in the hippocampus and greater memory and learning deficits. These results suggest that LP-BM5 infection in gp120tg(+) mice may display more severe HAND pathology and can be used as an additional HAND model.

Supported by NIH COBRE award 1P20GM103643 (PD Meng), and the NIH/NINDS R01 award, 1R01NS098426 (Cao)

W4.Neurocognitive Impairment in the HIV-1 Transgenic Rat at an Advanced Age

Balog, EM, BS[1], McLaurin, KA, MS[1], Booze, RM, Ph.D.[1], Mactutus, CF, Ph.D.[1]; [1]Program in Behavioral Neuroscience, Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States.

The introduction of combination antiretroviral therapy has expanded the lifespan of HIV+ individuals. The percentage of HIV+ patients over the age of 50 is projected to reach 73% in 2030. To establish deficits in sustained attention, flexibility, and inhibition at an advanced age we compared intact HIV-1 transgenic (Tg; male, n=15; female, n=18) and control (male, n=16; female, n=18) rats using a signal detection task. All animals had previous experience with signal detection tasks at varying durations (100-1000 msec). At 18 months of age, animals were challenged with shorter signal durations (10, 100, & 1000 msec) for 5 consecutive days. Subsequently, reversal learning was assessed in all animals, regardless of their previous performance, until they met criteria (70% accuracy for 5 consecutive days or 7 days total) or completed 60 days. Analyses showed a significant genotype effect [$F(1,63)=6.3$, $p\leq0.015$], which was moderated by biologic sex [$F(1,63)=8.8$, $p\leq0.01$] for the 18-month task. More specifically, the signal duration at which animals failed to distinguish between hits and misses shifted rightward from 25 msec in controls to 70 msec in HIV-1 Tg animals; a shift which was more pronounced in the males. In the reversal task, the signal duration at which animals failed to distinguish between hits and misses shifted rightward from 400 msec in controls to an indeterminate value greater than 1000 msec in HIV-1 Tg animals. Prominent neurocognitive impairment in the HIV-1 Tg rat at an advanced age suggests a clear progression of cognitive dysfunction across the lifespan.

Supported by Supported by NIH grants DA013137, HD043680, MH106392

W5.PERK Haplotype Function in HIV-Associated Neurocognitive Disorders

Bond, S., BS[1], Stutzbach, L., Ph.D.[1], Gannon, P., Ph.D.[1], Akay-Espinoza, C., MD[1], Jordan-Sciutto, K., Ph.D.[1]; [1]Department of Pathology, University of Pennsylvania, Philadelphia, PA, 19104 United States.

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

Supported by National Institute of Mental Health

W6.TAARgeting Astrocyte Mitochondrial Dysfunction during HIV-associated Neuroinflammation and METH Exposure.

Borgmann, K, BS[1], Ghorpade, A, Ph.D.[1]; [1]Institute For Molecular Medicine, University of North Texas Health Science Center, Fort Worth, TX, 76107 United States.

Methamphetamine (METH) use exacerbates HIV-1 infection, accelerating the severity and onset of HIV-associated neurocognitive disorders (HAND), along with immune dysfunction and resistance to antiretroviral therapy. Neurocognitive impairment is more prevalent in HIV+ METH users than either HIV+ or METH+ alone. A common neurotoxic mechanism during HIV CNS infection is mitochondrial impairment leading to oxidative stress. METH directly and indirectly contributes to mitochondrial impairment; however, the mechanisms regulating mitochondrial homeostasis and overall oxidative burden in astrocytes are not well understood in the context of HIV-associated neuroinflammation and METH abuse. We have reported that astrocyte-trace amine associated receptor 1 (TAAR1) is induced by HAND-relevant stimuli and binds METH, leading to cAMP/calcium signaling and impaired glutamate clearance during HIV. We hypothesize that METH-abuse in HAND modulates astrocyte-TAAR1 levels and activity, regulating astrocyte-mediated neurotoxic outcomes, including mitochondrial damage and increased oxidative burden. Here we report METH-mediated impairment of astrocyte mitochondrial recycling during prolonged exposure in the context of HIV, including enlarged mitochondrial size, mitofusin recruitment, increased oxygen consumption and resulting oxidative burden. Further, astrocyte TAAR1 appears to regulate mitochondrial recycling, indicating that it may be a valid therapeutic strategy to target astrocyte-mediated neurodegeneration in HAND and METH abuse.

Supported by NIH/NIDA: 5R01DA039789-02 to AG

W7.Pathologic changes in the NAc post experimental TBI and susceptibility to the rewarding effects of a subthreshold dose of cocaine following brain injury

Cannella, L.A., MS[2], Maynard, M.[1], Andrews, A.A., Ph.D.[1], Rawls, S.M., Ph.D.[2], Ramirez, S.H., Ph.D.[3]; [1]Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine Temple University, Philadelphia, PA, 19140 United States. [2]Center of Substance Abuse Research, Lewis Katz School of Medicine Temple University, Philadelphia, PA, 19140 United States. [3]Shriners Hospitals Pediatric Research Center, Lewis Katz School of Medicine Temple University, Philadelphia, PA, 19140 United States.

A chronic comorbidity seen in traumatic brain injury (TBI) patients is the development of a substance use disorder. Previously we found that moderate TBI during adolescence increased susceptibility to the rewarding effects of cocaine during adulthood. Here we investigated whether early life TBI enhances the effects of a subthreshold dose of cocaine. Furthermore, pathologic findings reveal that the blood-brain barrier (BBB) status, in areas of the reward pathway, appears altered. The implication of BBB changes post brain injury, as a component of neuroinflammation, may explain how the rewarding effects of cocaine may shift as a consequence of TBI. Experimental TBI was performed using a controlled cortical impactor set to induce a moderate or mild TBI in 6 week old, adolescent or 8 week old, young adult male C57BL/6 mice. Drug seeking behavior was assessed using 2.5 mg/kg cocaine in the CPP assay two weeks after injury. Histology and gene expression assays were used for pathologic indices. Moderate TBI during adolescence, but not during young adulthood, augmented place preference shift indicative of enhanced sensitivity. We detected increased immune response-associated gene expression in the PFC and disrupted BBB properties in vessels from the cortex and NAc of TBI animals. Our studies suggest that TBI during adolescence may enhance the abuse liability of cocaine in adulthood. Moreover, key pathologic findings such as BBB changes in areas of the reward pathway support the notion that neuroinflammation may contribute to how rewarding effects of cocaine post-TBI are affected.

Supported by NIH/NIDA: F32 DA041282, T32 DA007237, P30 DA013429-16; NINDS: R01 NS086570-01; Shriners Hospital for Children: 85110-PHI-14

W8.Computational characterization of off-target effect on HIV-1 excision gRNAs in the human genome

Chung, C-H[1], Nonnemacher, M[1], Wigdahl, B[1], Dampier, W[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

Despite antiretroviral therapy, HIV-1 infection remains a lifelong clinical problem due to reservoirs harboring proviral DNA in a latent/persistent form. Recently, gene-editing strategies utilizing the CRISPR/Cas9 system (CC9) have been developed to eradicate the HIV-1 genome from infected cells that approach a cure for HIV infection. However, due to the promiscuity of the guide RNA (gRNA) targeting, one area of interest has focused on off-target cleavage sites that may cause unwanted DNA damage to the human genome. This is further complicated by the uncertainty of DNA accessibility such as chromatin state and target sequence availability in a given cellular genome. In order to predict the off-target effect, a new database has been developed containing all potential cleavage sites within the entire human genome along with

all known single nucleotide polymorphisms in dbSNP. With the availability of unbiased identification of double-stranded breaks enabled by sequencing (GUIDE-seq) in HEK293T cells, our results have shown that, on a given genomic region, the DNA accessibility, implied by DNase-I hypersensitivity, positively correlated to the frequency of CC9 activity. Integrating quantitative off-target data available from publications with two-layer stacked regression models allowed a better understanding of generalizable factors that contribute to off-target effect. Compiling these results will greatly increase the ability of researchers to design gRNAs that cleave HIV-1 while avoiding off-target effects.

Supported by This work is supported by NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32

W9.Unravelling the role of beta-adrenergic signaling in gastric cancer

Coelho, MC, MS[1], Rasini, ER, BS[1], Luini, AL, BS[1], Legnaro, ML, BS[1], Marino, FM, Ph.D.[1], Ribeiro, LR, Ph.D.[2], Cosentino, MC, MD, Ph.D.[1]; [1]Center for Research in Medical Pharmacology, University of Insubria, Varese, Varese, 21100 Italy. [2]Department of Biochemistry, Faculty of Medicine, University of Porto, Porto, Porto, 4200-319 Portugal.

Studies addressing the relationship between stress-activated pathways and cancer progression suggest that adrenaline and noradrenaline besides affecting the anti-tumor immune response also display direct tumor-promoting effects in several types of tumors and that beta-adrenoceptors (AR) are key mediators of these effects. Indeed, cancer patients taking beta-AR antagonists may have increased survival and in vitro many of these drugs have anti-tumor effects, for instance inhibiting pro-survival cancer cell pathways. The underlying mechanisms of such effects are however so far poorly understood. The aim of this work is to study the expression and the role of beta-AR in gastric cancer cells, investigating the signaling pathways and identifying which beta-AR antagonists can be used to affect cancer growth. We found that the gastric cancer cell lines KATOIII and MKN45 express both beta1 and beta2-AR, at both mRNA and surface protein levels, and also mRNA for tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of adrenaline and noradrenaline. Expression of beta-AR and TH is higher in KATOIII in comparison to MKN45 and seems to correlate with cell growth. We are now testing beta-AR agonists (adrenaline, noradrenaline and isoprenaline) and antagonists (propranolol, ICI-118,551 and CGP20712A) on cell growth, migration and angiogenic potential. Results will clarify the role of beta-AR pathways in gastric cancer cells.

Supported by Marisa Coelho has a PhD scholarship in Clinical and Experimental Medicine and Medical Humanities by the University of Insubria

W10.A microstructure behavioral analysis of voluntary wheel running in HIV-1 Tg rats: evidence for dysregulation of motivation.

Cranston, MN, MS[1], Booze, RM, Ph.D.[1], Harrod, SB, Ph.D.[1], Mactutus, CF, Ph.D.[1]; [1]Program in Behavioral Neuroscience, Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States.

An estimated 30-50% of HIV-1+ individuals exhibit reductions in motivation (apathy). Given that rodent wheel running occurs in distinct, defined "bouts" of continuous running followed by inter-bout rest intervals (i.e.-disengagement of the running wheel or a cessation of running), we examined the microstructure of this behavior within the wheel running session. Adult, ovariectomized HIV-1 Tg (n=21) and F344N controls (n=24) rats were allowed nocturnal access to a voluntary running wheel (66min/day for 49 consecutive days). HIV-1 Tg animals were not significantly different from controls in the latency to initiate a wheel running session nor in maximal running speed. The number of running bouts, but not the inter-bout interval length, was significantly reduced ($p \leq .05$) in the HIV-1 Tg rats, relative to controls. Further, the HIV-1 Tg animals were also more likely have run significantly further during their bouts (~ 3-fold higher, $p \leq .05$) relative to controls. Overall, there was no evidence for motor impairments in the HIV-1 Tg rats (i.e., no difference in initiation of running, in maximum speed, or inter-bout interval length). However, the HIV-1 Tg rats had altered running profiles (significantly fewer running bouts but significantly greater distance during their bouts) which suggests dysregulaton of motivational processes with preservation of motor function. Moreover, these findings indicate that analysis of the microstructure of voluntary wheel running behavior is valuable in studies of motivational dysfunction.

Supported by NIH DA013137, HD043680, MH106392, GM081740

W11.Circadian Disruption Changes Gut Microbiome Taxa and Functional Gene Composition

Deaver, JA[1], Toborek, M, Ph.D.[1], Eum, SY, Ph.D.[1]; [1]Department of Biochemistry, University of Miami, Miami, FL, 33136 United States.

Disrupted circadian rhythms lead to increased susceptibility to diseases and metabolic disorders. Alteration of the gut microbiome composition and community gene expression is

suspected to play a role in the amplified incidence of disease caused by these disturbances. The purpose of this research was to identify whether circadian rhythm disruption by abnormal light-dark cycles affects microbiome composition and host vulnerability to disease. Stool samples were collected from two groups of mice at two time points each; before and after either a four week period of 24-hour light or of normal 12-hour light/dark cycles. A metatranscriptomic analysis revealed an increase in Ruminococcus torques, a bacterial species known to decrease gut barrier integrity, and a decrease in Lactobacillus johnsonii, a beneficial bacterium that helps maintain the intestinal epithelial cell layer, after circadian rhythm disruption. In addition, genes involved in pathways promoting beneficial immune responses were downregulated, while genes involved in the synthesis and transportation of the endotoxin lipopolysaccharide were upregulated. Mice with disrupted circadian cycles were also more prone to dysfunction of the intestinal barrier. These results further elucidate the impact of light-cycle disruption on the gut microbiome and its connection with increased incidence of disease in response to circadian rhythm disturbances.

Supported by National Institutes of Health (CA133257) and Alltech Nutrigenomics

W12.TGF-beta suppresses CFTR biogenesis and function by post-transcriptional gene silencing.

Dutta, R, MS[1], Chinnapaiyan, S, Ph.D.[1], Nair, M, Ph.D.[1], Unwalla, H, Ph.D.[1]; [1]Department of Immunology, Florida International University, Miami, FL, 33199 United States.

Respiratory symptoms are common in the HIV-infected population, particularly in HIV-infected persons who smoke, and obstructive lung disease is an increasing cause of morbidity and mortality even in the era of combination antiretroviral therapy (cART). HIV infection has been reported to suppress nasal mucociliary clearance (MCC). Since the primary components driving nasal MCC and bronchial MCC are identical, it is possible that bronchial MCC is affected as well. Effective MCC requires optimal ciliary beating which depends on the maintenance of the airway surface liquid (ASL), a function of cystic fibrosis transmembrane conductance regulator (CFTR) activity and the integrity of the signaling mechanism that regulates ciliary beating and fluid secretion. Impairment of either component of the MCC apparatus can compromise its efficacy and promote microbial colonization. TGF-beta signaling is enhanced by HIV Tat and Cigarette smoke. We demonstrate that HIV Tat and cigarette smoke suppress CFTR biogenesis and function in primary bronchial epithelial cells via a common pathway involving TGF-β signaling. RNA Pol II CHIP analysis demonstrates that transcription from the CFTR promoter is unaffected and blocking the miRNA processing pathway rescues CFTR suppression by TGF-β. A microRNA array TGF-β signaling induces several microRNAs capable of targeting CFTR. The implications of these findings will be discussed.

Supported by Flight Attendant Medical research institute (CIA150086)

W13.Red/Green Astrocytes Mimic CNS Viral Reservoirs in post ART HAND: Implications for Meth abuse

Edara, VVC, MS[1], Ghorpade, A, Ph.D.[1]; [1]Institute for Molecular Medicine, University of North Texas Health Science Center, Fort Worth, TX, 76107 United States.

Though anti-retroviral therapy (ART) has increased the life expectancy of the HIV-1 infected individuals, the quest for eradication of latent reservoirs continues. Several studies have shown the validity of astrocytes as a major reservoir of HIV-1 in the CNS. Meth abuse and HIV-1 infection increase neuroinflammation through several molecular and cellular mechanisms. We hypothesize that astrocyte HIV-1 reservoirs contribute to HAND pathogenesis, and are regulated via Meth abuse during HIV-1 infection. A doubly labeled fluorescent reporter Red/Green-HIV-1 (R/G-HIV-1) was used to model latency in primary human astrocytes. Pseudotyped R/G-HIV-1 infected astrocytes showed latency establishment over a period of 21 days. Latency establishment studies were also conducted with pre- and/or post-Meth treatment. Active (mCherry+/GFP+) and latently infected (mCherry+/GFP-) astrocytes were enriched using fluorescence activated cell sorting. Latently infected astrocytes were devoid of late viral proteins such as p24, indicating a functionally silent HIV-1 LTR. Vorinostat, an HDAC inhibitor, reactivated the silenced HIV-1 LTR in a mixed population of pseudotyped R/G-HIV-1-infected astrocytes. This suggests R/G-HIV-1 could be used as a relevant model of latency in astrocytes since it mimics virus reactivation in inflammation leading to viral proteins expression. We anticipate that healthy versus latently infected astrocytes respond differentially to inflammation. Investigating the underlying mechanisms will help in assessing the role of HIV-1 astrocyte reservoirs in HAND pathogenesis.

W14.In vivo manipulation of the CXCL12/CXCR4 signaling axis increases dendritic spine density and enhances cognitive flexibility in wild-type and HIV-Tg rats via the Rac1/PAK pathway

Festa, L, MS[1], Tian, Y, BS[1], Platt, B, BS[1], Floresco, S, Ph.D.[2], Meucci, O, MD, Ph.D.[1]; [1]Department of Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Psychology, University of British Columbia, Vancouver, BC, V6T 1Z4 Canada.

Sub-lethal synaptodendritic injury is correlated with neurocognitive impairment during HIV infection and is thought to be an underlying mechanism of HIV-associated neurocognitive disorders (HAND). The neuroprotective chemokine-receptor pair, CXCL12/CXCR4, is critical for maintaining dendritic spine density on excitatory neurons; however, the mechanism by which this occurs is currently unknown. Additionally, CXCL12/CXCR4 signaling is disrupted during HAND, suggesting that restoration of this pathway might ameliorate cognitive deficits in HIV+ individuals. In this study, we sought to determine the molecular mechanisms regulating CXCL12-mediated spine alterations, as well as whether ICV administration of CXCL12 could alter dendritic spines and cognitive function. Our studies demonstrated that the Rac1/PAK pathway, a known spine stabilization pathway, is activated by CXCL12 in cultured cortical neurons and this effect is blocked by pre-treatment with the CXCR4 antagonist, AMD3100. In vivo treatment of CXCL12 (25ng/5uL once per day) in WT and HIV-Tg rats significantly upregulated dendritic spine density in layer II/III pyramidal neurons in the medial prefrontal cortex (mPFC). Importantly, rodents treated with CXCL12 displayed an improvement in cognitive flexibility, which was correlated with the increase in dendritic spine number. Taken together, this study suggests that enhancement or restoration of CXCL12/CXCR4 signaling may have therapeutic potential in HAND, as well as in other neurocognitive disorders characterized by synaptodendritic alterations.

Supported by NIH DA15014, NIH DA 032444

W15.Metabolomic analysis of astrocytes transfected with HIV-1 gp120

Garcia-Justiniano, J., BS[1], Rodriguez-Santiago, R. E., BS[1], Garcia-Flores, Y., BS[1], Pabon-Cruz, E., BS[1], Rivera-Amill, V., Ph.D.[1]; [1]Ponce Research Institute, Ponce Health Sciences University, Ponce, PR, 00716 United States.

The human immunodeficiency virus (HIV) is able to enter the brain and is known to cause cognitive, behavioral, and motor difficulties collectively known as HIV-associated neurocognitive disorders (HAND). The envelope glycoprotein gp120 is one of the HIV-1 proteins that has neurotoxic effects. Active release of virotoxins, such as gp120, by glial cells in the brain leads to the activation of uninfected adjacent cells that may in turn result in the development of HAND. Gp120 alters important metabolic pathways such as the glutamine-glutamate cycle in astrocytes. These alterations stimulate a cytotoxic environment for the surrounding cells, therefore compromising their normal functions. In this study, we analyzed the metabolic composition of astrocytes transfected with gp120 using gas chromatography followed by mass spectrometry. Metabolites were first extracted from astrocytes either transiently transfected with a vector expressing gp120, an empty vector and mock-transfected. Peak intensity and retention time were obtained and analyzed with the Automated Mass Spectral Deconvolution and Identification System (AMDIS) Chromatogram to identify the metabolites in each sample. Identified and filtered metabolites were then analyzed using Metaboanalyst and correlations between them were obtained.

Supported by NIMHD / G12MD007579, NIMHD / U54MD007587

W16.Nutraceutical Apigenin regulates DC function in a RelB-dependent manner during neuroinflammation

Ginwala, R.[1], McTish, E[1], Moore, P.[1], Revuri, N.[1], Raman, C.[2], Singh, N.[3], Nagarkatti, M.[3], Nagarkatti, P.[3], Kranz, V.A.[4], Houle, J.D.[4], Jain, P.[1], Khan, Z.K.[1]; [1]Department of Microbiology and Immunology, and the Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, 19129 United States. [2]Division of Clinical Immunology and Rheumatology, University of Alabama School of Medicine, Birmingham, AL, 35294 United States. [3]Department of Pathology, Microbiology and Immunology, University of South Carolina, Columbia, SC, 29208 United States. [4]Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia, PA, 19129 United States.

Apigenin, a natural plant flavonoid, with anti-oxidant and anti-inflammatory properties has been in use for centuries as a medicinal approach to treat inflammatory disorders. However, there are significant gaps in knowledge regarding its effect on dendritic cell (DC) function in immunospecialized locations like the central nervous system (CNS). In order to establish the potential utility of Apigenin as a therapeutic agent against neuroinflammatory diseases, we tested and found that Apigenin treatment ameliorated disease severity, progression and relapse after onset of experimental autoimmune encephalomyelitis (EAE) in C57BL/6 and SJL mouse models of multiple sclerosis. Increased numbers of DCs and other myeloid cells in the periphery correlated with decreased immune cell infiltration and demyelination in the CNS of treated mice. Mechanistically, Apigenin reduced RelB expression in LPS-treated human peripheral blood DCs, which is central to DC maturation, its antigen presentation capabilities and DC-mediated T cell activation. IL-12A and IL-23, pro-inflammatory targets of RelB were reduced upon Apigenin treatment in these cells. Further, RelB causes a metabolic switch in immune cells upon inflammation, which was seen as a decrease in glucose uptake and lactate production (glycolysis), and an increase in mitochondrial activity when LPS-induced DCs were treated with Apigenin. These results

indicate a protective role of Apigenin against DC-regulated neurodegenerative effects through a RelB mediated pathway thus implicating a potential therapy for neuroinflammatory disease.

Supported by NIH/NINDS (R01CA054559, R56AI077414 and 1R01NS097147-01A1)

W17.Chronic effects of alcohol on CYP2E1 mediated oxidative stress and HIV replication in primary macrophages and corroboration of this finding with clinical samples of HIV-infected alcohol drinkers

Gong, Y[1], Sinha, N[1], Rao, PSS, Ph.D.[1], Kumar, S, Ph.D.[1]; [1]Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN, 38163 United States.

Since the consumption of alcohol in HIV+ population is prevalent, it is important to study the interactions between alcohol and HIV replication. Our previous study has shown that alcohol exposure causes cellular toxicity through induction of cytochrome P450 2E1 (CYP2E1) followed by CYP2E1-mediated oxidative stress in U937 monocytic cells and SVGA cells. This study was designed to examine the role of CYP2E1 and oxidative stress in alcohol-mediated HIV replication in human primary macrophages. We exposed HIV-infected macrophages to 20 mM alcohol for 14 days. We measured mRNA and protein expression of CYP2E1 and antioxidant enzymes (AOEs); PRDX6, SOD1, SOD2, and Catalase, and HIV replication. Our results showed that chronic exposure of alcohol increased HIV P24 level (~3-fold) from 4 different donors. Alcohol also increased the level of CYP2E1, while it did not alter the level of AOEs, suggesting an overall increase in oxidative stress. Further, inhibition of CYP2E1 by SiRNA showed a decrease in P24 level. General antioxidants Vitamin E and Vitamin C also showed reduced level of P24. We are now in process of determining the potential role of oxidative stress (generated through CYP2E1 pathway) in HIV replication. Our in vitro results are consistent with our clinical data, in which, the levels of CYP2E1 and oxidative stress in blood monocytes were induced, while the level of alcohol in the plasma was reduced in HIV-infected alcohol users compared to alcohol users. These results are clinically relevant for finding effective treatment strategy for HIV-infected alcohol users.

Supported by R01AA022063

W18.The role of CD40 in calcitonin gene related protein (CGRP) mediated antiviral effect in glial cells

Grlickova-Duzevik, E[1], Cao, L, MD, Ph.D.[2]; [1]Graduate School of Biomedical Sciences and Engeneering, University of Maine, Orono, ME, 04469 United States. [2]Biomedical Sciences, University of New England, Biddeford, ME, 04005 United States.

LP-BM5 is a murine retrovirus that induces peripheral neuropathy and AIDS like immune deficiency in B6 mice. Upon infection, primary sensory neurons in the spinal cord express elevated levels of calcitonin gene related protein (CGRP). Previously, we showed that CGRP induced reduction of LP-BM5 viral loads in primary mixed glial cells. The purpose of this current study was to investigate possible CGRP downstream targets that may contribute to its antiretroviral response. We hypothesized that CGRP decreases the viral load in glial cells by modifying the CD40 expression; increasing in CD40 signaling may promote production of cytokines and chemokines with antiretroviral properties. Using CD40 knockout mice we showed that CD40 is required for CGRP mediated antiretroviral response in mixed glia. In microglial cell line, CD40 expression was elevated after treatment with CGRP. Similar effect was seen in microglia from spinal cord derived mixed glial cultures. While LP-BM5 increased microglial CD40 expression gradually over time up to 7 days post-infection in primary mixed glia, CGRP caused an early elevation (24 hr post-infection) of microglial CD40 expression in microglia and stayed at steady levels 7 days after infection. CGRP also promoted chemokine CXCL1(KC) production in mixed glia. Our data suggest that activation of CD40 mediated signaling may result in increased production of chemokines that may interfere with retroviral replication. Future studies will be directed to specifically delineate CD40 downstream target involved in antiretroviral response in glial cells.

Supported by NIH COBRE grant 1P20GM103643; R01 NS098426

W19.Heme oxygenase-1 protects against TNF-alpha-induced brain endothelial cell barrier permeability

Gruenewald, AL, BS[1], Gill, AJ, Ph.D.[1], Alvarez, JI, Ph.D.[2], Kolson, DL, MD, Ph.D.[1]; [1]Department of Neurology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, 19104 United States. [2]Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, 19104 United States.

Persistent systemic and CNS inflammation and oxidative stress are consequences of HIV infection and likely contributors to pathogenesis of HIV-associated neurocognitive disorders (HAND). The blood-brain barrier (BBB) in particular is vulnerable to HIV-mediated inflammation and oxidative stress. We have previously identified a reduction in expression of heme oxygenase-1 (HO-1), a key mediator of the host antioxidant and anti-inflammatory response, in brains of

individuals with HAND. HO-1 is expressed in brain microvascular endothelial cells (BMVEC), and has been linked to endothelial cell barrier function. We hypothesize that dysregulation of HO-1 expression at the BBB contributes to BBB dysfunction in individuals with HAND. In a large HIV-positive brain autopsy cohort (n=90), we have shown that brain HO-1 protein expression correlates positively with RNA expression of two endothelial cell markers, platelet endothelial cell adhesion molecule-1 (PECAM-1) and von Willebrand factor (vWF) ($p<0.0001$, $p=0.02$), thus demonstrating a relationship between CNS HO-1 expression and endothelial activation/function in HIV infected individuals. In complementary in vitro transwell studies, we have shown that induction of HO-1 in BMVECs prevents disruption of the BMVEC barrier by the pro-inflammatory cytokine tumor necrosis factor-alpha ($p=0.01$). Together, these results suggest that HO-1 plays a protective role against HIV-mediated BBB dysfunction and further suggest that HO-1 may be a viable therapeutic target to prevent against BBB disruption in HIV-infected individuals.

Supported by R01 MH095671, T32AI007632

W20.TLR3 Activation of Human Intestinal Epithelial Cells Inhibits HIV Replication in Macrophages

Guo, Le, MS[1], Zhou, Li, Ph.D.[2], Xu, Xiqiu, BS[2], Li, Jieliang, Ph.D.[1], Wang, Xu, Ph.D.[1], Liu, Jinbiao, Ph.D.[2], Zhuang, Ke, Ph.D.[2], Liu, Hang, MS[2], Zhang, Biao, MS[2], Ho, Wenzhe, MD, M.Ph.[1]; [1]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA, 19140 United States. [2]School of Basic Medical Sciences, Wuhan University, Wuhan, Hubei, 430071 China.

The gastrointestinal (GI) tract is a major site of HIV infection/replication. The mucosal surface of the GI tract serves as a physiological and immunological barrier against HIV infection. We investigate the role of the epithelial cells of GI tract in protecting macrophages from HIV infection. We demonstrated that human IECs possess a functional toll-like receptor 3 (TLR3) signaling system, activation of which induces expression of endogenous IFN-β, IFN-γ and CC chemokines (RANTES, MIP-1α, MIP-1β), the ligands of HIV entry co-receptor CCR5. The TLR3 activation of IECs also induced the phosphorylation of interferon regulatory transcription factor 3 (IRF3) and IRF7, the key regulators of the IFN signaling pathway. When infected macrophages were incubated in media conditioned with SN from Poly I:C-activated IECs cultures, HIV replication in macrophages was significantly suppressed. This SN action of Poly I:C-activated IECs cultures on HIV-1 was mainly mediated through IFN-β and IFN-γ, an antibody to IFN-β and IFN-γ could block SN-mediated anti-HIV effect. In addition, SN from IECs cultures induced the expression of IFN-stimulated genes (ISGs: ISG15, ISG56, OAS-1, OAS-2, MxA and MxB) in macrophages. These findings suggest that the intestinal epithelial cells could act as an important bystander, participating in the GI innate immunity against HIV infection.

Supported by NIH (DA022177, DA036163 and DA040329) and NNSF of China (81301428, 81471943ï¼‰

W21.PF3845 Attenuates HIV-1 Tat Mediated Neurotoxicity through Cannabinoid Signaling on Neurons and Astrocytes

Hermes, D.J.[1], Xu, C.[1], Niphakis, M.J.[2], Cravatt, B.F.[2], Mackie, K.[3], Lichtman, A.[4], Ignatowska-Jankowska, B.[5], Fitting, S.[1]; [1]Department of Psychology & Neuroscience, University of North Carolina Chapel Hill, Chapel Hill, NC, 27599-3270 United States. [2]Department of Chemical Physiology, Scripps Research Institute, La Jolla, CA, 92037 United States. [3]Department of Psychology & Brain Science, Indiana University, Bloomington, IN, 47405-7000 United States. [4]Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23220 United States. [5]Edmond and Lily Safra Center for Brain Science, Hebrew University of Jerusalem, Jerusalem, Jerusalem, 9190401 Israel.

The HIV-1 virus is known to propagate in macrophages and microglia, which results in chronic neuroinflammatory conditions and neuronal damage. The HIV-1 transactivator of transcription (Tat) protein is detectable in the brains of AIDS patients receiving combined antiretroviral treatment (cART), and has been shown to be neurotoxic in in vitro and in vivo assays. Drugs targeting the degradative enzymes of endogenous cannabinoids have shown promise in reducing pain and inflammation with minimal side effects in rodents. Here we demonstrate that inhibiting the degradation of anandamide, an endogenous cannabinoid ligand, with PF3845 in murine neuronal cultures blunts the neurotoxic effects of Tat. Ca2+ imaging, cell survival assays, and dendritic process volumetric analysis with IMARIS show that neurons incubated with PF3845 before Tat exposure show less Ca2+ signaling dysregulation, cell death and dendritic degeneration compared to Tat treated neurons. Considering that the severity of neuroinflammation best predicts loss of cognitive function in AIDS patients, we also wanted to assess the effect of PF3845 through astrocyte cannabinoid signaling on the release of neurotoxic soluble factors. We exposed cultured murine astrocytes to Tat with or without incubation with PF3845. After 24 hours of exposure, conditioned media was collected from Tat treated astrocytes for neuron exposure experiments. Pure neuron cultures were exposed to a range of conditioned media

dilutions, and neurotoxicity was assessed using Ca2+ imaging and cell survival assays. Data collection is still in progress.

Supported by NIDA R21 DA041903, R00 DA033878 and T32 DA007244

W22.Lipid-raft tool gone viral- US9 lends a HAND

Hill, G[1], Nash, B[1], Brandimarti, R, Ph.D.[1], Meucci, O, MD, Ph.D.[1]; [1]Department of Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

Combination anti-retroviral therapy has improved and prolonged the lives of HIV+ individuals, yet a spectrum of neurocognitive disorders associated with HIV (HAND) persists. Recent work suggests that HIV proteins facilitate Amyloid Precursor Protein (APP) processing to Amyloid-beta, and also alter the stability and composition of the lipid-raft cellular micro-domain where this processing occurs. Our group seeks to use the lipid-raft targeting ability of Herpes Simplex Virus transport protein US9 to assess how enhanced Amyloid-beta production and parallel alterations in lipid-raft physiology contribute to HAND. The present studies test the hypothesis that US9 can be used both to identify lipid raft changes induced by HIV neurotoxins, and to target non-raft proteins to lipid-raft micro-domains for 'interventional purpose'. Here we show that fusing US9 with GFP, or with a protease, does not affect US9's distribution or alter its targeting ability. Further, the cleaving function of the attached protease remains intact, and is specific to substrates present in the lipid raft. Lastly, lentiviruses harboring GFP-US9 fusion proteins efficiently transduce target cells and show appropriate distribution profiles. Future in vivo studies will utilize these lentiviruses to explore HIV-induced alterations in lipid-raft physiology. Further, nonpathogenic APP-cleaving US9-ADAM10 constructs generated here will be used to blunt Amyloid-beta production. By targeting ADAM10 to the lipid raft to compete with Amyloid-beta-producing BACE1, we can identify contributions by Amyloid-beta to HAND.

Supported by DA040519

W23.Effects of GPR55 activation on neural stem cell proliferation, differentiation, and immune responses to chronic inflammation

Hill, JD[1], Zuluaga-Ramirez, V[1], Winfield, M[1], Gajghate, S[1], Persidsky, Y, MD, Ph.D.[1]; [1]Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

New neurons are produced by neural stem cells (NSCs) within the adult hippocampus. Numerous diseases including major depressive disorder and HIV-1 associated neurocognitive disorder are associated with decreased rates of adult neurogenesis. A hallmark of these conditions is a chronic release of neuroinflammatory mediators by activated resident glia. Recent studies have shown a neuroprotective role on NSCs of cannabinoid receptor activation. Yet little is known about the effects of GPR55 activation on neurogenesis especially in response to inflammation and HIV-1 infection. In the present study we examined NSCs exposed to HIV-1 and inflammatory cytokines to assess inflammation-caused effects on NSC proliferation and differentiation and the ability of GPR55 agonists to attenuate NSC injury. Protective effects of GPR55 agonists were assessed after treating human NSCs with inflammatory cytokines and HIV-1 related neurotoxic proteins (gp120, tat). NSC proliferation was evaluated via BrdU incorporation. NSC differentiation was determined via FACS analysis of NSC markers (Nestin, Sox2, DCX, GFAP, NeuN). Results showed an increase in proliferation rates induced by GPR55 agonist treatment. Neurogenesis rates were rescued after co-treatment with inflammatory mediators and GPR55 agonists. In vivo studies showed direct intrahippocampal administration of GPR55 agonist increased NSC proliferation and neurogenesis. These results suggest a neuroprotective role of GPR55 activation on NSCs in vitro while in vivo studies demonstrate a necessity for GPR55 signaling under homeostatic conditions.

Supported by AA15913, DA007237

W24.Buprenorphine decreases CCL2-Mediated Mature Monocyte Migration in the context of NeuroAIDS

Jaureguiberry-Bravo, M, MS[1], Kelschenbach, JL, Ph.D.[2], Carvallo, L, Ph.D.[2], Gu, CJ, Ph.D.[2], Volsky, DJ, Ph.D.[2], Berman, JW, Ph.D.[1]; [1]Deparment of Pathology, Albert Einstein College of Medicine, New York, NY, 10461 United States. [2]Department of Medicine, Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 United States.

HIV entry into the CNS mediates viral seeding and inflammation that lead to HIV associated neurocognitive disorders (HAND). Opioids have been shown to exacerbate the neuropathology of HAND. HIV enters the CNS by transmigration of infected monocytes across the BBB. A mature population of peripheral blood monocytes that is CD14+CD16+ is increased in number in infected people and is even higher in HIV+ drug abusers. Mature monocytes transmigrate preferentially across the BBB in response to CCL2, a chemokine increased in the CNS of HIV+ people even when on ART. Buprenorphine, a partial agonist of MOR and a full antagonist

of KOR, is an opiate derivate therapeutic for opioid dependency. Its effects on mature monocyte migration and its subsequent impact on neuroinflammation have not been studied. We showed that mature monocytes express MOR and KOR, and that these mature monocytes in the PBMC of HIV infected people express higher levels of both receptors compared to monocytes of uninfected individuals. We demonstrated that buprenorphine treatment of mature monocytes significantly reduced their adhesion to brain microvascular endothelial cells, an early step in diapedesis across the BBB, as well as their chemotaxis to CCL2. We demonstrated in vivo that buprenorphine eliminated cognitive impairment in the ECOHIV mouse model. Additionally, buprenorphine reduced monocyte migration into the CNS in this model. Our findings indicate that buprenorphine may be neuroprotective by limiting monocyte CNS entry and neuroinflammation, and therefore may be a more broadly useful therapeutic.

Supported by NIH R01MH075679-07 and NIH T32AI007501-23

W25.Exosome mediated intercellular communication in chronic pain

Jean-Toussaint, R, MS[1], McDonald, M, Ph.D.[1], Sacan, A, Ph.D.[2], Ajit, S, Ph.D.[1]; [1]Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, 19104 United States.

Chronic neuropathic pain resulting from nerve injury is difficult to treat. Â The mechanisms underlying chronic pain are influenced by signals from neurons, microglia and the peripheral immune system. Exosomes are 30-100 nm vesicles that carry mRNAs, miRNAs and proteins to recipient cells via circulation. The molecules present on the surface of these vesicles enable them to target recipient cells. The exosomal contents vary depending on the source and the physiological conditions of cells releasing them. Exosome uptake results in modulation of gene expression in recipient cells and represents a novel mechanism of cellular communication. We have previously shown that a single injection of exosomes derived from RAW 264.7 cells can attenuate thermal hyperalgesia in a mouse model of inflammatory pain, suggesting an immunoprotective role for macrophage-derived exosomes. Here, we sought to determine if there is a preferential uptake of RAW 264.7 cell-derived exosomes by primary mouse microglia and neurons in vitro. Our preliminary data suggest a more robust gene expression change in microglia following exosome uptake. We also characterized exosomes from mouse model of neuropathic pain and observed a distinct miRNA and protein signature compared to control. We hypothesize that alterations in exosomal composition following nerve injury render them pro-nociceptive and contribute to the maintenance of chronic neuropathic pain. Our findings provide a strong basis for further investigating exosome mediated neuroimmune communication and their role in modulating chronic pain.

Supported by Clinical and Translational Research Institute (CTRI)

W26.Polymeric Nanoparticles (NPs)-Mediated Gene Delivery to Human Astrocytes

Joshi, C.R., MS[1], Labhasetwar, V, Ph.D.[2], Ghorpade, A., Ph.D.[1]; [1]Institute for Molecular Medicine, University of North Texas Health Science Center, Fort Worth, TX, 76107 United States. [2]Department of Biomedical Engineering, Cleveland Clinic Lerner Research Institute, Cleveland, OH, 44195 United States.

Astrocyte tissue-inhibitor of metalloproteinases-1 (TIMP-1) protects neurons during HIV-1-induced apoptosis. However, TIMP-1 levels decrease during chronic inflammation typical of HIV-associated neurocognitive disorders (HAND). We propose that astrocyte-targeted TIMP-1-gene delivery could be a suitable therapeutic for HAND. To test this hypothesis, obtaining a safe and effective gene delivery system is in order. Nanoparticle (NP)-mediated gene delivery is a viable approach since genes can be delivered to specific brain cell types and NPs are less immunogenic than viral vectors. For proof-of-concept, we tested arginine-based polyethylenimine (PEI) analogs (AnPn) and poly-lactic-co-glycolic-acid (PLGA) in vitro (primary human cells) and in vivo (mice) using a luciferase-reporter plasmid (pLuc). PLGA NPs delivered pLuc across astrocyte membrane but failed to induce protein expression. In parallel, A5P50, a PEI analog, efficiently expressed pLuc in astrocytes. Yet, its neuronal biocompatibility was not optimal. Combining low doses of AnPn with pLuc-loaded-PLGA NPs lead to high gene expression in all cell types including astrocytes. Live imaging indicated that AnPn facilitated PLGA-released-pLuc delivery across the nuclear membrane by an unknown mechanism. Consequently, optimally biocompatible PEI analogs were also synthesized and tested. Our data indicate that AnPn-PLGA combination and new AnPn analogs overcome both neuronal biocompatibility and astrocyte-specific gene expression issues promising clinical translations for HAND treatment in future.

Supported by NIH R01 NS048837 to AG

W27.HIV-1 Tat induced acid store operated calcium entry (aSOCE) in primary cultured neurons

Lakpa, L, BS[1], Liang, H., MD, Ph.D.[1], Geiger, J. D., Ph.D.[1], Chen, X., MD, Ph.D.[1]; [1]School of Medicine and Health Sciences, University of North Dakota, Grand Forks, ND, 58202 United States.

Increased lifespan of human immunodeficiency virus type-1 (HIV-1) patients, as a result of combined anti-retroviral therapeutics, is accompanied by a high prevalence of HIV-1 associated neurocognitive disorders (HAND). Although the pathogenesis of HAND remains elusive, disruption of neuronal calcium homeostasis by HIV-1 viral proteins, such as transactivator of transcription (Tat), has been implicated. We and others have demonstrated that HIV-1 Tat perturbs calcium homeostasis through influencing NMDA receptor and endoplasmic reticulum. However, little is known on whether HIV-1 Tat affects calcium homeostasis of endolysosome, a newly-identified acidic calcium store. Recently, we have reported that elevation of neuronal endolysosome pH induces endolysosome calcium release, which results in extracellular calcium influx across the plasma membrane, a phenomenon we term as acid store operated-calcium entry ("aSOCE"). Currently, it is known that HIV-1 Tat enters endolysosome via receptor-medicated endocytosis, and we have shown that such endolysosome accumulation of HIV-1 Tat raises endolysosome pH. Here, we tested the hypothesis that HIV-1 Tat induces aSOCE in primary cultured rat neurons. We found that HIV-1 Tat could cause endolysosome calcium release followed by extracellular calcium influx. Such HIV-1 Tat-induced aSOCE might play an important role in disturbing calcium homeostasis in neurons, which might contribute to HAND.

Supported by P30GM103329, and R01MH100972, and R01MH105329

W28.Nef increases mitochondrial staining in U87 astrocytes

Lamboy, R, MS[1], Noel, R, Ph.D.[1]; [1]Ponce Research Institute, Ponce Health Sciences University, Ponce, Puerto Rico, 00716 Puerto Rico.

HIV-infected individuals can suffer from a wide range of neurocognitive impairments collectively termed HIV-associated neurocognitive disorders (HAND). HIV causes neurotoxicity through the expression of several viral proteins including Nef - a neurotoxin that affects both glial cells and neurons. Previous work has shown that HIV-infected cells are protected from apoptosis, but the mechanisms of this protective capacity are uncertain. We propose that in infection of astrocytes, Nef is involved in the protection of these infected astrocytes through the upregulation of mitochondrial activity that could lead to an increase in energy production and cell survival. To test if Nef influences mitochondrial activity, U87 and U87-CD4 astrocytes were transfected with a plasmid encoding Nef and were grown to different time points in co-culture with un-transfected astrocytes. At each time point (24, 48 and 72h), cells were stained with Mitotracker probes to assess mitochondrial intensity staining. After 72 hours, quantitative analysis suggests that astrocytes endogenously producing Nef have greater mitochondrial staining when compared to mock treated cells and astrocytes that were grown in co-culture with astrocytes transfected to produce Nef. Future experiments will be directed to understanding of the impact of Nef on mitochondrial activity and cell survival.

Supported by NIGMS RISE 3R25GM082406, G12 MD007579, GM106970.

W29.Autophagy is cytoprotective in neurons and necessary against Tat and morphine-induced toxicity in autophagy-deficient mice

Lapierre, J, MS[1], Rodiguez, M, Ph.D.[1], El-Hage, N, Ph.D.[1]; [1]Department of Immunology, Florida International University, Miami, FL, 33199 United States.

HIV-infected patients who abuse opiates often show acceleration of HAND. Our lab and others have shown interactive effects between the opiate morphine and HIV protein Tat in the brain. Given that neuronal autophagy is found to be dysregulated in many neurodegenerative diseases, studies suggest that HIV infection in the CNS along with viral protein release can alter autophagy. However, the mechanisms of neurotoxicity mediated by autophagy remain unclear. In this study, neurons derived from Becn1-deficient mice (Becn1+/-) were exposed to HIV-Tat and morphine to assess the role of autophagy as a cytotoxic or cytoprotective mechanism in neuronal viability, morphology, and calcium signaling. Becn1-deficient mice are heterozygous for the Becn1 allele and show reduced autophagy. Reduced levels of autophagy were shown to be detrimental to neuronal survival as compared to C57BL/J wild type controls. Treatment with Tat reduced neuronal survival for both strains, with co-administration of morphine enhancing toxic effects in wild type neurons, whereas this effect was negated in Becn1+/- neurons. Analysis of intracellular calcium also showed that Becn1+/- neurons can inhibit Tat/morphine effects. However, upon examination of morphology, wild type and mutant neurons showed enhanced neurite beading with Tat/morphine. This data confirms the role of autophagy as a cytoprotective mechanism and that basal levels of autophagy are necessary for neuronal

survival. Further, this suggests the autophagy pathway may play a role in mediating the combined Tat/morphine toxic effect to neurons.

Supported by National Institutes of Health (NIH)-National Institute on Drug Abuse (NIDA) grants R01 DA036154 and R21DA041287

W30.Role of Autophagy in HIV Tat-mediated Disruption of Blood-Brain Barrier

Liao, K, MS[1], Niu, F, MS[1], Hu, G, Ph.D.[1], Buch, S, Ph.D.[1]; [1]Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, omaha, NE, 68198 United States.

The blood brain barrier (BBB) is a tight barrier that prevents the entry of various pathogens such as HIV into the brain. HIV Tat protein are known to disrupt the tight junctions at the BBB. Autophagy is an intracellular degradation process that involves degradation and recycling of damaged organelles to the lysosome. The role of autophagy in HIV Tat-mediated BBB disruption however, remains unknown. In the present study, we demonstrate that exposure of human brain microvessel endothelial cells (hBMECs) to HIV Tat results in induction of autopahgy in a dose- and time-dependent manner with upregulation of Beclin1, ATG5, and LC3B. Furthermore, HIV Tat also results in the induction of autophagosomes in hBMECs. Intriguingly, HIV Tat mediated induction of autophagy correlated with a concomitant down-regulation of tight junction proteins ZO-1 and occludin, leading in turn, to increased cellular permeability in an in vitro BBB model. Pharmacological and genetic inhibition of autophagy resulted in abrogation of Tat-mediated induction of LC3II with a concomitant restoration of tight junction proteins, thereby underscoring the role of autophagy in HIV Tat-mediated breach of BBB. Furthermore, these findings were also validated in microvessels isolated from the brains of HIV Tg26 mice and lysates isolated from the frontal cortex of HIV-infected autopsied brains that exhibited increased autophagy markers and decreased expression of tight junction proteins. Overall, our findings identify autophagy an important mechanism underlying HIV Tat-mediated disruption of the BBB.

Supported by DA027729; DA036157

W31.Computationally Driven Rational Design of HIV-1-LTR-Targeted CRISPR/Cas9 Therapy Dismisses HERVs as a Primary Source of Toxicity

Link, R, BS[1], Nonnemacher, MR, Ph.D.[2], Wigdahl, B, Ph.D.[2], Dampier, W, Ph.D.[3]; [1]School of Biomedical Engineering and Health Systems, Drexel university, Philadelphia, PA, 19104 United States. [2]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [3]Center for Molecular Virology and Translational Neuroscience, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

Despite effective antiretroviral treatment (ART), long-term chronic HIV-1 disease is characterized by the retention of the HIV-1 provirus in an integrated form in host cell chromatin resulting in a persistent/latent infection. Recently, CRISPR/Cas9 has been used as a therapeutic avenue to target HIV-1 to cause complete excision of the proviral genome. Excision of the proviral genome is of particular interest in the brain where ART still permits the production of persistent infectious virus and neurotoxic HIV-1 proteins. The long terminal repeat (LTR) regions are an appealing CRISPR/Cas9 target because the symmetry between the 5' and 3' LTRs ensure that the entire latent HIV sequence is excised. However, due to the promiscuity of CRISPR/Cas9 binding, more research needs to be performed investigating possible off-target effects. Utilizing a k-mer approach in which 20bp segments across the LTR and along multiple subtypes, we identified a position dependent likelihood of off-target risk. Human endogenous retrovirus (HERV) LTR sequences were initially thought to be the most likely candidates in the human genome to share homology with HIV-1 LTR sequences. However, the results obtained in this regard have indicated that the HERV genomic regions do not share extensive homology with the HIV-1 LTR. Even with permissive investigative parameters, it was clear that HIV-1 5' LTRs and HERV 5' LTRs were dissimilar when compared on a global scale. These studies will be useful to facilitate the rational design of HIV-1-targeted CRISPR/Cas9 therapy to avoid potential off-target effects.

Supported by NIMH R01 MH110360 (PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32 MH079785 (Drexel Component PI, BW)

W32.Acute administration of endothelial-targeted catalase attenuates oxidative stress and reduces neuroinflammation in experimental traumatic brain injury

Lutton, EM[1], Razmpour, RR[1], Shuvaev, VV, Ph.D.[2], Muzykantov, VR, MD, Ph.D.[2], Ramirez, SH, Ph.D.[1]; [1]Department of Pathology and Laboratory Medicine, Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States. [2]ITMAT, University of Pennsylvania, Philadelphia, PA, 19104 United States.

TBI is a major clinical concern that contributes to one third of all injury related deaths in the US. Secondary mechanisms of injury in TBI, such as inflammation, are points at which intervention may improve functional recovery. Current treatment strategies for TBI are supportive, and the pathophysiology is not fully understood. Evidence suggests that reactive oxygen species (ROS) and oxidative stress propagate blood-brain barrier (BBB) hyperpermeability and inflammation following TBI. Such inflammation is associated with activated microglia that have been shown to persist for years after injury in the human brain. The use of endothelial-targeted catalase in TBI is hypothesized to quench ROS at their source to limit inflammation and protect BBB function. Preliminary data demonstrates a time dependent increase in vascular expression of endothelial ICAM-1 after TBI. To evaluate targeted antioxidant enzyme efficacy in TBI, catalase was conjugated to anti-ICAM-1 antibodies and administered to 6wk old C57BL6 mice 30min after moderate controlled cortical impact injury. Results suggest that catalase targeted to ICAM-1 reduces neuroinflammatory indices and BBB permeability and reduces levels of the ROS hydrogen peroxide in injured brains. Furthermore, the study of microglia in situ by multiphoton microscopy following TBI revealed that anti-ICAM-1/catalase attenuates microglia transition to an activated phenotype. These results demonstrate an effective proof-of-concept approach to acute TBI management that may also be applicable to other neuroinflammatory conditions.

Supported by NIH/NINDS NS086570-01 (SHR)

W33.Treatment with VDLP induction chemotherapy in acute lymphoblastic leukemia with TEL-AML1 and TEL-ABL rearrangements

Mao, Shuting, MD[1], Liu, Yufeng, MD, Ph.D.[1]; [1]the First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan, 450000 China.

TEL is an important hematopoietic regulatory component, and TEL rearrangement is observed in a variety of hematological malignancies including myelodysplastic syndromes, acute leukemias, and myeloproliferative disorders. We identify a case of a 3-year-old girl with acute lymphoblastic leukemia with TEL-AML1 and TEL-ABL rearrangements. Gene fusion events and TEL-ABL transcript levels were detected and quantified by multi-fusion gene qRT-PCR screening. Treatment with VDLP induction chemotherapy was administered following diagnosis. Upon completion of the induction therapy regimen, TEL-AML1 transcript levels were found to be reduced and TEL-ABL transcripts were undetectable, accompanied by hematological remission. We report here early identification of a rare TEL-AML1/TEL-ABL double positive ALL case using a fluorescent qRT-PCR-based 43 Fusion Gene Screening Kit. Due to early detection and prescribed induction chemotherapy treatment, the patient achieved hematological remission with reduction of both TEL-AML1 and TEL-ABL transcripts. Importantly, TEL-ABL levels were reduced to undetectable levels following treatment. As t(9;12) chromosomal rearrangements may not be detected using cytogenetic methods, this case underscores the efficacy of qRT-PCR detection methods to accurately identify gene fusions such as the TEL-AML1/TEL-ABL double fusion described above. Future identification of more concurrent double gene fusion events may give indication to the prognostic value of multiple fusions in ALL.

Supported by the youth fund of the First Affiliated Hospital, Zhengzhou University

W34.Treatment with VDLP induction chemotherapy in acute lymphoblastic leukemia with TEL-AML1 and TEL-ABL rearrangements

Mao, Shuting, MD[1], Liu, Yufeng, MD, Ph.D.[1]; [1]the First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan, 450000 China.

TEL is an important hematopoietic regulatory component, and TEL rearrangement is observed in a variety of hematological malignancies including myelodysplastic syndromes, acute leukemias, and myeloproliferative disorders. We identify a case of a 3-year-old girl with acute lymphoblastic leukemia with TEL-AML1 and TEL-ABL rearrangements. Gene fusion events and TEL-ABL transcript levels were detected and quantified by multi-fusion gene qRT-PCR screening. Treatment with VDLP induction chemotherapy was administered following diagnosis. Upon completion of the induction therapy regimen, TEL-AML1 transcript levels were found to be reduced and TEL-ABL transcripts were undetectable, accompanied by hematological remission. We report here early identification of a rare TEL-AML1/TEL-ABL double positive ALL case using a fluorescent qRT-PCR-based 43 Fusion Gene Screening Kit. Due to early detection and prescribed induction chemotherapy treatment, the patient achieved hematological remission with reduction of both TEL-AML1 and TEL-ABL transcripts. Importantly, TEL-ABL levels were reduced to undetectable levels following treatment. As t(9;12) chromosomal rearrangements may not be detected using cytogenetic methods, this case underscores the efficacy of qRT-PCR detection methods to accurately identify gene fusions such as the TEL-AML1/TEL-ABL double fusion described above. Future identification of more concurrent double gene fusion events may give indication to the prognostic value of multiple fusions in ALL.

Supported by the youth fund of the First Affiliated Hospital, Zhengzhou University

W35.HIV-1 Nef differentially modulates the TGFβ pathway in astrocytes and neurons

Martinez-Orengo, N., BS[1], Cruz, M., BS[1], Noel, R., Ph.D.[1]; [1]Ponce Research Institute, Ponce Health Sciences University, Ponce, PR, 00716 United States.

Astrocytes help to maintain neuronal function. The close relationship between neurons and astrocytes makes the latter a possible therapeutic target in neurological diseases. HIV-1 patients suffer neurocognitive impairments regardless of the efficacy of their antiretroviral therapy. In previous studies we have demonstrated that TGFβ and inflammatory cytokines are increased in astrocytes transfected with HIV-1 Nef. However, neurons that have been exposed to astrocytes expressing Nef do not show significant apoptosis. TGFβ is a cytokine involved in apoptosis and inflammatory responses that can have contrasting effects in terms of conferring protection. In this study we aim to measure the effect of Nef on astrocytes and neurons and how this early HIV-1 protein modulates the TGFβ pathway. Lysates of astrocytes transfected with Nef were assayed using a TGFβ signaling pathway panel. Supernatants from these astrocytes were collected at different time points and added to neurons. Lysates of neurons were tested using the TGFβ panel. P-SMAD2 and p-SMAD3 were increased in Nef-exposed neurons when compared to astrocytes. Treatment with a TGFβRI antagonist prevented the increase in phosphorylation of SMAD2/3. Total SMAD4 was increased in neurons and it was not decreased by the inhibitor. TGFβRII, p-ERK1/2 and p-AKT were increased in astrocytes when compared to neurons. Our data suggests that Nef may be modulating the TGFβ pathway in a cell type dependent manner. The activation of the non-canonical TGFβ pathway in astrocytes can be a possible response to prevent neuronal loss.

Supported by 1F31GM118014-01 (F31), R25GM082406 (RISE), GM106970 (To RJN), MD007579 (MAGIC)

W36.Tight junction complex deregulation by morphine and HIV-1 Tat exposure in an in vitro blood-brain barrier system

Maubert, M[1], Kercher, K[1], Strazza, M[1], Pirrone, V[1], Lin, W[2], Feng, R[2], Nonnemacher, M[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Biostatistics and Epidemiology, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104 United States.

Many of the pathological observations made in human immunodeficiency virus type 1 (HIV-1)-associated neurocognitive disorders (HAND) have been attributed to compromise of blood-brain barrier (BBB) integrity, and selected viral proteins have been implicated in deregulation of the BBB, including the HIV-1 transactivator of transcription (Tat). In addition, illicit drug use is a known confounder of disease in HIV-1-infected individuals. Importantly, opioid abuse within this population enhances disease progression in multiple ways, including enhanced incidence and severity of neurocognitive impairment including dementia, as compared to non-users. Studies have suggested that exposure to both HIV-1 Tat protein and μ-opioids disrupts BBB homeostasis and permeability in primary cells, including an increased pro-inflammatory state, as well as augmented cellular transmigration, and enhanced barrier leakiness. In this study, a human brain microvascular endothelial cell line, hCMEC/D3, was utilized to establish an in vitro model of the BBB to investigate the effects of Tat or morphine exposure on BBB compromise. Changes in mRNA transcripts of tight junction proteins (TJP) were observed throughout the course of exposure. Differences in TJP expression and localization were also observed at the protein level following cellular fractionation and western immunoblot analysis. These studies demonstrate that exposure to Tat or morphine compromises BBB integrity by inducing alterations in molecular expression at both the mRNA and protein levels.

Supported by This work is supported by, NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32 MH079785 (Drexel Component PI, BW)

W37.Temporal Processing Deficits in the HIV-1 Tg Rat: Dopamine Transporter Dysfunction

McLaurin, KA, MS[1], Li, H, MD, Ph.D.[1], Booze, RM, Ph.D.[1], Mactutus, CF, Ph.D.[1]; [1]Program in Behavioral Neuroscience, Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States.

Given the extensive track record of psychostimulant use to improve cognitive disorders, the potential therapeutic use of methylphenidate (MPH), an inhibitor of the dopamine (DA) transporter, was assessed in HIV-1 transgenic (Tg) rats. Using the clinical relevant route, oral self-administration (OSA) of MPH (2.4 ± 0.2 mg/kg) was used in adult (8-9 months), ovariectomized female HIV-1 Tg (n=19) and F344/N control rats (n=20). First, a pre-post design was used to determine the acute effect of OSA of MPH on temporal processing, which may be a critical underlying dimension of HAND. OSA of MPH significantly altered auditory gap threshold detection in control animals ($p \leq 0.001$), but not in HIV-1 Tg animals. Second, a history (14 days) of OSA of MPH affected dendritic spine morphology in layers II-III pyramidal neurons in the medial prefrontal cortex. Preliminary analyses (HIV-1 Tg

(n=7); control (n=7)) suggested a history of MPH exposure produced a population shift towards shorter spines with increased volume in control animals; a shift which was not as robust in HIV-1 Tg animals. Third, control animals exhibited a linear decrease in auditory startle response at 5 msec as dendritic spine volume increased; a brain/behavior relationship not observed in HIV-1 Tg animals. Collectively, these results provide strong evidence for DA system dysfunction in the adult HIV-1 Tg rat, which is not ameliorated by oral self-administration of MPH, and suggest that targeting the DA transporter is unlikely to be an efficacious therapeutic treatment for HAND in adult HIV-1 seropositive individuals.

Supported by Funded by NIH grants DA013137, HD043680, and MH106392

W38.HIV-1 Tat variants derived from neurocognitively impaired patients may differentially transactivate HIV and host cell gene promoters

Mele, A[1], Antell, G[2], Dampier, W[1], Pirrone, V[1], Aiamkitsumrit, B[1], Williams, J[1], Passic, S[1], Kercher, K[1], Zhong, W[1], Szep, S[2], Jacobson, J[3], Wigdahl, B[1], Hershberg, U[2], Nonnemacher, M[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Medicine, Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [3]Department of Medicine, Section of Infectious Disease, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

HIV-1 mortality has decreased; however, the incidence of neurocognitive impairment has increased. Previous studies examined the HIV-1 protein Tat and detected expression, even in patients currently on ART with low-to-undetectable viral titers. Tat has been shown to be neurotoxic and a causative agent of CNS inflammation. The current studies seek to identify and characterize predominant variations within Tat, correlated with neurocognitive impairment, and understand their role in neuropathogenesis. HIV-1 Tat sequences were obtained from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. The sequences were amplified from PBMCs, translated, and aligned to HIV-1 subtype B genome to identify amino acid substitutions. Multiple positional hotspots of high variation in Tat were identified within the cysteine-rich and glutamate-rich domain, and second exon. Statistical analyses were applied to amino acid positions and variants associated with the patient's neurocognitive impairment status. To assess these variant functionally, five neurocognitively impaired and five non-impaired patients were selected based on their Global Deficit Score (GDS). Tat exon quasispecies were quantified by their frequency per patient. The predominant Tat exon 1 and 2 sequences were reconstructed into full-length Tat expression vectors. These recombinant vectors were used to examine structural and functional alterations in vitro. Initial results concluded no difference in HIV-1 promoter transactivation. Studies are now focused on the interaction of Tat with host promoters.

Supported by This work is supported by NIMH P30 MH092177 and NIMH T32 MH079785 (Drexel Component PI, BW) and NINDS R01 NS089435 (PI, MRN)

W39.Investigating the RNA-binding domain of transcription factor E2F1 in the neuronal context

Meurice, C, BS[1], Doa, AQ, BS[2], Jackson, DP, Ph.D.[2], Cho, G, BS[3], Zyskind, JW, Ph.D.[1], Jordan-Sciutto, KL, Ph.D.[2]; [1]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104 United States. [2]School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, 19104 United States. [3]College of Arts and Sciences, University of Pennsylvania, Philadelphia, PA, 19104 United States.

Despite its well-known role as a transcription factor involved in cell proliferation cycle, E2F1 has been linked to neuronal death during neurodegenerative diseases such as Alzheimer Disease, Parkinson's Disease, and HIV Encephalitis. Interestingly, E2F1 has been observed to be predominantly cytoplasmic in post-mitotic neurons, which is counterpoint to its well-known role as a transcriptional regulator. A previous study demonstrating that E2F1 has a higher affinity for RNA in its monomeric form led us to hypothesize that E2F1 may play a role in neuronal survival during neurodegenerative diseases by regulating RNA through direct binding. By mutating key residues in the nucleic acid binding domain that regulate E2F:DNA interaction when heterodimerized with DP1 as well as a key amino acid in this domain that differs between E2F1 and other E2F family members, we examined affinity for DNA and RNA using electrophoretic mobility shift assay (EMSA). Using this approach, we confirmed that E2F1 has a higher affinity for RNA over DNA in neuronal lysates. Further, we found that amino acid R125 and N152 may be critical for interactions between E2F1 and RNA. Further, analysis of this distinct function for E2F1 in neurons will provide insight into the role of this protein in neurodegenerative diseases.

Supported by NINDS and NIMH

W40.Increased neuronal iron following exposure of cortical cultures to morphine drives upregulation of the Ferritin Heavy Chain protein, but iron loading is not sufficient to reproduce morphine's inhibition of CXCR4 signaling.

Nash, B. S., BS[1], Tarn, K., MS[1], Nolan, R.A., BS[1], Meucci, O., MD, Ph.D.[1]; [1]Department of Pharmacology & Physiology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

HIV associated neurocognitive disorders are exacerbated by opioid drug use in many patients. Previous work from our lab shows that opioids inhibit neuronal homeostatic CXCR4 chemokine receptor signaling via upregulation of Ferritin Heavy Chain (FHC); an event that is mediated by mu-opioid receptors and correlates with disease progression. The aim of this work is to understand the mechanism of opioid mediated FHC upregulation in cortical neurons. Over 24 hours, morphine up regulates FHC in neuronal cultures in a G-protein and dose-dependent manner. However, it does not alter FHC transcript levels, suggesting a post-transcriptional regulation of FHC. Since small changes in iron levels also post-transcriptionally regulate FHC expression, iron levels after morphine treatment were measured, and indeed were slightly increased in neurons. Additionally, iron loading and morphine treatment both up-regulate Ferritin Light Chain, which self-assembles with FHC to form the mature iron storage protein. Furthermore, chelation of intracellular (but not extracellular) iron blocks morphine-induced FHC upregulation. Iron loading neurons (or non-neuronal cell lines) will robustly up regulate FHC. Nevertheless, downstream CXCR4 signals in these cells are not blocked - suggesting that FHC loses its CXCR4 regulatory ability when cells are overloaded with iron. The data imply that FHC behaves differently when induced by morphine vs extracellular iron uptake, but iron levels remain a crucial component of both pathways.

Supported by NIDA DA32444, DA15014

W41.Immunosuppressive Effects of Cannabidiol in Mild and Moderate Disease States of Experimental Autoimmune Encephalomyelitis

Nichols, JM[1], Gilfeather, K, BS[1], Yray, G[1], Kummari, E, Ph.D.[1], Dhital, S, Ph.D.[1], Kaplan, BLF, Ph.D.[1]; [1]Department of Basic Sciences, Colllege of Veterinary Medicine, Mississippi State University, Mississippi State, MS, 39762 United States.

Exploration of the pharmacological effects of cannabidiol (CBD) has shown that this compound exhibits immunosuppressive properties; however, it lacks the psychoactive effects seen with Δ9-tetrahydrocannabinol (THC). For these reasons CBD is being investigated for its therapeutic potential for diseases such as multiple sclerosis (MS), which is a complex progressive neurodegenerative autoimmune disease seen in middle aged adults. Currently our lab is working with the experimental autoimmune encephalomyelitis (EAE) model of MS to explore the immunosuppressive effects of orally administered cannabidiol (CBD). We use a variation of this model without pertussis toxin (PTX), since PTX blocks cannabinoid receptors (CB1 and CB2) and could potentially confound any studies in which cannabinoids are being investigated. It was hypothesized that treatment with oral CBD would slow the progression of disease in the PTX-free model of EAE. Oral administration of CBD for 5 days after the initiation of mild or moderate disease with high and low levels of MOG peptide, respectively, delayed onset of EAE for both mild and moderate disease, and ultimately lessened the disease severity for mild disease. However, cultures of lymphocytes from secondary lymphoid tissues revealed no significant difference in production of IL17 or IFNγ between groups, and measurements of in vivo levels of MOG specific IgG, CD8+ IL17A+ cells, and CD4+IL17A+ cells did not vary significantly between groups. These results suggest that the effects of CBD on EAE are not mediated by CBD acting on peripheral T lymphocytes.

Supported by NIH/NIGMS P20GM103646

W42.A comparison of DRD2 transcript and protein expression in undifferentiated and differentiated neuronal cells.

Nolan, R[1], Tarn, K[1], Gaskill, P, Ph.D.[1], Meucci, O, MD, Ph.D.[1]; [1]Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

Alterations in dopamine (DA) neurotransmission have been linked to HIV-Associated Neurocognitive Disorders (HAND). On the other hand, synaptic changes may occur to maintain DA neurotransmission within an optimal range. For instance, studies in the prefrontal cortex of HIV patients suggest that down regulation of type 2 DA receptor (DRD2) correlates with improved cognitive function. However, contradictory data exist on DRD2 transcript/protein changes in HAND and the role of DA signaling in HIV cognitive impairment. Also undefined is the relationship of DA signaling with underlying inflammation or sub threshold immune activation. To shed some light on these issues, we asked whether neuroimmune mediators involved with HAND, i.e. the neuroprotective chemokine CXCL12, alter DRD2. As a first necessary step, our initial combined intent was to test quality of available reagents and examine DRD2 changes under different experimental

conditions, in both human and rat neuronal cultures. We found significant up regulation of DRD2 mRNA and protein in human SH-SY5Y neuroblastoma cells upon differentiation with retinoic acid, either alone or in combination with 12-O-tetradecanoyl-phorbol-13-acetate. DRD2 mRNA and protein are also expressed at different levels in rat cortical neurons - depending on the age of culture (i.e. maturation). Ongoing in vitro and in vivo experiments will extend these analyses to primary human neurons, determine if CXCL12 regulates neuronal DRD2 expression in HAND animal models, and examine physical/functional interactions between DRs and the CXCL12 receptor CXCR4.

Supported by NIH/NIDA: DA15014, DA3244, DA40519; NIH/NIMH: T32 MH079785

W43.Methamphetamine augment HIV-1 Tat mediated memory deficits by altering the expression of synaptic proteins and neurotrophic factors

Nookala, Anantha Ra, BS[1], Kumar, Anil, Ph.D.[1]; [1]Division of Pharmacology and Toxicology, University of Missouri-Kansas city, Kansas City, MO, 64108 United States.

Methamphetamine (METH) abuse is common among individuals infected with HIV-1 and has been shown to affect disease progression towards AIDS. HIV-1 Tat, a viral protein released during HIV-1 replication has been implicated in HIV-associated neurotoxicity. However, combined effect of METH and HIV-1 Tat on neurocognition and its potential effect on neuroplasticity mechanisms is not well documented. The present study was undertaken to determine the combined effect of METH and HIV-1 Tat on behavior and neuroplasticity markers. Doxycycline inducible HIV-1 Tat (1-86) transgenic mice were administered 6mg/kg MA twice a day for 3 weeks before commencement of behavior. We employed Y maze and Morris water maze to determine cognition in these mice. Working memory, indicated by number of spontaneous alterations, was decreased significantly in Tat mice that were administered METH. In the water maze task, there was a significant increase in escape latency in all the groups compared to control mice, and the memory deficit was larger in Tat transgenic mice that were administered MA. There is significant decrease in the protein expression of synapsin1, synaptophysin, arg3.1, PSD95 and BDNF in different brain regions. We also looked at the expression levels of a second marker of synaptodendritic integrity, CaMKII and found that it is significantly decreased in HIV-1 Tat mice that were treated with METH in both sexes. This study therefore provides novel insights into the interaction of HIV-1 Tat and METH on the dysregulated expression of various plastic proteins in different brain regions.

Supported by NIDA and NIAAA

W44.Self-administered methamphetamine by HIV-1 transgenic rats alters markers of neuronal activity and plasticity in the limbic brain

Ohene-Nyako, M, MS[1], Persons, A, Ph.D.[1], Napier, T. C, Ph.D.[1]; [1]Department of Pharmacology, Rush University, Chicago, IL, 60612 United States.

Methamphetamine (meth) abuse co-occurs with HIV infection, and the comorbid condition presents a greater pathology than either condition alone. Extracellular regulated kinase (ERK), a mitogen-activated protein kinase (MAPK), and the downstream transcription factor ΔFosB, play vital roles in activity-dependent neuronal plasticity associated with chronic exposure to stimulants, including meth. Both meth and HIV-1 proteins alter MAPK signaling in brain regions involved in addiction, but little is known about the comorbid effects of HIV-1 proteins and meth. To address this gap, we studied young adult Fischer 344 HIV-1 transgenic (Tg) and non-Tg rats that self-administered meth (0.02-0.04mg/kg/0.05ml iv infusion) 2h/day for 21 days; controls were saline-yoked. One day following the last operant session, rats were killed; the nucleus accumbens (NAc) and ventral pallidum (VP) were dissected out and prepared for immunoblotting. ERK data were analyzed as a ratio of phosphorylated ERK (pERK) to total ERK. We observed a significant interaction between genotype and meth for pERK1/ERK1 in the NAc. Post hoc analysis indicated an increase in pERK1/ERK1 in Tg-meth rats compared to Tg saline controls. There also was an effect of meth for pERK2/ERK2 and ΔFosB in the NAc. (p

Supported by NIH R21ES025920, P30A1082151

W45.Lipocalin-2 in HIV-induced neuronal damage

Ojeda-Juárez, D, B.S.[1], Shah, RL[1], Sanchez, AB, Ph.D.[1], Kaul, M; Ph.D.[1], [1]Infectious and Inflammatory Disease Center, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, 92037 United States.

Brain injury and cognitive impairment is one of the many challenges people living with human immunodeficiency virus-1 (HIV-1) face, and the pathological mechanism is incompletely understood. Transgenic (tg) mice expressing HIV envelope protein gp120 in their central nervous system (CNS) provide a model of HIV-associated brain injury. These gp120tg mice present neuropathological features observed in AIDS brain, including astrocytosis, microgliosis, and decreased synaptic connections and dendritic processes. The acute phase protein lipocalin-2 (LCN2) is up-regulated in gp120tg brains, but its function is incompletely understood. In order to gain insight into the role of LCN2 in HIV-induced brain injury, we cross-bred gp120tg mice with a genetic knock

out of lipocalin-2 (LCN2KO) or a double knock out of LCN2 and HIV co-receptor CCR5 (LCN2KO/CCR5KO). The brains of these animals were analyzed for GFAP which is a marker for astrocytosis. The analysis showed that LCN2KO animals and LCN2KO/CCR5KO had lower levels of GFAP protein compared to wild-type (wt) controls. LCN2KO or LCN2KO/CCR5KO expressing gp120 showed GFAP levels higher than wt controls but not different from gp120tg mice that are wt for LCN2 and CCR5. These findings suggest that LCN2 is necessary to maintain normal levels of GFAP in the brain and knocking out LCN2 and/or CCR5 is not sufficient to prevent astrocytosis caused by HIV gp120. A better understanding of the role of astrocytosis in HIV-Induced brain injury is expected to help the identification of new therapeutic targets.

Supported by NIH R01 MH087332, MH104131 and MH105330

W46.Drug-drug interaction of cART and Morphine mediated by autophagy exacerbates the overall effect of cART in brain

Ojha, CR, MS[1], Lapierre, J, MS[1], Estrada, H, BS[1], Rodriguez, M, Ph.D.[1], El-Hage, N, Ph.D.[1]; [1]Department of Immunology, College of medicine, Florida international university, Miami, FL, 33199 United States.

Human Immunodeficiency Virus 1 (HIV-1) penetrates the central nervous system early after transmission and frequently leads to HIV associated neurocognitive disorders (HAND) despite combination antiretroviral therapy (cART). cART-related toxicity, limited permiabilization through the blood brain barrier and comorbidity with drug of abuse particularly opiate are likely contributors to HAND. In this study, the underlying mechanism regulating drug-drug interactions between opioid and cART leading to neuronal dysfunction was analyzed. HIV-1 infected human astrocytes were treated with cART ± morphine. HIV-1 replication, as measured by P24 levels, was decreased in astrocytes exposed with cART alone, while co-exposure with morphine caused a significant increase in viral titer. Inflammatory responses were suppressed by exposure of cART, while co-exposure with morphine significantly reverted and heightened the responses. To investigate the mechanism underlying the effects of drug-drug interaction, we performed mRNA expression profile for cytotoxicity and found increased levels of sequestosome 1 (p62) mRNA expression, which was further confirmed by western blotting and RFP-p62 cell based assay, suggesting possible blockade of autophagy pathway as a result of drug-drug interaction. Images of Map2-positive dendrites showed increased beading in HIV-Tat treated neurons exposed to cART and morphine. Our findings indicate that the overall positive effect of cART is counteracted by morphine, and imbalances in the autophagy pathway may aggravate cART and morphine interactive affect.

Supported by National Institutes of Health (NIH)-National Institute on Drug Abuse (NIDA) grants R01 DA036154 and R21DA041287

W47.Methamphetamine (METH) and HIV-tat mediated oxidative stress contributes to Mitochondrial dysfunction in microglia.

Parikh, NU, MD[1], Aalinkeel, R, Ph.D.[1], Reynolds, J, Ph.D.[1], Quaye, E[1], Cwiklinski, K, BS[1], Skyes, D, Ph.D.[1], Mammen, M, MD[1], Sharikova, A, Ph.D.[2], Khmaladze, A, Ph.D.[2], Schwartz, S, MD, Ph.D.[1], Mahajan, S, Ph.D.[1]; [1]Division of Allergy, Immunology and Rheumatology, University at Buffalo, Buffalo, NY, 14203 United States. [2]Department of Physics, University at Albany, Albany, NY, 12222 United States.

Methamphetamine (METH) exacerbates the severity and onset of HIV-1 associated neurocognitive disorders (HAND). Exposure to both HIV and METH increases oxidative stress and apoptosis in the brain and leads to neurological impairment. Oxidative stress can lead to an increase in ROS production and cause mitochondrial DNA (mtDNA) damage. Altered mitochondrial membrane permeability results in activation of factors leading to apoptosis. Human microglial cells (CHME5/HIV) immortalized with SV40 T-antigen and co-transfected with an HIV LTR reporter and the HIV Tat gene were treated with METH (10-100μM) and/or HIV tat (10-100ng/ml) and we evaluated the expression levels of pro-apoptotic proteins APAF-1, BAX, Caspases and measured the levels of Mt DNA using real-time qPCR. Our results demonstrate that METH treatment induced LTR activation, which was potentiated in the presence of Tat. Both METH and Tat had additive effects on microglial apoptosis as measured in real time by TUNEL staining and Digital Holographic Microscopy. METH (50μM) alone resulted in a significant increase in the gene expression levels of pro-apoptotic proteins APAF-1 (21.2 fold increase, $p<0.001$), BAX (2.6 fold increase, $p<0.01$), Caspase 3/7 (56% increase, $p<0.05$) and significantly decreased mtDNA content (96% decrease, $p<0.001$). Meth and Tat also increased expression of mitochondrial proteins, Cytochrome C, COX 4 and transcriptional factor NFkB indicating an inflammatory response. Both METH and Tat induce mitochondrial dysfunction, initiating CNS cytotoxicity due to microglial apoptosis.

W48.Functional effects of chronic alcohol stress in human monocyte-derived dendritic cells and the immuno-protective role of trichostatin A as demonstrated by single cell imaging flow cytometry

Parira, Tiyash, MS[1], Granado, Sherly[1], Figueroa, Gloria, BS[1], Agudelo, Marisela, Ph.D.[1]; [1]Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States.

The neuro-protective effects of trichostatin A (TSA) in human CNS cells under acute ethanol (EtOH) exposure has been previously reported by our lab. Recently, we also reported that TSA modulates oxidative stress related genes in human monocyte-derived dendritic cells (MDDCs) acutely treated with EtOH. However, the functional effects of chronic EtOH treatment on the production of reactive oxygen species (ROS) by MDDCs and the protective role of TSA has not been documented yet. In this study, MDDCs were chronically (5 days) treated with 0.2% EtOH in the presence or absence of TSA. Post treatment, ROS was measured using dichlorofluorescin diacetate and single cell imaging flow cytometry. Inflammatory cytokine secretion was also measured from supernatants of treated MDDCs. Our results show EtOH (0.1-0.2%) treatments increase ROS production when compared to control while TSA decreases ROS. In addition, pretreatment with TSA inhibits the effects of alcohol by decreasing EtOH-induced ROS. Cytokine array studies also show TSA reduces secretion of inflammatory cytokines like ICAM-1 and MIP-1β upregulated due to EtOH. Additionally, EtOH and TSA also modulated Nrf2 expression. This study shows TSA effectively reduced alcohol-induced ROS production confirming the anti-oxidant properties of TSA and bringing into light the immuno-protective role of TSA in MDDCs chronically treated with alcohol. Overall, this study highlights TSA as a potential anti-oxidative stress and anti-inflammatory therapeutic drug target.

Supported by National Institute on Alcohol Abuse and Alcoholism, award R00AA021264 and partially by Presidential Fellowship, FIU.

W49.Potential Role of Exosomes in Defense against Ethanol-Induced Oxidative Stress in the Central Nervous System (CNS)

Patters, BJ, BS[1], Kumar, S, Ph.D.[1]; [1]Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN, 38163 United States.

Ethanol exposure to cells of the CNS is associated with oxidative stress generated by multiple sources. To counteract this stress, cells produce an antioxidant response, upregulating and enhancing production of antioxidant elements (AOEs), i.e., antioxidant enzymes, proteins, and small molecules such as glutathione. Exosomes are small (<100nm) membrane-bound vesicles derived from the endocytic pathway, produced and received by many different cell types. We propose that astrocytes and macrophages, two cell types found in the CNS, package AOEs into exosomes as part of their antioxidant response to ethanol exposure. These exosomes are secreted and then picked up by neighboring cells, particularly neurons. We further hypothesize that these exosomes, having delivered their AOE content, will improve the survival of recipient cells in a similar environment of ethanol-induced oxidative stress. We treated U937 monocyte-derived macrophages and SVGA astrocytes with different doses of ethanol, then collected exosomes from the supernatants of the treated cells and co-treated recipient cells (SVGA and SH-SY5Y neuroblastoma cells) with exosomes and ethanol. Data show that exosomes obtained from alcohol-treated donor cells had greater protective effects on recipient cells than exosomes from untreated donors, in an environment of alcohol exposure. We will further characterize the contents of those exosomes through proteomic, metabolomic, and RNAseq analyses to identify the specific exosomal AOEs, providing valuable understanding of a previously unexplored mechanism of antioxidant defense.
Supported by NIH 1R21DA042374-01

W50.Mutation of human dopamine transporter aspartic acid206 displays a neutral effect on basal dopamine transport and attenuates Tat-induced inhibition of transport function

Quizon, PM[1], Yuan, Y[2], Sun, WL[1], Strauss, MJ[1], Zhu, Y[1], Zhou, Y[1], Midde, NM[1], Zhan, CG[2], Zhu, J[1]; [1]Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC, 29208 United States. [2]Molecular Modeling and Biopharmaceutical Center, and Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY, 40536 United States.

HIV-1-associated neurocognitive disorders are attributed to dysfunctional dopaminergic neurotransmission. HIV-1 Tat inhibits the human dopamine transporter (hDAT), leading to increased synaptic dopamine (DA) levels, resulting in dopaminergic neurodegeneration. We identified key Tat recognition binding residues on DAT through integrated computational modeling prediction and experimental validation. This study determined the effect of mutations of aspartic acid206 (D206L) and aspartic acid381 (D381L) on the extracellular loop 2 and 4 regions of hDAT, respectively, on basal and Tat-induced inhibition of DA transport function. Compared to wild-type (WT) hDAT, D206L did not show any changes to Vmax, Km, Bmax, Kd, or IC50 for DA, cocaine, and

GBR12909, but attenuated Tat inhibitory effect on DA transport. In contrast, D381L decreased Vmax by 64%, altered potencies for DA, cocaine, and GBR12909, and did not attenuate Tat inhibition on [3H]DA uptake. Both mutants did not change zinc-induced modulation of either [3H]DA uptake or [3H]WIN35,428 binding as well as basal DA efflux relative to WT hDAT, suggesting no alteration of transporter conformational transitions. These findings suggest that D206L mutant displays a neural effect on basal DA transport, but attenuates Tat-induced inhibition of DA uptake, which provides mechanistic insights into identifying targets on DAT for Tat binding and improving DAT-mediated DA dysfunction. Further characterizing the intermolecular interactions of identified residues on hDAT for Tat binding using multiple mutations is an important ongoing study.

Supported by NIH/NIDA DA035714 and DA041932

W51.Potential Role of Cytochrome P450s and Oxidative Stress in Benzop(a)pyrene-mediated HIV-1 Replication in Monocytic cells

Ranjit, Sabina[1], Sinha, N.[1], Midde, N. M., Ph.D.[1], Kumar, S., Ph.D.[1]; [1]Department of Pharmaceutical Sciences, University of Tennessee Health Science center, Memphis, TN, 38163 United States.

The mechanism for smoking-induced HIV-1 pathogenesis is unknown. Benzo(a)pyrene (BaP), a major carcinogen in cigarette smoke, requires metabolic activation through cytochrome P450s (CYPs) to exert its toxic effects. We propose that HIV-1 replication is aggravated by oxidative stress from reactive oxygen species (ROS), generated in course of CYP-mediated metabolism of BaP. Recently, we have shown that chronic exposure of BaP to U937 monocytic cells and human primary macrophages, induce CYP and antioxidant expression, increase ROS and ultimately cause cell toxicity. Next, we examined the chronic effect of BaP in U1 cells (HIV-1 infected U937 cells), where we observed ~4 fold increase in HIV-1 replication along with an induction of CYP1A1 expression and enzymatic activity. Elevated ROS and massive cell death were also observed. When BaP-exposed U1 cells were treated with antioxidants and CYP1A1 inhibitors, HIV-1 replication decreased. To further confirm the role of CYP1A1 and 1B1 in BaP-induced oxidative stress pathway and HIV-1 replication, we knocked down the gene expression of CYP1A1 and 1B1 in HIV-1 infected human primary macrophages, using SiRNA. Our results showed decrease in the viral replication in CYP1A1 and 1B1 knock-out cells upon BaP exposure. We will further determine oxidative DNA damage and quantify BaP metabolites in HIV-1 infected primary macrophages, using LC-Mass Spectrometry. Our results are clinically relevant as they would help to find a novel therapeutic target to improve drug therapy outcomes in HIV positive smokers.

Supported by UTHSC

W52.Astrocyte expression of Nef can activate a pro-inflammatory response in the small intestine by macrophages

Rivera Ortiz, J, MS[1], Cruz Rentas, M, BS[1], Noel, R, Ph.D.[1]; [1]Biochemistry, Ponce Health and Science University, Ponce, PR, 00732 United States.

Patients diagnosed with the human immune deficiency virus (HIV) and treated with combined Antiretroviral Therapy (cART) often manifest with secondary complications that currently have no identifiable cause. The continued production of viral proteins, such as Nef, is thought to be a cause of the pathologies observed in individuals suffering from HIV even though they are being treated with cART. Nef is an HIV neurotoxin and is known to cause neurological damage and induce systemic damage. Previous work in our lab helped develop a model of learning impairment by expression of Nef by astroctyes in the hippocampus of Sprague Dawley rats. Using this model, we found that Nef can disrupt the blood brain barrier (BBB) and exert peripheral pathology. We have extended this work to gain a better understanding of the toxicity in brain and the small intestine due to Nef. Rats were infused with astrocytes transfected to produce Nef, GFP (control), and naïve. Two days after surgery, the rats were sacrificed and brain and small intestine were collected. We found a qualitative increase in MMP9 expression in brain from Nef treated group. Furthermore, in the small intestine, the Nef group contained more proliferation of pro-inflammatory macrophages. These results suggest that an increase in MMP9 may contribute to the BBB disruption and activate the release of pro-inflammatory cytokines that could contribute to the systemic pathology. Finally, an increase in pro-inflammatory macrophages in the small intestine contributes to the pathogenesis of the organ.

Supported by MD007579, GM106970, R25GM082406

W53.Integrative Model of Medical Past, Inflammation Markers, and Microbiome in HIV Subjects with Sustained Virological Control

Rodriguez-Santiago, RE, BS[1], Sanchez, R, BS[1], Garcia-Justiniano, J, BS[1], Pabon-Cruz, E, BS[1], Yamamura, Y, Ph.D.[1], Rivera-Amill, V, Ph.D.[1]; [1]Ponce Research Institute, Ponce Health Sciences University, Ponce, PR, 00716 United States.

Systemic inflammation due to HIV infection and antiretroviral therapy is a common occurrence in HIV infected subjects. This systemic inflammation has been correlated with a spectrum of comorbidities including mental illnesses. In our study, we seek to link the systemic inflammation in HIV infected subjects with the gut, plasma, and saliva microbiomes along with the comorbidities presented by the subjects. Our methods included sequencing bacterial 16S rRNA, quantification of inflammatory cytokines, and recompilation of medical information of HIV infected subjects. The data collected from these methods were used to characterize the fecal, saliva, and plasma sample bacterial composition and predict correlations between the microbiome composition, microbial metabolism, cytokines, and clinical manifestations of HIV infected subjects. With these observations, we want to correlate microbial translocation/dysbiosis with HIV infection, comorbid diseases, and inflammatory cytokines to produce a model that can be used to develop microbiome based therapeutics for HIV infected subjects.

Supported by NIGMS / 5R25GM082406, NIMHD / G12MD007579, NIMHD/ U54MD007587

W54.The Effects of Antiretroviral Therapies on Oligodendrocyte Growth and Maturation

Roth, LR, BS[3], Zidane, BZ, BS[1], Jensen, BJ, Ph.D.[1], Perlegos, AP, BS[1], Grinspan, JG, Ph.D.[2], Jordan-Sciutto, KJS, Ph.D.[1]; [1]Department of Pathology, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, 19104 United States. [2]Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, 19104 United States. [3]Pharmacology Graduate Group, University of Pennsylvania, Philadelphia, PA, 19104 United States.

About half of HIV+ individuals develop HIV-associated neurocognitive disorder (HAND), a spectrum of cognitive, motor, and behavioral disturbances of varying severity. Combined antiretroviral therapy (cART) has led to significant decrease in the most severe form of HAND; however, less severe forms of the disorder persist in 30-50% of patients. In this post-ART era, white matter pathologies are prevalent, including thinning of the corpus callosum, and reduction in white matter volumes. Moreover, white matter loss is correlated to the duration of ART exposure. A recent transcriptome study comparing untreated and ART-treated patients with HAND showed downregulation of genes critical for oligodendrocyte differentiation and myelin production in patients treated with ART. We hypothesize that ART compounds alter oligodendrocyte differentiation and function, influencing the persistence of HAND. In our lab, previous studies have shown that HIV antiretroviral compounds, Lopinavir and Ritonavir, both of the protease inhibitor class, inhibited oligodendrocyte precursor differentiation, while zidovudine, a nucleoside reverse transcriptase inhibitor, did not. Preliminary studies testing the newest class of HIV antiretroviral compounds, the integrase inhibitors, Raltegravir and Elvitegravir, suggest that Elvitegravir also decreases differentiation of oligodendrocyte precursors while Raltegravir does not. Investigation of the effects of these compounds will provide insights into the white matter changes seen in HAND patients with implications for their contribution to cognitive impairment.

Supported by R01 MH098742 (KJS & JBG)

W55.Prescription opioid Hydromorphone use disrupts gut microbiome resulting in gut barrier compromise and persistent bacterial translocation

Sharma, U, Ph.D.[1], Khatri, R, MD[2], Banerjee, S, Ph.D.[1], Meng, J, Ph.D.[1], Zhang, L, MS[1], Roy, S, Ph.D.[1]; [1]Department of Surgery, University of Miami, Miami, FL, 33136 United States. [2]Department of Surgery, University of Minnesota, Minneapolis, MN, 55455 United States.

Morphine is the gold standard for pain management. Chronic morphine treatment disrupts intestinal epithelial barrier and suppress immune system, which contribute to induction of sepsis. In the current study we investigated the consequence of chronic prescription opioids (fentanyl, oxycodone, buprenorphine, hydromorphone and morphine) use on gut microbial homeostasis and barrier integrity. Wild type mice were treated with prescription opioids for 72 hours. Our results show that hydromorphone treatment resulted in body weight loss, intestinal barrier disruption and consequently increased bacterial translocation. We next investigated the effect of hydromorphone on gut microbiome using bacterial 16s rDNA amplification and miseq250 sequencing. Hydromorphone decreased gut microbial richness, and altered bacterial composition when compared to control. Taxonomic profiling of fecal microbiota demonstrated increase in Proteobacteria and Verrucomicrobia with significant enrichment of Enterobacteriaceae while a decrease in Firmicutes when compared with control. These data indicate that hydromorphone used for pain management may result in the dysregulation of the gut microbiota leading to sustained inflammation and promote disease severity. Increased intestinal permeability results in the invasion of normal microflora and deregulation of the immune response against indigenous microbiota, leading to faster disease development. We represented for first time the effect of hydromorphone on intestinal microbiota dysbiosis in a murine model.

Supported by R01DA037843, R01DA034582, R01DA031202, K05 DA033881

W56.Role of Eph-Ephrin Signaling in perturbing Neuron-Glia Interaction in HIV-1 Induced Neurodegeneration.

SINGAL, M.S.C, MS[1], SETH, P, Ph.D.[1]; [1]CELLULAR AND MOLECULAR NEUROSCIENCE, NATIONAL BRAIN RESEARCH CENTRE, GURGAON, HARYANA, 122051 India.

Glia-Neuron communication is crucial for maintaining brain homeostasis. Eph-Ephrin signaling is pivotal for neuron-glia crosstalk in the brain. EphA4 receptor and its ligand EphrinA3 alter spine density in neurons and glutamate levels in the synapse during neuron-astrocyte crosstalk. Exposure of astrocytes to HIV downregulates the expression of glutamate transporters leading to an increase in the levels of glutamate in the synapse, causing glutamate excitotoxicity. Dendritic spines in neurons are altered in HIV patients. We hypothesized that since Eph-ephrin signaling is regulating the two major phenomena which are the sole reason behind HIV associated dementia, then probably there is some disruption in this pathway due to HIV exposure. These receptors are present on the neurons and its ligand on astrocytes, hence we adopted human Glia-Neuron co-cultures. Astrocytes and neurons were differentiated from human fetal derived primary neural stem cells. The differentiated cells expressed EphA4 receptor and EphrinA3 ligand. These co-cultures when exposed to HIV-1 Tat B protein altered these proteins. We have also observed that glutamate concentrations as well as glutamate transporters on these cells were altered following Tat exposure and it seems to be mediated via the EphA4/EphrinA3 signaling. We are currently employing siRNA approaches to gain novel insights into the role of EphA4/EphrinA3 signaling in HIV-1 induced neurodegeneration, we plan to correlate these in vitro findings with autopsy tissue sections from HIV-1 patients.

Supported by National Brain Research Centre, Core funding

W57.APP cleavage by BACE1 mediates HIV-associated neurotoxicity

Stern, AL, BS[1], Gannon, P, Ph.D.[1], Gelman, B, MD, Ph.D.[2], Kolson, D, MD, Ph.D.[1], Jordan-Sciutto, K, Ph.D.[1]; [1]Perelman School of Medicine, The University of Pennsylvania, Philadelphia, PA, 19104 United States. [2]Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555 United States.

HIV-associated neurocognitive disorder (HAND) persists in 30-50% of HIV+ patients despite viral suppression by antiretroviral therapy. HAND has some symptoms and neuropathological features in common with Alzheimer's Disease (AD). One key feature of AD is accumulation of amyloid-β (Aβ) oligomers, which are generated from amyloid precursor protein (APP) cleavage by BACE1. BACE1 is increased in AD, and BACE1 inhibitors reverse neuron loss and cognitive decline in animal AD models with human trials pending. APP processing is altered in HIV+ patients, but it is unknown whether increased BACE1 or oligomeric Aβ are a feature of HAND. HIV neurotoxicity is attributed to factors released from HIV-infected macrophages including glutamate, and NMDA receptors (NMDARs) mediate HIV neurotoxicity in vitro. Herein, we hypothesize that HIV neurotoxicity is mediated by increased BACE1 and altered APP processing. In support, we observed increased BACE1 and Aβ oligomers in CNS of HIV+ patients. As a model of HIV neurotoxicity, we treated primary rat neurons with supernatants from HIV-infected monocyte derived macrophages (HIV/MDMs) and observed an NMDAR-dependent BACE1 increase. NMDA treatment also increased BACE1 and Aβ. Both pharmacological BACE1 inhibition and genetic loss of APP partially protected neurons from NMDA-induced toxicity. In APP-/- neurons, toxicity was BACE1-independent, indicating that the toxic role of BACE1 is related to APP cleavage. These data suggest that BACE1 and Aβ may contribute to HAND neuropathology, and BACE1 inhibition may have therapeutic potential in HAND.

Supported by R01 MH106967

W58.Pharmacological profile of dopaminergic transmission in HIV-1 inducible Tat transgenic mice

Strauss, MJ[1], Denehy, ED[2], Zhou, L, Ph.D.[1], Sun, WL, Ph.D.[1], Quizon, PM[1], Eans, SO[3], Cirino, TJ[3], Bardo, MT[2], McLaughlin, JP, Ph.D.[3], Zhu, J, MD, Ph.D.[1]; [1]Drug Discovery and Biomedical Sciences, University of South Carolina, Columbia, SC, 29208 United States. [2]Department of Psychology, University of Kentucky, Lexington, KY, 40506 United States. [3]Department of Pharmacodynamics, college of Pharmacy, University of Florida, Gainesville, FL, 32611 United States.

The inducible HIV-1 Tat transgenic (iTat-Tg) mouse model recapitulates many aspects of neurocognitive impairments observed in HIV infected individuals. Tat and cocaine synergistically increase synaptic dopamine (DA) levels by directly inhibiting DA transporter (DAT) activity, ultimately leading to dopaminergic neuron damage. This study determined the pharmacological profile of DA transmission in iTat-Tg mice following a 7-d doxycycline (Dox) treatment. In Experiment 1, HPLC analysis revealed that DA content in the prefrontal cortex (PFC) and nucleus accumbens (NAc) of iTat-Tg mice were increased by 92% and 37%, respectively, compared to control mice. Consistently, DA/DOPAC in the PFC and NAc of iTat-Tg mice was increased by 44% and 26%, respectively. In Experiment 2, the Vmax of [3H]DA uptake in whole brain synaptosomes was increased (29%) in control mice and 9% in iTat-Tg mice following a 7-d Dox treatment. No difference in the Vmax was

found between iTat-Tg and control mice in saline group. These findings demonstrate that Dox itself may increase DAT uptake function. However, the Dox-mediated increase in DA uptake was diminished in iTat-Tg mice, consistent with the enhanced DA content, adding to evidence that DAT function is inhibited by Tat. Moreover, a 14-d exposure to Tat protein was found to potentiate cocaine-conditioned place preference in iTat-Tg, but not control mice. Therefore, the iTat-Tg model offers specific advantages to study the mechanism(s) underlying the HIV associated dysregulation of DA system, cognitive deficits, and increased cocaine-mediated behavior.

Supported by Supported by NIH grant DA035714 and DA041932

W59.Determining on- and off- target excision events of HIV-1 specific gRNAs using GUIDE-Seq

Sullivan, N[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States.

Current antiretroviral therapy has reduced viral loads to undetectable levels, but it does not remove the integrated HIV proviral DNA from the latent reservoirs. The CRISPR/Cas9 gene-editing systems has moved closer towards an HIV cure based on the excision of the entire HIV genome from infected cells by targeting the long terminal repeats (LTRs) at each end of the integrated viral genome. We have deep-sequenced utilizing next generation sequencing (NGS) LTRs from 269 patients enrolled in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. These sequences were used to design packages of 4 and 10 selected molecular gRNA targets (SMRT). The SMRT-4 and -10 packages in silico are able to excise all subtype B LTR in the Los Alamos HIV database and from the CARES Cohort, as well as 4/5 LTRs representing the CNS reservoir. Thus far, current HIV-specific gRNAs tested do not appear to have any off-target excision events, but extensive sequencing is vital to confirm this observation. To understand if there are unwanted of-target events, genome-wide, unbiased identification of DSBs enabled by sequencing (GUIDE-Seq) is being used to determine on- and off-target excision of the previously published Temple A-D and the newly designed SMRT gRNAs. GUIDE-seq allows a short 35bp oligonucleotide (linker) to be inserted where Cas9 induces double-stranded DNA breaks. Sequencing has demonstrated that all gRNAs cut the LTR at their specific location and insert the linker in TZMbl cells. Further NGS analysis will determine any off-target excision outside the LTR.

Supported by This work is supported by NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32

W60.Withaferin A suppresses Beta amyloid in APP expressing cells: Studies for neurocognitive dysfunctions

Tiwari, Sneham, MS[1], Kaushik, A, Ph.D.[1], Jayant, RD, Ph.D.[1], Yndart, A, MS[1], Nair, M, Ph.D.[1]; [1]Department of Immunology, HWCOM, Florida International University, Miami, FL, 33199 United States.

Alzheimer's disease (AD) is a highly prevalent neurodegenerative disease affecting ~36 million globally, characterized by memory loss and progressive neurocognitive dysfunctions. The histopathological hallmarks are the accumulation of extracellular aggregates of amyloid beta (Aβ) and intracellular neurofibrillary tangles of hyper-phosphorylated Tau protein in the CNS. Aβ plaques aggravate in HIV-1 infection and contribute in HAND. To address the issue of toxic plaque aggregation in AD patients and its possible association with HIV infection, we propose a potent compound Withaferin-A (WA), a purified steroidal lactone (active moiety of Withania Somnifera). Aim of our study was to test the efficacy of WA in reducing Aβ aggregation and assess its possible efficacy against HIV-associated neurocognitive deficits. Dose optimization study showed, lower concentrations (0.5-2μM) of WA significantly reduces the Aβ40 production, without any cytotoxic effects in SHAPP cells (neuroblastoma cell line stably overexpressing human APP751). AD array studied showed that WA significantly modulates genes involved in AD signaling pathway. Also, preliminary efficacy study using flow cytometry showed that Aβ production increased significantly in SHAPP cells exposed to HIV. However, an optimized dose of WA (2μM) was able to reduced Aβ to control basal levels. Due to the larger size and hydrophobic nature of WA, it is incapable to cross BBB, we are employing nanotechnology-based approaches for its efficient BBB transmigration and to achieve therapeutic efficacy.

Supported by RO1DA034547-01

W61.Cenicriviroc, anti-JAM-A, and anti-ALCAM are potential therapeutics to prevent preferential transmigration across the BBB of HIV+CD14+CD16+ monocytes: reducing CNS viral seeding and HAND in the ART era

Veenstra, M., MS[1], Byrd, D., Ph.D.[2], Inglese, M., MD, Ph.D.[3], Gama, L., Ph.D.[4], Clements, J., Ph.D.[4], Shiramizu, B., MD[5], Anastos, K., MD[6], Morgello, S., MD[7], Berman, J.W., Ph.D.[8]; [1]Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, 10461 United States. [2]Departments of Neurology, and Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 United States. [3]Departments of Neurology, Neuroscience, and Radiology, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 United States. [4]Molecular and Comparative

Pathobiology, Johns Hopkins School of Medicine, Baltimore, MD, 21205 United States. [5]Departments of Pediatrics, and Medicine, John A. Burns School of Medicine at University of Hawaii, Manoa, Honolulu, HI, 96813 United States. [6]Departments of Medicine, and Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, NY, 10461 United States. [7]Departments of Neurology, Neuroscience, and Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 United States. [8]Departments of Pathology, and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY, 10461 United States.

CD14+CD16+ monocytes enter the CNS in response to chemokines, including CCL2. When these cells are infected, this leads to establishment and reseeding of viral reservoirs, even when people have undetectable viral loads due to ART. Viral reservoirs release early viral proteins and cytokines, despite active ART, that mediate persistent neuroinflammation and neuronal damage leading to HIV associated neurocognitive disorders (HAND). To develop therapeutics that can limit viral (re)seeding of the CNS and subsequent HAND, we examined the mechanism of entry of infected monocytes into the CNS. We show for the first time that HIV+CD14+CD16+ monocytes preferentially transmigrate across the BBB in comparison to uninfected but HIV-exposed HIVexpCD14+CD16+ monocytes. We found that Cenicriviroc, a CCR2/CCR5 dual inhibitor, as well as JAM-A blocking antibody prevent specifically the preferential transmigration of HIV+CD14+CD16+ monocytes, and that ALCAM blocking antibody reduces their preferential transmigration. This indicates that CCR2 and JAM-A may be potential therapeutic targets for HAND. In addition, we found that CCR2 is specifically increased on CD14+CD16+ monocytes from HIV-infected people with HAND, and not on those from people with impairment due to other causes or unimpaired. We also show that increased CCR2 on CD14+CD16+ monocytes correlates with increased peripheral PBMC HIV DNA, and using neuroimaging, that increased CCR2 correlates with decreased neuronal activity in the basal ganglia. This provides strong evidence that CCR2 may be a biomarker for HAND.

Supported by Burroughs Wellcome Fund, NIMH

W62.HIV-1 Vpr induces CCL5 and CXCL10 upregulation in astrocytes mediated by p38-MAPK and NFkB signaling pathways

Velázquez-Pérez, B., BS[1], Noel, R., Ph.D.[1]; [1]Ponce Research Institute, Ponce Health Sciences University, Ponce, PR, 00716 United States.

Even when properly treated, HIV infection produces chronic inflammation producing morbidities like cognitive impairment. Growing evidence suggests that viral gene expression in the brain contributes to progressive neurological damage. Vpr is a neurotoxic HIV protein that can cause neuronal apoptosis and cell cycle arrest. We hypothesize that Vpr also induces inflammatory gene expression in astrocytes and want to understand the signaling pathways that drive this process. The purpose of this study is to determine the role of two different signaling pathways in upregulation of the proinflammatory chemokines CCL5 and CXCL10. Glioblastoma cells (U87) were transfected with a plasmid pEGFP-Vpr and the cells were collected at 24 and 48 hours. In addition, we used chemical inhibitors for p38-MAPK (SB203580) and NFÎ°B (SC-514) to test the role of these signaling pathways in chemokine expression. The mRNA was used to quantify CCL5 and CXCL10 expression by RTPCR. Vpr upregulates CCL5 and CXCL10 mRNA at both 24 and 48 hours. CCL5 levels in supernatants are increased by Vpr at both time points; however, CXCL10 levels were below detection by ELISA. The increases in CCL5 were not prevented by either pathway inhibitor alone; however, Vpr-induced CXCL10 levels were reduced by the NF-kB inhibitor at both 24 and 48 hours and by the p38 inhibitor only at 24 hours. Our data suggest that Vpr induces inflammatory cytokine expression in astrocytes and that the CCL5 induction uses redundant signaling pathways while for CXCL10 the p38 and NF-kB pathways are both required for full induction.

Supported by GM106970, MD007579

W63.Sigma-1r and cocaine interplay in cathepsin b secretion in HIV-1 infected macrophages

Vélez López, O., BS[1], Segarra Marrero, A, Ph.D.[1], Meléndez Aponte, L, Ph.D.[1]; [1]Department of Microbiology Medical School , University of Puerto Rico Medical Sciences Campus, San Juan, PR, 00921 United States.

Pathogenesis of HIV-1 associated neurocognitive disorders (HAND) is mediated through the infiltration of perivascular macrophages into the brain that secrete viral proteins, neurotoxic and inflammatory factors. One of these factors is cathepsin B (CATB), a lysosomal cysteine protease that induces neuronal apoptosis. Interestingly, cocaine potentiates CATB secretion and neurotoxicity in vitro. Cocaine modulates sigma-1 (Sig1R), a non-opioid receptor, and correlates with several effects in macrophages: increased viral replication, oxidative stress and inflammatory cytokines. However, the role of Sig1R in CATB secretion and HIV-1 replication in macrophages is unknown. Our goal is to determine if Sig1R modulation with pharmacological agents can reduce CATB secretion from macrophages. Monocyte derived-macrophages (MDM's) from seven (n=7) seronegative human donors were isolated from blood samples, differentiated and

infected with HIV-1ADA, treated with Sig1R antagonist (BD1047) prior to cocaine treatment for 3, 6, 9 and 12 days post-infection. The same procedure was followed with PRE-084, a specific Sig1R agonist. HIV infection and cathepsin B secretion levels were assessed from supernatants by ELISA. Results demonstrate that activation of Sig1R by PRE- 084 1μM significantly decreased CATB secretion at 11 days post-infection in HIV+ vs. HIV+PRE-084 groups ($p \leq 0.05$) while having no effect on infection. No further differences in infection or CATB secretion after Sig1R antagonist treatments. PRE-084 might be used as a potential therapeutics for HIV-1 cocaine abusing populations.

Supported by NIGMS SC1GM113691, NIMH G12-MD007600 (Translational Proteomics Center) (LMM), R25GM061838 (RISE) and NIDA 1R21DA041018-01

W64.Wnt7a skews macrophage phenotype: Relevance to NeuroAIDS

Wallace, JW, MS[1], Al-Harthi, LA, Ph.D.[1]; [1]Immunology and Microbiology, Rush University Medical Center, Chicago, IL, 60612 United States.

Monocytes infiltrate the central nervous system (CNS) for pathological and/or homeostatic purposes and differentiate into proinflammatory (M1), alternative (M2) macrophages, or a variation of intermediate phenotypes. In HIV infection macrophage/microglia phenotype has been shown to correlate with neuropathological events. One such example is the increased expression of the CD163 scavenger receptor with increasing HIV encephalitic lesion size. Published data from our lab illustrates that beta-catenin; a protein primarily regulated by a family of morphogenetic glycoproteins known as Wnts, is a restrictive factor for productive HIV infection of monocytes. Beta-catenin decreases as monocytes mature, rendering monocyte derived macrophages (MDMs) more susceptible to infection. Our objective is to further evaluate the impact of Wnts on macrophage phenotype and function, specifically as it relates to HIV neuropathogenesis. Culturing monocytes for 7 days with Wnts 1, or 7a recombinant human proteins revealed that Wnt7a inhibited the expression of CD14+CD16-, CD14+CD16+, and CD163 relative to M1 and M2 MDMs. Wnt7a also inhibited secretion of IL1b and DKK1 compared to M1 and M2 MDMs. These data suggest that Wnt7a influences MDM phenotype; ongoing studies continue to assess the phenotype and functionality of Wnt7a treated MDMs. Ultimately, this study will provide a better understanding of how Wnts influence the phenotype and function of MDMs in the CNS.

W65.Testis-specific lactate dehydrogenase (LDH-C4) expressed in lung cancer may accelerate tumor metastasis to the brain

Wang, Yang, MD, Ph.D.[1], Zhang, Hui, MD[1], Wang, Jing, MD, Ph.D.[1]; [1]Department of respiratory and critical care medicine, First affiliated hospital, Zhengzhou University, Zhengzhou, Henan, 450000 China.

LDH-C4 in mammals was previously thought to be expressed only in testis and spermatozoa, further studies has revealed that some kinds of cancers, including lung cancer, breast cancer and melanoma, express LDH-C4. Among them LDH-C4 expresses highest in lung cancer, and it accounts for about half in proportion in tissue samples. These properties of LDH-C4 are beneficial to catalyze the conversion pyruvate to lactate even in the high concentration of lactate. LDH-C4 expressed in cancer may play the crucial role in anaerobic glycolysis and generate ATP rapidly since this is the role of LDH-A4 in common tissues excluding testies and sperms. It is well known lung cancer is apt to spread to the brain, most in parietal lobe and frontal lobe. Edema around tumor is a common CT manifestation of single brain metastases, which infringes mainly of cerebral white matter. Surpringly, we discovered that more lung cancers expressed LDH-C4 have metastasis to the brain. The brain consumes a great amount of ATP and produce a large concentration of lactate. It is thought that an activity-regulated lactate shuttle from astrocytes to neurons would allow neurons to benefit from lactate, thus LDH-C4 may contribute to the tumor metastasis to the brain. These findings could bring an inspiration that the inhibition of LDH-C4 as therapeutic medicine may prevent tumor metastasis of lung cancer to the brain.

Supported by Youth Natural Science Fund in the First affiliated hospital, Zhengzhou University, China.

W66.Gp120 Alters Stability of Microtubules and Interrupts the Transport of Essential Organelles

Wenzel, ED, BS[1], Avdoshina, V, MD, Ph.D.[2], Taraballi, F, Ph.D.[3], Mocchetti, I, Ph.D.[2]; [1]Department of Pharmacology, Georgetown University, Washington, DC, 20057 United States. [2]Department of Neuroscience, Georgetown University, Washington, DC, 20057 United States. [3]Department of Nanomedicine, Houston Methodist Research Institute, Hourson, TX, 77030 United States.

Despite successful antiretroviral drug therapy in the periphery, a subset of Human Immunodeficiency Virus-1 (HIV) positive individuals still display synapto-dendritic simplifications and functional cognitive impairments. These symptoms are referred to HIV-associated neurological disorders.

Considerable experimental evidence indicates that HIV proteins, including gp120, can cause neurological damage similar to that of HIV. However, the full mechanism of gp120 neurotoxicity is still unknown. Recently, our lab demonstrated that intracellular gp120 can bind to assembled neuronal microtubules and can be neurotoxic even without activation of the associated chemokine receptors, CXCR4 or CCR5. We hypothesize that the destabilization of microtubules by gp120 causes impairments in the transport of organelles and essential nutrients for neurons. To test this hypothesis, we used primary rat cortical neurons to test whether gp120 causes alterations in post-translational modifications and therefore changes microtubule stability. We found that gp120 causes a decrease in the acetylation of neuronal specific tubulin and therefore promotes destabilization. To examine the functional consequences of this destabilization, we examined the expression and distribution of essential organelles such as lysosomes (LAMP2) and autophagosomes (LC3). We found that these organelles are aberrantly localized, even after short exposure to gp120. Our data suggest that gp120 alters microtubule stability and transport of organelles which are essential for a functional neuron.

Supported by NINDS/NS079172

W67.Human cervical epithelial cells inhibit HIV replication in macrophages

Xu, Xiqiu, BS[1], Guo, Le, MS[2], Li, Jieliang, Ph.D.[2], Wang, Xu, Ph.D.[2], Liu, Jinbiao, Ph.D.[1], Zhuang, Ke, Ph.D.[1], Gu, Jun, BS[1], Liu, Hang, BS[2], Ho, Wenzhe, MD, M.Ph.[2], Zhou, Li, Ph.D.[1]; [1]School of Basic Medical Sciences, Wuhan University, Wuhan, Hubei, 430071 China. [2]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA, 19140 United States.

Human cervical epithelial cells at the surface of the female reproductive tract participate in the mucosal innate immunity against viral infections. Our early work showed that cervical epithelial cells possess a functional TLR3/RIG-I signaling system, the activation of which can mount an Interferon-γ (IFN-γ)-mediated antiviral response to herpes simplex virus 2 (HSV-2) infection. The present study examined whether the cervical epithelial cell lines End1/E6E7 cell can be immunologically activated and produce cellular antiviral factors that inhibit HIV infection of macrophages, a key target and reservoir for the virus. We found that supernatant (SN) from TLR3 or RIG-I activated cervical epithelial cells cultures could potently inhibit HIV Bal infection of macrophage. This SN action of cervical epithelial cells on HIV was mediated through both IFN-β and IFN-γ, as the antibodies to IFN receptors blocked the SN-mediated anti-HIV effect. Further studies showed that treatment of the cells with SN from cervical epithelial cells cultures induced the expression of IFN-stimulated genes (ISGs: ISG15, ISG56, OAS-1, OAS-2, MxA, and MxB, Tetherin) in macrophages. These observations highlight the importance of human cervical epithelial cells in mucosal innate immunity against HIV infection.

Supported by National Natural Science Foundation of China (81301428 and 81271334) and Development Program of China (“973”, 2012CB518900)

W68.Morphine induced analgesic tolerance is modulated by disruption of gut microbiome and sustained inflammation

Zhang, L, MS[1], Ramakrishnan, S, Ph.D.[2], Roy, S, Ph.D.[2]; [1]Department of Pharmacology, University of Minnesota, Minneapolis , MN, 55455 United States. [2]Department of Surgery, University of Miami, Miami, FL, 33442 United States.

Long term morphine use, as a commonly prescribed opioid analgesic, is associated with multiple negative consequences including analgesic tolerance. We had previously reported that chronic morphine use results in microbial dysbiosis and compromises intestinal epithelial integrity, consequently leading to intestinal bacterial translocation and systemic immune cells activation. In the current study we tested the hypothesis, that sustained inflammation as a consequence of microbial dysbiosis and gut leakiness contributes to morphine-induced analgesic tolerance. We demonstrate that analgesic tolerance was significantly attenuated in TLR4KO mice and TLR2KO mice. In addition, morphine-induced bacterial translocation into liver was abolished in TLR2KO and TLR4KO mice. The pro-inflammatory cytokine, IL-6 and IL1-β, which were significantly higher in the liver homogenates of morphine treated WT animals were lower in morphine-treated TLR2KO and TLR4KO mice. When animals were gavaged with the probiotics VSL#3, which is a cocktail of bacterial communities that were decreased or depleted following morphine treatment, into wild type mice, show that probiotics pretreatment inhibited morphine-induced analgesic tolerance and decreased IL-6 and IL-1β levels in liver in the morphine + probiotics treatment group. Our studies demonstrate that morphine induced microbial dysbiosis results in the dysregulation of immune system through a mechanism that involves TLR2 and 4 implicating that restoration of gut microbiota through probiotics may be a potential adjunct therapy to delay opioid tolerance.

Supported by R01DA037843, R01DA034582, R01DA031202, K05 DA033881

W69.Small molecule ONC201/TIC10 inhibits HIV-1 replication in reservoir cell types in vitro and in mouse brains xenotransplanted with human macrophages

Zhao, RZ, BS[1], Wu, BW, MD[1], Zheng, JCZ, MD[1], Huang, YH, MD, Ph.D.[1]; [1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198-5700 United States.

HIV-1 continues to retain reservoirs in central nervous system, representing a significant challenge for viral eradication. FOXO3a, a transcription factor critical for immune homeostasis, is known to inhibit latent HIV-1 reservoirs. TIC10 is the first potent and stable small molecule FOXO3a activator capable of crossing blood brain barrier. We hypothesize that targeting FOXO3a through TIC10 will inhibit HIV-1 in its reservoir cell types. Viral infection and integration were monitored by HIV-1 reverse transcriptase activity, p24, and two step Alu-based nested PCR, respectively in human primary microglia, monocyte-derived macrophages and peripheral lymphocytes. HIV-1-infected macrophages were intracranially injected into the basal ganglia of NOD/scid-IL-2Rgcnull (NSG) mice. Mice were injected intraperitoneally with TIC10. Brain tissues were homogenized for detection of HIV-1 p24 by Western blot. TIC10, but not its isomer, potently inhibited HIV-1 replication and reduced integrated DNA in infected macrophages, microglia, and lymphocytes in a dose-dependent manner, suggesting that the antiviral activity is specific to TIC10. The reduced levels of HIV-1 replication and integration in the infected cells were associated with FOXO3a activation and the cleavages of PARP and caspase 3, indicating that TIC10 inhibits HIV-1 replication through modulation of FOXO3a and the related apoptotic signaling. TIC10 treatment significantly reduced the levels of HIV-1 p24 in the mouse brains compared with those of the control group, suggesting that there is an antiviral activity of TIC10 in vivo.

Supported by NIH/R03 NS094071

W70.Soybean Lectin Inhibits HIV Infection of Macrophages

Zhou, RH, MS[1], Wang, X, Ph.D.[1], Ma, TC, Ph.D.[1], Wang, H, MD, Ph.D.[1], Ho, WZ, MD, M.Ph.[1], Li, JL, Ph.D.[1]; [1]Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States.

HIV/AIDS continues to be one of the most severe global health problems. Although highly active antiretroviral therapy (HAART) can suppress plasma HIV to undetectable levels, the viruses remain in their reservoirs. In addition, HAART causes the adverse effects and drug resistance viruses. Therefore, it is necessary to develop new and effective agents to prevent and/or treat HIV. We here report that a natural product from soybean, soybean-derived lectin (SBL) could effectively inhibit HIV infection of macrophages in a dose-dependent manner. Mechanistically, SBL enhanced the phosphorylation of IFN regulatory factor 3 (IRF3) in macrophages and induced the expression of IFN-β, which then triggered the JAK/STAT signaling pathway, resulting in the upregulation of IFN stimulated genes (ISGs), including ISG15, ISG56, OAS-1, Viperin and Mx2. In addition, SBL decreased the expression of HIV receptor CD4 and coreceptor CCR5 on macrophage surfaces. Moreover, SBL upregulated CC chemokines (MIP-1α/β and Rantes), the natural ligands of HIV entry coreceptor CCR5. Importantly, SBL had no effect on PBMCs activation. Therefore, SBL could suppress HIV at several steps of its replication, while would be difficult for HIV to develop resistance. These findings indicate the potential of SBL as a therapeutic agent for HIV treatment. However, future studies are needed in order to determine the ex vivo and in vivo impact of SBL on HIV infection.

Supported by National Institutes of Health: DA022177; DA041302; DA040329

W71.Effects of HIV anti-retroviral drugs on oligodendrocyte differentiation via SREBP1 pathway

Zidane, B, MS[1], Monnerie, H, Ph.D.[2], Jordan-Sciutto, K, Ph.D.[1], Grinspan, J, Ph.D.[2]; [1]2Department of Pathology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, 19104 United States. [2]1Department of Neurology, Children's Hospital of Philadelphia, Philadelphia, PA, 19104 United States.

Despite effective viral suppression and decreased mortality rate through combined antiretroviral therapy (cART), approximately half of HIV-positive patients on cART have HIV-associated neurocognitive disorders (HAND). A consistent finding in studies of antiretroviral-treated HIV-positive patients is persistent white matter abnormalities. We have previously shown that the differentiation of oligodendrocyte precursor cells (OPCs) is negatively affected by specific cART agents, but the mechanism behind this is unknown. Ritonavir increases both fatty acid and cholesterol synthesis in adipose tissue by transcriptional activation of lipid metabolism genes via the sterol regulatory element-binding protein (SREBP). We have shown that inhibition of SREBP1 activation in oligodendrocytes reduces the number of differentiated oligodendrocytes suggesting that lipid metabolism contributes to oligodendrocyte differentiation. We hypothesized Ritonavir may inhibit oligodendrocyte differentiation by disrupting SREBP pathway. Immunoblot of oligodendrocytes treated with Ritonavir showed an increase of both SREBP1, and lipid

metabolism enzyme Fatty Acid Synthase (FASN). These findings suggest that Ritonavir may cause altered lipid metabolism in oligodendrocytes via changes in SREBP1 which then interferes with the ability of the oligodendrocytes to fully differentiate. Further studies will elucidate the effects of Ritonavir on SREBP processing and its contribution to the defect in oligodendrocyte differentiation caused by Ritonavir.

3) Posters - Thursday

T1.MRI-assisted magnetically guided CNS delivery of magneto-electro nanoparticles in non-human primate

Ajeet Kaushik, AK, Ph.D.[1], Jose Rodriguez, JR, MS[2], Daniel Rothen, DR, Ph.D.[3], Vinay Bhardwaj, VB, Ph.D.[4], Rahul Dev Jayant, RDJ, Ph.D.[1], Roozbbeh Nikkhah-Moshaie, RNM, Ph.D.[1], Andria Yndart, AY, MS[1], Beatriz Fuentes, BF, Ph.D.[2], Hitendra Chand, HC, Ph.D.[1], Varan Govind, VG, Ph.D.[5], Nazira El-Hage, NE-H, Ph.D.[1], Norma S Kenyon, NK, Ph.D.[6], Madhavan Nair, MN, Ph.D.[1]; [1]Center of Personalized Nanomedicine, Institute of Neuroimmune Pharmacology, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University , Miami, FL, 33199 United States. [2]Interdisciplinary Stem Cell Institute,Miller School of Medicine , University of Miami, Miami, FL, 33136 United States. [3]Division of Veterinary Resources, Miller School of Medicine , University of Miami, Miami, FL, 33136 United States. [4]Department of Biomedical Engineering, Rutgers University, Piscataway, NJ, 08901 United States. [5]Department of Radiology, Miller School of Medicine, University of Miam, Miami, FL, 33136 United States. [6]Cell Transplant Center, Diabetes Research Institute, University of Miam, Miami, FL, 33136 United States.

Magnetically-guided brain delivery of magneto-electro nanoparticles (MENPs ± 25 nm), and established drug nanocarriers capable to exhibit a. c. magnetic field stimuli responsive on-demand drug release, in C57Bl/J mice has been demonstrated earlier by our research group. Our findings confirm uniform distribution of MENP in the brain of mice without causing clinical toxicity and altering neurologic behavior. However, the translation of this brain delivery method for humans is not yet developed due to a mismatch of available static magnet dimension in relation to the human brain size and shape. Aiming to develop personalized nanomedicine to eradicate neuro-HIV/AIDS, we demonstrated magnetically-guided brain delivery of MENPs to the brain of an adult female baboon (Papio hamadryas) using magnetic resonance imaging (MRI) as a navigation tool. An optimized dose of MENP (22 mg/13 kg) suspended in 100 mL PBS was injected into the baboon vasculature via the saphenous vein with a flow rate of 220 mL/hr. After injection, the baboon was placed under static MRI magnetic exposure for 3 hours to achieve magnetically-guided brain delivery. MRI image analysis confirmed MENPs distribution within the brain regions such as basal ganglia, hemisphere, and vertex. The results of histopathology and blood toxicity profile studies confirmed that injected MENPs did not cause any toxicity or metabolic abnormalities. We propose utilizing MRI as a potential navigation tool for the brain delivery of magnetic therapeutic formulations to treat brain diseases for personalized health care.

Supported by RO1-DA042706, RO1-DA027049, R21-MH 101025, RO1-DA 034547, R01-DA037838, and 1R01-DA-040537

T2.DIFFERENTIAL IN VITRO NEUROTOXICITY OF ANTIRETROVIRAL DRUGS

Akay-Espinoza, C, MD[1], STERN, A, BS[1], LEE, RN, BS[1], PANVELKER, N, BS[1], LI, JJ, BS[1], JORDAN-SCIUTTO, KL, Ph.D.[1]; [1]Department of Pathology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, 19104 United States.

Mounting evidence suggests antiretroviral drugs (ARVs) may potentially contribute to the persistence and evolution of clinical and pathologic presentation of HIV-associated neurocognitive disorders (HAND) which impacts 30% - 50% of HIV+ patients in the antiretroviral (ART) era. We previously reported that oxidative stress and unfolded protein response (UPR), with subsequent synaptic damage and neuronal death, induced by two first generation HIV protease inhibitors (PIs), ritonavir and saquinavir, were alleviated by augmentation of the endogenous antioxidant response (EAR). Herein, we determined the potential neurotoxicity of two newer PIs, darunavir and lopinavir as well as integrase inhibitor (II) class ARVs (raltegravir, dolutegravir, and elvitegravir) in vitro. Primary rat neuroglial cultures at 14 - 21 days in vitro were treated with increasing therapeutically relevant doses of ARVs for 4 h - 8 days. Within the II class, only elvitegravir was neurotoxic in a time- and dose-dependent manner. Among PIs, lopinavir but not darunavir was neurotoxic after 2 days. Moreover, lopinavir increased BiP, a UPR marker, and heme-oxygenase 1 (HO-1), an EAR protein induced in response to oxidative stress, whereas mitochondrial membrane potential was decreased with lopinavir treatment. These effects of lopinavir were partially ameliorated by HO-1 induction by CDDO-Im. These findings extend our previous observations in PIs, could be alleviated by EAR induction and provide new information on the potential class- and drug-specific neurotoxic effects of ARVs.

Supported by NIH MH109382 to KJS

T3.Neutral sphingomyelinase 2 regulates amyloid beta levels and size of blood-brain barrier extracellular vesicles in the presence of HIV-1

Andras, IE[1], Garcia-Contreras, M[2], Toborek, M[1]; [1]Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, 33136 United States. [2]Diabetes Research Institute, University of Miami School of Medicine, Miami, FL, 33136 United States.

Elevated amyloid beta deposition was demonstrated in the brains of HIV-infected patients. Not long ago, extracellular vesicles (ECV) were proposed to be significant players in neurodegenerative diseases, particularly in amyloid beta pathology. Moreover, HIV-1 can use the exosomal pathway to its advantage leading to increased viral spread. The blood-brain barrier (BBB) is critical for amyloid beta homeostasis and contributes to amyloid beta accumulation in the brain. Our previous data have shown that HIV-1 robustly increased ECV shedding from human brain microvascular endothelial cells (HBMEC) and elevated ECV exogenous amyloid beta levels. Because ceramide was shown to interfere with exosome production, we investigated the role of a ceramide-generating enzyme, neutral sphingomyelinase 2 (nSMAse 2), in the HIV-1 induced increase in ECV shedding and ECV-amyloid beta levels. Pretreatment of HBMEC with GW 4869, a nSMAse 2 inhibitor, effectively blocked ECV-amyloid beta level increase evoked by HIV-1. Interestingly, this effect was not accompanied by an ECV number decrease, as determined by nanoparticle tracking analysis. Instead, GW 4869 significantly reduced the mean ECV size in the presence of HIV-1+amyloid beta. In summary, these data show that nSMAse 2 deeply affects BBB-derived ECV exogenous amyloid beta levels and ECV size in the context of HIV-1. Our data may contribute to a better understanding of the role of BBB-derived ECV in the HIV-1 related amyloid beta pathology of the brain.

Supported by MH098891, MH072567, DA039576, DA027569, HL126559 and MH063022.

T4.PCR amplification of PBMC-derived proviral HIV-1 infection for next-generation sequencing and defining mutagenic process during latent/persistent infection

Atkins, A[1], Dampier, W[1], Kercher, K[1], Passic, S[1], Zhong, W[1], Williams, J[1], Balashov, S[1], Mell, J[1], Earl, J[1], Pirrone, V[1], Szep, Z[2], Jacobson, J[3], Nonnemacher, M[1], Wigdahl, B[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Medicine, Division of Infectious Disease and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [3]Department of Medicine, Section of Infectious Disease, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

Interruption of antiretroviral therapy (ART) has been shown to result in resurgence of systemic viral load due to activation of latent provirus. However, recent success has been achieved in the removal of HIV-1 from infected cell lines and primary cells from infected patients suggesting a new approach to targeted elimination of latent infection. Bioinformatic analysis of NGS data can be used to design gRNAs that target persistent HIV-1 DNA using the CRISPR/Cas9 system. To pursue this strategy, it is necessary to collect sequence data from ART-suppressed patients. Furthermore, it is important to track genetic variation over time in HIV-1-infected patients; the genetic sequences of latent HIV proviral DNA pools are likely not static in the PBMC compartment or other viral reservoirs. To address this problem, we utilized a collection of patient samples from the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort to develop a series of longitudinal samples from a group of patients facilitating the observation of HIV-1 mutation over time. Presented here is a strategy we have developed to address whole-genome sequencing of latent HIV infection in ART-suppressed patients. We have determined that an effective strategy is to subdivide the HIV-1 genome into overlapping fragments of 1000 (+/-200) bp allowing PCR amplification of each fragment. We then use NGS to acquire sequence data with sufficient depth to identify genetic variants making up less than 1% of the quasispecies in the sampled genome, allowing effective design of CRISPR/Cas9-based cure strategies.

Supported by This work is supported by NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Component PI, BW), NIMH T32

T5.Inflammasome Gene Expression and their role in HIV and Cocaine Induced Apoptosis

Atluri, VSR, Ph.D.[1], Pilakka-Kanthikeel, S, Ph.D.[1], Garcia, G, BS[1], Jayant, RD, Ph.D.[1], Sagar, V, Ph.D.[1], Thangavel, S, Ph.D.[1], Yndart, A, BS[1], Nair, M, Ph.D.[1]; [1]Department of Immunology, Institute of NeuroImmune Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States.

Inflammasomes are known to activate pro-inflammatory cytokines and HIV infection exacerbates the inflammasomes, although the precise mechanisms are yet to be clearly elucidated. In the present report, we have analyzed inflammasome gene expression profile and their role in induction of apoptosis in HIV infected macrophages in combination with cocaine to explore the mechanisms in the HIV associated neuro-immune pathogenesis. We have observed significantly increased HIV infection in HIV infected macrophages in the presence of cocaine that could be due to the downregulation of BST2 restriction factor in these cells. In human inflammasome PCR array,

among different genes involved in inflammasome formation, in HIV infected macrophages in the presence of cocaine, we have observed significant upregulation of NLRP3, AIM2 genes and downstream genes IL-1β and PTGS2. Among various NOD like receptors, NOD2 was significantly upregulated in both HIV alone and HIV plus cocaine treated cells. We have also observed significant ROS production (in HIV and/or cocaine treated cells) which is one of the indirect-activators of inflammasomes formation. Further, we have observed early apoptosis in HIV alone and HIV plus cocaine treated macrophages which may be resultant of inflammasome formation and caspase-1 activation. These results indicate that in case of HIV infected macrophages exposed to cocaine, increased ROS production and IL-1β transcription serve as an activator for the formation of NLRP3 and AIM2 mediated inflammasomes that leads to caspase 1 mediated apoptosis.

Supported by 5R01DA034547

T6.Targeting latent HIV-1 in the brain using state of the art nanotechnology

Avila, Diana, Ph.D.[1], Dikici, E, Ph.D.[1], Yndart, A, BS[2], Tomitaka, A, Ph.D.[2], Daunert, S, Ph.D.[1], Madhavan, N, Ph.D.[2], Toborek, M, MD, Ph.D.[1]; [1]Department of Biochemistry and Molecular Biology, University of Miami, Miami, FL, 33136 United States. [2]Department of Immunology, Institute of NeuroImmune Pharmacology, Florida International University, Miami, FL, 33199 United States.

Brain HIV-1 infection occurs early after the virus reaches the blood, and eradication of brain HIV reservoirs poses numerous challenges. Different approaches have been used to eradicate HIV-1 reservoirs; however, one of the most promising is the use of nanotechnology. Our central goal focuses on the eradication of HIV-1 reservoirs by nanoparticle drug delivery and the reactivation of HIV-1 in latently infected brain reservoirs. We use two different approaches to achieve this goal. In collaboration with Dr. Nair's group (Florida International University), we use magneto-electric nanoparticles (MENPs). In addition, we use G-5 PAMAM dendrimers in combination with SIRT-1 inhibitor (HR-73). The latter approach is based on our recent study on the involvement of SIRT-1 in NFkB activity and HIV-1 replication (Castro et al., FASEB J, 2016). We observed a strong binding between HR-73 and the G-5 PAMAM dendrimer. Importantly, SIRT-1 inhibition increased protein levels of p24 and reduced activation of pSIRT-1 in latently infected brain pericytes. These results suggest that SIRT-1 inhibition could be used as method for HIV reactivation from latent reservoirs. We also examined the cytotoxicity effects of MENPs on primary pericytes and astrocytes, and demonstrated that MENPs +/-Tenofovir have low cytotoxicity at the dose of up to 20 μg/ml in vitro. These results imply that pharmaceutical agents in combination with nanoparticles (MENPs or G-5 PAMAM dendrimers) may be used as therapeutic approaches to deliver HIV-1 activators and/or anti-retroviral drugs into the brain reservoirs.

Supported by R01DA040537 and R01DA039576

T7.Restoration of Rev nuclear-cytoplasm shuttling increases EcoHIV infectivity in vitro and in vivo.

Bertrand, L, Ph.D.[1], Velichkovska, M, BS[1], Leda, A, Ph.D.[1], Toborek, M, MD, Ph.D.[1]; [1]Biochemistry and molecular biology, University of Miami - Miller School of Medicine, Miami, FL, 33136 United States.

HIV has a very selective host tropism, which limits the generation of a suitable animal model. Most models either use high sentient animals (non-human primates) and the simian immunodeficiency virus or use severe combined immune deficiency (SCID) mice reconstituted with a human immune system. Recently, a mouse adapted strain of HIV, EcoHIV, was generated and demonstrated to successfully infect wild type mice and display similar tropism as HIV. This was achieved by replacing gp120 with gp80 of murine leukemia virus. While the change enables efficient HIV entry into mouse cells, blocks to HIV replication remain, in particular due to the function of Rev. In mouse cells, Rev is inefficiently transported between the cytoplasm and nucleus, leading to a deficiency in viral mRNA transport. We hypothesized that restoring sub-cellular transport of Rev may increase HIV replication both in vitro and in vivo. To address this hypothesis, we modified the sequence of Rev using site-directed mutagenesis to add a nuclear export signal to the Rev open reading frame. This modification restored cellular localization for Rev and increased viral replication in vitro. Furthermore, we were able to demonstrate a substantial (over 100 folds in the brain) increase in viral replication in vivo in mouse tissues as compared to the unmodified virus. These results indicate that restoration of Rev Function significantly increases EcoHIV infectivity in mice and produces a robust infection.

Supported by Supported in part by HL126559, DA039576, MH098891, MH63022, MH072567, DA027569, and the Miami Center for AIDS Research funded by

T8.Gesicle mediated delivery of LTR targeted CRISPR/Cas9 decreases HIV proviral activity in HIV-nanoLuc CHME-5 microglia

Campbell, LA, Ph.D.[1], Richie, C, Ph.D.[1], Harvey, BK, Ph.D.[1]; [1]Molecular Mechanisms of Cellular Stress and Inflammation, National Institute on Drug Abuse, Baltimore, MD, 21224 United States.

CRISPR/Cas9 gene editing technology has enhanced our ability to efficiently modify genomic DNA. For example, investigators have utilized virus mediated transgene delivery of CRISPR/Cas9 to target well conserved regions of the Human Immunodeficiency Virus (HIV), leading to decreased infectivity and pathogenesis in vitro and ex vivo. However, viral delivery methods result in extended expression of Cas9 endonuclease, potentially leading to unwanted off-target mutation events. Thus, alternative delivery strategies to enhance the safety of this technology would benefit its translation into human therapies. We have utilized a specialized microvesicle termed a 'gesicle'• to efficiently and transiently deliver CRISPR/Cas9 components targeting the HIV long terminal repeat (LTR). Gesicles are produced by overexpression of vesicular stomatitis virus glycoprotein and package protein as their cargo, thus bypassing the need for transgene delivery. Using both NanoSight particle analysis and western blotting with a specific Cas9 antibody, we verify production of Cas9 containing gesicles by HEK293 cells. Application of gesicles to CHME-5 microglia results in rapid but transient transfer of Cas9, peaking at 4 hours but undetectable at 24 hours post treatment. Finally, gesicle delivery of Cas9 + LTR gRNA to HIV-nanoLuc CHME-5 cells results in site specific mutation of the LTR, reduced basal and LPS/TNF-α stimulated proviral activity, and decreased expression of HIV viral protein Nef. These data suggest that gesicles are a viable alternative approach to deliver CRISPR/Cas9 technology.

T9.Mechanisms of cathepsin B / serum amyloid P complex neuronal dysfunction in HIV infection

Cantres-Rosario, YM, Ph.D.[1], Ogando Vélez, A[2], Plaud, M, MS[1], Gorantla, S, Ph.D.[3], Melendez, LM, Ph.D.[1]; [1]Microbiology and Medical Zoology Department, University of Puerto Rico, Medical Sciences Campus, San Juan, PR, 00935 United States. [2]NeuroID Program, University of Puerto Rico, Rio Piedras Campus, San Juan, PR, 00935 United States. [3]Durham Research Center, University of Nebraska Medical Center, Omaha, NE, 68198-5880 United States.

HIV-associated neurocognitive disorders (HAND) prevail, despite antiretroviral therapy. HIV-infected macrophages infiltrate the brain, induce apoptosis, and secrete cathepsin B (CATB), a lysosomal protease that interacts with serum amyloid p component (SAPC). We have shown that pre-treating macrophage-conditioned media (MCM) with CATB and SAPC antibodies reduces apoptosis. Additionally, we found that recombinant active CATB is internalized by neurons in vitro, depending on the levels of HIV infectivity in MCM. We exposed SK-N-SH neuroblastoma cells to histidine-tagged CATB in culture media alone or with anti-CATB antibody, and localized the histidine tag in neurons by intracellular flow cytometry. CATB was internalized by neurons (52.0%), while pre-treating the media with CATB antibody reduced CATB internalization (34.9%). We then examined the presence of CATB and SAPC in exosomes isolated from uninfected and HIV-infected MCM. Western blots and ELISA showed exosomes contain CATB, SAPC, and express the exosome markers: CD63, CD81 and Hsp70. Finally, we identified CATB/SAPC in brain tissue from an HIV-encephalitis (HIVE) mouse model, and we found increased CATB and cleaved caspase-3 in mice inoculated with HIV-infected MDM. Our results suggest a novel mechanism of neuronal dysfunction in which CATB: (1) is internalized by neurons, a mechanism lessened by cathepsin antibodies; (2) is secreted from macrophages in exosomes that could facilitate neurotoxicity; and (3) is expressed in the brain of HIVE mice, the model that we will use to test CATB and SAPC inhibitors against HAND.

Supported by NIH R24 OD 018546-01, NIGMS SC1GM113691, NIMH G12MD007600, NIH ENDURE NeuroID Program R25NS080687

T10.Critical role of NLRP3 inflammasome in cocaine-mediated activation of microglia: Implications in exacerbated neuroinflammation in HAND

Chivero, ET, Ph.D.[1], Periyasamy, P, Ph.D.[1], Callen, S, BS[1], Guo, ML, Ph.D.[1], Buch, S, Ph.D.[1]; [1]Pharmacology & Experimental Neuroscience, UNMC, Omaha, NE, 68198 United States.

Drug abuse & HIV infection are two linked global health crises since needle sharing is a well-known mode of HIV transmission. Cocaine, often abused by HIV-infected individuals is known to promote HIV replication & glial activation. Detailed molecular mechanisms underlying this phenomenon however, remain unknown. While NLRP3 inflammasome has been implicated in HIV-induced neuroinflammation, the effects of cocaine on this pathway remain unexplored. Since cocaine is known to induce both microglial activation as well as reactive oxygen species (ROS), we hypothesized that cocaine-mediated microglial activation involves ROS & NLRP3 signaling pathways. We examined canonical & non-canonical NLRP3 pathways in microglia & found a dose- & time-dependent induction of NLRP3 expression in cocaine exposed microglia. Cocaine exposure also time-dependently increased mature caspase-1, caspase11 & IL-1β levels suggesting activation of both canonical & non-canonical pathways. Blockade of ROS (NAC) attenuated cocaine-induced NLRP3 priming. Pharmacological inhibition of NLRP3 (MCC950) attenuated IL-1β release. These studies were validated in an in-vivo mouse model administered cocaine for 7 days. NLRP3 & IL-1β were significantly upregulated in the

hippocampus, striatum & cortical regions of these mice. Collectively, these findings suggest that cocaine activates ROS/NLRP3 inflammasome axis in microglia thereby contributing to inflammation. NLRP3 can thus be envisioned as a therapeutic target to alleviate HIV & cocaine-mediated neuroinflammation & likely, subsequent cognitive decline.

Supported by NIDA-ANRS-IAS; R01DA036157

T11.HIV-1 alters expression of gap junctions and adherens junctions in human brain pericytes

Cho, HJ, Ph.D.[1], Toborek, M, MD, Ph.D.[1]; [1]Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, 33136 United States.

Pericytes, uniquely positioned in the neurovascular unit (NVU), have recently drawn more attention given the vital and active nature of their newly identified participation in brain physiology, such as maturation and maintenance of the blood-blood barrier (BBB). Although studies on the BBB have primarily focused on endothelial barrier functions via astrocytes and brain endothelial cells, we reported that low replication of HIV-1 in human BBB pericytes resulted in BBB disruption via increased endothelial permeability (Nakagawa et al., 2012). In the present study, we hypothesize that injury signals are propagated from infected pericytes to neighboring cells, resulting in a general disruption of BBB functions. To address this notion, we investigated these bystander effects by focusing on the role of gap junctions (GJs) and adherens junctions (AJs) between pericytes. A dye coupling assay monitoring the dye transfer between adjacent cells demonstrated a significant decrease after HIV infection. These results suggested that HIV infection alters intercellular communication between non-infected and HIV-infected cells. To determine the effect of HIV infection on expression GJs and AJs in human pericytes, mRNA and protein expression levels of connexins (Cxs, GJ proteins) and N-cadherin (Ncad, AJ protein) were examined using quantitative real-time RT-PCR and immunoblotting. Our results demonstrated a marked upregulation of Cxs and Ncad in HIV-infected human pericytes. In conclusion, HIV infection markedly affects intercellular communication via GJs and AJs in BBB pericytes.

Supported by HL126559, DA039576, MH098891, MH63022, MH072567, DA027569, P30AI073961

T12.Nef Expression in Astrocytes Promotes Astrogliosis and Synaptic Dysfunction

Cruz, ML[1], Cintron, K[2], Rodriguez, Y[1], Valles, Y[1], Noel Jr, RJ, Ph.D.[1]; [1]Department of Basic Sciences, Ponce Health Sciences University-Ponce Research Institute, Ponce, PR, 00717 United States. [2]Research - Biology, University of Puerto Rico - Ponce, Ponce, PR, 00717 United States.

Astrocytes play a crucial role in metabolic support and neuronal function and help prevent excitotoxicity in the CNS. Upon infection with HIV, astrocytes are characterized for producing early viral proteins such as Nef, which is known to cause neurotoxicity. We investigated astrocytes transfected with Nef to understand the effect on learning, astrocyte morphology and neuronal synapses. Primary rat astrocytes expressing Nef or GFP were infused in the hippocampus of 30-day-old Sprague Dawley rats. Learning was assessed by Morris water maze (5 and 10 days after infusion). Brain tissues were collected for double immunofluorescence staining and the morphology of astrocytes and synaptophysin expression in CA3 and dentate gyrus of the hippocampus were assessed using Image J software. GFAP area fraction was significantly increased (P

Supported by GM106970, MD007579, GM0969

T13.Selection of gRNAs to target the HIV-1 quasispecies with CRISPR/cas9

Dampier, W[1], Nonnemacher, M[1], Chung, C-H[1], Allen, A[1], Sullivan, N[1], Desimone, M[2], Pirrone, V[1], Kercher, K[1], Passic, S[1], Williams, J[1], Wigdahl, B[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]School of Biomedical Engineering and Health Systems, Drexel University, Philadelphia, PA, 19104 United States.

Latent HIV-1 persistence even after long-term antiretroviral therapy is a major hurdle to a cure. Genomic editing techniques, like the CRISPR/Cas9 system, hold promise to permanently excise the integrated virus from a host cell. Targets are defined by a 20 nucleotide guide RNA (gRNA) complementary to the desired genomic region. However, due to the rapid mutation rate intrinsic to HIV-1 replication, the virus in patients exists as a collection of distinct genomic variants, termed quasispecies. Presented here is a computational methodology for designing gRNA sequences to cleave a patient's HIV-1 quasispecies. PBMC genomic DNA was isolated from patients in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort and the long terminal repeat (LTR) of the HIV-1 quasispecies was sampled using Next Generation Sequencing (NGS). gRNAs were computationally selected by examining their binding potential across a random training set of 100 CARES patient samples. This screening selected a package of 4 or 10 gRNAs, which cleaved the entire detectable quasispecies of the remaining, unseen, CARES samples an average of 3.4 +/- 1.7 or 5.4 +/- 3 times, respectively. The package was further tested against a national sampling of subtype B North American LTRs from

the Los Alamos National Database and was shown to cleave all sequences. Functional expression of the gRNAs in a TZMbl model have shown knockdown of LTR activity. This work presents a step towards understanding the complex task of using excision therapy to target HIV-1 quasispecies in the infected patient population.

Supported by This work is supported by NIMH R01 MH110360 (Contact PI, BW), NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC,

T14.Examining the Role of Cocaine Abuse on HIV-1 Pathogenesis

Dash, C, Ph.D.[1], Pandhare, J, Ph.D.[1], Jennings, R, BS[1], Berthaud, V, MD[1], Kalams, S, MD[2]; [1]Center for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN, 372 United States. [2]Division of Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN, 37235 United States.

Over 1.2 million people in the United States are infected with the human immunodeficiency virus (HIV). African-Americans continue to bear the disproportionate burden of HIV/AIDS of all racial and ethnic groups. They account for more than half of the HIV-infected persons in the United States. Tremendous progress has been made over the past three decades on many fronts in the prevention and treatment of HIV-1 disease. However, HIV-1 infection is incurable and antiretroviral drugs continue to remain the only effective treatment option for HIV infected patients. Unfortunately, only three out of ten HIV-1 infected individuals in the US have the virus under control. Thus, majority of HIV-1 infected individuals in the US are either unaware of their infection status or not connected/retained to care or are non-adherent to antiretroviral therapy (ART). This national public health crisis, as well as the ongoing global HIV/AIDS pandemic, is further exacerbated by substance abuse, which serves as a powerful cofactor at every stage of HIV/AIDS including transmission, diagnosis, pathogenesis, and treatment. Cocaine is one of the most commonly abused substances among HIV infected individuals. Cocaine abuse has been associated with increased infection, accelerated disease progression and AIDS-related mortality. However, the mechanisms by which cocaine use accentuates CD4+ T cell decline remain largely unclear. Therefore, the goal of this project is to delineate effects of cocaine abuse on HIV-1 associated CD4+ T cell death using peripheral mononuclear cells (PBMCs) from HIV-1 infected coca

Supported by NIDA/NIH

T15.Exosomes released from astrocytes in response to morphine and inflammatory cytokines deconstruct synaptic connections through modulation of microRNA cargo

Datta Chaudhuri, A, Ph.D.[1], Wang, S, Ph.D.[1], Khuder, S, Ph.D.[1], Dastgheyb, R, Ph.D.[1], Trout, A, Ph.D.[2], Yoo, S, Ph.D.[1], Talbot, C, Ph.D.[3], Hao, H, Ph.D.[3], Haughey, N, Ph.D.[1]; [1]Department of Neurology, Richard T Johnson Division of Neuroimmunology and Neurological Infections, Johns Hopkins University School of Medicine, Baltimore, MD, 21287 United States. [2]Sanders-Brown Center of Aging, University of Kentucky, Lexington, KY, 40536 United States. [3]Institute of Basic Biomedical Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, 21287 United States.

Persistent CNS inflammation associated with HIV infection and opiate abuse can impair gliotransmission. Here we investigated how inflammatory cytokines and morphine regulate the release and molecular cargo of astrocyte-derived extracellular vesicles (ADEV), and effects on neuronal structure/function. ADEV shed in response to ATP enhanced dendritic complexity, synaptic maturation, and increased neural network activity. Treatment of neurons with ADEV shed in response to supernatant from HIV-infected macrophages, IL-1β, and morphine impaired neurite outgrowth, reduced dendritic complexity, and neural network activity. To understand the mechanism for this deconstruction of synaptic connections, we determined the microRNA cargo of ADEV. Ten microRNAs (including miR-125a-5p and miR-16) were enriched in ADEV shed in response to IL-1β, and miR-501 was enriched in ADEV secreted in response to morphine. Bioinformatic analysis identified the neurotrophin receptor TrkC and its downstream effector Bcl2 as putative targets of miR-125a-5p and miR-16. ADEV shed in response to IL-1β decreased neuronal TrkC and Bcl2 protein expression. Inhibition of miR-125a-5p and miR-16-5p in ADEV prevented rescued TrkC, Bcl2 expression, and dendritic outgrowth/complexity. These data suggests that morphine may interfere with the trophic response of ADEVs by slowing release, and inflammatory stimuli modify the miRNA cargo of ADEVs to deconstruct neural networks.

Supported by R01MH110246, R01MH077542, R01DA040390

T16.CRISPR/Cas9 mediated activation of astrocytic glutamate transporter, EAAT2 gene

Datta, PK, Ph.D.[1], Shekarabi, M, Ph.D.[1], Deshmane, S, Ph.D.[1]; [1]Neuroscience, Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States.

Solute carrier family 1, member 2 (SLC1A2) encodes the glutamate transporter 2 (EAAT2) protein primarily expressed in astrocytes that reuptakes excess glutamate from the synaptic cleft to prevent excitotoxicity. EAAT2 plays an essential role in cognitive functions and decreased expression of EAAT2 protein is observed in NeuroAIDS. In the current study, we investigated whether engineered transcriptional activation systems based on CRISPR/Cas9 can be harnessed to activate HIV-1 Tat mediated dysregulation of EAAT2 expression in astrocytes. We have developed a stable astrocytic cell line that expresses the deactivated Cas9 (dCas9) protein which includes dCas9 cassette in frame with the catalytic domain of p300 protein. We designed guide RNAs to target the promoter and induce the expression of EAAT2 protein. Using multiple techniques, we demonstrate that the SLC1A2 promoter is induced by the gRNAs in presence dCas9-p300 in the human glioma cell line and primary human fetal brain astrocytes. Induction of SLC1A2 promoter led to increase in EAAT2 mRNA and protein expression. In addition, we demonstrate that the co-transfection of gRNAs with dCas9-p300 can mitigate HIV-1 Tat induced down regulation of EAAT2 RNA in the glioma cell line. Collectively, these results demonstrate that CRISPR/Cas9 system can be used for potential induction of EAAT2 expression not only in NeuroAIDS but also in other neurodegenerative diseases such as ALS and Alzheimer's disease.

Supported by NIH/NIDA

T17.TGF-beta signaling in BMPR2 under-expressing hyper-proliferative smooth muscle cells exposed to cocaine and HIV-protein(s).

Dhillon, NK[1], Dalvi, P[1], Konstantinova, T[1], Sanderson, M[1], O'Brien-Ladner, A[1]; [1]Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS, 66160 United States.

Abstract text not printed at request of authors.

Supported by R01DAO34542, R56HL129875 and R03DAO31589

T18.Transport of acute-phase isoforms of serum amyloid A across the blood-brain barrier

Erickson, MA, Ph.D.[1], Rhea, EM, Ph.D.[1], Salameh, TS, Ph.D.[1], Fernandez, EG, BS[1], Banks, WA, MD[1]; [1]Geriatric Research, Education and Clinical Center, VA Medical Center, Seattle, WA, 98108 United States.

Serum amyloid A (SAA) isoforms 1 and 2 are major acute phase lipoproteins that are primarily produced by the liver in humans and mice. SAA in blood can increase dramatically during infection, and elevated plasma levels of SAA have been observed in HIV patients, drug users, metabolic disease, COPD, rheumatoid arthritis, and many other conditions associated with systemic inflammation. SAA is detectable in the brains of humans and animals with stroke and Alzheimer's disease, and SAA that is overexpressed by the liver can enter the brain and contribute to neurovascular pathology in mice. These data suggest that SAA accumulation in the brain could be a pathologically relevant event in CNS diseases. It is therefore important to develop a better understanding of the mechanisms that regulate SAA entry into the brain. Here, we show that both SAA1 and SAA2 cross the undisrupted blood-brain barrier (BBB) of healthy mice, but the rate of SAA2 transport was approximately 5 times faster than SAA1. Both SAA1 and SAA2 associated with a high molecular weight species in blood, suggesting that both proteins rapidly associate with HDL, their normal chaperone in blood. Transport of SAA1 and SAA2 across the BBB was not saturable, but induction of systemic inflammation by LPS significantly increased the rate of SAA2 transport across the BBB. Our results demonstrate that circulating SAA1 and SAA2 can enter the CNS by crossing the BBB, and indicate that systemic inflammation could potentiate entry of SAA2 into the CNS by increasing its blood levels and its rate of transport across the BBB.

Supported by NIH/R21NS093368 and R01AG046619

T19.Triggering receptor expressed on myeloid cells 2 levels are altered in the brain during HIV-associated neurocognitive disorders

Fields, JA, Ph.D.[1], Spencer, B, Ph.D.[1], Martin, E, Ph.D.[1], Alexeeva, A, BS[1], Gough, S[1], Flores, I[1], Achim, C, MD, Ph.D.[1], Desplats, P, Ph.D.[1]; [1]Department of Psychiatry, University of California, San Diego, San Diego, CA, 93093 United States.

HIV-associated neurocognitive disorders (HAND) affect 50% of HIV+ patients, even those on combined antiretroviral therapy (cART). As HIV+ patients live longer with low-level viral loads, the innate immune system may contribute to neurodegeneration in HAND. Triggering receptor-expressed on myeloid cells 2 (TREM2) activity modulates neuroinflammation and microglial phagocytic activity in several neurodegenerative diseases including Alzheimer's disease and Parkinsons disease. Here, we hypothesized that TREM2 expression may be altered in brains of HAND donors due to HIV-induced inflammation. The TREM2 promoter includes 25 binding sites for the prolific transcription factor CCAAT enhancer binding protein beta (C/EBPB) and both proteins are increased in the frontal cortex of HAND donors. TREM2 and C/EBPB had similar patterns of expression in the brain; both

proteins are increased in neurons and in glia in HIV+ compared to HIV-, but decreased in neurons in more severe HAND. In vitro, TNF-a, but not IL1B, induced increases in TREM2 and CEBPB in neurons. Overexpression of C/EBPB increases TREM2 in neurons, astroglia and microglia; knockdown of C/EBPB reduced TREM2 levels. These studies show that TREM2 levels are altered brains of HAND donors, and TREM2 is expressed in all three cell types in vivo and in vitro. We also show that C/EBPB facilitates transcription of TREM2 downstream of TNF-a in neurons and astroglia. Future studies will focus on the role of TREM2 in these different cell types during HAND.

Supported by NIMH

T20.Mass Spectrometry and Single Cell Imaging Flow Cytometry as screening tools to detect H3 and H4 post-translational modifications after acute and chronic alcohol exposure of human monocyte-derived dendritic cells (MDDC)

Figueroa, G., BS[1], Parira, T, MS[1], Casteleiro, G.[1], Alejandra, L.[1], Gomez-Hernandez, M., Ph.D.[2], Fernandez-Lima, F., Ph.D.[2], Nair, M., Ph.D.[1], Agudelo, M., Ph.D.[1]; [1]Herbert Wertheim College of Medicine, Florida International University (FIU), Miami, FL, 33199 United States. [2]Department of Chemistry and Biochemistry, FIU, Miami, FL, 33199 United States.

Acute alcohol exposure and binge drinking have been shown to increase histone deacetylases (HDACs) in human monocyte-derived dendritic cells. However, whether this increased in HDACs is functionally affecting MDDCs and altering histone modifications has not been elucidated yet. In addition, it is evident that acute and chronic alcohol consumption exert differential effects on our immune system, and these effects may be driven by epigenetic mechanisms. The goal of the current study is to exploit novel tools such as mass spectrometry and single cell imaging flow cytometry to elucidate the effects of acute and chronic alcohol exposure on histone quantity and post-translational modifications (PTMs), which are modulated by epigenetic enzymes like HDACs and histone acetyl transferases (HATs). Therefore, human MDDCs were treated acutely and chronically with alcohol (0.1-0.2%). Total H3 and H4 quantification was assessed by colorimetric assay revealing acute alcohol exposure induces H3 quantity while chronic alcohol has an effect on both H3 and H4 quantity. Besides histone quantity, chronic alcohol significantly upregulated H4 lysine (K) 12 acetylation (ac). Mass spectrometry and single cell imaging flow cytometry were used as screening tools to detect H4K12ac and other PTMs in MDDCs. In summary, this study provides novel insights into the beneficial and detrimental epigenetic effects of acute versus chronic alcohol exposure in human peripheral cells and may elucidate novel epigenetic biomarkers of alcohol abuse.

Supported by NIAAA award # R00AA021264. Startup funds: Department of Immunology, INIP, HWCOM, FIU. GC and AL are funded by R25GM061347

T21.Interferon-beta decreases macrophage heme oxygenase-1 expression: role in HIV neuropathogenesis

Garza, RG, BS[1], Rossi, FP, Ph.D.[1], Gill, AJ, MD, Ph.D.[1], Kolson, DL, MD, Ph.D.[1]; [1]Department of Neurology, University of Pennsylvania, Philadelphia, PA, 19104 United States.

We previously demonstrated that loss of the critical anti-inflammatory enzyme heme oxygenase-1 (HO-1) in the brains of HIV-infected individuals correlates with increased type I interferon responses, neuroinflammation, and neurocognitive impairment. We now show that this loss of HO-1 in HIV-infected brain associates with increased HO-1 RNA expression, and we hypothesize that HIV-associated inflammatory signaling reduces HO-1 protein levels by post-transcriptional mechanisms. We have shown that HIV infection of macrophages decreases HO-1 protein expression and increases release of neurotoxic levels of glutamate. To test whether HIV inflammatory modulators also alter HO-1 expression and glutamate production in macrophages, we assessed effects of selected proinflammatory modulators on HO-1 protein and mRNA expression, glutamate release, and neurotoxicity in human monocyte-derived macrophage (MDM) cultures. Prolonged stimulation of MDM with TNF-alpha and GM-CSF significantly decreased HO-1 protein and RNA expression. Strikingly, prolonged stimulation with interferon-beta decreased HO-1 protein expression (p

Supported by NIH

T22.Notch3/VEGF-A axis is involved in proliferation of pulmonary artery smooth muscle: implications in HIV-associated PAH

Guo, ML, Ph.D.[1], Kook, YH, MS[1], Callen, S, BS[1], Buch, S, Ph.D.[1]; [1]Pharmacology & Experimental Neuroscience, Univeristy of Nebraska Medical Center, Omaha, NE, 68198 United States.

The incidence of pulmonary arterial hypertension (PAH) in HIV (+) individuals is signficantly higher than in HIV negative population. Pulmonary artery smooth muscle cell (PASMC) has been observed with increased proliferation in the lung of HIV infected persons and suggested to play critical roles in the pathogenesis of HIV-associated PAH. However,

the detailed molecular mechanisms underlying HIV-associated PASMC proliferation remain much unknown. In this study, we investigated the effects of HIV-TAT on PASMCs and the pathways underlying these effects. Our results demonstrated that TAT significantly increased the proliferation of human PASMCs. Notch3 was activated during this process and inhibition of Notch3 signaling by either pharmacological (γ-secretase inhibitor, DAPT) or genetic approach (Notch3 siRNA) blocked TAT-mediated proliferation. Vascular endothelial growth factor A (VEGF-A) was identified as a novel downstream molecule following Notch activation. Findings from in vitro studies were further validated by using our archived simian immunodeficiency virus (SIV)-infected monkey lung tissues. Increased activation of Nothch3 signaling and enhanced expression of VEGF-A were revealed in SIV-infected lung tissues compared to SIV(-) controls. Taken together, we demonstrated that HIV-TAT increased the proliferation of PASMC through the Notch3/VEGF-A axis which may be responsible for the increased incidence of PAH in HIV(+) individuals. Our findings implied that targeting the Notch3/VEGF-A axis could be a potential therapeutic approach for HIV-associated PAH.

Supported by NIH/NIDA DA033614, DA035203, DA036157

T23.Regulation of Programmed cell Death Protein 1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) in HIV-1 infection, and Therapeutic Prevention

Haorah, James, Ph.D.[1], Mishra , Vikas, Ph.D.[1], Schuetz, Heather, BS[1]; [1]Department of Biomedical Engineering, New Jersey Institute of Technology, Newark, NJ, 07102 United States.

Combined active antiretroviral therapy (cART) is effective in controlling HIV/AIDS, but not cure. A cure may be achieved by understanding the mechanisms of HIV-infected cell death and discovery of ARTs that can eliminate HIV-1 infection, persistence, and resurgent. We examined these two approaches for possible prevention of HIV/AIDS. We demonstrate that interaction of PD-1/PD-L1 plays critical role in HIV-1 infected cell death. Normal macrophages and microglia express very low levels of PD-1/PD-L1, but we observed a significant upregulation of PD-1/PD-L1 when infected with HIV-1 virus, suggesting the dampening of innate immune response in infected host cells. Conversely, endogenous levels of PD-1/PD-L1 expressed in astrocytes were diminished by HIV-1 infection. This differential expression in microglia/macrophage and astrocytes indicate the mechanisms of PD-1/PD-L1 regulated cell death and defense in HIV-1 infection. Intriguingly, our new antiviral Drug-S not only prevented the HIV-induced upregulation of PD-1/PD-L1, but also effectively inhibited HIV-1 infection and persistence at the low concentration without causing any cell toxicity. Highly permeability of Drug-S across the blood-brain barrier indicates the potential of this drug to inhibit the replication of HIV reservoir in the CNS. Examination of HIV-1 viral components in cell free, elimination of latently infected HIV persistence, and the host cellular signaling pathways during active HIV-1 infection suggest that our antiviral Drug-S could be a novel candidate for a possible HIV-1 elimination.

Supported by Institutional internal support

T24.Inhibition of extracellular vesicle biogenesis halts productive Zika virus infection in human fetal astrocytes

Huang, Y., Ph.D.[1], Li, Y.[1], Zhang, H.[1], Jing, R.[1], Xu, Y.[1], Zhao, R.[1], He, M.[1], Peer, J.[1], Zheng, J.[1]; [1]Department of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198-5930 United States.

Zika virus (ZIKV) is a neurotrophic flavivirus that is capable of infecting humans leading to possible neurological disorders and causing brain abnormalities during fetal development. The ZIKV infectivity in neural target cells remains poorly understood. Here, we found that ZIKV efficiently infected predominantly GFAP-positive primary human astrocytes derived from fetal brains. The infected astrocytes released competent viral particles and exhibited diverse morphologies, indicating a broad spectrum of susceptibility to ZIKV infection. Furthermore, both ZIKV-infected and bystander astrocytes manifested programmed cell death and a progressive cytopathic effect. Interestingly, ZIKV infection in human fetal astrocytes induced a significant increase of extracellular vesicle (EV) biogenesis. EVs from infected cultures generated higher infection levels compared with EVs from mock infected cultures incubated with an equivalent dose of ZIKV, suggesting that ZIKV exploits EVs for efficient viral replication. Consistent with these result, GW4869, a specific inhibitor of neutral sphingomyelinase-2 known to disrupt the biogenesis of EVs, suppressed ZIKV infection and reduced the release of infectious ZIKV virions in astrocytes. Therefore, ZIKV appears to have close association with EVs biogenesis in primary human fetal astrocytes. Strategies aiming to decrease EV biogenesis might be useful in halting ZIKV infection.

Supported by National Institutes of Health: 2R56NS041858-15A1 (JZ), 1R01NS097195-01 (JZ/YH), and R03 NS094071-01 (YH).

T25.Establishment of CRISPR/Cas9 stable astrocytes for gene-editing study for HIV eradication

Huang, Z, Ph.D.[1], Nair, M, Ph.D.[1]; [1]Immunology Department, Florida International University, Miami, FL, 33199 United States.

Human immunodeficiency virus (HIV) infection and associated syndrome (AIDS) remain as a major challenge to health despite the progress made in HIV/AIDS research front and therapeutics. The major reason is that HIV remains in reservoirs and the anti-HIV drugs cannot penetrate to the CNS/brain which serve as major HIV reservoirs. Astrocytes are predominant cells in the brain hosting latent HIV provirus. To remove or disrupt HIV provirus in latently infected astrocytes, one top option is the application of gene-editing technique especially with CRISPR/Cas9 gene-editing technique. We report for the first time, the development of a stable CRISPR/Cas9 astrocytes as a research tool for gene-editing study for HIV eradication. In detail, astrocytes were transfected with a Cas9 mammalian expression plasmid which carries a Cas9 coding sequence and a puromycin selection cassette. Single clones were selected by the selection of puromycin and then the clones were expanded. Cell genomic DNA of the clones were extracted and examined for Cas9 gene genomic integration with multiple PCRs. Cell proteins of the clones were extracted and examined for Cas9 protein expression with SDS-PAGE and Western blot analysis. Furthermore, nuclear localization of Cas9 protein was confirmed by fluorescent immunocytochemistry. In summary, we have successfully established stable Cas9 astrocyte clones for further gene-editing studies of HIV provirus in astrocytes. Moreover, these Cas9 stable cells can be used as novel research tool to study gene functions with a Cas9 mediated gene knockout approach.

Supported by NIH

T26.Role of de-acidified endolysosome in antiretroviral drug-induced amyloidogenesis

Hui, L, MD, Ph.D.[1], Lakpa, L, BS[1], Geiger, J. D, Ph.D.[1], Chen, X, MD, Ph.D.[1]; [1]Department of Biomedical Sciences, University of North Dakota, School of Medicine & Health Science, Grand Forks, ND, 58203 United States.

Combined antiretroviral therapeutic (ART) strategies have effectively increased the long-term survival of HIV-1 infected individuals. However, along with increased longevity comes age-related neurological disorders including a very high prevalence of HIV-1 associated neurocognitive disorders (HAND), as well as, clinical manifestations and pathological features of Alzheimer's disease (AD). However, the pathogenesis of HAND remains unclear, and little is known about how AD-like pathology is developed as a result of HIV-1 infection and/or long-term use of ART drugs. Physicochemically, ART drugs can be classified as being either weak acids or weak bases. This physicochemical property may be of central importance to ART drug-induced AD-like pathology, because weak bases tend to accumulate in endolysosomes and cause de-acidification, whereas weak acids have no apparent effect on endolysosome pH. Here, we tested the effects of a subset of ART drugs on endolysosome pH and amyloid beta (Aβ) protein generation in rat primary cultured neurons. We demonstrated that only those ART drugs that de-acidified endolysosomes, but not those ART drugs that do not affect endolysosome pH, increased Aβ levels. Furthermore, we demonstrated that an agent that acidifies endolysosomes (MLSA-1) blocked ART drugs-induced increases in Aβ levels. Collectively, our data suggest endolysosome de-acidification plays a critical role in ART drug-induced amyloidogenesis.

Supported by This work was supported by, P30GM103329, and R01MH100972, and R01MH105329.

T27.Novel E2F1 splice variants in the Rat CNS

Jackson, DP, Ph.D.[1], Ting, JH, Ph.D.[1], Schleidt, S, BS[1], Lymberopoulos, J, BS[1], Jordan-Sciutto, K, Ph.D.[1]; [1]School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, 19128 United States.

The transcription factor E2F1 classically functions in the cell-cycle G1/S transition and in apoptosis. While predominantly localized to the nucleus in non-neuronal cells, cytoplasmic localization of E2F1 is reported in post-mitotic cells including neurons, cardiac myocytes, and keratinocytes. Investigation of E2F1 levels in the CNS of patients with neurodegenerative diseases including Alzheimer disease, Parkinson Disease, ALS and Huntington disease revealed increased E2F1 levels in neurons of brain regions, suggesting a role for E2F1 in neurodegeneration. We identified two E2F1 alternatively spliced transcripts: one lacks exon 6 and another retains intron 5. We have shown these variants are specific to neuronal cell types including neurons, astrocytes, and oligodendrocytes. Treatment with cyclohexamide or thapsigargin decreased expression of all E2F1 transcripts; however, treatment with anisomycin did not reduce expression of intron 5 retaining E2F1 transcript, despite reducing expression of the other two transcripts. Increasing neuronal activity with KCl led to increased expression of the intron 5 retaining E2F1 transcript, but did not alter expression of the other two E2F1 transcripts. Given the altered sub-cellular localization of E2F1 in neurons and neurodegenerative diseases, its possible E2F1 has an unknown function in the brain. Our data suggest the regulation

of two E2F1 transcripts may be distinct from that of the full-length transcript, underscoring the need for further studies to understand the role E2F1 plays in neurons and neurodegenerative diseases.

Supported by National Institutes of Health

T28.Effects of HIV-1 Tat within the Go/No-Go Task using a Transgenic Mouse Model

Jacobs, I.R., MS[1], Antonucci, A.G.[1], Fergusson, A.B.[1], Manjarres, M.[1], Sabater, S.M., BS[1], Ignatowska-Jankowska, B., Ph.D.[2], Fitting, S., Ph.D.[1]; [1]Department of Behavioral Neuroscience, University of North Carolina, Chapel Hill, NC, 27599 United States. [2]Edmond and Lily Safra Center for Brain Science, Hebrew University of Jerusalem, Jerusalem, Jerusalem, 9190401 Israel.

Due to combined antiretroviral therapy (cART), HIV-1 is now considered a chronic disease with a high prevalence of mild forms of neurocognitive impairments, including problems in memory consolidation, attention, and impulsivity. Using our Tat transgenic (tg) mouse model the present study investigated the effects of HIV-1 Tat on behavior by using the operant conditioning Go/No-Go (GNG) task. Previous in vitro research in our lab demonstrated Tat-induced deficits in dendritic growth and morphology of cultured primary prefrontal cortex (PFC) neurons. The PFC is involved in regulating attention and inhibition; therefore, the GNG task, which places demands on those systems, served as an appropriate task to further investigate Tat effects in vivo. We hypothesized that Tat interacts with impulsivity, indicated by Tat expressing mice (Tat+) that show less inhibition than their wild-type counterparts (Tat-). 26 Tat tg mice were free fed a doxycycline (DOX) diet for two weeks to activate Tat expression. During the following week mice were food deprived to 85% of their initial body weights where they remained throughout the rest of the study. Using standard MED operant chambers, experimenters trained mice on the GNG task for three months over four phases. A 6 sec temporal discrimination differentiated Go and No-Go trials where the No-Go trials involved a 6 sec trace period. A measure of inhibition was taken in a fifth and final phase by comparing the proportion of correct omissions from No-Go trials to the number of incorrect omissions from Go trials. Analyses are on-going.

Supported by NIDA R21 DA041903 and R00 DA033878

T29.FDC-TFH Interactions within CNS Draining Cervical Lymph Nodes of SIV-Infected Rhesus Macaques

Jain, P.[1], Dave, R.S.[1], Sharma, R.K.[2], Muir, R.R.[3], Haddad, E.[3], Gumber, S.[4], Villinger, F.[5], Nehra, A.[1], Khan, Z.K.[1], Wigdahl, B.[1], Ansari, A.[4], Byrareddy, S.[6]; [1]Department of Microbiology and Immunology, and the Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, 19129 United States. [2]Advanced Eye Center, Post Graduate Institute of Medical Education and Research, Chandigarh, Chandigarh, 160 012 India. [3]Division of Infectious Disease and HIV Medicine, Department of Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [4]Department of Pathology & Laboratory Medicine, School of Medicine and Emory Vaccine Center, Emory University, Atlanta, GA, 30322 United States. [5]New Iberia Research Center, University of Louisiana at Lafayette, New Iberia, LA, 70560 United States. [6]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198 United States.

Neuroinvasion by HIV (Human immunodeficiency virus) leads to neurodegeneration and associated neurocognitive disorders. Reservoirs of HIV-1 in the central nervous system (CNS) provide a major challenge for cure strategies. T cells and dendritic cells are thought to gain access and survey the CNS through recently discovered functional lymphatic vessels lining the dural sinuses that are connected to the deep cervical lymph nodes (CLNs). However, the role of CLNs in serving as source of HIV reservoirs remains an open question especially in context to HIV-CNS infection. In this respect, we analyzed CLNs of chronically infected rhesus macaques and detected significant viral burden in CLNs as compared to plasma viral loads. SIV virion trapping was observed on follicular dendritic cells (FDCs) localized in the follicular regions of CLNs. The entrapment of SIV on FDCs within deep CLN germinal centers (GCs) was further demonstrated by immunohistochemistry. Further, the interaction of FDCs with B cells and TFHs (CD4+ T follicular helper cells) involving SIV as antigen was demonstrated within GCs. Interestingly, we found between 73% to 90% of the TFHs cells within CLNs were SIV infected (SIVp27+). Hence, CLN FDCs not only retained SIV but likely exchange the virus with TFHs and B cells. Based on these observations, we infer a novel role for CLNs' FDCs in SIV entrapment and a putative source of viral reservoirs that could traffic from CLNs to the CNS.

Supported by NIAID (R56AI077414)

T30.Development and Delivery of Intranasal Cas9/gRNA Aerosol Nanoformulation to Eradicate Latent HIV-1 in CNS

Jayant, R.D., Ph.D.[1], Kaushik, A, Ph.D.[1], Yndart, A, MS[1], Chand, H, Ph.D.[1], Raghavan, V, Ph.D.[2], Labhasetwar, V, Ph.D.[2], Kuehl, P, Ph.D.[3], Khalili, K, Ph.D.[4], Nair, M,

Ph.D.[1]; [1]Center for Personalized Nanomedicine, Institute of NeuroImmune Pharmacology, Dept. of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States. [2]Dept. of Biomedical Engineering,, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, 44195 United States. [3]Dept. of Biomedical Engineering,, Lovelace Biomedical and Environmental Research Institute, Albuquerque, NM, 87108 United States. [4]Dept. of Neuroscience,, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

The aim of the current study was to develop the Cas-9/gRNA aerosol nanoformulation (NF) for direct CNS delivery for the complete eradication of CNS latent HIV-1 reservoirs. Cas-9/gRNA NF was prepared by simultaneous spray (SS) novel technique using nontoxic PEI-modified polyplex i.e. P(SiDAAr)5P3 transfecting agent as a non-viral vector. The NF was characterized and optimized for different parameter i.e. effects of pressure, distance, nozzle aperture, N/P ratios and time of incubation with respect to transfection efficiency. Also, the effects of Cas9/gRNA plasmid spraying with respect to damage analysis, P(SiDAAr)5P3 condensation ability, cell uptake, cytotoxicity and NF efficacy studies were performed in latent HIV-1 CHME-5 cells. Results showed that volume mean diameters of the polyplex prepared by SS (0.3 mm spray nozzle, N/P-5, air pressure- 5 bar with20 ml/min airflow rate) was 185±7.5 nm in size with +18±2.5 mV surface charge. Further, no degradation of SS sprayed Cas-9/gRNA plasmid was observed compared to non-sprayed plasmid control. NF showed high cell uptake, 70% reduction in HIV-1 LTR levels with the transfection efficiency of > 50±5 %. P(SiDAAr)5P3 polyplex also exhibited lower cytotoxicity (> 90% cell viability) compared to standard jetPEI polyplex (> 65% cell viability) tested in latent CHME-5 cells after 48 hr treatment. Thus, the developed NF is nontoxic in nature and safe for in-vivo use. Currently, we are developing the intranasal aerosol NF and will test the efficacy in BLT mice model as a future personalized nanomedicine for HIV-1 treatment.

Supported by NIH grants: RO1-DA042706-A (MN); RO1-DA037838 (MN) and R01NS092033 (VL)

T31.Cannabidiol (CBD) induces functional CD4+CD25+ FOXP3+ Tregs in response to low level T cell activation

Kaplan, BLF, Ph.D.[1], Dhital, S, Ph.D.[1], Stokes, JV, MS[1], Park, N, Ph.D.[1], Seo, KS, Ph.D.[1]; [1]Department of Basic Sciences, Colllege of Veterinary Medicine, Mississippi State University, Mississippi State, MS, 39762 United States.

It is well known that cannabinoids are immunomodulatory and can either suppress or enhance cytokine production depending on antigen type or amount, cannabinoid concentration, or time of delivery relative to antigen. We had previously reported that CBD induced strong IL-2 production in response to low level T cell stimulation so in this work, we investigated downstream effects of CBD-induced IL-2 production. We hypothesized that CBD would induce Tregs since IL-2 plus TGF-β can induce Tregs in vitro. We compared various concentrations of phorbol ester and calcium ionophore, which we designated suboptimal and ultrasuboptimal (S/o, 4 nM PMA/0.05 μM ionomycin; Us/o, 1 nM PMA/0.0125 μM ionomycin) as low level T cell activators. CBD + Us/o induced CD25 and FOXP3 expression on CD4+ splenocytes, and the effect was more robust using enriched CD4+ cells. We also determined that CBD + Us/o increased FOXP3 expression if pre-purified CD4+CD25- and CD4+CD25+ cells were used. Finally, we demonstrated that CBD + Us/o-induced CD4+CD25+ cells suppressed proliferation of responder splenocytes but CBD + Us/o-induced CD4+CD25- cells were not able to suppress proliferation. Together these data demonstrate that CBD induces Tregs under conditions of low level T cell stimulation.

Supported by NIH/NIGMS P20GM103646

T32.Chronic morphine administration facilitates EcoHIV-induced neurocognitive impairments in mice

Kelschenbach, J, Ph.D.[1], Borjabad, A, Ph.D.[1], Kim, B-H, Ph.D.[1], Gu, C-J, Ph.D.[1], He, H, Ph.D.[1], Chao, W, MS[1], Arancio, O, MD, Ph.D.[2], Potash, M J, Ph.D.[1], Volsky, D J, Ph.D.[1]; [1]Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 United States. [2]Pathology and Cell Biology, Columbia University, New York, NY, 10027 United States.

Mild HIV associated neurocognitive disorders continue to occur at high frequency despite the success of antiretroviral therapies in alleviating immunological abnormalities of HIV infection and delaying onset of AIDS. Data suggest that the molecular processes underlying HAND are triggered without overt symptoms early, possibly during primary infection when absence of adaptive antiviral immunity facilitates virus expansion, neuroinvasion, and establishment of viral reservoirs. Factors, such as drugs of abuse, may facilitate these processes and enhance HAND pathogenesis. Conventional mice infected with a mouse-tropic HIV, EcoHIV, suggests that chronic morphine administration prior to primary HIV infection accelerates HIV neuroinvasion and induction of neurocognitive impairments as assessed by radial arm water maze testing. This finding was accompanied by a significant increase in brain viral load, suggesting that morphine may facilitate viral transit into the brain. Both NCI and the virological changes were reversed in the presence of naltrexone, indicating that

these findings are opiate dependent. We hypothesize that chronic morphine exposure facilitates induction of HAND pathogenesis early in HIV infection when brain reservoirs are being established and molecular processes that predetermine NCI are initiated. The goal of this work is to maximize currently available therapeutics to help individuals at risk for the devastating neurocognitive complications associated with HIV infection, with a focus on drug abusing HIV populations, which are both prevalent and underserved.

Supported by DA037611, MH104145, and DA017618

T33.APOE isoform-dependently affects Tat-mediated HIV-1 LTR transactivation in astrocytes

Khan, Nabab, Ph.D.[1]; [1]Department of Basic sciences, School of Medicine and Health Sciences, Grand Forks, ND, 58203 United States.

Currently 36 million people worldwide are infected with HIV-1, and it is estimated that 50% of those HIV-1 infected individuals have HIV-1 associated neurocognitive disorders (HAND). As the major cholesterol carrier in the brain, apoE genotype has been shown to affects HIV infection and HAND, and apoE4 allele is associated with higher steady-state viral load and accelerated rate of cognitive decline; however, the underlying mechanisms are not clear. HIV-1 transactivator of transcription (Tat), an essential viral protein for HIV-1 viral replication, can be secreted from infected or transfected cells and taken up by bystander cells from extracellular environment via receptor-mediated endocytosis. Because Tat can bind to LRP, one of the major receptors that mediate apoE-cholesterol uptake in the brain, we determined the extent to which different isoforms of apoE affects Tat-mediated HIV-1 LTR transactivation in U87MG astrocytoma cells expressing the LTR-driven luciferase. Compared to apoE2-cholesterol and apoE3-cholesterol, apoE4-cholesterol (co-incubation or post-incubation) is less effective in preventing Tat-mediated HIV-1 LTR transactivation. Similar effects were observed when cells were treated with isoforms of apoE without the incorporation of cholesterol. Furthermore, we demonstrate that a small peptide that contains the apoE receptor-binding region attenuated the Tat-mediated HIV-1 LTR transactivation. These findings contribute to our understanding of the effects of apoE on HIV-1 infection and associated neurocognitive disorders. Thus, apoE mimetic peptide might be

Supported by This work was supported by R01MH100972, and R01MH105329

T34.HTLV-1 infection and neuropathogenesis in the context of Rag1-/-γc-/- (RAG1) and BLT mice

Khan, Z.K.[1], Ginwala, R.[1], Caruso, B.[2], Chew, G.M.[2], Pattekar, A.[1], Corley, M.J.[3], Loonawat, R.[1], Jacobson, S.[2], Sreedhar, S.[1], Ndhlovu, L.C.[3], Jain, P.[1]; [1]Department of Microbiology and Immunology, and the Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, 19129 United States. [2]Department of Microbiology, Immunology and Pathology,, Colorado State University, Fort Collins, CO, 80523 United States. [3]Viral Immunology Section, Neuroimmunology Branch, National Institutes of Health, Bethesda, MD, 20892 United States.

To date, the lack of a suitable small animal model has hindered our quest to understand the immuno- and neuropathogenesis of HTLV-1, the causative agent of chronic disabling neuroinflammatory disease HAM/TSP. Host immune response that plays a critical role in the outcome of HTLV-1 infection could be better tested in the context of humanized (hu) mice. Thus, we infected neonatal and adult Balb/c-Rag1-/-γc-/- (Rag1) as well as Bone marrow-Liver-Thymic (BLT) mice with HTLV-1. Proviral load (PVL) was determined in the peripheral blood, spleen, and other organs by droplet digital PCR. Within blood, PVL and viral protein Tax was detected as early as 2 weeks post-infection (wpi). Tax showed peak expression at 14 wpi in Rag1 mice with continued expression until 16 weeks. Both PVL and Tax expression was considerably higher in the adult Rag1 mice as compared to the neonates with the latter showing less than 20% PVL in the peripheral blood, brain, and liver. Moreover, signs of lymphocytic infiltration with concomitant Tax expression and resulting myelin disruption were observed in the spinal cord and brain of infected mice. Thus far, several members of the CD28:B7 family of co-signaling molecules have been associated with T-cell dysfunctions in HTLV-infected patients. We found increased expression of PD-1, TIGIT and TIM-3 on CD8+ T cells in the CNS of infected hu-mice. This represents the first attempt to establish HTLV-1 neuropathogenesis in the context of Rag-1 and BLT hu-mice suggesting the possibility of developing a small animal model of HAM/TSP for testing innovative therapies.

Supported by NIAID (R56AI077414)

T35.Loss of TH-phenotype in the substantia nigra but not ventral tegmental following HIV-Tat exposure

Khoshbouei, Habibeh, MD, Ph.D.[1]; [1]Department of Neuroscience, University of Florida, Gainesville, FL, 32605 United States.

This study focuses on the effects of HIV-Tat on the excitability of dopamine neurons in the substantia nigra pars compacta (SNc) vs. ventral tegmental area (VTA). Contrary to our hypothesis, in wildtype C57BL6 mice, we found dopamine neurons in the SNc receive less microglial support than VTA dopamine neurons and that HIV-1 Tat exposure decreased firing activity of dopamine neurons, reduced TH immunoreactivity in the SNc but not in the VTA. In addition, we found a 7-day HIV-1 Tat induction did not induce microglial activation in the SNc or VTA, it decreased microglia-neuronal interaction and decreased TH immunoreactivity in the SNc without an effect on the total number of neurons in the SNc. The observed loss of TH immunoreactivity in the SNc might be due to the loss of TH-phenotype in these neurons rather than neuronal death - at least following short-term exposure to HIV-Tat. Utilizing functional assays, our ongoing experiments examine the nature of neuronal-microglial interactions in the VTA and SNc, important in understanding differential susceptibility of dopamine neurons in these brain regions.

Supported by NIDA

T36.Impaired Insulin Sensitivity Indicates Worsening Cognitive Function in HIV-infected Patients

Khuder, S., Ph.D.[1], Kim, S., Ph.D.[1], Letendre , S., MD[2], Marcotte, I., MD[2], Saktor, N., MD[1], McArthur, JC., MD[1], Dickens, AM, Ph.D.[1], Haughey, NJ., Ph.D.[1]; [1]Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD, 21287 United States. [2]HIV Neurobehavioral Research Program and Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, CA, 92093 United States.

HIV and cART are independently associated with increased risk of developing metabolic abnormalities that include altered fat metabolism and insulin resistance. Insulin resistance is characterized by lipolysis of adipocytes, release of fatty acids into circulation and redistribution to ectopic tissues. Here, we sought to determine if insulin sensitivity was associated with cognitive function in HIV. We conducted untargeted lipidomic analysis, targeted clinical and metabolic measures in longitudinal plasma samples from eighty-nine HIV patients from the CHARTER/Hopkins HIV neurology cohort exhibiting longitudinal changes in cognitive status. Baseline hyperinsulinemia, elevated triacylglycerols (TAG), and monoalkyldiacylglycerols (MADAG) with decreased phospholipid (PL) levels were associated with cognitive decline (CD). Elevated plasma c-peptide with a normal c-peptide: insulin ratio suggested that CD was associated with enhanced insulin production, with normal insulin clearance. Low HDL, high LDL/HDL, and an elevated cholesterol/HDL ratio at baseline further supported an association of insulin resistance with CD. Patients with cognitive improvement (CI) showed lower baseline levels of TAG and MADAG, and PL levels compared to patients with stably impaired (SI) cognition. Circulating insulin, c-peptide, and lipoprotein levels were similar in CI and SI, suggesting and association of insulin sensitivity with CI. These results suggest that CD is associated with a pre-insulin resistance phenotype while CI is associated with restoration of insulin sensitivity in HIV infected patients

Supported by NIMH Center for Novel Therapeutics MH075673 R01MH110246 R01MH077542 R01DA040390

T37.Role of CXCR4 in a rat model of cocaine seeking behaviors

Kim, JK, Ph.D.[1]; Unterwald, EM, Ph.D.[1], Ramirez, SH, Ph.D.[1], Razmpour, R[1], Meissler, JJ[1], Connelly, KL[1], Doura, MD, Ph.D.[1], Eisenstein, TK, Ph.D.[1], Rawls, SM, Ph.D.[1], [1]Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140 United States.

The function of chemokines in the central nervous system is not restricted to chemotaxis and neuroinflammation but extends to neuromodulatory actions on circuits that subserve behavior including responses to drugs of abuse. Our study investigated the interplay between neural and immune mechanisms in a rat model of cocaine reinforcement and relapse, as well as the impact of cocaine exposure on the chemokine CXCL12/CXCR4 system in brain reward pathways. Results demonstrate that four days of cocaine place conditioning in adult male rats caused a significant place preference that was dose-dependently antagonized by the selective CXCR4 antagonist AMD3100, and also produced an increase in CXCL12 gene expression in the ventral tegmental area (VTA). In rats trained to self-administer iv cocaine, AMD3100 dose-dependently reduced the number of cocaine infusions obtained. In a model of relapse, AMD3100 attenuated reinstatement to cocaine seeking behaviors produced by a cocaine prime and/or by cocaine associated cues. Further investigation showed that the levels of CXCR4 in the prefrontal cortex and VTA were significantly increased 30 days after cessation of cocaine self-administration. In rats that were injected with cocaine for 7 days followed by a 30-day withdrawal period, CXCL12 in the prefrontal cortex was significantly lower than that in rats injected with saline. These results demonstrate a potential role of CXCL12/CXCR4 in cocaine reinforcement and relapse and highlight the interplay between neuroimmune and brain reward systems.

Supported by NIDA/NIH: P30-DA013429 & T32-DA007237

T38.CIGARETTE SMOKE CONDENSATE (CSC) ALTERS THE CHARACTERISTICS OF MONOCYTE-DERIVED EXOSOMES AND DIFFERENTIALLY REGULATES EXOSOMAL ANTIOXIDANR ENZYNES

Kumar, S, Ph.D.[1], Sinha, N, BS[1], Midde, NM, Ph.D.[1], Haque, S, MS[1], Kashanchi, F, Ph.D.[2]; [1]Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN, 38163 United States. [2]National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, VA, 20110 United States.

Smoking is known to affect the CNS cells including monocyte-derived cells. However, its underlying mechanism is poorly known, except the fact that oxidative stress, perhaps through cytochrome P450 (CYP), is associated with smoking-mediated cytotoxicity. Exosomes, the membrane bound nano-vesicles, produced by many cell types, play a crucial role in inter-cellular cell signaling and are hypothesized to be involved in cytotoxicity. In the present study, we studied the role of cigarette smoke condensate (CSC) on the secretion of U937 monocytic cells-derived exosomes and exosomal proteins. First, we showed that mRNA level of CYP1A1 is 5-fold higher, whereas mRNA of CYP2A6 is 20-fold higher in exosomes than U937 cells. Likewise, exosomal AOEs namely SOD1 exhibited 5-fold, whereas, catalase, GSTK1 and PRDX6 showed a dramatic 100-500-fold increase in mRNA in exosomes compared to U937 cells. Results from western blot showed that only catalase and PRDX6 are detectable in U937 cells-derived exosomes. Further CSC treatment to U937 cells showed altered physical and biochemical characteristics of U937-derived exosomes. Importantly, CSC treatment showed ~2 fold increase in catalase protein, but almost completely abolished PRDX6 protein in the exosomes without affecting their levels in U937 cells. Finally, exposure of these exosomes to naÃ¯ve U937 and SH-SY5Y neuronal cells showed altered oxidative stress and marker proteins of oxidative stress and apoptosis in these cells. Overall, these results suggest an important role of exosomes in mediating cell-cell interaction in the CNS cells.

Supported by NIDA DA042374

T39.The involvement of the endocannabinoid system in HIV-induced amyloid-beta pathology

Leda, AR, Ph.D.[1], Andras, IE, MD[1], Toborek, M, MD, Ph.D.[1]; [1]Department of Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, 33136 United States.

Neurodegeneration and dementia in HIV-associated neurodegenerative disorders (HAND) and Alzheimer's disease (AD) share common pathogenic mechanisms, such as neuroinflammation, amyloid-beta (Abeta) accumulation, and disruption of the blood-brain barrier. Here, we sought to determine the role of the endocannabinoid system in the context of HIV-induced Abeta pathology. HMC3 cells, a human microglia cell line, were pre-treated with Abeta 1-42 peptide and infected with a molecular clone of HIV-1. Cell lysates were collected three and five days post-infection. The results indicated a 50% increase in the cannabinoid receptor 2 (CB2) expression in cells exposed to Abeta alone, compared to untreated controls. At day 5 post-infection, elevated CB2 protein levels were observed in the HIV-1 and HIV-1 plus Abeta groups. There was also a trend of decreased MAGL protein expression in the HIV-1 group as compared to the HIV-1 plus Abeta group. CB2 and MAGL protein expression was also evaluated in the brain of 5XFAD mice, which overexpress mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations, as well as human PS1 harboring two FAD mutations, M146L and L286V. 5XFAD mice are characterized by substantial Abeta accumulation in the brain at the age of 12 months. Our data indicated that protein levels of CB2 and MAGL were decreased in 5XFAD mice compared to wild type mice. Overall, these results suggest a synergistic role of HIV-1 infection and Abeta pathology in initiating endocannabinoid responses.

Supported by NIH MH072567 and MH098891

T40.The potential effects of abnormal β-Amyloid aggregation on HIV-related cognitive disorder in aged rats

Li, HL, MD, Ph.D.[1], Likins, B, MS[1], Mactutus, CF, Ph.D.[1], Chang, SL, Ph.D.[2], Booze, RM, Ph.D.[1]; [1]Department of Psychology, University of South Carolina, Columbia, SC, 29208 United States. [2]Institute of NeuroImmune Pharmacology and Department of Biological Sciences, Seton Hall University, South Orange, NJ, 07079 United States.

The HIV-related cognitive impairment has shown prevalent in aged patients. However, the cause of the worsening cognitive disorder is still unknown. To investigate the neurodegenerative process associated with HIV, we used HIV-1 transgenic (HIV-1Tg) rats (with its background strain, Fisher 344) as a model to study the cognitive deficits and abnormal protein aggregates (β-Amyloid) in the brain. The data of immunohistochemistry staining indicated that an abnormal intraneuronal β-Amyloid accumulation was found in hippocampal CA3 region (1.34 fold increase) and cortex (4.06 fold increase) in HIV-1 Tg rats compared with the F344 control rats. Interestingly, a higher amount of amyloid precursor protein was detected in CA3 region of hippocampus in F344 control

rats (3.81 fold increase) relative to the HIV-1 Tg rats. However, there was no significant difference of amyloid precursor protein expression in cortex between the F344 control and HIV-1 Tg rats. The Western Blot data additionally proved the abnormal increase of β-Amyloid in HIV-1 Tg rats. Further experiments will elucidate the potential effects of intraneuronal β-Amyloid accumulation on the HIV-induced neuronal dysfunction. Collectively, in HIV patients, an accumulation of β-Amyloid suggests that long-term survival with HIV might interfere with the elimination of harmful proteins like β-Amyloid that might worsen the neurodegenerative process and cognitive impairment.

Supported by DA013137, DA026356, HD043680, MH106392

T41.Methamphetamine compromisesTLR3-mediated antiviral response of astrocytes via induction of autophagy

Li, Jieliang[1], Wang, X, Ph.D.[1], Zhou, RH, MS[1], Ho, WZ, MD, M.Ph.[1]; [1]Jieliang Li, Temple University, Philadelphia, PA, 19140 United States.

Methamphetamine (METH), a highly-addictive psychostimulant, has been implicated as a comorbidity risk for neurocognitive dysfunction in HIV-positive individuals. METH users were shown to have greater exhaustion of the immune system. In the present study, we investigated the effect of METH on TLR3-mediated antiviral response of astrocytes and the underlying mechanism(s). METH pretreatment of human primary astrocytes attenuated PolyI:C-induced expression of interferon-stimulated genes (ISGs). Investigation of the mechanism showed that METH increased the expression of Beclin1 and LC3, two key ATGs for the formation of the autophagosome. METH also dose- and time-dependently triggered the endogenous expression and conversion of the autophagic flux marker from MAP1LC3B-I to MAP1LC3B-II. The LC3 puncta were increased in METH-treated pEGFP-LC3 transfected astrocytes. METH-induced autophagy could be blocked by the phosphatidylinositol-3 kinase (PI3K) inhibitor 3-MA, which could also partially reverse the inhibitory effect of METH on TLR3-induced ISGs expression. On the contrary, in the presence of Bafilomycin A1, a proton pump inhibitor that blocks the lysosomal acidification and the autophagosome (AP)-lysosome fusion, the effect of METH on LC3B-II was abolished, indicating that the upregulated conversion was due to the impaired autophagic flux. Collectively, these findings suggest that METH-mediated astrocytes contributes to the dowregulation of antiviral response in astrocytes, which may have implications in the disturbance of innate immunity of the CNS.

Supported by MH109385, DA040329, DA041302

T42.Emodin Inhibits HIV-1 Infection of Macrophages through the JAK-STAT Pathway

Luo, F[1], Wang, XK[1], Wang, H[1], Shi, JC[1], Zheng, WJ[1], Xiong, HR[1], Feng, Y[1], Wang, X[2], Ho, WZ[2], Hou, W[1]; [1]State Key Laboratory of Virology,Institute of Medical Virology,School of Basic Medical Sciences, Wuhan University, Wuhan, Hubei, 430071 China. [2]Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States.

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is one of the natural anthraquinone derivatives obtained from the root and rhizome of polygonum cuspidatum. Previous studies have shown that emodin exhibits a great variety of anti-viruses properties. We thus initiated this study to investigate its antiviral activity on HIV-1 infection and explore the possible mechanisms of the anti-HIV-1 action in human monocytes-derived macrophages (MDM). It was demonstrated that emodin can inhibit HIV-1 replication in a time- and dose-dependent manner after HIV-1 infection. Especially, eight days after HIV-1 challenge, addition of emodin to chronically infected MDM cultures can significantly down-regulated the level of p24 during the subsequent course of infection. Further study showed that emodin potentiated the production of type I IFN(IFN-αand IFN-β) and induction of multiple antiviral cellular factors(ISG56, APOBEC3G/F and TRIM-5α). The more data identified that emodin promotered the expression and phosphorylation of STAT-1 and STAT-2 in JAK-STAT signaling pathway. In conclusion, emodin represents a potential anti-HIV-1 agent that induces type I IFN expression and activation of JAK-STAT signaling pathway.

Supported by Natural Science Foundation of Hubei Province of China(Key Program)

T43.Human transplanted astrocytes into NSG-HuPBMCs mice demonstrate astrocyte-initiated HIV spread from the brain to other tissues

Lutgen, V., Ph.D.[1], Narasipura, S.D., Ph.D.[1], Richards, M., Ph.D.[1], Robinson, K., BS[1], Al-Harthi, L., Ph.D.[1]; [1]Immunology/Microbiology, Rush University Medical Center, Chicago, IL, 60612 United States.

Given the inability for invasive studies to examine the role of astrocytes as a reservoir for HIV in humans coupled with the inability of HIV to infect non-human astrocytes, we developed a novel humanized astrocyte/human peripheral blood mononuclear cells (PBMCs) NOD/scid-IL-2Rgc null (NSG) mouse models using either neonatal pups or adult mice. Neonatal (PND1) NSG pups were injected with normal human astrocytes (NHAs) either uninfected or infected with HIV-GFP

(NLENG1-IRES 70), with or without VSVG pseudotyping. Pups were reconstituted at 6 weeks. By 10 weeks, NHAs continue to survive, proliferate, and migrate throughout many areas of the forebrain. To circumvent time required for neonatal studies, we bilaterally microinjected HIV-infected or uninfected NHAs into the adult (5-6 weeks old) NSG striatum, reconstituted with huPBMCs and sacrificed 4 weeks later. NHAs survived in adult mouse, however, there is less proliferation and little migration at this time point. We found HIV in brain, blood, spleen and lymph nodes in about 80% of the infected animals by HIV DNA or RNA detection or GFP expression. Further, injection of free virus into the brain did not lead to HIV detection in the mouse. These data demonstrate that HIV-1 infected astrocytes are capable of harboring replication competent virus in vivo that can egress from the brain to seed the periphery. Given that astrocytes make up ~60% of the brain cells and rate of infection is around 1-2%, astrocytes can be a significant reservoir for HIV that is not compartmentalized in the brain but can disseminate to other tissues.

Supported by NINDS, NIMH

T44.Human Immunodeficiency Virus infection compromises the inositol phosphate metabolism in astrocytes: Role in survival of HIV CNS reservoirs

Malik, S, Ph.D.[1], Eugenin, EA, Ph.D.[1]; [1]Public Health Research Institute, New Jersey Medical School-Rutgers University, Newark, NJ, 07103 United States.

HIV/AIDS has claimed more than 35 million lives worldwide. Combined anti-retroviral therapy has been tremendously successful in reducing peripheral viral load in HIV infected individuals, but complete eradication of the virus has not been achieved. Prevalence of HIV-Associated Neurocognitive Disorders in HIV infected individuals with no detectable viral replication indicates persistence of virus in brain cells including perivascular macrophages, microglia and a small population of astrocytes. Our laboratory has shown that apoptotic signals (Ca2+ and Inositol triphosphate, IP3) generated in these few HIV infected astrocytes are transferred to uninfected cells through gap junctions (GJ) leading to aggravation of HIV neuropathogenesis. In the present study, we observed that HIV infected astrocytes had elevated levels of IP3, a cellular second messenger, as compared to uninfected cells. The increased levels of IP3 did not result in calcium release suggesting that HIV alters the IP3/ IP3 receptor axis to survive infection. Furthermore, we detected that HIV infection increased expression of inositol hexakisphosphate kinase-1 (IP6K1) and inositol multikinase (IPMK), two enzymes key in IP processing. Analysis of IP3 receptors revealed altered expression and localization. Blocking GJ channels between the few HIV infected astrocytes and uninfected cells did not prevent increase in IP3 in HIV infected cells, but prevented diffusion and apoptosis in uninfected neighbouring cells. Our data has identified several new mechanisms of bystander damage and survival of HIV infected astrocytes.

Supported by NIH MH096625

T45.Compromised intestinal barrier function by opioids accelerates HIV disease progression

Meng, J, Ph.D.[2], Banerjee, S, Ph.D.[2], Zhang, L, MS[1], Sindberg , G, Ph.D.[1], Buch, S, Ph.D.[3], Segura, B, MD, Ph.D.[4], Bauman, B, MD[4], Roy, S, Ph.D.[2]; [1]Department of Pharmacology, University of Minnesota, Minneapolis, MN, 55455 United States. [2]Department of Surgery, University of Miami, Miami, FL, 33442 United States. [3]Department of Pharmacology and Experimental Neuroscience, Nebraska Medical Center, Omah, NE, 68198 United States. [4]Department of Surgery, University of Minnesota, Minneapolis, MN, 55455 United States.

Substantial epidemiologic and clinical studies have established the correlation between opioid abuse and HIV infection. Emerging studies also demonstrate that opioids promote disease progression in patients infected with HIV. However, the mechanisms underlying the complex interactions between opioid use and HIV infection remain elusive. Recent studies reveal that compromised gut barrier integrity at early stage HIV infection is linked to chronic inflammation and disease progression. In the current study, the gut tissues from patients, rhesus macaques (RMs) and BLT humanized mice were analyzed. The results show that opioids compromise intestinal epithelial barrier function in infected individuals by inhibiting GDNF production, the major growth factor promoting intestinal barrier maturation. Compromised barrier function induced intestinal inflammation was associated with goblet cell hyperplasia in the gut epithelium. The imbalance between tight junction and mucin expression in the intestinal epithelium were consistently observed in the HIV-infected patients abusing opioids and HIV-infected humanized BLT mice treated with morphine, validating that humanized BLT mouse is a good model to investigate disease progression during HIV infection.

Supported by R01DA037843, R01DA034582, R01DA031202, K05 DA033881

T46.Assessment of hepatic clearance, CYP3A4 inhibition, and cellular pharmacokinetics of darunavir in the presence of alcohol

Midde, NM, Ph.D.[1], Gong, Y, MS[1], Li, J, MS[2], Li, W, Ph.D.[2], Meibohm, B, Ph.D.[1], Kumar, S, Ph.D.[1]; [1]Pharmaceutical

Sciences, University of Tennessee Health Science Center, Memphis, TN, 38163 United States. [2]Shanghai Key Laboratory of New Drug Design, East China University of Science and Technology, Shanghai, Shanghai, 200237 China. The prevalence mild to moderate level alcohol consumption is 2.5 times higher in HIV+ people than general public. Studies showed that alcohol use reduces the response and adherence to antiretroviral therapy (ART), but no study on how alcohol manipulates the metabolism of darunaivr (DRV) has been reported. Current study examined the effects of physiological concentration of alcohol (20 mM) on Cytochrome P450 (CYP) 3A4-medaited DRV metabolism with or without its pharmacoenhancer ritonavir (RTV). All in vitro work was performed in either human liver microsomes or HIV-infected monocytic (U1) cells. CYP3A4 and DRV/RTV docking was performed using GOLD suite. Alcohol treatment significantly decreased the apparent half-life and increased degradation rate constant of RTV-boosted DRV but not for DRV alone. Similarly, alcohol exposure increased the hepatic intrinsic clearance for RTV-boosted DRV with no significant influence on DRV treated microsomes. In addition, inhibitory potencies of DRV were significantly reduced in the presence of alcohol suggesting that alcohol alters DRV-CYP3A4 interaction. Alcohol exposure decreased intracellular DRV exposure levels with limited effect on HIV p24 levels in HIV infected monocytic cells. Our docking results projected that alcohol increases the average distance between DRV and heme iron of CYP3A4. Taken together, these outcomes suggest that alcohol influence may have greater effect on DRV metabolism in the liver than at the cellular level. Future studies in HIV infected human primary macrophages are needed to clinically validate these findings.

Supported by NIAAA/NIH AA-022063

T47.Oncostatin M is a potential therapeutic target in HIV-associated neuronal excitotoxicity

Moidunny, S., Ph.D.[1], Matos, M., Ph.D.[2], Wesseling, E., MS[3], Banerjee, S., Ph.D.[1], Volsky, D.J., Ph.D.[1], Cunha, R.A., Ph.D.[2], Agostinho, P., Ph.D.[2], Boddeke, H.W., Ph.D.[3], Roy, S., Ph.D.[1]; [1]Department of Surgery, Miller School of Medicine, University of Miami, Miami, FL, 33136 United States. [2]Center for Neuroscience of Coimbra, University of Coimbra, Coimbra, Coimbra, 3004-517 Portugal. [3]Department of Neuroscience, University Medical Center Groningen, University of Groningen, Groningen, Groningen, 9713GZ Netherlands.

Elevated levels of oncostatin M (OSM), an interleukin-6 cytokine family member, have been observed in HIV-1-associated neurocognitive disorders (HAND) and Alzheimer's disease. However, the function of OSM in these disease conditions is unclear. Since deficient glutamate uptake by astrocytes is instrumental in HAND-associated neurotoxicity, we hypothesized that OSM impairs glutamate uptake in astrocytes and thereby promotes neuronal excitotoxicity. Primary cultures of mouse cortical astrocytes, neurons, microglia, and BV2 cell line were used. The expression of glutamate transporters (GLAST/EAAT1 and GLT-1/EAAT2) was investigated using real-time PCR and Western blot, and their activity was assessed by measuring 3H-D-aspartate uptake. Neuronal toxicity was measured using the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl-) 2,5-diphenyltetrazolium bromide) assay and immunocytochemistry. A chimeric HIV-1 that infects murine cells (EcoHIV) was used to investigate whether the virus induces OSM, OSMR-β (OSM receptor), gp130, GLT-1, GLAST (mRNA and protein) and OSM release (ELISA) in cultured BV2 cells, primary microglia or astrocytes. Statistical analyses of the data were performed using one-way ANOVA (to allow multiple comparisons) and two-tailed Student's t-test. OSM treatment (10 ng/mL) time-dependently reduced GLAST and GLT-1 expression, and inhibited 3H-D-aspartate uptake in cultured astrocytes in a concentration-dependent manner, an effect prevented by the JAK/STAT3 inhibitor AG490. Down-regulation of astrocytic glutamate transport by OSM resulted in NMDA receptor-dependent ex

Supported by NIH - R01 DA031202

T48.SDG Mediated HO-1 Induction and Partial Inhibition of Viral Replication in HIV-infected Human Macrophages

Nieves-Rosado, H, BS[1], WIlliams, K.S., Ph.D.[1], Pu, S, BS[1], Jordan-Sciutto, K, Ph.D.[1]; [1]Pathology, University of Pennsylvania, Philadelphia, PA, 19104 United States.

Under normal conditions, macrophages/microglia (M/M) activate the endogenous antioxidant response pathway to decrease oxidative stress and inflammation by increasing heme oxygenase-1 (HO-1) levels. However, HO-1 levels have been shown to be reduced in HIV (+) patients with HIV associated Neurocognitive Disorders (HAND). Further, prolonged HO-1 deficiency in macrophages has been negatively correlated with increased HIV replication in human macrophages. In this study, we investigated the role of a flaxseed lignin, secoisolariciresinol diglucose (SDG), on oxidative stress in HIV-infected macrophages. To evaluate HO-1 expression, human monocyte derived macrophages were infected with HIV over the duration of a 15 day infection. By immunoblot, HIV increased HO-1 levels during early infection but suppressed HO-1 levels in macrophages by 12 days post infection. Also, a decline in HO-1 levels correlated with increased HIV replication. To determine if SDG can reverse prolonged HO-1 deficits, macrophages were pretreated with SDG for 1 hour prior

to infection and replenished every day. SDG treatment increased HO-1 protein levels after 12 days post infection. Also, pretreatment of SDG partially suppressed viral replication 12 days post infection in human macrophages, assessed by a reverse transcriptase assay. Given these data, SDG has the potential to increase HO-1 levels and reduce productive viral replication in macrophages.

Supported by NIH/R25MH080663, NIH/MH106967

T49.Nanoelectroporation of magneto-electric nanoparticles as drug nano-carriers into the brain cells

Nikkhah-Moshaie, Roozbeh, Ph.D.[1], Kaushik, A, Ph.D.[1], Sinha, R, MS[2], Bhardwaj, V, Ph.D.[3], Atluri, V, Ph.D.[1], Jayant, R.D., Ph.D.[1], Yndart, A, MS[1], Pala, N, Ph.D.[2], Nair, M, Ph.D.[1]; [1]Florida International University, Herbert Wertheim College of Medicine, Department of Immunology, Miami, FL, 33199 United States. [2]Florida International University, College of Engineering and Computing, Department of Electrical and Computing Engineering, Miami, FL, 33174 United States. [3]Rutgers University, Department of Biomedical Engineering, Piscataway, NJ, 08854 United States.

Significant advanced researches have been conducted towards the development of an effective personalized nanomedicine to cure a targeted disease. The efficacy of designed therapeutic formulations has been improved by high cell uptake through nanoelectroporation (NEP). Various methodologies have been established to achieve NEP, however the related detailed mechanism is not demonstrated yet. In this research, for the first time, we report cell uptake of magneto-electric nanoparticles (MENPs), a potential drug nano-carrier, through NEP under alternating current (a.c.) magnetic field. The NEP of MENP is confirmed by TEM of thin layers of the cells prepared by dual focused ion beam (FIB). In addition, the findings are validated by a numerical simulation model. We developed an in-vitro model using microglial cells, very sensitive cells to viral infection, to perform NEP using a customized electromagnetic coil. The a.c. magnetic field of various strength was applied for NEP of MENP, and cells were processed for TEM confirmed biodistribution of MENP inside the cells. The cytotoxicity study indicates that applied a.c. magnetic field ≤ 60 Oe was non-toxic, however a.c. magnetic field of 80 Oe caused toxicity resulting in cell damage confirmed by SEM. The FIB-assisted TEM experiments revealed the uniform distribution of MENP inside the cell without aggregation and compromising the chemical integrity. We believe that demonstrated NEP of MENP can be utilized at large scales to achieve high cell uptake of MENP-based personalized nanomedicine with on-demand controlled release.

Supported by NIH/RO1-DA027049 - NIH/RO1-DA 034547

T50.Alcohol influences HAND via astrocyte-TLR4 and cPLA2 cross-talk

Pandey, R, Ph.D.[1], Ghorpade, A, Ph.D.[1]; [1]Institute of Molecular Medicine, University of North Texas Health Science Center, FORT WORTH, TX, 76107 United States.

Over the past few decades, ~25 million people died with human immunodeficiency virus (HIV)-1 disease. About 70% of HIV patients suffer from HIV-associated neurocognitive disorders (HAND), & the prevalence of alcohol abuse among HIV-1+ve individuals is estimated to be 2-3 times that of the general population in the US. HIV patient brains harbor up to 20% HIV-infected astrocytes, thus making them critical players in HAND. Previously, we reported that HIV-1 &/or alcohol activated astrocytes induced inflammation via cytosolic phospholipase A2 (cPLA2) activation. How EtOH regulates HIV-1-mediated inflammatory episodes initiated at the cell surface level is still unclear. Toll-like receptor (TLR) signaling in immune cells, astrocytes, microglia and neurons may play roles in pathogenesis of multiple diseases including HIV-1. Here, we showed EtOH, HIV-1, IL-1β & anti-retroviral (ARV) drugs significantly upregulated TLR4 in human astrocytes. Our results established that EtOH+/- HIV-1 activated TLR4 signaling leads to IRAK4 phosphorylation followed by NF-kB activation, ultimately leading to excessive production of inflammatory mediators such as COX2 & CCL2. EtOH &/or HIV-1 increased inflammatory molecules in MyD88-dependent manner. TLR4-RNAi studies reversed EtOH &/or HIV-1-regulated effects. Moreover, on silencing TLR4, the increase in EtOH+/-HIV-1-induced cPLA2 phosphorylation was not observed. Thus our study demonstrated the cross-talk of EtOH-mediated TLR4 & cPLA2 cascade in HAND. Hence, TLR4 could be the master regulator of alcohol-induced astrocyte inflammation with HIV-1.

T51.HIV and methamphetamine-induced brain oxidative stress is mediated by reduced level of Mn-superoxide dismutase (SOD2)

Park, M, Ph.D.[1], Leda, A, Ph.D.[1], Barral, E[1], Cambow, D[1], Levine, H[1], Toborek, M, MD, Ph.D.[1]; [1]Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, 33136 United States.

Although HIV-positive methamphetamine (METH) users exhibit more severe neurological complications than non-users, the mechanisms of the combined effects of HIV and METH are still unclear. We hypothesize that mitochondrial damage caused by HIV and METH exposure plays a role in the development of neurotoxicity. This study was based on a chronic exposure to METH (5 days with escalating doses at 3 h intervals), followed by EcoHIV infusion into C57BL/6J mouse

brain. Brains were collected one week post EcoHIV infusion. Strong immunoreactivity for GFAP (a marker for astrocyte activation) was detected in the hippocampus and caudate-putamen regions in METH-EcoHIV group compared to control, or single-treated groups. A distinct pool of GFAP-positive cells was detected in proximity of brain capillaries, suggesting disruption of blood-brain barrier (BBB) integrity. Indeed, BBB permeability in the hippocampi of METH-EcoHIV group was increased compared to other groups. Next, we evaluated the function of antioxidant defense mechanisms by measuring activity of the superoxide disputase (SOD) family. The brain homogenates were divided into the cytoplasmic and the mitochondrial fractions to measure the activity of SOD1 and SOD2, respectively. There was no difference in SOD1 activity among groups; however, both SOD2 activity and protein expression were significantly reduced in the METH-EcoHIV group compared to the control group. These results suggest that HIV-METH-induced SOD2 dysfunction can contribute to oxidative stress, resulting in BBB disruption and astrocyte activation.

Supported by DA039576, HL126559, MH098891, MH63022, MH072567, DA027569, and the Miami Center for AIDS Research funded by the NIH grant P30AI0

T52.Co-expression pattern analyses of immune checkpoint receptors and ligands on T cells and dendritic cells from HTLV-1 infected individuals and humanized mice

Pattekar, A.[1], Ginwala, R.[1], Khan, Z.K.[1], Chew, G.M..[2], Charlins, P.[3], Akkina, R.[3], Caruso, B.[4], Jacobson, S.[4], Ndhlovu, L.C.[2], Jain, P.[1]; [1]Department of Microbiology and Immunology, and the Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, 19129 United States. [2]Department of Tropical Medicine, Medical Microbiology and Pharmacology, University of Hawaii, Honolulu, HI, 96813 United States. [3]Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO, 80523 United States. [4]Viral Immunology Section, Neuroimmunology Branch, National Institutes of Health, Bethesda, MD, 20892 United States.

Targeting inhibitory receptors is an established treatment strategy in cancer and chronic infection and could be highly significant in infection-associated neuroinflammatory diseases as well. HTLV-1 is the etiologic agent of adult T-cell leukemia (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) both of which have no effective therapy. Despite the high frequency of virus-specific CD8 T cells, the resultant vigorous cellular immune response is ineffective in controlling viral loads in HAM/TSP. Our initial studies implicated programmed death (PD)-1 receptor and its ligand, PD-L1 as potential underlying factors for immune cells' dysfunction leading to disease progression, primarily in HAM/TSP patients. Further studies with 10 HTLV-1 negative and 10 positive subjects revealed increased co-expression of negative regulators PD-1 and TIGIT on both CD4 and CD8 T cells from infected samples. This co-expression was more pronounced on Tax11-19-Pentamer positive T cells. Further, we noticed that Tax11-19-specific CD8 T cells from HAM/TSP patients over expressed TIM-3 and 2B4 along with PD-1. Gene expression analysis revealed slight increases in expression of PD-1, TIGIT, 2B4 in patients as compared to controls. Immunofluorescence studies on organ sections of HTLV-1 infected humanized mice show increased PD-1, TIGIT and TIM-3 as compared to controls. Analyses of the co-expression patterns of negative regulators will help standardize a blockade strategy aimed at restoring polyfunctionality and cytolytic potential of antigen specific T cells in HAM/TSP patients.

Supported by NIAID (R56AI077414)

T53.HIV-1 TAT-mediated epigenetic downregulation of miR-124 promotes microglial activation via MeCP2-STAT3 axis

Periyasamy, Palsamy, Ph.D.[1], Guo, Ming-Lei, Ph.D.[1], Buch, Shilpa, Ph.D.[1]; [1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198 United States.

The present study demonstrated HIV-1 TAT-mediated epigenetic downregulation of microglial (Mg) miR-124 & its possible association with Mg activation. Exposure of Mg to TAT decreased the primary miR-124 as well as mature miR-124 levels. Also, TAT exposure increased the expression levels of DNA methylation enzymes, such as DNA methyltransferase (DNMT)-1, DNMT3A, & DNMT3B thereby increased the global DNA methylation levels in Mg cells. As an outcome, the DNA methylation of the pri-miR-124 promoter was significantly increased. Methyl CpG binding protein 2 (MECP2), was identified as a novel 3'-UTR target of miR-124. We further confirmed that TAT-mediated downregulation of miR-124 resulted in upregulation of MECP2, which in turn, repressed the expression of miR-124. Besides MECP2, miR-124 also modulated the levels of signal transducer & activator of transcription 3 (STAT3) through targeting 3'-UTR, resulting in Mg activation. Luciferase assays & Ago2 immunoprecipitation determined the direct binding between miR-124 & 3'-UTRs of both MECP2 & STAT3. Silencing of MECP2 as well as DNMT1 further upregulated the expression of miR-124 while decreasing Mg activation. Reciprocally, overexpression of miR-124 in Mg blocked TAT-mediated activation of Mg. In summary, our findings demonstrate a novel

mechanism of TAT-mediated activation of Mg via downregulation of miR-124, leading ultimately to increased MECP2 & STAT3 activation. In summary, these studies will form the basis for the future development of epigenetic targets as adjunctive therapeutic modalities for treating HAND.

Supported by NIH (DA043138, DA040397)

T54.IFN-induced transmembrane (IFITM) protein mediates restricted HIV entry in astrocytes.

Pilakka Kanthikeel, S, Ph.D.[1], Sagar, V, Ph.D.[1], Ghasemloian, J, BS[1], Nair, M, Ph.D.[1]; [1]Department of Immunology, Florida International University, Miami, FL, 33199 United States.

Recent reports suggest that IFN-induced transmembrane (IFITM) protein, a recent addition to the antiviral repertoire of so-called 'HIV-1 restriction factors', acts at viral entry. However, the mechanism of IFITM antiviral activity is currently not elucidated yet in astrocytes. Moreover, the role of IFITMs in the CNS is largely unknown, except for few reports of IFITM3 expression in the patient's brains with certain neurologic diseases. Also, modulation of IFITM with drugs of abuse has also not been explored yet. We hypothesize that higher expression of IFITM serves as a restriction factor in astrocytes, in which the main restriction to HIV-1 is believed to be at entry and cocaine modulates the expression of IFITM, thereby increasing the infectivity. We found that astrocytes express high IFITM compared to microglia, and transfection of astrocytes with IFITM siRNA (50nm) increases the HIV infection significantly. Further, cocaine (1-10nM) decreased the IFITM expression in astrocytes in a dose dependent manner, with corresponding increase in HIV infection. These results suggest that IFITM plays an important role in restricting the HIV infection in astrocytes and cocaine relieves this restriction by downregulating IFITM expression. Upregulation of IFITM in combination with other molecular targets will be of preventive and /or therapeutic significance against HIV infection in cocaine users.

Supported by NIH (RDA037782A, R37DA025576)

T55.Cannabinoids modulate cytokine profiles within HIV-1-infected individuals in the Drexel Medicine CARES Cohort

Pirrone, V[1], Dampier, W[1], Parikh, N[1], Passic, S[1], Blakey, B[1], Szep, Z[2], Jacobson, J[3], Nonnemacher, M[1], Wigdahl, B[1]; [1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [2]Department of Medicine, Division of Infectious Diseases and HIV Medicine, Drexel University College of Medicine, Philadelphia, PA, 19102 United States. [3]Department o Medicine, Section of Infectious Disease, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, 19140 United States.

Cannabinoids are often prescribed for HIV-1-infected patients with peripheral neuropathy. In addition, medical cannabis has become legalized in an increasing number of states now numbering 30. This study was performed to evaluate the relationship between the use of cannabinoids and impact on cytokine modulation and HIV-1 disease severity in HIV-1-infected patients enrolled in the Drexel Medicine CNS AIDS Research and Eradication Study (CARES) Cohort. Within the Cohort, patients are assessed approximately every 6 months for HIV-1 clinical markers and history of illicit drug, alcohol, tobacco and medication use. The Luminex human cytokine 30-plex panel was used for cytokine quantitation. Analysis was performed using a newly developed biostatistical model. Substance abuse was found to be common within the cohort. Utilizing the drug screens at the time of visit, subjects in the Cohort were categorized as preferential nonusers, cannabinoid users, and multidrug users. Clinical parameters such as viral load, CD4+ and CD8+ T-cell counts as well as CD4:CD8 ratio were not impacted by increased cannabinoid use. Among the 30 cytokines investigated, IL-1β, IL2, IL-1A, and IL-5 are positively associated with cannabinoid use and MCP-1 is negatively associated. In conclusion, cannabinoids do not appear to facilitate HIV-1 disease progression based on these assessments. Due to the connection between MCP-1 and neurocognitive impairment, and the fact that MCP-1 was downregulated in cannabinoid users, future studies will examine if global dementia scores are effected by cannabinoid use.
Supported by This work is supported by NIDA R01 DA19807 (PI, BW),NIMH R01 MH110360 (Contact PI, BW), NIMH P30 MH092177 (CNAC/CTRSC, Drexel Co

T56.The blood-brain barrier as a source of extracellular vesicles; emerging concepts in vascular remodeling in neurotrauma, substance abuse and HIV CNS infiltration

Ramirez, SH, Ph.D.[1], Lutton, EM, BS[1], Rawls, SM, Ph.D.[2], Razmpour, R, BS[1], Andrews, AM, Ph.D.[1]; [1]Department of Pathology & Laboratory Medicine, The Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140 United States. [2]Center for Substance Abuse Research, The Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140 United States.

Throughout the HIV epidemic, drug use has remained a primary risk factor for contracting, transmitting and worsening the outcomes of HIV despite treatment with cART. In fact, neuropsychological decline is greater in individuals who are cocaine users and HIV-seropositive. Extracellular microvesicles (EVs) have emerged as an intriguing

phenomenon with expanding biological roles and appear to be released by virtually every cell type in the body. We have shown that EVs from brain endothelial cells (ECs) are shed during brain injury and are highly enriched with key protein constituents of the BBB. Similarly, a hallmark feature of HIV-related CNS damage involves BBB disruption. Our latest analysis has revealed that the formation of EVs from brain ECs is associated with vascular destabilization and that HIV and psychostimulants trigger EV release. Thus, we hypothesize that HIV infection and/or exposure to drugs of abuse contributes to BBB alteration via brain endothelial EV release, and thus facilitates BBB breach and immune access to the CNS. Results presented here from primary cells, animal models and clinical samples support the notion that brain EC-derived EVs are a fundamental part of the inflammatory response at the brain endothelium. Thus, the case is made for brain EC-derived EVs to have a functional role in neuropathology and a utility as biosignatures for evaluation of BBB status. Furthermore, analysis with syngenic cultures and a newly developed BBB-microfluidic model reveals the potential for therapeutic blocking of EV biogenesis in preserving BBB integrity.

Supported by R01 NS086570-01 (SHR), and The Shriners Hospitals for Children 85110-PHI-14 (SHR)

T57.Development of a polydrug exosome-based mimetic to treat HIV-associated neuroinflammation/neurotoxicity and opiate abuse.

Raymond, A, Ph.D.[1], Brito, D, BS[1], Tomitaka, A, Ph.D.[1], Lapierre, J, MS[1], Yndarta-Arias, A, BS[1], El Hage, N, Ph.D.[1], Nair, M, Ph.D.[1]; [1]Department of Immunology, Herbert Wertheim College of Medicine Florida International University, Miami, FL, 33199 United States.

Human Immunodeficiency virus(HIV) Negative factor (Nef) detected in exosomes play a role in HIV neuropathology. Nef+ exosomes (exNef) damage both neurons and the blood-brain-barrier(BBB) while promoting neuroinflammation. Here we study the efficacy of an exosome-based mimetic to treat exNef effects in the context of HIV and opiates. The exosome-mimetic (Exo-ML) consists of a liposome cocktail containing molecular targets miRNA29b and Nef-peptide #14 (NP-14) to disrupt Nef, CTOP, a mu-receptor antagonist to block morphine, and an anti-HIV drug Tenofovir to restrict HIV. Exo-ML was added to Nef-transduced microglia cell line CHME-5(CHME-5NG) in the absence/presence of morphine (10 nM). Exo-ML restricted morphine-induced increase in CHME-5NG exNef. Although morphine had no significant effect, exNef reduced BBB integrity as measured by transendothelial electrical resistance (TEER) and increased permeability (Dextran-FITC transport assay) in an in vitro BBB model. Apical Exo-ML BBB administration restored TEER and permeability. Exo-ML inhibited exNef increase in monocyte BBB transmigration. ExNef exposure of SH-SY5, neuronal cell line increased intracellular calcium (Ca2+) and lowered viability as measured by Fura-2 and XTT assay, respectively. Pre-treatment with Exo-ML, inhibited exNef-mediated effects on intracellular Ca2+ and viability. Exo-ML reduced viral replication in HIV-infection in CHME-5 co-cultured with the BBB. Overall, Exo-ML blocked HIV replication and exNef thereby providing a proof-of-concept for treatment of neuropathology associated with HIV and opiate use.

Supported by NIH/NIDA grant# R01DA027049-04S1; NIH/NIDA grant# R01DA027049

T58.Intranasal administration of small interfering (si) RNA Beclin1 encapsulated with linear polyethylenimine (PEI) nano-plexes to attenuate HIV-1 infection in the brain

Rodriguez, M, Ph.D.[1], Lapierre, J, MS[1], Ojha, C, MS[1], Batrakova, E, Ph.D.[2], Kashanchi, F, Ph.D.[3], Dever, S, Ph.D.[1], El-Hage, N, Ph.D.[1]; [1]Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States. [2]Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, 27599 United States. [3]Laboratory of Molecular Virology , School of Systems Biology, George Mason University, Manassas, VA, 20110 United States.

Despite the success of antiretroviral drugs on HIV, the interlinked epidemic between HIV and opiates is still prevalent. We have discovered that activation of the host autophagic protein, Beclin1, by HIV-1 infection represents an essential mechanism in controlling HIV-replication and viral-induced inflammatory responses in microglial cells. Using external magnetic force as a delivery tool we transmigrated siBeclin1 bound to an electro-magnetic nano-particle across an artificial blood-brain-barrier to achieve reductions in HIV titer and HIV-induced cytokines in microglial cells. In the present study, we used non-invasive, intranasal delivery of siBelin1 encapsulated with cationic polyethylenimine (PEI), a biodegradable linear polymer, as gene carrier for delivery of siBeclin1 to the brain. As a proof-of-concept, FITC-labeled control siRNA complexed with PEI was delivered intranasally in adult mice. Fluorescence-tagged siRNA was detected in several brain regions after 4 and 24 hour of administration, and morphological assessments of these regions did not show any characteristic indication of neurotoxicity Next, PEI-siBeclin1 nano-plex was delivered intranasally in adult mice. Western blot analysis of brain tissues showed significantly downregulation of the Beclin1 protein after 24 and 48 hours with no significant evidence of toxicity. Overall, our data

shows that intranasal delivery of PEI-siBeclin1 nano-plex is a safe and effective methodology to deliver drugs to the brain, although the interaction of Beclin1 with opiates needs to be further discerned.

Supported by National Institutes of Health (NIH)-National Institute on Drug Abuse (NIDA) grants R01DA036154 and R21DA041287

T59.NIR-stimuli sensitive 'smart' magnetic nanoagent for on demand drug delivery

Sagar, VIDYA, Ph.D.[1], Atluri, V.S.R., Ph.D.[1], Tomitak, A. , Ph.D.[1], Nagasetti, A., Ph.D.[1], Vashisit, A., Ph.D.[1], Pilakka-Kanthikeel, S., Ph.D.[1], El-Hage, N., Ph.D.[1], McGoron, A., Ph.D.[1], Nair, M., Ph.D.[1]; [1]Vidya Sagar, Florida International University, Miami, FL, 33174 United States.

Investigations involving coupling of magnetic nanoparticles (MNPs) and near infrared ray (NIR) have been little explored for their potential brain therapeutics. We examined the exposure of 808 nm NIR (power ~1.5 W/cm2) for controlled drug release from magnetic nanocarrier. Our study suggest size-dependent absorbance peak at 808 nm: while MNPs with < 15 nm size displayed absorption near to bottom line i.e. extremely low, significant absorption peak is observed for particles of ~ 35 nm size. This size-dependent photoexcitation property of MNPs in the NIR region was explored for drug release study. Exposure of 808 NIR up to 2 min resulted in ~24% release of AZTTP (anti-HIV drugs) immobilized on the MNPs of ~35 nm size. MNPs were characterized by TEM, XRD, DLS, and VSM. Further, NIR exposed different brain cells treated with MNPs (50μg MNPs/ml & for 10e6 cells) did not show signs of cytotoxicity upon exposure to 808 nm NIR light up to 2 minutes as determined by MTT assay. Also, cell resistance/impedance (Ω) measurement in an Electric Cell-substrate Impedance Sensing system suggests a steady growth behavior of astrocytes up to10 hr post-NIR treatment. Similarly, confocal microscopy reveals no harmful changes in dendrite and spine morphology (synaptic plasticity) of SKNMC cells upon NIR exposure with or without MNPs treatment. Importantly, this exposure did not cause a temperature rise of the cell culture milieu. Thus, our study has led to discovering a dissipation-free, transient NIR biophotonic approach for controlled drug delivery that can be applicable for brain targeting.

T60.Methamphetamine and HIV-1/gp120 protein affect neurotransmitter systems in vivo through lasting changes in CNS gene expression

Sanchez, AB, Ph.D.[1], Yuan, NY, Ph.D.[1], Maung, R[1], Kaul, M, Ph.D.[1], TMARC Group, [2]; [1]Infectious and Inflammatory Disease Center, Sanford Burnham Prebys Discovery Medical Institute, La Jolla, CA, 92037 United States. [2]Translational Methamphetamine AIDS Research Center, University of California San Diego, San Diego, CA, 92093 United States.

Among individuals afflicted with human immunodeficiency virus type-1 (HIV-1), between 10 to 15% reportedly abuse methamphetamine (METH). The combination of viral infection and METH in the central nervous system (CNS) is suspected to exacerbate HIV-associated neurocognitive disorders (HAND). METH abuse alone can lead to irreversible damage in the brain, causing neuroinflammation and compromising several neurotransmitter systems. However, the combined effects of HIV-1 and METH on the brain are incompletely understood at the molecular level. We recently treated 3-4 months old HIV-1/gp120 transgenic (gp120tg) and wild type (wt) mice with an escalating METH binge regimen for 25 days. At 10-12 months of age, HIV-1/gp120tg and METH-exposed animals showed significant impairment in spatial learning and memory and neuropathology. METH-treated HIV-1gp120tg mice were the most severely affected. In order to investigate underlying mechanisms in the brain, RT^2 Profiler PCR Arrays were utilized to determine the changes in expression of genes related to the dopaminergic, serotonergic, GABAergic, and glutamatergic neurotransmission systems. Comparisons between the four experimental groups revealed significant gene regulation due to METH exposure and chronic HIV-1/gp120 expression: 1) WT Saline (SAL) versus (vs.) WT METH; 2) WT SAL vs. gp120 SAL; 3) WT METH vs. gp120 METH; 4) gp120 SAL vs. gp120 METH. In summary, histopathology and impaired spatial learning and memory due to METH exposure and HIV-1/gp120 expression are associated with significant alterations of neurotransmission systems.

Supported by Supported by NIH, MH087332, MH104131, MH105330 and DA026306 (to M.K.)

T61.β-arrestin 2 regulates anti-inflammatory effects of Salmeterol in lipopolysaccharide stimulated BV2 cells

Sharma, Monika, MS[1], Flood, Patrick, Ph.D.[1]; [1]Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, T6G 2E1 Canada.

Microglial activation contributes to chronic inflammation and neuronal loss in progressive neurodegenerative disorders like Parkinson`s disease (PD). Thus, mediators suppressing microglial activation may have therapeutic benefits to lessen the progression of neurodegenerative diseases. Our previous findings show that Salmeterol, a long-acting β2-adrenergic receptor (β2-AR) agonist, is neuroprotective in animal models of PD. In the present study, we assessed the anti-inflammatory

effects of Salmeterol on activation induced by lipopolysaccharide (LPS) in murine microglial BV2 cells. Salmeterol inhibited LPS-induced release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and nitric oxide (NO) in BV2 cells. Moreover, Salmeterol suppressed nuclear factor kappa-B (NFκB) p65 activation and its nuclear translocation by inhibiting the IκB-α degradation and TAK1 phosphorylation. We have also found that Salmeterol increases the expression of β-arrestin2 and enhances the interaction between β-arrestin2 and TAB1 (TAK1-binding protein), disrupting TAK1-TAB1 mediated activation of NFκB and expression of pro-inflammatory genes. In addition, silencing of β-arrestin2, abrogate the anti-inflammatory effects of Salmeterol in LPS-stimulated BV2 cells. Taken together, our findings suggest that anti-inflammatory properties of Salmeterol is β-arrestin2 dependent and also offers novel drug targets in the convergent β2AR/β-arrestin2 and inflammatory pathways to prevent microglial cell activation and neuronal loss in neuroinflammatory diseases like PD.

Supported by Parkinson's Alberta

T62.Morphine-mediated astrogliosis involves dysregulated autophagy

Sil, S, Ph.D.[1], Periyasamy, P, Ph.D.[1], Guo, ML, Ph.D.[1], Callen, S, BS[1], Buch, S, Ph.D.[1]; [1]Department of Pharmacology and Experimental Neuroscience, UNMC, Omaha, NE, 68198 United States.

Though morphine is a highly effective painkiller, its long-term use can lead to tolerance, addiction & cognitive impairments. A recent study from our lab has revealed a link between morphine-mediated autophagy & synaptic alterations. The current study was aimed at investigating whether morphine-mediated induction of astrogliosis involved ER stress/autophagy axis. Our in vitro findings demonstrated a dose- & time-dependent upregulation of the autophagy markers (Beclin1, LC3-II & p62) in morphine-exposed human A172 astrocytoma cells. Morphine exposure significantly increased autophagosome formation with a concomitant defect in the autopahgic flux as evdienced by the LC3-turnover, p62 degradation & mRFP-EGFP-LC3 overexpression analyses. Pharmacological blocking of the μ-opioid receptor underscored the role of opioid receptor in this process. Using both pharmacological & gene-silencing approaches it was demosntrated that morphine-mediated dysregulation of autophagy involved upstream activation of ER stress with sequential downstream activation of GFAP and generation of proinflammatory cytokines. These results were also validated in the various brain regions isolated from morphine dependent rhesus monkeys. Our findings showed preferential activation of ER stress/autophagy axis and inflammation in the basal ganglia, frontal cortex, occipital cortex & cerebellum in morphine administered macaques. Interventions aimed at blocking either μ-opioid receptor or ER stress could thus provide promising therapeutic targets for abrogating morphine-mediated astrogliosis.

Supported by NIH DA033150, DA035203, DA036157

T63.Methamphetamine increases HIV-1 infectivity in neural stem and progenitor cells

Skowronska, M, Ph.D.[1], McDonald, M, MS[2], Toborek, M, MD, Ph.D.[1]; [1]Biochemistry and Molecular Biology, University of Miami Miller School of Medicine, Miami, FL, 33136 United States. [2]Department of Marine Biology, University of Hawaii at Manoa, Honolulu, HI, 96822 United States.

HIV infection and METH abuse are often comorbid. Since active METH users were shown to have higher HIV viral load and more severe neurological complications than non-users, synergistic pathological effects were suggested. We hypothesize that one of the mechanisms by which METH enhances HIV-related neurodegeneration is METH-potentiated HIV production in neural stem cells (NSCs) that results in impaired neurogenesis. Mouse (NE4C) and human (ReNcell) NSCs were infected either with EcoHIV/NL4-3, a chimeric HIV-1 that infects mice or HIV/NL4-3, which is infectious to humans. METH was added simultaneously or 24h before infection. To determine the mechanism underlying METH-induced HIV production, NE4C cells were transfected with different variants of HIV-LTR promoters and then exposed to METH. To assess the effects of METH and/or HIV on neurogenesis, differentiation of primary mouse NSCs was analyzed by immunostaining and FACS. Our results demonstrate that pretreatment with METH significantly increased EcoHIV and HIV infectivity in mouse and human NSCs, respectively. We found that METH induced LTR activation, and that expression from the HIV LTR required both NF-kB and Sp1 signaling. In addition, pretreatment with METH decreased neuronal differentiation of EcoHIV infected NSCs and reduced the amount of differentiated glia and neurons. This study suggests that METH increases HIV infectivity in NSCs, through NF-kB/Sp1 dependent activation of HIV-LTR and subsequent increased viral genes expression. Such events may underlie METH-induced progression of HIV-associated neurodegeneration.

Supported by DA039576, DA027569, HL126559, MH098891, MH072567, MH063022

T64.Kallikrein-kinin system and Type I Interferons in Neurolupus

Sriram, U[1], Zuluaga-Ramirez, V[1], Merkel, SF[1], Winfield, M[1], Fernandes, NC[1], Gajghate, S[1], Gallucci, S[2], Persidsky, Y[1]; [1]Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States. [2]Department of Microbiology and Immunology, Temple University Lewis Katz School of Medicine, Philadelphia, PA, 19140 United States.

Neuropsychiatric lupus is one of the most common manifestations of human systemic lupus erythematosus (SLE), causing depression in many. SLE is an autoimmune disease characterized by multi-organ damage including the brain and Interferon-α (IFN-α) is a central mediator in SLE pathogenesis. Excessive production/response to Type I IFNs is a hallmark of SLE. The mechanism of how IFN causes depression remain poorly understood; however, it is known that IFN-α administration in certain chronic viral infections and some cancers causes the development of depressive symptoms in a high percentage of patients. The Kallikrein-Kinin System (KKS) that is comprised of kallikreins (klks), bradykinins (bk), angiotensin converting enzyme (ACE), and many other molecules, has classically known to be involved in a variety of physiological processes, including coagulation, angiogenesis and control of blood pressure. The KKS has been explored recently for their regulation of brain functions. We hypothesize that IFN-α may cause some of the symptoms in neurolupus patients and the KKS can ameliorate these effects. In this study, we used the MRL/lpr lupus-prone mouse model and showed that exposing MRL mice to IFN-α (a) increased depressive-like behavior, (b) decreased klk expression, and (c) enhanced ACE expression in the brain. Administering captopril (a commonly prescribed ACE inhibitor) decreased IFN-induced gene expression in the brain. The role of key KKS effectors and their interactions with the IFN pathway may provide a rationale for therapeutic use of KKS molecules for treatment of neurolupus.

Supported by NIAMS / RO3AR065157

T65.Role of TGF-β/Smad signaling pathway in CD8+ T cell function impairment in HIV/AIDS patients

Su, Q J S, Ph.D.[1], Xiao, J X, Ph.D.[2], Lu, Z Z L, Ph.D.[1], Shi, D X S[1], Liang, A H L[1]; [1]Ruikang Hospital, Guangxi University of Chinese Medicine, Nanning, Guangxi, 530011 China. [2]School of Basic Medical Science, Guangxi Medical University, Nanning, Guangxi, 530200 China.

Objective To explore the role of TGF-β/Smad signaling pathway in the impairment of CD8+ T cell function. **Methods** Thirty patients were recruited from AIDS patients ($CD4^+$ T cell counts<200/μl) and HIV infected patients ($CD4^+$ T cell counts≥200/μl), respectively, and thirty healthy people were recruited as control. Peripheral venous blood were collected and were tested for CD28 molecule and phosphorylated Smad2/3 protein (p-Smad2/3) of CD8+ T cells using Flow Cytometry, and for plasma TGF-β1, IFN-γ, and TNF-α using enzyme linked immunosorbent assay (ELISA). **Results** $CD8^+CD28^+$ double-positive cells accounted for 47.75%, 47.40% and 36.20% (median) of the total $CD8^+$ T cells in healthy people, HIV infected patients and AIDS patients, respectively. AIDS patients had a lower proportion of $CD8^+CD28^+$ double-positive T cells compared with healthy people and HIV infected patients (P<0.05). 79.15%, 55.60% and 60.60% of $CD8^+$ T cells was positive for p-Smad2/3 in healthy people, HIV infected patients and AIDS patients, respectively. This proportion was lower for HIV infected patients and AIDS patients than for healthy people (P<0.05). Plasma TNF-α was (147.92±1.47)pg/ml,(60.04±1.78)pg/ml and(54.43±2.03)pg/ml respectively in the three groups, and it was lower in HIV infected patients and AIDS patients than in healthy people (P<0.05). There were not significant differences in Plasma TGF-β1 and INF-γ across groups. **Conclusion** TGF-β/Smad signaling pathway may be involved in the functional impairment and abnormal activation of $CD8^+$ T cell.

T66.Effects of human dopamine transporter histidine547, tyrosine88 and lysine92 intermolecular interactions on basal and Tat-inhibited dopamine transport

Sun, WL, Ph.D.[1], Quizon, PM, BS[1], Yuan, Y, Ph.D.[2], Strauss, M, BS[1], McCain Jr, R, BS[1], Zhan, CG, Ph.D.[2], Zhu, J, MD, Ph.D.[1]; [1]Department of Drug Discovery and Biomedical Sciences, University of South Carolina, Columbia, SC, 29208 United States. [2]Molecular Modeling and Biopharmaceutical Center, and Department of Pharmaceutical Sciences, University of Kentucky, Lexington, KY, 40536 United States.

Abnormal dopaminergic transmission is implicated as a risk determinant of HIV-associated neurocognitive disorders. HIV-1 Tat protein increases synaptic dopamine (DA) levels by directly inhibiting human DA transporter (hDAT) activity, ultimately leading to dopaminergic neuron damage. Through integrated computational modeling prediction and experimental validation, we have identified that histidine547, tyrosine88 and lysine92 in hDAT are key residues for Tat binding and critical for Tat-induced inhibition of DAT and transporter conformational transitions. This study evaluated the effect of double (H547A/Y88F) and triple (H547A/Y88F/K92M) mutations of these residues on basal and Tat-induced inhibition of DAT function. Compared to WT hDAT, H547A and H547A/

Y88F displayed a similar increase (190%) in the Vmax, whereas the Vmax was decreased by 97% in H547A/Y88F/K92M and K92M, but not altered in Y88F. Recombinant Tat1-86 induced a 31% reduction of DA uptake in WT hDAT, which was attenuated in H547A, H547A/Y88F, Y88F and K92M. Consistent with H547A, H547A/Y88F displayed a differential sensitivity to PMA-induced activation of DAT function relative to WT hDAT, indicating a critical role of His547 in basal PKC-mediated DAT activity. In addition, H547A/Y88F attenuated zinc-mediated binding sites of [3H]WIN35,428, augmented basal DA efflux and amphetamine-induced DA efflux. These findings demonstrate that among these residues histidine547 plays a key role in intermolecular interactions of Tat-DAT for stabilizing basal DA transport and Tat-induced inhibition of DA transport.

Supported by NIH/NIDA DA035714, DA041932

T67.Wnt5a is a neuron-to-astrocyte signal regulating the pathogenesis of HIV-1/AIDS-associated neuropathic pain

Tang, S.-J., Ph.D.[1], Liu, X., Ph.D.[1]; [1]Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX, 77555 United States.

Neuropathic pain is a common neurological complication in HIV-1/AIDS patients, but effective therapies are not available. We have been investigating the pathogenic mechanism for the ultimate development of rationale-based therapeutic approaches. Our previous analysis of postmortem tissues reveal that astrocytes are specifically activated in the spinal dorsal horn (SDH) in the 'pain-positive' HIV patients but not in the 'pain-negative' patients, indicating a critical role of reactive astrocytes in the pathogenesis of HIV-associated pain. The current study is to elucidate how the astrocytes are activated during the pathogenesis. Using a mouse model that develops similar pain pathologies to that of 'pain-positive' HIV patients, we show that HIV-1 gp120 induces rapid Wnt5a up-regulation and subsequent astrocyte activation in the SDH. To test the hypothesis that Wnt5a is secreted from SDH neurons (the major cell type expressing Wnt5a) to activate astrocytes via its co-receptor ROR2, we deleted Wnt5a in neurons or ROR2 in astrocytes. Our results show that neuronal Wnt5a and astrocytic ROR2 are critical for gp120 to induce pain and astrocyte activation. The findings suggest that Wnt5a is a neuron-to-astrocyte signal that is critical for HIV-associated astrocyte activation and pain pathogenesis.

Supported by R01NS079166; R01DA036165

T68.Cocaine-mediated activation of microglia: an implication of defective mitophagy

Thangaraj, Annadurai, Ph.D.[1], Periyasamy, Palsamy, Ph.D.[1], Buch, Shilpa, Ph.D.[1]; [1]Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, 68198 United States.

Though cocaine is known to induce neuroinflammation by enhanced activation of glial cells such as microglia, the mechanism remains unclear. The present study was aimed to explore whether cocaine-mediated mitophagy involved in microglial activation. Our results demonstrated that exposure of mouse primary microglial cells to cocaine resulted in decreased mitochondrial membrane potential, accompanied by increased expression of mitophagy markers, Pink1 & Parkin. The results also demonstrated that exposure of microglia to cocaine increased the expression of DRP1, thereby intensifying the fragmentation of depolarized mitochondria for its clearance via mitophagy. Exposure of microglia to cocaine upregulated the expression of autophagosome markers, Beclin1 & LC3-II, indicating that increased mitophagosome formation. These findings were validated by imaging studies which demonstrated increased co-localization of damaged mitochondria with the LC3 puncta. Instead, cocaine exposure also increased the expression of p62, suggesting that a possible blockade of mitophagy flux & accumulation of mitophagosomes. Further, we also demonstrated the cocaine-mediated increased expression of TNF-α, IL-1β, & IL-6, characteristic features of microglial cells activation. Using pharmacological approaches to block sigma receptor as well as either autophagy/mitophagy demonstrated that cocaine-mediated activation of microglia involved sequential activation of mitophagy with a poor clearance. In conclusion, cocaine exposure induces microglial activation via mitochondrial damage & its poor clearance.

Supported by NIH (DA040397; DA035203)

T69.Cocaine and HIV Upregulate Glycolytic Enzymes and Mitochondrial Biogenesis in Astrocytes.

Thangavel, S, Ph.D.[1], Atluri, VSR, Ph.D.[1], Nair, M. P, Ph.D.[1]; [1]Department of Immunology, Institute of NeuroImmune Pharmacology, College of Medicine, Florida International University, Miami, FL, 33176 United States.

Previous studies have demonstrated that HIV and cocaine act synergistically to trigger HIV-1 disease progression and neuronal dysfunctions. Astrocytes are the major regulators for energy storage, utilization and metabolic function in the central nervous system (CNS). Cocaine and HIV are also known to disrupt brain energy metabolism; however the mechanisms have not been clearly elucidated yet. We hypothesize that HIV-Tat with cocaine synergistically dysregulate the energy metabolism by converting most glucose to lactate regardless of the availability of O2, diverting glucose metabolites from energy production to anabolic (generate enough ATP to

maintain cell function) process and generate biomass to accelerate glycolytic enzymes such as Hexokinase II, pyruvate kinase 1/2 (PKM1/2), monocarboxylate transports 1 & 4 (MCT-1and MCT-4) leading to mitochondrial biogenesis of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and mitochondrial transcription factor AFTM leading to disease progression. Primary astrocytes treated with HIV Tat and cocaine and cell lysates were analyzed by western blotting to determine glycolytic protein expression in HK-I, HK-II, PKM ½, MCT1 & 4 and PGC-1α, and AFTM. Our results indicated that HIV-Tat significantly upregulated glycolytic enzymes and mitochondrial biogenesis expression and these effects were accelerated by cocaine. These results suggest that cocaine and HIV synergistically accelerate glycolytic and mitochondrial biogenesis expression and subsequently impact astrocyte energy storage, utilization and energy transfer metabolism exacerbating neurodegeneration of HIV infected cocaine users. These studies have translational significance for therapeutic targeting by controlling the energy metabolism in HIV-infected cocaine users.

Supported by MH08529, MH101025 and DA034547

T70.Magneto-plasmonic nanoparticles for image-guided brain targeting with enhanced blood-brain barrier transmigration

Tomitaka, A, Ph.D.[1], Arami, A, Ph.D.[2], Raymond, A, Ph.D.[1], Yndart, A, BS[1], Takemura, Y, Ph.D.[3], Toborek, M, MD, Ph.D.[4], Nair, M, Ph.D.[1]; [1]Department of Immunology, Institute of NeuroImmune Pharmacology, Centre for Personalized Nanomedicine, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States. [2]Molecular Imaging Program at Stanford (MIPS), The James H Clark Center, Stanford University, Stanford, CA, 94305 United States. [3]Department of Electrical and Computer Engineering, Yokohama National University, Yokohama, Kanagawa, 240-8501 Japan. [4]Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL, 33136 United States.

Nanoparticles have been gaining considerable attention for drug delivery. In spite of the progress in nanomedicine, drug delivery to the brain remains challenging due to the presence of blood-brain barrier (BBB). Among various nanoparticles developed for biomedical applications, magnetic nanoparticles have shown promise for brain targeting and magnetic resonance imaging (MRI) due to their unique magnetic properties. Gold nanoparticles have excellent properties for various imaging modalities including X-ray computed tomography (CT) and optical imaging systems. The integration of magnetic and gold nanoparticles gives multi-functionalities which enable multi-modal imaging and multi-modal image-guided therapies. In this study, we synthesized magneto-plasmonic nanoparticles which consist magnetic core and gold shell for image-guided brain targeting. Magnetic nanoparticles were synthesized by co-precipitation and they were coated with gold shell through citrate reduction. The synthesized magneto-plasmonic nanoparticles exhibited superparamagnetic property which contributed to the imaging contrast in magnetic resonance imaging (MRI). Also, the gold shell part of magneto-plasmonic nanoparticles showed imaging contrast in X-ray computed tomography (CT). The transmigration study using an in vitro blood-brain barrier (BBB) model proved enhanced transmigration of magneto-plasmonic nanoparticles. These results indicate that developed multifunctional magneto-plasmonic nanoparticles can be used for theranostic purpose for brain diseases and neurological disorders.

Supported by NIH grants 1R01DA037838-01, 1R01DA040537-01, and R01DA034537

T71.Development of novel biopolymer based magnetic micro/nano hydrogels for targeted drug delivery and theranostic applications

Vashist, Arti, Ph.D.[1], Kaushik, Ajeet, Ph.D.[1], Atluri, Venkata, Ph.D.[1], Nikkhah-Moshaie, Roozbeh, Ph.D.[1], Tomitaka, Asahi, Ph.D.[1], Sagar , Vidya , Ph.D.[1], Nair, Madhavan, Ph.D.[1]; [1]Center for Personalized Nanomedicine, Institute of NeuroImmune Pharmacology, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States.

Micro/nanogels are the swollen micro/nano sized particles developed using hydrophilic biopolymers. Recently, they are in demand owing to their application in delivery of various drugs and biologically active molecules. However, existing micro/nano hydrogel requires significant improvement for specific cell or organ targeting or for diagnostic purpose and which can be visualized through non-invasive imaging. In the present work, a novel magnetic micro/nano hydrogels were developed using biopolymers chitosan and hydroxyethyl cellulose (HEC) along with nanoparticle of iron oxide (III) deploying water in oil emulsion polymerization technique. Iron oxide nanoparticles were used with an aim to impart magnetic properties to these gels for fetching targeted delivery of the drug. Polyol was used to introduce element of hydrophobicity and increased stability of micro/nanogels. Tenovofir disopropxil was used as model drug for studying in vitro drug loading and release kinetics. The formation of the crosslinked gels and drug binding was confirmed using FT-IR and Raman analysis. Structural characterization of the magnetic micro/nano gels was evaluated using SEM and TEM analysis.

Biocompatibility of developed micro/nanogels (1-100 ng) was confirmed by performing the XTT analysis in PBMCs and Microglia cells. It is anticipated that both the hydrophobic modification and the magnetic character of the gels will facilitate their entry to brain by traversing the blood brain barrier and may serve as novel targeted on demand drug delivery vehicle and for theranostic purpose.

Supported by R01DA40537-01; R01DA34547-01.

T72.Epigallocatechin gallate inhibits macaque SEVI-mediated enhancement of SIV or SHIV infection

Wang, X, Ph.D.[1], Zhou, RH, MS[2], Guo, L, MS[2], Liu, JB, Ph.D.[2], Liu, H, MS[2], Hou, W, MD, Ph.D.[2], Ho, WZ, MD, M.Ph.[1], Li, JL, Ph.D.[1]; [1]Pathology and Laboratory Medicine, Temple University, PHILADELPHIA, PA, 19140 United States. [2]School of Basic Medical Sciences/State Key Laboratory of Virology, Wuhan University, Wuhan, Hubei, 430071 China.

Background: Human semen contains a factor that can enhance HIV infection up to 10^5-fold in cultures. This factor is termed SEVI (Semen-derived Enhancer of Virus Infection), which is composed of proteolytic fragments (PAP248-286) from prostatic acid phosphatase (PAP) in semen. Here, we examined whether macaque SEVI can facilitate simian immunodeficiency virus (SIV) or chimeric simian/human immunodeficiency virus (SHIV) infection. We also studied the effect of EGCG on macaque SEVI-mediated SIV or SHIV enhancement. Methods: SIV or SHIV was mixed with different concentrations of macaque SEVI in the presence or absence of epigallocatechin gallate (EGCG). The mixture was added to cultures of TZM-bl cells or macaque PBMC. The effect of EGCG on macaque SEVI was measured by Congo-red staining assay, thioflavin T (ThT) fluorescence assay and visualized by a transmission electron microscope. Results: We identified that there is one amino acid difference at the site of 277 between human PAP248-286 and macaque PAP248-286. Macaque SEVI significantly enhanced SIV or SHIV infection of TZM-bl cells and macaque PBMC. EGCG could block macaque SEVI-mediated enhancement of SIV or SHIV infection. Mechanistically, EGCG could degrade the formation of macaque SEVI amyloid fibrils that facilitates HIV attachment to the target cells. Conclusions: The finding that macaque SEVI could enhance SIV or SHIV infection indicates the possibility to use the macaque SEVI in vivo studies with the macaque models. In addition, future studies are necessary to examine whether EGCG can be used as an effective m (truncated due to character limit)

Supported by NIH/DA022177 and DA041302 to WZH; MH109385 and DA040329 to JL

T73.HIV and Catecholamine Induced β-Arrestin Inhibits Interferon-α in Macrophages: Implications for Neurotropic HIV Infection in the Context of Substance Abuse

Williams, DW[1], Askew, L[1], Clements, JE[1]; [1]School of Medicine, Johns Hopkins University, Baltimore, MD, 21201 United States.

HIV infects the CNS within days of primary infection. Type I Interferon, a potent antiviral pathway, is dysregulated in brain during HIV infection and contributes to HIV-associated cognitive disorders; however, the mechanisms by which this occurs are not fully understood. We previously showed that Interferon-alpha (IFN-α) is selectively decreased by CCL2 in brain, but not peripheral organs, in a simian immunodeficiency virus model. In our current study, we used monocyte-derived macrophages (MDM) to more completely characterize the mechanisms by which CCL2 decreases IFN-α. We determined that CCL2 decreased HIV-induced IFN-α, but not IFN-β, MxA or TRAIL, key Interferon stimulated genes, suggesting that the effects of CCL2 occurred through a non-canonical signaling pathway. This was not specific to HIV infection, as CCL2 also decreased endogenous IFN-α in uninfected MDM and also upon exposure to poly I:C a ligand of TLR3. CCR2, the only known receptor for CCL2 on MDM, was required as blockade abrogated the effect. Tight CCR2 engagement was also required, as weak ligands CCL5, CCL7, and CCL8 had no effect on IFN-α. We determined that β-Arrestin decreased IFN-α in a G-protein coupled Kinase 5 (GRK5) and Erk dependent manner. β-Arrestin activation by the β-adrenergic receptor and TAAR1 also decreased IFN-α. These data indicate β-Arrestin as an inhibitor of antiviral responses in brain during HIV infection. Additionally, our findings suggest that catecholamine receptors may limit an integral antiviral pathway and may contribute to the brain as a viral reservoir.

Supported by R01 AI127142, R01 NS076357, R01 NS077869, R25 MH080661, JHU Provost's Diversity Postdoctoral Fellowship

T74.Induction of the Endogenous Antioxidant Response Pathway by Secoisolariciresinol diglucoside in HIV- infected Human Macrophages

Williams, KS, Ph.D.[1], Pu, S, BS[1], Nieves, H, BS[1], Guerrero, A, BS[1], Jordan-Sciutto, KJ, Ph.D.[1]; [1]School of Dental Medicine, University of Pennsylvania, Philadelphia, PA, 19104 United States.

Macrophages and microglia (M/M) play pivotal roles in the pathogenesis of HIV associated neurocognitive disorders. The

ensuing inflammatory M/M activation causes neuronal damage. Studies utilizing exogenous anti-inflammatory and antioxidants to mitigate disease progression have been unsuccessful; however, targeting endogenous antioxidant pathways such as the endogenous antioxidant response (EAR) pathway, which upregulates key antioxidant enzymes, including heme oxygenase 1 (HO-1) may be useful. In this study, we investigated the role of a flaxseed lignin, secoisolariciresinol diglucose (SDG), on oxidative stress in HIV-infected macrophages. To evaluate EAR, human monocyte derived macrophages were infected with HIV and/or SDG for 10, 30 and 60mins and Nrf2 expression was assessed by immunoblot or Immunocytochemistry to evaluate its translocation from the cytoplasm to the nucleus. Treatment with SDG alone increased Nrf2 translocation to the nucleus while HIV resulted in a small increase in Nrf2 translocation. Concurrent treatment with SDG and HIV enhanced translocation of Nrf2 over HIV infection alone. Consistent with these findings, macrophages infected with HIV for one day slightly increased HO-1 expression, however, HO-1 was suppressed by 12 days post infection. To determine if SDG can reverse the prolonged HO-1 deficits, macrophages were pretreated with SDG for 1hr prior to infection and replenished every day. SDG treatment increased HO-1 protein levels throughout infection. Given these data, SDG increases endogenous antioxidants pathways and may be a possible adjunctive

Supported by NIH/R25MH080663; NIH/MH106967

T75.Exploring benefits of Methanandamide: towards developing a nanoformulation to combat against cannabinoids induced effects in HIV patients

Yndart A, AY, BS[1], Kaushik A, KA, Ph.D.[1], Tomitaka A, TA, Ph.D.[1], Tiwari S, TS, MS[1], Agudelo M, AM, Ph.D.[1], Munoz K, MK, MS[1], Nair M, NM, Ph.D.[1]; [1]Center for Personalized Nanomedicine, Institute of Neuro immune Pharmacology, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, 33199 United States.

Methanandamide (mAEA) is a stable synthetic analog of endocannabinoid, anandamide (EA) and has been used as a therapeutic agent to improve motor functions and to down regulate the production of inflammatory mediators. HIV-infected cannabinoid users manifest significant impairments in neurocognition, neurodegeneration, and behavioral alteration. In this study, we explored the effect of mAEA on the cAMP, MAPK pathway and neurite outgrowth. The activation of cAMP, MAPK and neurite outgrowth produced by mAEA was reverted by AM281 an antagonist of CB1 receptor. Although mAEA is impenetrable across blood brain barrier (BBB), effective delivery of mAEA to the brain may help to revert cannabinoid and HIV induced deleterious effect. We have previously reported that magneto-electric nanoparticles (MENPs) can be used as nanocarrier to deliver therapeutic drugs across the BBB to brain effectively and to release the drugs on-demand. Therefore, in the present study we report the development of MENP-liposome-mAEA based nanoformulation (NF) and its further characterization. Our initial results confirmed that NF is non-toxic and did not affect the integrity of the BBB. Development of an effective NF containing mAEA, for brain delivery, may be able to combat cannabinoid induced deleterious effects in HIV-infected patients.

Supported by NIH/NIDA R01DA034547; 1R01DA037838; 1R01DA040537; 1R01DA042706

T76.Activation of the transient receptor potential mucolipin-1 receptor restores lysosomal biogenesis in model of HIV-related endolysosomal dysfunction

Yoo, SW, Ph.D.[1], Lisinicchia, J, BS[2], Lovett, J, BS[1], Gelman, BB, MD, Ph.D.[2], Haughey, NJ, Ph.D.[1]; [1]Department of Neurology, Richard T Johnson DIvision of Neuroimmunology and Neurological Infections, Johns Hopkins University School of Medicine, Baltimore, MD, 21287 United States. [2]Department of Pathology, Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, TX, 77555 United States.

Disturbances of endolysosomal systems have been implicated in cognitive impairment (CI) associated with HIV-infection. Here we examined the Coordinated Lysosomal Expression And Regulation (CLEAR) network, and its master transcription factor EB (TFEB) in frontal neocortices obtained from the National NeuroAIDS Tissue Consortium. CI patients (MCMD, n=36 and HAD, n=29) showed increased expression of TFEB, a negative regulator of transient receptor potential mucolipin-1 (TRPML1; regulates lysosomal calcium efflux and lysosomal acidification), Lamp1, a lysosomal structural protein, and several lysosomal hydrolases compared with cognitively normal (CN) HIV+ patients (n=41), or HIV- controls (n=68). We also observed decreased expression of TRPML1, an aspartyl protease, and a glucosidase in CI compared with CN and HIV- patients. Alterations in the expression of TFEB, Lamp1, and CTSD were confirmed by immunoblot. Based on these human data, we next used an animal model in which gp120 facilitates the accumulation of amyloid peptides, sphingomyelin, and calcium in lysosomes (gp120/APP/PS1 mice) to determine if pharmacological facilitation of luminal acidification could rescue lysosomal function. Luminal acidification by intraventricular infusion of ML-SA1 (a TRPML1 agonist) normalized CLEAR gene expression, sphingomyelin and Aβ deposition in hippocampus.

These suggest that CI in HIV infection is associated with dysfuncitons in the CLEAR network, and suggest that therapeutic approaches to restore lysosomal functions may protect the CNS in HIV infection.

Supported by R01MH110246, R01MH077542, P01MH105280

T77.Limited impact of alcoholism comorbidity in HIV on peripheral cytokine levels

Zahr, N.M., Ph.D.[1], Asok, P., MD[1], Sullivan, E.V., Ph.D.[2], Pfefferbaum, A., MD[1]; [1]Neuroscience, SRI International, Menlo Park, CA, 94025 United States. [2]Psychiatry and Behavioral Sciences, Stanford University, Stanford, CA, 94305 United States.

To determine if alcohol use disorders (AUD) contribute to disrupted cytokine levels in HIV, plasma were analyzed in 4 groups: controls (C, n=56, 26 women, 50.6 ±11.2yrs), AUD (E, n=82, 27 women, 50.8 ±9.1yrs), HIV infected (H, n=45, 16 women, 55.7 ±7.2yrs), and AUD+HIV comorbidity (HE, n=43, 16 women, 55.5 ±6.3yrs]. The Human Immune Monitoring Center (http://iti.stanford.edu/himc/) performed assays (EMD Millipore kits) on samples (n=226) collected between March 2013 and October 2016. Studentized residuals of the median fluorescence intensity of each cytokine considered kit number, Age, Sex, SES, and Ethnicity. Of 41 analytes, 9 showed diagnoses effects: IL12P40 (p=.03), IL15 (p=.03), IL1A (p=.03), IL9 (p=.007), IL1B (p=.008), and IL2 (p=.03) were lower and IP10 (p Supported by AA017347; AA005965; AA017168