ABSTRACT BOOK

12th SNIP CONFERENCE

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POSTER SESSION 1

- Young Investigator Session -

WP–1:

THE HIV gp120 PROTEIN DOES NOT INTERNALIZE CXCR4 IN SH-SY5Y CELLS. G. Bardi, J. Patel, R. Sengupta, M.Z. Khan, and O. Meucci; Department of Pharmacology & Physiology, Drexel Univ. College of Medicine, Philadelphia.

CXCR4, the specific receptor for CXCL12, is expressed by most cells in the brain including neurons and regulates important neuronal and glial function. CXCR4 also acts as HIV co-receptor and is involved in HIV neuropathology. In cultures of primary neurons the HIV envelope protein gp120 binds CXCR4 and activates signal transduction pathways leading to apoptosis, whereas CXCL12 promotes neuronal survival signaling through its receptor. To investigate the possibility of a different action of the two ligands on CXCR4 trafficking we used the human SH-SY5Y neuronal cell line and compared the effect of CXCL12 and gp120 (IIIB and SF2) on CXCR4 expression by flow cytometry. As expected, increasing concentrations of CXCL12 induce concentration-dependent receptor internalization. On the contrary, gp120 (both monomeric and oligomeric, with or w/o human CD4) did not affect CXCR4 surface expression, despite its ability to induce cell death and activate various intracellular pathways in SY5Y cells. Our results show that the events induced by gp120 in these cells do not require CXCR4 internalization.

WP-2

EFFECT OF ULTRAVIOLET LIGHT ON MURINE CUTANEOUS NERVES: VISUALIZATION AND GROWTH CHARACTERISTICS OF YFP-TAGGED NERVES IN A MOUSE MODEL. Elhabib Benlhabib PhD[a], Maria K. Hordinsky MD[a], George L. Wilcox PhD[a,b], Marna E. Ericson PhD[a]; Departments of [a]Dermatology and [b]Neuroscience, University of Minnesota, Medical school, Minneapolis, MN, USA 55455.

Loss or malformation of epidermal nerve fibers is a hallmark sign of neuropathic pain in humans and in animal models; understanding the conditions under which these nerve fibers die back would enhance our understanding of the etiology and possibly therapy of neuropathic pain. Ultraviolet (UV) irradiation is known to induce many changes in skin structure, function and interaction with nerves, and can induce both inflammation and alterations in the skin immune system. The aim of the present study is to develop a model allowing visualization of changes in skin/nerve structure and nerve-tissue interactions over time in mice whose neurons are tagged with yellow fluorescent protein (YFP) (compared with wildtype C57BL/6J mice as controls) by visualizing and quantifying growth characteristics of epidermal and dermal nerves after UV-induced insult. We used mice expressing YFP in peripheral nerve, B6.Cg-Tg(Thy1-YFP)16Jrs/J, and wildtype, C57BL/6-TgN (ACTbEGFP) 10 sb, from Jackson Labs in Bar Harbor, ME. This variant expresses YFP in motor and sensory neurons that is fluorescent into adulthood and amenable to aldehyde fixation. No fluorescence is detectable in non-neural cells (Feng G et al., 2000 *Neuron* 28:41). We chose this mouse line to increase our ability to accurately monitor morphological, functional, and signaling changes with intra-vital imaging of both dermal and epidermal nerves as well as inflammatory responses in skin.

Intravital imaging using a multi-photon laser confocal microscope (LSCM, Bio-Rad MRC 1024 for UV) of an anesthetized mouse allowed repeated monitoring of changes in innervation in dermis and epidermis after UV insult. Prior to imaging, male mice were anaesthetized with ketamine-acepromazine (final doses of ketamine [40 mg/kg] and acepromazine [1mg/kg]). Analysis of intravital imaging and fixed immunostained (PGP 9.5, CGRP, and collagen type IV) biopsy sections (60 microns) of non-irradiated or UVB-irradiated male YFP and wildtype mouse ear skin after a single exposure to UV light (10 mJ/cm^2) showed decrease in nerve density compared to naïve ears within 24 hours.

WP-3

THE CHEMOKINE CCL2 MODULATES CELL JUNCTIONAL PROTEINS IMPORTANT IN HIV INFECTED LEUKOCYTE TRANSMIGRATION ACROSS THE BLOOD BRAIN BARRIER. C.M.Buckner, E.A. Eugenin, L. Lopez, T. Belbin and J.W. Berman; Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

We demonstrated that HIV infected human leukocytes have an enhanced capacity to transmigrate across our tissue culture model of the human BBB in response to CCL2, resulting in increased BBB permeability and reduced tight junction protein (TJP) expression. To determine the effects of CCL2 on the BBB, we treated human brain microvascular endothelial cells (BMVEC) and astrocytes with CCL2 and performed Western blot analyses for cell junctional proteins. The TJPs ZO-1 and occludin decreased at 2h post-treatment, with ZO-1 remaining decreased for up to 12h in BMVEC. PECAM, CD99 and JAM-1, proteins involved in leukocyte transmigration, increased in BMVEC at early timepoints, with PECAM-1 expression decreasing after longer treatment with CCL2. In astrocytes, CCL2 induced an early increase in occludin and a late decrease in ZO-1. Preliminary studies using cDNA microarray analyses of untreated and CCL2 treated BMVEC demonstrated modulation of cell matrix adhesion, migration and proliferation genes. These data indicate that CCL2 alters proteins on BBB cells that mediate leukocyte transmigration, and therefore CCL2 may play an important role in the pathology characteristic of NeuroAIDS.

WP-4

THE ROLE OF OSTEOPONTIN IN NEUROAIDS. T. H. Burdo, M. Zandonatti, C. Flynn, D. Watry, and H. S. Fox; Molecular and Integrative Neurosciences Program, The Scripps Research Institute, La Jolla, CA 92037.

HIV-1 dementia has been shown to correlate with macrophage abundance in the brain. We have developed a novel transendothelial migration model that allows us to test the ability of chemokines to alter monocyte trafficking. Based on our microarray analyses that showed osteopontin (OPN), a unique type of chemoattractant protein, was upregulated in the brains of SIV encephalitis (SIVE) cases, we have further investigated the role of osteopontin. In our model, OPN decreased the percentage of macrophage capable of reverse transmigration (exiting the brain), thus potentially aiding in macrophage accumulation in the brain. In addition, real-time PCR analysis has revealed that OPN is significantly upregulated in frontal lobes of SIVE animals. Immunostaining indicated that OPN was also highly upregulated in perivascular regions of SIVE animals, and colocalized with CD163 (a marker for macrophages/microglia in neuroAIDS). Surprisingly, longitudinal examination of OPN in infected animals revealed a prominent correlation of increased levels of plasma OPN with

encephalitis. In SIVE, OPN levels in the plasma increased post-infection, and quite strikingly preceding the development of clinical symptomatology requiring sacrifice.

WP-5

SYNERGISTIC EFFECT OF COCAINE AND HIV ON FACTORS MEDIATING HIV-DEMENTIA. Dhillon N., Nicolay B., Gadgil M., Tsai Y-J., Dhillon S., Kenjale H., Li C., Narayan O., Buch S.; Kansas Univ. Med. Center,Kansas City, KS.

The role of cocaine in development of HIV-1-associated dementia (HAD), is becoming increasingly important. In this study we focused on the effect of cocaine on virus replication in monocyte-derived macrophages (MDM), and up regulation of factors that may lead to apoptosis of bystander neuronal cells in brain. Cocaine markedly enhanced virus production from SHIV-infected monocyte derived macrophages and from chronically infected promonocytic cell line (U1) as seen by ELISA and immunocytochemistry. Cocaine-treated macrophages transfected with HIV-LTRgag-GFP expressed higher levels of GFP compared untreated transfected cells. Analyses of chemokines in cocaine-treated macrophages by Realtime RT-PCR and Luminex assays suggested an increase in chemokines CXCL10 &, CCR2 and cytokine, IL-10, all of which are known to promote HIV replication in MDMs. In addition, cocaine caused upregulation of activation markers HLA-DR and CD40 and the viral co-receptor CXCR4. The results suggest that cocaine operates at multiple pathways to accelerate HAD.

WP-6

PREGNANCY-ASSOCIATED CHANGES OF IMMUNITY, A MODEL OF THE POSSIBLE CONTROL OF SOME AUTOIMMUNE DISEASES. S. R. Draca. Clinic "Dr M.Zotovic", Sokobanjska 13, Belgrade 11 000, Serbia and Montenegro.

The operational immune system is an *in vivo* open complex system, which can be incredibly sensitive to external factors, including hormones. Physiological states, pregnancy and parturition, are accompanied by remarkable endocrine-immunological alterations, and, undoubtedly associated with either remission or aggravation of the most of the autoimmune diseases. In the last decade, numerous studies suggested that immunological phenomena developing during pregnancy and postpartum period permits identification of the mechanism by which autoimmune diseases are modified. The potential of that interrelationship, as much as the role of "causative" or "trigger" agents should be further investigated in relation to new therapy approaches.

WP-7

HIV-1 INFECTED HUMAN ASTROCYTES USE GAP JUNCTION COMMUNICATIONS TO INDUCE APOPTOSIS OF UNINFECTED CELLS. Eugenin E.A. and Berman J.W. Dept of Pathology, Albert Einstein College of Medicine, Bronx, New York.

HIV infection of the CNS occurs early in the disease process and despite HAART, infection persists. Although there is no evidence of direct infection of neurons, neuronal damage and dropout can occur years after initial CNS infection and without apparent significant viral replication. The cellular reservoirs of the virus and its precise mechanism of neurotoxicity are still not fully identified. Astrocytes are a key cell of the BBB, and represent the majority of the cells of the CNS

parenchyma. Others and we determined that astrocytes can be infected with HIV, at a low percentage of infection. We used mixed cultures of human astrocytes and neurons and R5 or X4 HIV isolates and demonstrated that 2-3% of the cells were HIV infected. This low level of infection induced apoptosis in 40 ± 7 % of neurons and 11 ± 3.8 % of astrocytes. This apoptosis was dependent on gap junctional communication, as treatment of infected cultures with gap junction blockers abolished the spread of apoptosis. HIV infected astrocytes may mediate extensive CNS damage through gap junction communication.

WP-8

CD163 EXPRESSION IN CNS SUGGESTS TRAFFICKING OF HIV-1 INFECTED MONONUCLEAR PHAGOCYTES IN HIVE. T. Fischer-Smith[1], S. Croul[1, 2], C. Bell[1], K. Khalili[1], and J. Rappaport[1]; [1]Center for Neurovirology, Temple University School of Medicine, Philadelphia, PA, USA; [2]Drexel University College of Medicine, Philadelphia, PA USA

CD163, a monocyte/macrophage specific scavenger receptor for hemoglobin-haptoglobin complex, is reported to be expressed in the CNS by perivascular macrophages but not by resident microglia in human brain. We utilized this disparity in the expression pattern of CD163 by brain macrophages to further characterize the mononuclear phagocytes (MPs) in the CNS in HIVE and in HIV associated Progressive Multifocal Leukoencephalopathy (PML). Our results demonstrate $CD163^+$ cells with microglial morphology in HIVE, but not PML. Both parenchymal microglia and perivascular macrophages were $CD163^+$ in HIVE. In PML, only foamy macrophages associated with areas of demyelinating lesions were $CD163^+$. Results of these studies, demonstrating accumulation of $CD163^+$ MPs in CNS and visceral organs provides strong evidence for the role of increased monocyte/macrophage trafficking in the pathogenesis of HIVE.

WP-9

TIMP-1 PROMOTER REGULATION IN ASTROCYTES DURING CHRONIC NEUROINFLAMMATION. J.A. Gardner, L. Wu, A. Ghorpade; Pharmacology and Experimental Neuroscience, Univ. of Nebraska Medical Center, Omaha, NE

The pathogenesis of many neurodegenerative disorders is exacerbated by an imbalance between metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). We previously reported differential TIMP-1 expression in acute versus chronic activation of astrocytes, and in brain tissue of patients with HIV-1-associated dementia (HAD). To investigate TIMP-1 promoter regulation we used TIMP-1-luciferase reporter constructs in transfected astrocytes and interleukin (IL)-1beta as a model proinflammatory stimulus. Our results demonstrated that promoter regulation is an important mechanism for TIMP-1 chronic downregulation in astrocytes. TIMP-1 promoter activity was particularly responsive to activating protein (AP)-1 and polyoma enhancer A3 (PEA3) elements. Other cytokines including tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma, along with HIV-1, enhance the effects of IL-1beta on astrocyte-TIMP-1 expression. These data are important for unraveling the mechanisms underlying astrocyte responses during chronic inflammation.

WP-10

CELLULAR ORIGIN ALTERS VIRION INFECTIVITY. Peter J Gaskill, Michelle Zandonatti, Jenn Boyd and Howard Fox, Molecular and Integrative Neurosciences Department, TSRI, La Jolla, CA, 92037

Macrophage infection remains key to the pathogenesis of neuroAIDS. Here we report that the infectivity of viral particles is influenced by cellular origin, with virions derived from macrophages showing enhanced infectivity relative to those derived from CD4+ T lymphocytes. This was not due to viral genotype, as molecularly cloned virus exhibited the effect, and infectivity was dependent entirely on the cell type the virion had been generated from. Examination of this cell-type based variability in viral particle infectivity revealed that it was not due to variation in attachment conditions, cell-type specific soluble factors or cell-type specific incorporation of host cell proteins. Treatment of viral stocks derived from both macrophages and T-cells with a variety of glycosidases established that the infectivity of T-cell derived viral particles but not macrophage derived virions was consistently enhanced through deglycosylation, suggesting that cell-type based variability in viral infectivity could be due to differential glycosylation pathways in macrophages and T-cells. These data help to explain the correlation of macrophage tropism with brain disease.

WP-11

ALPHA-SYNUCLEIN MEDIATED MICROGLIAL ACTIVATION. J. Glanzer*, A. Reynolds*, I. Kadiu*, M. Ricardo-Dukelow*, L. Shlyakhtenko, M. Thomas, R.L. Mosley, H.E. Gendelman**; Dept. Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68132.

Activated microglia play a pathogenic role in Parkinson's disease (PD). Activation is incited, in part, by aggregated nitrated alpha-synuclein released from Lewy bodies contained within dead or dying dopaminergic neurons. We defined a fingerprint for these microglia using combinations of genomic (gene arrays), and proteomic (SELDI-TOF and liquid chromatography-tandem mass spectrometry) methods and identified secretory changes in transcriptional regulation (NF*K*B activation) and secretion (inflammation, lipid binding and lipid metabolism proteins) that follow alpha-synuclein stimulation. This fingerprint is unique when compared to other known microglial stimulators (lipopolysaccharide, latex beads). The data, strengthened by both genomic and proteomic data, provides evidence for a unique pathway for microglia-mediated inflammation in the pathogenesis of PD.

WP-12

TRAIL-MEDIATED INHIBITION OF AKT-1 PHOSPHORYLATION INDUCES APOPTOSIS IN HIV-1 INFECTED MACROPHAGE: INNATE CONTROL OF A PRINCIPAL VIRAL RESERVOIR. Y. Huang, N. Erdmann, H. Peng, S. Herek, J. Davis, X. Luo, T. Ikezu, and J. Zheng; University of Nebraska Medical Center, Omaha, NE

HIV-1 enters the brain within weeks of initial infection as Mononuclear Phagocytes (MP) cross the blood brain barrier. These HIV-1 infected MP are thought to be a reservoir for viral replication, and thus drive the pathogenesis of HIV-1 associated dementia (HAD). In this work we examine the effect and signaling cascade of TRAIL, a newly identified death ligand, in HIV-1 infected MP. Our

results demonstrated TRAIL induced death in HIV-1 infected human monocyte-derived macrophage (MDM); TRAIL mediated cell death observed in uninfected cells was at significantly lower levels. TRAIL induced activation of caspase-3, -8, and -9 in HIV-1 infected MDM, while caspase inhibition blocked apoptosis. TRAIL exposure significantly decreased Akt-1 phosphorylation in HIV-1 infected MDM. Transfection with a dominant-negative Akt-1 adenovirus potentiated TRAIL-induced apoptosis, while constitutively active Akt-1 blocked TRAIL-induced apoptosis in HIV-infected MDM. From this data we conclude TRAIL preferentially provokes apoptosis of HIV-1 infected MDM, and the mechanism is reliant upon the inhibition of Akt-1 phosphorylation.

WP-13

OPIOID INDUCED ELEVATIONS OF SPINAL PROINFLAMMATORY CYTOKINES ARE MEDIATED VIA NON-CLASSICAL OPIOID MECHANISMS. M.R. Hutchinson, E.D. Milligan, B. Jekich, B. Coats, S. Lewis, S.F. Maier, & L.R. Watkins. Dept. Psychology & Center for Neuroscience, Univ. of Colorado at Boulder.

Spinal glial proinflammatory cytokines (PICs) are powerful pro-nociceptive signals which compromise acute & chronic morphine analgesia. No study has addressed if other clinically relevant opioids release spinal PICs, or if opioids exert their effects in a classical opioid fashion. Methods: Rats received R- (RM), S-methadone (SM), morphine (Mor) or saline intrathecally for 7 days. On days 1, 4 & 7, mechanical (Von Frey) & thermal (radiant heat: tailflick & hind paws) were recorded. On day 7, tissues were collected for mRNA & protein analysis. Results & Discussion: On day 1, RM & Mor produced profound analgesia with significant tolerance developed to RM & Mor analgesia by day 7. RM, SM or Mor all produced significant hyperalgesia & allodynia. Analysis of dorsal spinal cord samples revealed both opioid active (Mor & RM) & inactive (SM) treatments produced elevations of Interleukin-1beta mRNA corresponding to changes in nociception. Further studies characterizing protein & mRNA changes in other PICs are underway.

WP-14

SUPPRESSION OF PROINFLAMMATORY CHEMOKINE EXPRESSION BY NOCICEPTIN. D.E. Kaminsky* and T.J. Rogers, Depts. of Microbiology and Immunology, and Pharmacology, Temple University, Philadelphia, PA 19140.

Various physiological functions of opioids are regulated through the activation of an opioid-like receptor (ORL1). Activation of ORL1 modulates immune system function by inducing the chemotaxis of certain immune cells, participating in cross-talk with, and desensitization of, the mu-opioid receptor, and modulation of PHA-induced PBMC proliferation. The focus of the current work was to study the regulation of pro-inflammatory chemokine expression in N/OFQ-treated human monocytes. Our previous results showed that N/OFQ suppressed the basal and LPS-induced expression of MCP-1 and RANTES protein in human monocytes. However, MCP-1 and RANTES mRNA expression were not reduced in response to N/OFQ administration. Analysis of intracellular MCP-1 through flow cytometry also revealed that N/OFQ failed to inhibit the secretion or translation of MCP-1 protein. These data suggest a potentially unique role for N/OFQ in the post-translational suppression of pro-inflammatory chemokines in human monocytes. Supported by NIH grants DA-14230, DA-13249, DA-06650, and DA-16544.

WP-15

A ROLE FOR IL-12 IN MORPHINE WITHDRAWAL MEDIATED TH2 DIFFERENTIATION. Jennifer Kelschenbach and Sabita Roy, U of Minnesota, Minneapolis 55455

The consequences that drug withdrawal has on immune functioning has only recently been appreciated, however given the wide variety of opiate analgesic use understanding the decrements to immune function that withdrawal from these drugs cause is of crucial importance. In current studies, it was hypothesized that morphine withdrawal mediated Th2 differentiation results from suppression of IL-12 production. To gain insight into how morphine withdrawal is regulating IL-12 synthesis an *in vitro* model of morphine withdrawal was developed. Both primary macrophages and CRL-2019 macrophages displayed decreased levels of IL-12 after being treated using this paradigm. Additionally, analysis of TLR-4 expression was performed following *in vitro* withdrawal treatment, and it was demonstrated that withdrawal decreased the expression of TLR-4. Finally, a withdrawal time course study was performed *in vivo* to gain further understanding of the mechanisms that control IL-12 production following withdrawal. It was demonstrated that withdrawal not only decreased IL-12 levels in a time-dependent manner, but also decreased NF-KB, AP-1, and C/EBP binding to DNA response elements. Ongoing studies are examining the effects of withdrawal on IL-12 p40 promoter activity, to gain further insight into how withdrawal regulates IL-12 production. This work was supported by sponsored grants from NIH (R-01 DA12104, KO-2 DA015349, and P50 DA11806) and the Pharmaconeuroimmunology Training Grant from NIDA (T32 DA 07097).

WP-16

THE KOR INFLUENCE ON THE LEVEL OF IL-7R IN MURINE THYMOCYTES IS MATURATION STAGE-SPECIFIC. M. Khimich, J.M. Bidlack; Dept. of Pharmacology and Physiology, U Rochester, Sch Medicine and Dentistry, Rochester, NY 14642.

Endogenous stimulation of the kappa opioid receptor (KOR) has been shown to provide a positive signal for intrathymic T-cell maturation. However, *in vitro* studies using primary mouse thymocytes suggest that the κ-selective agonist U50,488 decreases the level of expression of IL-7R, the receptor which plays a major role in early T-cell development primarily by promoting thymocyte survival. To further elucidate the role of KOR in thymocyte development, in current study, we investigated the influence of U50,488 administration on IL-7R alpha chain transcription in the EL4.IL-2 and R1.1 thymoma cell lines corresponding to II and IV stages of intrathymic T-cell development. Using RT-PCR, we showed that U50,488 had opposite effects on these cell lines: U50,488 decreased IL-7R mRNA expression in EL4.IL-2 cells, while increased it in the R1.1 cells in PHA – dependent manner. The effects were completely blocked by the kappa-selective antagonist nor-BNI. The data obtained suggest that the effect of opioids may depend on the stage of thymocyte differentiation. (Supported by grants K05-DA00360 and DA04355).

WP-17

MECHANISMS OF HIV-TAT TOXICITY IN HUMAN NEURONS. J.E. King, E.A. Eugenin, T.M. Calderon, and J.W. Berman; Dept of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

HIV infection of the CNS can result in significant neurologic dysfunction. NeuroAIDS is characterized by neuronal injury and loss, yet there is no evidence of HIV positive neurons. Neuronal damage must therefore be due to elaboration of factors by other CNS infected cells, including the viral protein tat. We and others demonstrated that tat induces apoptosis in neurons, and this process is dependent on NMDA receptor (NMDAR) activation. We demonstrated formation of a newly identified complex including tat, LRP, PSD95, NMDAR, Pyk2 and nNOS on the surface of tat treated neurons that mediates the early events of tat induced apoptosis. We also demonstrated that tat treatment of neurons causes NMDAR phosphorylation on multiple subunits. The NMDAR subunit 2A is phosphorylated 5 to 15 minutes after tat treatment in a Src kinase dependent manner. Using phosphorylation site specific antibodies, we showed that NMDAR1 is phosphorylated 30-60 minutes after tat treatment which is PKC, but not PKA, dependent. These data suggest that NMDAR phosphorylation is an important mechanism by which NMDAR activity is upregulated during apoptosis.

WP-18

CHRONIC MORPHINE INHIBITS IMMUNE CELL-MEDIATED BACTERIAL CLEARANCE AND ANGIOGENESIS FOLLOWING TISSUE INJURY. J.L. Martin and S. Roy; Department of Pharmacology, U of Minnesota, Minneapolis, MN.

A marked delay in tissue healing events with significant bacterial infection on board is present in injection sites of heroin addicted patient. Wound healing occurs following two sequential events; 1) bacterial clearance and 2) wound remodeling. We hypothesize that chronic morphine's suppressive effect on immune cells will lead to an inhibition of bacterial clearance and a delay in tissue remodeling. Preliminary data from our *in vitro* studies show significant inhibition of macrophage inflammatory protein-2 and vascular endothelial growth factor expression following chronic morphine treatment of macrophages induced with LPS. *In vivo* studies also show marked decreases in a) immune cell migration and function b) bacterial clearance and c) wound closure following morphine. However, angiogenesis is prematurely induced in mice treated with chronic morphine. Understanding the mechanisms by which these wound healing events are mediated in the presence of morphine is currently under investigation and will be presented at the conference.(Supported by RO-1 DA 12104 , KO2 DA015349, and P-50 DA11806).

WP-19

DISEASE PROGRESSION AND SIV TAT EVOLUTION IN CSF CORRELATE INVERSELY IN MACAQUE MODEL OF MORPHINE-DEPENDENCE AND AIDS. R.J. Noel Jr., Z. Marrero, R. Kumar, G. Chompre, Y. Yamamura, A. Kumar; Dept. Biochemistry, Dept. Microbiology, Ponce School Medicine, Ponce PR 00716.

Morphine abuse has been associated with greater replication and faster progression in a macaque model of AIDS. We have reported that 50% of morphine-addicted macaques progress rapidly and

2/3 of rapid progressors developed NeuroAIDS. Here we examined the sequence evolution of the SIV Tat in the cerebrospinal fluid (CSF) of morphine-dependent and control macaques. Tat evolution in CSF was inversely related to disease progression. The highly neuropathogenic inoculum clone is the prevalent CSF form in rapid progressors. Divergence from the inoculum clone was significantly greater in both morphine-dependent normal progressors and control macaques. Furthermore, morphine alters the type of mutation, showing an enhanced transition:transversion ratio. Rapid disease exacerbates this trend and appears to influence the distribution of mutations. Thus morphine abuse may change the nature and extent of mutations that drive viral evolution.

WP-20

MORPHINE SUPPRESSES MHC-II EXPRESSION ON CIRCULATING B LYMPHOCYTES. Alexandria L. Nugent and Barbara M. Bayer, Georgetown U.

Previous reports have demonstrated that a single exposure of rats to morphine (20 mg/kg) results in a decrease of both MHC-II mRNA and protein expression in circulating B lymphocytes. In the present study, we examined whether these effects were reproduced with a lower dose of morphine and, if so, whether they persisted upon chronic drug exposure. A significant decrease in basal MHC-II expression was observed 2 hours following morphine (10 mg/kg) administration. This was also accompanied by a significant decrease in interleukin 4 (IL-4) induction of MHC-II expression. However, following chronic exposure to morphine, the effects on basal and induced expression of MHC-II were no longer present. To determine whether *direct* activation of central pathways could be involved, naltrexone was microinjected into the lateral ventricle thirty minutes prior to administration of either morphine or saline. Central naltrexone pretreatment completely blocked the suppressive effects of morphine treatment on IL-4 induced MHC-II expression. These results suggest that central opioid receptors may be involved in both the acute and chronic effects of morphine on MHC-II expression.

WP-21

***IN-VIVO* MODEL FOR THE DETECTION OF MORPHINE INDUCED APOPTOSIS.** M. Olin, S. Roy, T. Molitor, and P. Peterson. College of Veterinary Med, University of Minnesota, St. Paul, MN 55108.

Opiates are known to have adverse affects on immunological processes such as apoptosis. Apoptosis is an important mechanism for the purging of infected cells, eliminating the ability of pathogens to replicate. Apoptosis is initiated through a complex pathway involving a family of cysteine proteases known as caspases. The detection of apoptosis is important for a complete understanding of adverse effects of drugs such as morphine. However, when cells or organs are removed from the host for analysis, cells naturally go into apoptosis, resulting in exaggerated positive results. The objective of this study was to develop an *in-vivo* model for the detection of apoptosis induced by morphine treatment. Mice were injected with placebo, LPS, or morphine and LPS. Subsequently, mice were injected with a poly-caspase probe through the tail vein. Spleen and tissue samples were harvested and analyzed for apoptosis by flow cytometry and fluorescent microscopy. It was concluded that in vivo administration of the polycaspase probe was a reliable-method for detecting apoptotic cells resulting from morphine exposure *in-vivo*.

WP-22

DEC-205 IS A NOVEL RECEPTOR FOR HIV-1 IN RENAL TUBULAR CELLS. R.Patel, I.Hatsukari, H.Schmidtmayerova. Feinstein Institute for Medical Research, Manhasset, NY, 11030

Renal biopsy data suggest that tubular cells may serve as a reservoir for HIV-1. The mechanism by which HIV-1 enters these cells has not been identified. The renal cells do not express any of the known HIV-1 receptors. Here we report that the C-type lectin DEC-205 acts as an HIV-1 receptor in human kidney tubular (HK2) cells. Interaction of HIV-1 with DEC-205 results in the internalization of the virus and establishment of a nonproductive persistent infection of HK2 cells. HIV-1-specific strong stop DNA is detected in the infected HK2 cells over an extended period of time, and the virus can be rescued by co-cultivation of HIV-1-infected HK2 cells with primary macrophages. HIV-1 infection is blocked by pre-treatment with specific anti-DEC-205 antibody. Moreover, expression of DEC-205 in cells lacking the DEC-205 receptor renders them susceptible to HIV-1 infection.

WP-23

PROTEIN KINASE C ZETA (PKCz) MEDIATES HETEROLOGOUS DESENSITIZATION BETWEEN THE MU OPIOID RECEPTOR (MOR) AND CCR5. R.T. Rahim, G. Bardi, *F. Bednar, and T. J. Rogers, Center for Substance Abuse Res., *Dept. of Micro. & Immunol. & Fels Institute of Mol. Bio. & Cancer Res., Temple Univ., Phila, PA 19140.

Previously, we have shown that DAMGO, a MOR-selective agonist, induces heterologous desensitization of CCR5. The present study examined the signaling events mediating cross-talk between MOR and CCR5. Cell lines expressing MOR and CCR5, and human macrophages were treated with DAMGO and used for these studies. Western blot analyses showed that PKCz associates with CCR5 and that phosphorylated PKCz (Thr 410) is upregulated by treatment with DAMGO. In further studies, desensitization of CCR5 by pretreatment with DAMGO led to inhibition of Ca^{2+} mobilization and a decreased chemotactic response to MIP1-beta. In addition, DAMGO induced phosphorylation of CCR5 and PKCz kinase activity were observed upon immunoprecipitation of CCR5. Use of a PKCz pseudo-substrate inhibitor reversed these effects. Overall, the data suggest that DAMGO-induced desensitization of CCR5 leads to phosphorylation of CCR5 and that PKCz mediates this process. Supported by NIDA grants DA16544, DA06654, DA14230, and P30-DA13429.

WP-24

EVOKED POTENTIALS IN A PRIMATE MODEL OF DRUG ABUSE & NEURO-AIDS. Riazi, M[1], Marcario, JK[1], Samson, FK[1], Kenjale, H[2], Adany, I[2], Narayan, O[2], & Cheney, PD[1]. Molecular & Integrative Physiology[1], Microbiology, Molecular Genetics & Immunology[2], U. Kansas Med Ctr, Kansas City, KS 66160.

The general objective of our work is to characterize the electrophysiological consequences of opiate (morphine) dependence in a nonhuman primate model of neuro-AIDS produced by bone marrow injection of SIVmac (R71/E17). Electrophysiological integrity was assessed by recording cortical and spinal cord motor evoked potentials (EPs), auditory brainstem responses, somatosensory EPs, and visual EPs in a cohort of 17 male, Indian origin rhesus macaques. The cohort was divided

into 3 groups: morphine only (n=5), SIV only (n=5), and SIV + morphine (n=6). Multimodal EPs were recorded during the morphine dependency period (26 weeks) and post-inoculation period (33 weeks). Viral load, CD4+/CD8+ cell counts and white blood cell counts were obtained monthly from blood samples. Viral RNA was recovered from plasma and CSF samples of all inoculated subjects (n=11) indicating productive infection. Preliminary results suggest that the severity of EP abnormalities is not substantially altered by chronic morphine exposure. (NIH NDA12827)

WP-25

BEHAVIORAL CHARACTERISTICS OF THE HIV-1 TRANSGENIC RAT. D.H. Root, M. Vigorito, A.L. LaShomb, J.A. Beltran, S. Swarup, S.L. Chang; Dept. of Psychology; Dept. of Biology, Seton Hall University, South Orange, NJ 07079.

The effects of HIV-1 infection on behavior and cognition are not completely understood but likely result from the actions of the virus itself and cytokines induced by HIV-1 infection (e.g.,TNF-alpha, IL-1beta, and IL-6). The new HIV-1 transgenic (Tg) rat model provides the opportunity to investigate the behavioral and physiological manifestations of HIV-1 and cytokines. In this study, behavioral characteristics and cytokine levels of the HIV-1 Tg rat were examined and compared to F344 control rats. Prior studies indicate that cytokine treatment causes anhedonia or a reduced response to reward. There was no difference in the serum levels of TNF-alpha, IL-1beta, and IL-6 between the HIV-1 Tg and F344 controls, and little evidence of anhedonia using sucrose as a reward. Surprisingly, when allowed access to a running wheel, the HIV-1 Tg rats ran significantly more than controls. Open field tests indicated that high running levels of HIV Tg rats may not be attributed to hyperactivity. These data suggest that HIV-1 infection may increase the "addictive" properties of some rewarding behaviors such as running (partially supported by DA007058 and DA 016149 to SLC).

WP-26

HIV-1 TAT CYTOTOXICITY AND MICROTUBULES. RL Self, KJ Smith, L Ghayoumi, and MA Prendergast; Psych., Univ. of Kentucky, Lexington, KY 40506.

Human Immunodeficiency Virus Type 1 (HIV-1) causes dementia in 1/3 of seropositive individuals. The viral transcription protein Tat may be a key component in HIV-related cytotoxicity. Tat interacts with alpha beta-tubulin dimers, microtubules, and LIS1 in T-cells. Studies examined whether Tat 1-72 (0.1-100nM) exposure (24-120 hrs.) would alter microtubule associated protein-2 (MAP-2) immunoreactivity (IR) and/or propidium iodide (PI) uptake in rat hippocampus. Subsequent experiments examined Tat (0.01-100nM) on tubulin polymerization. Tat did not induce changes in MAP-2 levels and increased PI uptake only in the CA1 at 10 and 100nM after 24hrs. At 72hrs, increases in MAP-2 IR and decreases in PI uptake were seen at all concentrations and in all regions (DG, CA3, and CA1). After 120 hrs, significant reductions in MAP-2 IR resulted at all Tat concentrations and in all regions, but PI uptake increased only at 0.1 and 1nM. Tat significantly decreased tubulin polymerization at all concentrations. Data suggests Tat disrupts microtubule function in a time-dependent manner and may target cytoskeleton structure to produce cytotoxicity, leading to cognitive impairment and/or HIV-associated dementia.

WP-27

IDENTIFICATION OF THE CANNABINOID RECEPTOR 2 GENE (*Cnr2*) ELEMENTS IN B-cells of C57BL/6 MICE. T.A. Sherwood, L. Nong, M. Agudelo, H. Friedman and T.W. Klein; Dept. Medical Microbiology & Immunology, Univ. South Florida, Tampa, FL 33612.

Searching of mouse databases revealed that the *Cnr2* gene is located on chromosome 4, is 24.7kb in length and produces two transcripts employing different first exons. There is much understanding of the coding region of the mouse *Cnr2* gene in terms of its nucleic acid sequence; however, the entire gene structure including the transcriptional start sites (TSS), promoter, and *cis*-factors that regulate its expression within the immune system have not been identified. We isolated B-cells from mouse splenocytes using the mouse EasySep B-cell enrichment kit (Stem Cell Technologies Inc) followed by RNA extraction (Tri-Reagent, Sigma) and finally employing the SMART RACE technique (Clontech) to identify three putative TSS. Furthermore, using web based programs such as ClustalW and Genomatrix we have taken a bioinformatics approach in identifying the putative promoter as well as putative *cis*-elements. Future reporter system studies will be performed to confirm promoter activity. Supported by DA10683 and DA03646 from NIDA.

WP-28

ROLE OF E2F1-DEPENDENT APOPTOTIC TARGETS IN HIV NEUROPATHOLOGY. S. Shimizu, M.Z. Khan, A. Parkar, and O. Meucci; Dept. Pharmacology and Physiology, Drexel University, College of Medicine, Philadelphia, PA 19102.

Recent *in vitro* and *in vivo* studies suggest the Rb/E2F1 pathway is involved in HIV neuropathogenesis. This study aims to test the hypothesis that up-regulation of E2F1-dependent proteins are involved in HIV-induced neuronal damage. To this end, we used an in vitro model of HIV neurotoxicity and human brain tissue samples from the following groups of patients: 1) HIV (+) with neurological deficits, 2) HIV (+) without neurological deficits and 3) normal control patients. Our data show that the expression of E2F1 is up-regulated in rat primary neuronal cultures exposed to the HIV envelope protein, gp120. Also, in these cultures gp120 treatment increased the transcriptional activity of E2F1, as determined by gene reporter assays. Furthermore, primary neurons derived from E2F1 deficient mice were resistant to gp120 induced neurotoxicity. The studies with brain tissue samples showed increased expression of E2F1 in the nucleus of neurons of HAD patients, as well as the expression of one of its transcriptional targets, cdc2. These data suggest that E2F1 transcriptional targets may play a crucial role in HIV neuropathology.

WP-29

LOW DOSES OF MORPHINE INHIBIT HIV-1 REPLICATION IN PRIMARY MACROPHAGES. P. Singh, N. Hitosugi, I. Hatsukari, H. Schmidtmayerova, and P. C. Singhal. Feinstein Institute for Medical Research, Manhasset, NY, 11030

Several studies have reported enhancement of HIV-1 replication in morphine-treated peripheral blood mononuclear cells (PBMC), but the effects of morphine on viral replication in primary macrophages are not clearly defined. In this study, we report dichotomous effects of morphine on HIV-1 replication in primary cultures of blood-derived macrophages (BDM). While high doses of morphine showed minimal effects on HIV-1 replication, a low dose of morphine inhibited HIV-1

replication in BDMs. The effect of morphine was reversed by opiate receptor antagonist, naloxone, indicating involvement of morphine-induced activation of opiate receptors in the inhibition of HIV-1 replication. Furthermore, inhibition of morphine-induced activation of p38 MAPK with SB202190 reversed morphine-induced suppression of HIV-1 replication. These data suggest that low concentration of morphine inhibit HIV-1 replication in BDM via activation of p38 MAPK pathway.

WP-30

DOWN-REGULATION OF DOR mRNA CAUSED BY ACTIVATION OF MOUSE MICROGLIAL CELL LINE. S. Sumagin, J. M. Bidlack Department of Pharmacology and Physiology University of Rochester School of Medicine and Dentistry, Rochester, NY

Microglia play a vital role in a number of neuroinflammatory diseases. Because there are, as of yet, no well characterized human microglial cell lines, and primary microglial cultures are difficult to obtain, the mouse microglial cell line BV-2 has been used. Our hypothesis was that activation of the mouse microglial cell line BV-2 with proinflammatory molecules would lead to altered levels of opioid receptors. We used RT-PCR to detect mRNA levels for the three opioid receptors mu, delta, and kappa. Delta opioid receptor (DOR) mRNA was expressed in BV-2 cells in untreated cells but treatment for 3, 6, 12, and 24 hr, with LPS, IFN-gamma, or a combination of both, decreased the expression of DOR mRNA. Neither mu nor kappa opioid receptor mRNA was detected, regardless of treatment type, or time. DOR mRNA was also decreased by treatment of cells for 24 hr with TNF-alpha, and IL-6, but not by treatment with IL-1beta. The BV-2 mouse microglial cell line can be used as a model to study the regulation of DOR. (Supported by the grants K05-DA00360, DA04355, and T32 DA07232 from NIDA.)

WP-31

EFFECT OF CHRONIC MORPHINE AND MORPHINE WITHDRAWAL ON NK CELL ACTIVITY. A. Verma, J. J. Meissler, T. K. Eisenstein; Center for Substance Abuse Research and Dept. of Microbiology and Immunology, Temple Univ. School of Medicine, Philadelphia, PA

Literature on the effect of chronic administration of morphine or its withdrawal on Natural Killer (NK) cell activity is sparse, and results do not agree. Using spleen cells from mice implanted with morphine slow-release pellets, we quantified NK cell activity against YAC-1 targets and determined NK cell percentages by flow cytometry, with or without pre-treatment with alpha-galactosyl-ceramide (a-GalCer), an immune stimulator that increases NK cell numbers. At 48 hr post pellet implantation, morphine suppressed both NK cell percentages and activity, as compared to placebo. At 96 hr, NK cell levels returned to normal. When morphine pellets were removed (abrupt withdrawal), NK cell numbers and activity showed robust (40%) decreases at 15 and 24 hrs. Without a-GalCer, the trends were similar, but the differences were smaller. These results show that mice become tolerant to the depressive effect of morphine on NK cell numbers and activity, and that withdrawal reinstates depression of these parameters. Supported by NIDA grants DA14223 and DA13429.

WP-32

HEROIN-INDUCED ALTERATIONS OF LIPOPOLYSACCHARIDE (LPS)-INDUCED HYPERTHERMIA: TESTS OF DOSE DEPENDENCY. Alison F. Wagner, Donald T. Lysle; Psychology, Univ. North Carolina, Chapel Hill, NC.

Opioids are known to induce alterations in body temperature, but little is known about how these alterations interact with fever, an important component of the host's defense. In the present study, we evaluated the effects of different doses of heroin on LPS-induced fever using the Mini-Mitter biotelemetry system. The results showed that a low dose of heroin (1.0 mg/kg) produced hyperthermia after administration (1-3 hours), whereas a high dose of heroin (10 mg/kg) produced hypothermia during the same period of time. Furthermore, the results showed that both doses of heroin inhibited LPS-induced hyperthermia, which occurs 4-8 hours after administration. The results indicate an inhibitory effect of heroin on the LPS-induced fever that is not dependent on the nature of initial heroin-induced alterations in body temperature. Additional studies are providing further analysis of the mechanisms of these independent effects. The investigation provides important insights into opioid alterations of body temperature in response to infection.

WP-33

MORPHINE UP-REGULATES FUNCTIONAL EXPRESSION OF NEUROKININ-1 RECEPTOR IN NEURONS. Q. Wan, S.D. Douglas, X. Wang, D.L. Kolson, L.A. O'Donnell, and W-Z., Ho; Div. of Immunol., The Children's Hosp. of Phila, Dept. of Pediatr., Univ. of Penn. Sch. of Med., Phila., PA 19104.

Neurokinin-1 receptor (NK-1R), the neuropeptide substance P (SP) preferring receptor, is highly expressed in areas of the central nervous system that are implicated in behavior, especially in the management of depression, anxiety and stress. Repeated exposure to opioids may sensitize neuronal systems involved in stress response. In the present study, the effects of morphine, the principal metabolite of heroin, on the functional expression of NK-1R were assessed in primary rat cortical neurons. We demonstrated that mu-opioid receptor and NK-1R are co-expressed in rat cortical neurons. Morphine enhanced NK-1R expression in primary rat cortical neurons at both the mRNA and protein levels. The up-regulated NK-1R by morphine was functional, since morphine-treated cortical neurons had greater SP-induced Ca^{2+} mobilization than untreated cells. Blocking opioid receptors on the cortical neurons by naltrexone or CTAP (a mu-opioid receptor antagonist) abolished morphine's action. Investigation of the mechanism(s) responsible for morphine-mediated NK-1R up-regulation showed that morphine had the ability to activate NK-1R promoter. In addition, morphine induced the phosphorylation of p38 MAPK protein in rat cortical neurons. These data suggest a plausible cellular mechanism involved in opioid-mediated neurological disorders.

WP-34

EPIGENETIC AND TRANSCRIPTIONAL REGULATION OF IL-2 IN ACTIVATED T CELLS BY MORPHINE. Jinghua Wang, Roderick A. Barke, Sabita Roy. Depts of Pharmacology and Surgery, University of Minnesota

Chronic morphine inhibits IL-2 at both transcription and protein levels. Herein, we showed that morphine up-regulated CREM, and down-regulated p-CREB expression in activated T cells.

Transfection of an anti-sense CREM and CREB plasmid into T cells rescued inhibition of IL-2 promoter activity and protein production induced by morphine. Furthermore, morphine treatment resulted in an increase of cAMP and inducible cAMP early repressor (ICER) expression. ICER competed for p-CREB binding to the cAMP responsive elements (CREs), leading to the uncoupling of CBP/p300, and thereby abrogating early stages of transcription of IL-2. Moreover, CREM competitively substituted for pCREB, which decreased transcription of IL-2 in the late stage of T cells activation. In addition, we also found that morphine increased the acetylation of histones and decreased the accessibility of the IL-2 promoter to endo-nuclease. These findings suggest that morphine may function through both transcriptional and epigenetic mechanisms to inhibit IL-2 production. (This work was supported by grants RO-1 DA 12104 and KO2 DA015349, and P50 DA 11806, T32DA07097).

WP-35

MORPHINE WITHDRAWAL ENHANCES HIV INFECTION OF HUMAN T LYMPHOCYTES THROUGH THE INDUCTION OF SUBSTANCE P. X. Wang[1], J.S. Peng[3], S.D. Douglas[1], D. S. Metzger[2], D. J. Zhou[3], and W.Z. Ho[1] [1]Div.of Immunol., The Children's Hosp. of Phila, [2]Dept. of Pediatr., Univ. of Penn. Sch. of Med., Phia., PA 19104 [3] Virology Lab, Wuhan CDC, Wuhan, P.R.China

Opioid withdrawal is a crucial and recurring event during the course of opioid abuse, and has a negative impact on the immune system. In this study, we investigated whether morphine withdrawal (MW) and precipitated withdrawal (PW, blocking opioid receptors by nalxone after morphine cessation) potentiates HIV infection of human T lymphocytes. MW and PW enhanced HIV infection of peripheral blood lymphocytes (PBL) and T cell lines (Jurkat and CEMX174). In addition, both MW and PW induced HIV replication in a latently HIV infected human T cell line (J1.1). Investigation of mechanisms responsible for MW and PW action showed that MW and PW significantly enhanced expression of the neuropeptide substance P (SP), an important immunomodulator, by both PBL and the T-cell lines. Exogenous SP, when added to T-cell cultures, also enhanced HIV replication. CP-96,345, a non peptide the SP receptor antagonist not only blocked exogenous SP mediated-enhancement of HIV infection, but also abolished MW- or PW-induced HIV replication in T cells. Furthermore, MW or PW activated HIV LTR promoter. These findings are additional evidence that opioids have a cofactor role in promoting HIV infection.

WP-36

DIFFERENTIAL REGULATION OF IFN-GAMMA INDUCED IDO AND WRS BY IL4 AND IL13 IN MICROGLIA. M.C. Yadav, C. Flynn, D. Watry, E.M.E Burudi and H.S. Fox; Dept. Molecular and Integrative Neurosciences, TSRI, San Diego, CA 92037.

Indoleamine 2,3 dioxygenase (IDO), the first and rate limiting enzyme in tryptophan catabolism, has been implicated in the pathogenesis of neuroAIDS. It can lead to neurotoxicity by generating quinolinic acid and inhibit the immune response through both tryptophan depletion and generating its catabolites. Here, we report the modulation of IDO by IL4 and IL13 in mouse microglia. Both IL4 and IL13 greatly enhanced IFN-gamma induced IDO expression. Also both IL4 and IL13 acted by a STAT6 independent mechanism. As tryptophenyl tRNA synthetase (WRS), which protects cells by allowing them to use low tryptophan levels is coinduced with IDO, we checked WRS expression

in parallel. Strikingly, IL4 and IL13 downregulated IFN-gamma induced WRS expression. Also, both wortmannin and LY294002 reduced IDO but enhanced WRS expression. These findings show discordance between modulation of expression of two distinct tryptophan-related–enzymes. Deciphering pathways regulating these enzymes in HIV-infected macrophages / microglia will allow these pathways to be manipulated therapeutically.

WP-37

INVESTIGATION OF THE ROLE OF CHEMOKINES RECEPTORS IN INFLAMMATION-INDUCED HYPERALGESIA. Ning Zhang and Joost J. Oppenheim; Laboratory of Molecular Immunoregulation, Intramural Research Support Program, Building 560, Room 21-89A, Frederick, MD 21702-1201, USA

Chemokine receptors serve as a bridge between the immune and neural systems. Neuropeptides, such as opioids, inhibit chemokine receptor function on leukocytes by activating Gi protein and calcium-independent protein kinase C. Conversely, during inflammation, chemokines similarly regulate neuronal sensing. Activation of chemokine receptors on neurons desensitizes the analgesic μ-opioid receptor and concomitantly enhances the sensitivity of a pain receptor, TRPV1, via protein kinase C-dependent phosphorylation, resulting in hyperalgesic effects.

POSTER SESSION 2

- General Session -

TP-1

NEUROAIDS AND OXIDATIVE STRESS: CHANGES IN NEURONAL OXIDATIVE STATUS ARE ESSENTIAL PART OF THE MECHANISM OF HIV-1 TAT NEUROTOXICITY. M.V. Aksenova, M. Aksenov, C.F. Mactutus and R. M. Booze. Depts. of Psychology, Physiology and Pharmacology, Program in Behavioral Neuroscience, USC, Columbia, SC, 29229.

HIV-1 protein Tat is neurotoxic and is believed to play an important role in pathogenesis of HIV dementia. As we report in this study, oxidative stress is early and essential event in the mechanism of Tat-mediated neurotoxicity. Changes in protein oxidation induced by Tat microinjections in rat striatum start occur early and coincide with the earliest detectable signs of neuronal degeneration. Recombinant Tat 1-72 induced mitochondrial depolarization, increased intracellular production of reactive oxygen species, protein oxidation, and neuronal degeneration in primary hippocampal and midbrain rat cell cultures. Biologically inactive analog of Tat protein, Tat (Delta 31-61) did not affect neuronal oxidative status and viability *in vivo* and *in vitro*. Oxidative stress and cell death in Tat-treated cell cultures were prevented by 10 mkM dose of antioxidant Trolox. This work supported by NIH grants DA 11337, DA 09160, DA 13137, HD 043680, DA 014401.

TP-2

NEUROAIDS AND DRUGS OF ABUSE: THE DOPAMINE TRANSPORTER (DAT) AND D1 DOPAMINE RECEPTOR PLAY A ROLE IN THE MECHANISM OF COCAINE-MEDIATED ENHANCEMENT OF HIV-1 TAT NEUROTOXICITY. M. Aksenov , M.V. Aksenova, J. M. Silvers, C. F. Mactutus and R. M. Booze. Depts. of Psychology, Physiology and Pharmacology, Program in Behavioral Neuroscience, Univ of South Carolina, Columbia, SC, 29229.

Evidence is accumulating that administration of psychostimulative drugs of abuse may synergize with neurotoxic effects of HIV-1 proteins. Our studies demonstrate that physiologically relevant dose of cocaine significantly increases the toxicity of Tat and enhances biomarkers of oxidative stress in Tat-treated rat midbrain and hippocampal cell cultures. Cocaine-mediated increase of Tat neurotoxicity can not be completely prevented by antioxidant Trolox treatment. Our results demonstrate that selective blocker of DAT, GBR 12909, is able to emulate the cocaine-mediated enhancement of Tat toxicity. The antagonist of D1 receptors, SCH 23390, downgrades cocaine-mediated increase of Tat neurotoxicity. Our results provide evidence of the involvement of monoamine transmission systems in the cocaine-mediated increase of Tat neurotoxicity. This work is supported by NIH grants DA 11337, DA 09160, DA 13137, HD 043680, DA 014401.

TP-3

NEUROAIDS AND CHANGES IN PROTEIN MARKERS OF DOPAMINERGIC TRANSMISSION: POSSIBLE ROLE FOR THE NEUROTOXIC HIV-1 PROTEIN, TAT. J. Silvers, M.V. Aksenova, M.Y. Aksenov, C. F. Mactutus and R. M. Booze, Dept of Psychology, Behavioral Neuroscience Program, Univ. of South Carolina, Columbia, SC 29208.

Human Immunodeficiency Virus (HIV)-associated dementia (HAD) continues to be a serious complication even with the advent of highly active antiretroviral therapy (HAART). In the current study, we compared levels of the dopamine (DA) related proteins, dopamine transporter (DAT) and tyrosine hydroxylase (TH), in extracts prepared from the substantia nigra of HIV-infected donor tissue and seronegative controls. A follow-up study investigated effects of HIV-1 protein Tat on DAT-specific ligand binding and immunoreactivity in rat primary midbrain cell cultures. The analysis of protein levels of TH and DAT in the substantia nigra of HIV tissue and seronegative controls by immunoblotting demonstrated the reduction of TH protein with no concurrent change in DAT protein levels. In our cell culture studies, we determined that the exposure of rat midbrain cultures to Tat 1-72 did not affect DAT protein levels but significantly decreased binding of the DAT-specific ligand [3H]WIN 35428. These results further suggest involvement of the dopamine system in the neurotoxicity of HIV proteins.

TP-4

PROTEIN KINASE R (PKR) IMPACT ON GLYCOPROTEIN120 (GP120) INDUCING NEURODEGENERATION IN RAT CORTICAL CULTURES. M. Alirezaei, S.A. Lipton, H.S. Fox; Molecular and Integrative Neuroscience Department, The Scripps Research Institute, La Jolla, CA, 92003.

It has been demonstrated that HIV-1/gp120, shed from infected microglia/macrophages, induces neuronal death in brain. Using primary rat cortical cultures, we determined for the first time that the expression of PKR is induced, and the kinase activated after exposure to gp120. Such induction and activation leads to neurotoxicity through apoptosis. However, the expression of the protein activator of PKR (PACT) is not induced in cultures exposed to gp120. The Functional role of PKR in neurotoxicity was shown with two independent inhibitors of PKR activity, with could both abrogate neuronal death in cultures exposed to gp120. Furthermore, we have demonstrated a high level of the activated form of PKR in the brain sections from HIV associated dementia (HAD) compared to HIV-infected, but HAD negative cases.

TP-5

DEFINING THE EFFECTS OF COCAINE AND THC ON NITRIC OXIDE EFFECTOR PATHWAYS IN HUMAN MACROPHAGES. G.C. Baldwin, S. Uh, K. Whittaker, A. Uh, S. Woo, S.M. Kiertscher, D.P. Tashkin, and M.D. Roth. Dept of Medicine, UCLA, Los Angeles, CA 90095.

Our studies with human alveolar macrophages (AM) have identified nitric oxide (NO) as an important effector molecule involved in bacterial killing. Production of NO by these cells requires cytokine priming *in vivo* and this priming event appears to be compromised in the lungs of marijuana smokers and cocaine smokers. To characterize the mechanisms involved, we established an *in vitro*

assay in which human monocyte-derived macrophages (MACS) are stimulated by the bacteria *S. aureus*. Our results demonstrate that *S. aureus* engages TLR2 on the surface of human MACS, that cytokine priming is an essential cofactor in generating a response, and that the combined action of cytokine priming and TLR2 activation signals through both NF-KB and MAPK pathways to trigger NO production. Moreover, *ex vivo* exposure of MACS to either cocaine or THC leads to a dose-dependent impairment in signaling, diminished NO production and compromised microbicidal activity. Supported by NIDA #DA03018 and DA08254.

TP-6

DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING MEASURES NEURODEGENERATION IN THE MPTP RODENT MODEL OF PARKINSON'S DISEASE. Boska M[ab], Hasan K[c], Nelson J[ab], Kibuule D[ab], Uberti M[ab], Gendelman HE[a], and Mosley RL[a] ; [a]CNND and [b]Radiology, University of Nebraska Medical Center, Omaha NE 68198. [c]Diagnostic Imaging, University of Texas Medical Center, Houston, TX 77030

Diffusion Tensor Magnetic Resonance Imaging (DTI) can track neurodegeneration through its ability to measure changes in mean water diffusivity (D_{av}) and cell shape in individual image voxels (fractional anisotropy, FA). We now compared D_{av} and FA in the striatum, corpus callosum, and substantia nigra pars compacta (SNpc) in MPTP-intoxicated mice. Five mice were imaged before and 7 days after MPTP treatment. Paired t-test from region of interest analyses of striatum, corpus callosum, and SNpc demonstrated no change in FA or D_{av} in striatum or corpus callosum, but a significant increase in D_{av} ($p=0.036$) and decrease in FA ($p=0.002$) in the SNpc. We conclude that DTI can be used to track dopaminergic neurodegeneration in the SNpc and can be useful as a non-invasive system to assess therapies aimed at ameliorating Parkinson's disease.

TP-7

GLIAL CELL TYPE-SPECIFIC EFFECTS OF MORPHINE AND HIV-TAT ON CYTOKINE RELEASE AND OPIOID RECEPTORS. J. Turchan-Cholewo, K.F. Hauser, P.E. Knapp & A.J. Bruce-Keller. Univ. KY, Lexington, KY.

HIV-1 patients who abuse opiate-based drugs have increased rates of HIV dementia, which may be related to brain inflammation. To model the effects of morphine on HIV-related glial inflammation, we measured the effects of morphine and Tat on cytokine release from both microglia and astrocytes. While Tat significantly increased cytokine release from both microglia and astrocytes, morphine increased Tat-induced astrocyte cytokine release, but decreased release from microglia. Examination of cell-surface opioid receptor patterns revealed that microglia are particularly enriched in mu opioid receptors as compared to astrocytes. Furthermore, examination of opioid receptors following Tat/morphine exposure in microglia indicated that Tat prevented morphine-induced opioid receptor down-regulation. Our findings indicate that opioid receptors are differentially regulated in microglia as compared to astrocytes, and suggest that mu opioid receptors in microglia may influence inflammatory cytokine release. Supported by P01 DA19398 and RO1 NS046267.

TP-8

THE SECOND EXON OF HIV TAT ALTERS GENE EXPRESSION IN ASTROCTYES IN THE PRESENCE AND ABSENCE OF MORPHINE. G. Chompré, E. Rivera, and R.J. Noel Jr. Dept. of Biochemistry, Ponce School of Medicine, Ponce, PR 00731

Drug abuse is a risk factor for acquisition and progression of HIV infection. During HIV infection, opiates and viral proteins have both individual and synergistic effects on gene expression in CNS cells. It has been shown that the first exon of Tat (Tat72) works in synergy with morphine to alter astrocyte gene expression, which may have implications in HIV dementia. During the viral life cycle, Tat also is expressed in a full length form (Tat101), raising the possibility for additional effects of Tat. We transfected three naturally occurring forms of Tat (Tat72, Tat86 and Tat101) into SVGA astrocytes with or without morphine to measure the full capacity of this important viral protein to alter astrocyte gene expression. Inflammatory and chemotactic cytokines and apoptosis were measured by real time RT-PCR and Annexin V, respectively. We found that two exon forms of Tat produce greater apoptosis and enhanced cytokine expression, but that synergy with morphine was present with all Tat forms.

TP-9

ACTIVATION OF COMPLEMENT FACTOR 3 BY HIV-INDUCED CYTOKINES AND MORPHINE: ROLE IN HIV PATHOGENESIS. P.K. Datta, J. Maranto and J. Rappaport. Dept. Neuroscience, Temple University, Philadelphia, PA 19122.

HIV/SIV infection is known to induce the synthesis of complement factor 3 (C3) in brain and kidney and could contribute to the pathogenesis of NeuroAIDS and HIV-associated nephropathy (HIVAN). Since inhibition of complement synthesis and activation may represent a putative therapeutic approach, we analyzed in detail the mechanisms of HIV-induced modulation of C3 expression. Our studies indicate that HIV induced inflammatory cytokines IL-1 beta and TNF-alpha, and morphine stimulate expression of C3. Our studies utilizing C3 promoter-Luciferase construct demonstrate that viral protein Nef induces C3 promoter. Furthermore, C/EBP family of DNA binding transcription factors, C/EBP-beta and C/EBP-delta stimulate expression of the C3 promoter in astrocytes, and renal mesangial and tubular cells. Our results demonstrate that selective inhibition of p38 MAPK by SB202190-HCl inhibits C/EBP-delta mediated C3 promoter activity.

TP-10

A MACROPHAGE NANOPARTICLE SYSTEM FOR ANTI-RETROVIRAL DRUG. H. Dou[1], C. Destache[1], J. Morehead[1], RL. Mosley[1], M. Boska[1], J. Kingsley[1], B. Rabinow[2], and H E. Gendelman[1]* ; [1]Dept. of Pharma/Exp Neurosci. University of Nebraska Medical Center, [2]Baxter Healthcare Corporation.

Highly active antiretroviral therapy had affected long-term use and clinical efficacy by complex dosing regimens, side effects, limited biodistribution and variable drug pharmacokinetic patterns. We developed a nanoparticle indinavir (NP-IDV) formulation packaged into bone-marrow-derived-macrophages (BMM) as drug carriers that may overcome such limitations. Drug distribution and disease outcomes were assessed in viral challenged NOD/SCID mice reconstituted with hu-PBL. SPECT, MRI and histology demonstrated robust lung, liver, and spleen BMM distributions. Tissue

and sera IDV levels were ≥ 50 nM/ml up to 2 weeks of observation. NP-IDV-BMM treated mice showed reduced numbers of virus-infected cells and restored $CD4^+$ T cells. We conclude that a single dose of NP-IDV using BMM as a carrier is bio-available and effective for anti-retroviral therapy.

TP-11

MACROPHAGE-BASED ANTIRETROVIRAL DELIVERY FOR HIV-1 ASSOCIATED DEMENTIA. H. Dou[1], C. J. Destache[1], J. Kingsley[1], J. Morehead[1], M. Chauba[2], J. Rabinow[2], and H. E. Gendelman[1]* ; 1Dept. of Pharma/Exp Neurosci. University of Nebraska Medical Center, 2Baxter Healthcare Corporation.

Nanoparticles (NP) used for anti-microbial drug delivery can provide site-specific delivery, diminished toxicity, and prolonged dosage frequency. We posit that the same mononuclear phagocytes (MP) that serve as perpetrators of disease could also be used as vehicles for anti-retroviral therapy. To this end, bone marrow macrophages (BMM) were used as carriers for NP formulated indinavir (NP-IDV). NP-IDV loaded BMM was administered intravenously into SCID mice with HIV-1 encephalitis (HIVE). Drug delivery and antiviral efficacy was analyzed at day 10. BMM densities within and around the injection sites were easily visualized. No labeled BMM were found in unaffected brain tissue. NP-IDV BMM drug levels of 2 to 16ng and 10ng in the injected and control hemispheres respectively. The percent virus-infected MDM were reduced 2-fold in NP-IDV-BMM HIVE mice. These finding have significant implications for treatment of HIV-1-associated dementia.

TP-12

CONVERGENT $[Ca^{2+}]_i$ SIGNALS UNDERLIE SYNERGISM IN MORPHINE AND HIV-1 TAT INDUCED MCP-1 RELEASE BY ASTROCYTES. N. El-Hage, S.P. Zou, T.Y. Zhao, A.J. Bruce-Keller, P.E. Knapp, and K.F. Hauser, Dept. Anat. & Neurobiol., Univ. Kentucky, Lexington, KY 40536.

The mechanisms by which opiates synergistically increase monocyte chemoattractant protein (MCP-1/CCL2) release in HIV-1 Tat exposed astrocytes were explored. Tat-induced increases in MCP-1 were abolished by NF-kappaB (NF-kB) or PI3K inhibition, Ca^{2+} ionophore or thapsigargin, and reduced by JNK or p38 MAPK inhibitors. Although morphine alone did not affect MCP-1 release, it potentiated the effects of Tat and the interaction was negated by ERK1/2 inhibition or by blocking Ca^{2+}-induced Ca^{2+} release (CICR), but not by blocking JNK or p38 MAPK. PI3K or ERK inhibition blocked morphine's effects on $[Ca^{2+}]_i$. Thus, opiates potentiate Tat-induced MCP-1 production in astrocytes through actions at PI3K, ERK, and CICR, while Tat elevates $[Ca^{2+}]_i$ through another pathway involving NF-kB. Morphine and Tat signals converge at the level of $[Ca^{2+}]_i$ and morphine exacerbates Tat-induced MCP-1 production through regenerative amplification of the Ca^{2+} signal via CICR. Support: NIDA P01 DA19398.

TP-13

DELTA-OPIOID RECEPTOR ACTIVATION DECREASES INFLAMMATORY PAIN: STUDY WITH KNOCKOUT MICE. C. Gaveriaux-Ruff[1], L.A. Karchewski[2], B.L. Kieffer[1]; [1]Institut de Biologie Moleculaire et Cellulaire, Illkirch, 67400, France, [2]NRC-Institute for Biological Sciences, National Research Council, Ottawa, ON, K1A OR6 Canada

We analysed mu, delta and kappa-opioid receptor knockout mice in a model of inflammation-induced hyperalgesia induced by Complete Freund Adjuvant in the paw. Mechanical allodynia and thermal hyperalgesia were measured by the Von frey filaments and the plantar test, respectively. By comparing all three single opioid-receptor knockout animals to wild-types (WT), we found that delta-receptor mutants were more sensitive than WT, underlining a role for delta-receptor endogenous activation. The chronic treatment with a delta-selective agonist produced an analgesic effect in this inflammation model, and this effect was abolished in the delta-receptor knockout mice. Altogether, these data suggest that the delta-receptor may represent a potential therapeutic target for chronic inflammatory pain.

TP-14

IL-1β-ACTIVATED ASTROCYTE INFLAMMATORY PROFILES: UPREGULATION OF CD38 AND IMPLICATIONS FOR ASTROCYTE-MEDIATED NEUROTOXICITY IN HIV-1 DEMENTIA. A. Ghorpade, M. Deshpande, C-H Shao, L. Wu, K. Bidasee. Univ. of Nebraska Med Center, Omaha, NE 68198-5215

HIV-1-associated dementia (HAD) is an important complication of HIV-1 infection. Reactive astrogliosis is a pathological feature of neuroinflammatory injury. Our data showed that astrocytes activated with IL-1β, an HAD-relevant proinflammatory cytokine, induce neurotoxicity. These mechanisms are currently being investigated. Using gene microarrays, global changes in IL-1β-activated primary human astrocytes were studied. CD38 was the most upregulated gene (17-fold higher). Real-time PCR and western blot analyses confirmed CD38 upregulation in IL-1β-activated astrocytes and exposed to HIV-1_{ADA}. Brain tissue samples from HIV-1-positive patients confirmed these data. CD38 has an ADP-ribosyl cyclase activity and mediates the production of cyclic ADP ribose (cADPR) that modulates intracellular calcium levels. Our new preliminary data suggests that CD38 upregulation in astrocytes may lead to neurotoxicity, most likely, through disruption of intracellular calcium levels in IL-1β-activated astrocytes.

TP-15

IMMUNODEFICIENT MODELS OF HIV-1 ENCEPHALITIS (HIVE). S. Gorantla, E. Miller, H. Klasek, J. Morehead, L. Walters, H.E. Gendelman, L. Poluektova. University of Nebraska Med. Ctr., Omaha, NE, 68198.

Loss of NK and adaptive immune function are associated with progressive HIV-1 disease in infected humans. However, how each affects the development of neurological disease remains poorly understood. To this end, C.B.-17/*scid* and $Rag2^{-/-}gamma_c^{-/-}$ mice were used to model deficiencies in adaptive immunity and NK cell dysfunction. Mice were injected with HIV-1 infected

human monocyte -derived macrophages (MDM) into the basal ganglia and monitored for lymphocyte infiltration, glial inflammatory responses and neuronal loss by FACS, immunohistology and RT-PCR assays. Infiltration of CD3 cells was present in brains of CB-17/scid mice. NK cells were not found in the area of inflammation. Both models did not significantly affect pathological features of HIVE. Among the tested pro- and anti-inflammatory cytokines, only IL-1□ expression was higher in CB-17/scid mice. We have to conclude that NK cells do not affect the course of HIVE and functional innate immune responses of glial cells. The absence of interleukin-2 receptor gamma chain may be a unifying genetic event in pathologic responses in murine HIVE.

TP-16

COPAXONE IMMUNIZATION SUPPRESSES NEURO-INFLAMMATION AND ELICITS NEURONAL PROTECTION IN MURINE HIV-1 ENCEPHALITIS (HIVE). S. Gorantla, H. Klasek, L. Walters, H. Dou, T. Ikezu, D.J. Volsky, L. Poluektova, H. E. Gendelman. University of Nebraska Medical Center, Omaha, NE, USA.

Copaxone (Cop-1) vaccination can modulate microglial immunity and as such protect neurons. We investigated whether Cop-1 modulates innate and adaptive immunity and leads to neuroprotection in two murine models of HIVE. SCID/HIVE mice devoid of functional T and B lymphocytes were used to analyze the innate immune responses. To study the effect of Cop-1 induced adaptive immune responses, C57Bl/6 mice were injected intracranially with bone marrow derived mouse macrophages infected with HIV-1/VSV pseudotypes (HIV/VSV-HIVE). In HIV/VSV-HIVE mice, presence of adaptive immune response greatly enhanced Cop-1's ability to control microglial and astrocyte induced inflammation with reduced expression TNF-alpha, IL1-beta, IL-6, and iNOS. Cop-1 adaptive immune response restored neuroregeneration in the hippocampus of HIVE animals. Cop-1 regulates both innate and adaptive immune responses in experimental models of HIVE and shows potential promise for treatment of human disease.

TP-17

MORPHINE EXACERBATES HIV-1 TAT-INDUCED NEUROINFLAMMATION AND GLIAL ACTIVATION THROUGH CCL2-CCR2 INTERACTIONS. N. El-Hage, G. Wu, J. Ambati, A.J. Bruce-Keller, P.E. Knapp, and K.F. Hauser, Univ. Kentucky, Lexington, KY

Opiate abuse may exacerbate HIV-1 encephalitis (HIVE) by increasing monocyte chemoattractant protein-1 (MCP-1/CCL2) release by astrocytes. We assessed morphine and HIV-1 Tat interactions in wild type and CCR2 null mice. The effects of intrastriatal Tat and/or systemic morphine (25 mg time-release implant) on macrophage/microglial and astroglial activation were examined 7 days after Tat injection. Tat or morphine markedly increased the proportion of CCL2 immunoreactive astroglia, and together caused additive increases in CCL2+ astroglia, which were prevented by naltrexone. The number of F4/80+ macrophages/microglia and GFAP+ astroglia were significantly reduced in CCR2(-/-) compared to wild-type mice after exposure to Tat or morphine and Tat. CCR2 mediates increases in microglial and astroglial activation caused by Tat, and this is enhanced by opiates. Thus, opiates exacerbate HIVE by enhancing CCL2-CCR2 inflammatory signaling among astroglia and macrophages/microglia. Support: NIDA P01 DA19398.

TP-18

TRANSCRIPTIONAL AND POST-TRANSLATIONAL REGULATION OF OTK18 BY HIV-1. Tsuneya Ikezu, James Buescher, and Shinji Sato. Ctr. for Neurovirology and Neurodegenerative Disorders, Dpt. of Pharmacology and Experimental Neuroscience, Univ. of Nebraska Med. Ctr, Omaha, NE 68198-5880

OTK18, an HIV-1-inducible zinc finger protein, reduces progeny virion production in infected human macrophages. We have identified the HIV-1-inducible promoter region of OTK18 proximal to the transcriptional start site (-884/-580). Although VSV-G pseudotyped YU-2 virion significantly enhanced the promoter activity, infection with HIV-1 ADA or non-pseudotyped YU-2 had little. The upregulation of (-884/+1) was not dependent on HIV-1 rival protein expression. The OTK18 expression level is further regulated by its endoproteolysis in caspase-3 and calpain-I-dependent manner. The kinetics of its endoproteolysis has been characterized by a novel confocal live imaging system of transfected OTK18 fluorescent fusion protein constructs. These data demonstrate that OTK18 promoter is activated by HIV-1 infection but independent of HIV-1 co-receptor signaling or viral protein expression, and its translational product is further regulated by specific endoproteolysis in human mononuclear phagocytes.

TP-19

K^+ CHANNELS INFLUENCE TRANSENDOTHELIAL MIGRATION OF HIV-1 INFECTED MONOCYTES IN ARTIFICAL BLOOD-BRAIN BARRIER SYSTEMS. Kanmogne,G.D., Thomas, M.P., Morsey B., Persidsky Y., Gendelman H.E. Dept of Pharmacology & Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE.

HIV-1 encephalitis is characterized by brain infiltration of monocuclear phagocytes (MP) but the underlying mechanisms are poorly understood. Modulation of membrane ion channels can affect cell shape, volume and cell migration. We now demonstrate enhanced migration of HIV-1 infected monocytes (Mo) and to lesser degree monocyte-derived macrophages (MDM) through an artificial blood-brain barrier. Following a 6 and 12h exposure of Mo to HIV-1_{ADA} we observed increased cell migration of 108 to 140 and 7 to 11-fold respectively when compared to control uninfected Mo. Migration was potentiated by relevant chemokine, MCP-1. Exposure of MDM to virus at 6 and 12 h increased migration by 5 to 15 and 2 to 21-fold. The K^+ channel blockers charybdotoxin, kaliotoxin and margatoxin slowed Mo migration by 37, 35 and 67% respectively, while the Cl^- channel blockers NPPB and Flufenamic acid diminished Mo migration by 55 and 38% respectively. Electrophysiological recording confirmed the presence of these channels in Mo and MDM. These results demonstrate the importance of ion channels in Mo migration after viral infection, likely regulating cell volume and shape during cell trafficking and migration.

TP-20

HIV-1 ACTIVATES PRO-INFLAMMATORY AND OXIDATIVE STRESS RELATED GENES IN HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS (HBMECS). Kanmogne, G.D., Morsey, B., Schall K., Persidsky Y. Dept. Pharmacology & Exp. Neuroscience, Univ. Nebraska Med. Ctr. Omaha, NE.

The mechanisms underlying dysfunction of blood-brain barrier (BBB) seen in HIV-1 infection are poorly understood. We investigated HIV-1-induced genomic changes in HBMECs after co-culture for 12 h with infected or uninfected monocyte-derived macrophages (MDM) using a

transwell system. Genome expression determined using Affymetrix U133 Plus 2.0 arrays in HBMECs co-cultured with virus-infected MDM (group, G1), with non-infected MDM (G2), and untreated HBMECs (G3). Differentially expressed genes were determined by hierarchical modeling and clustering. 184 probe sets were differentially expressed in G1 compared to G2 and 1071 probe sets differentially expressed in G1 compared G3. Genes activated (up to 107-fold) in G1 included chemokines and cytokines of the IL-8 family (MIP-3alpha, MIP-2beta, MCP-1, IL-8, CX3CL1, CXCL6, CXCL1, CXCL10); IL-6, tumor necrosis factor-alpha-induced protein 2 (TNFAIP2), TNFAIP3, TNFAIP6, TNFAIP10 and TNF ligand superfamily-10, ICAM-1 and inhibitors of cyclin-dependent kinases, superoxide dismutase and oligoadenylate synthetase. Thus, HIV-1 can cause impairment of the BBB by mechanisms involving inflammation, increased adhesion and oxidative stress in HBMECs.

TP-21

MAGNETIC LABELED MACROPHAGES MIGRATE ACROSS THE BLOOD BRAIN BARRIER IN MICE WITH HIV-1 ENCEPHALITIS. Kibuule D[a,b], Dou, H. [a,b], Uberti M[b], Nelson J[a,b], Mellon M[b], Bradley J[b], Gendelman HE.[a,b] and Boska M[a,b] [a]Center for Neurovirology and Neurodegenerative Disorders and [b]Department of Radiology, University of Nebraska Medical Center, Omaha NE

Mononuclear phagocyte (MP)-induced inflammatory response underlies the neuropathogenesis of HIV infection. How MP cross into the brain and the means to track such activities have now been realized. Indeed, we now demonstrate non-invasive tracking of MP across the BBB in humanized mice. Ten severe combined immunodeficient mice were prescanned using T_2* weighted MRI 2 days after intracranial stereotactic injection of HIV-1 infected human monocyte-derived macrophages to induce an encephalitis. Feridex labeled bone marrow macrophages were then injected intravenously. Coregistration, signal normalization and subtraction (Analyze, Mayo) detected the presence of labeled macrophages and showed cells concentrated around the injection line and in the ventricles which was verified by histologic examinations of affected brain tissue. These results have implications for studies of disease pathogenesis and for therapeutic delivery.

TP-22

THC MODULATES THE ABILITY OF DENDRITIC CELLS (DC) TO STIMULATE T CELL RESPONSES. S.M. Kiertscher, D.P. Tashkin and M.D. Roth. Dept. of Medicine, UCLA, Los Angeles, CA 90095-1690.

Human monocytes express CB2, and exposure to THC alters their capacity to differentiate into DC. The resulting changes in cell surface molecule expression and cytokine production may impact their ability to stimulate antigen-specific T cell responses. To test the ability of THC exposed DC (THC-DC) to stimulate recall responses, control and THC-DC were pulsed with HBV surface antigen and used to stimulate T cells from HBV-immunized donors. Control DC stimulated strong proliferation of antigen-specific T cells, while THC-DC stimulated only limited proliferation. To measure the effect of THC exposure on primary responses, control and THC-DC were used to stimulate allogeneic naïve T cells in a mixed leukocyte reaction (MLR). CFSE-labeled $CD45RA^+$ T cells were analyzed following co-culture with DC. Unlike control DC, THC-DC were deficient in their ability to induce naïve T cell proliferation, and failed to promote the shift from naïve to

memory responses. Our results suggest that the cannabinoid receptor system plays an important role in regulating antigen presentation and T cell immunity. NIDA #DA03018.

TP-23

SILENCING THE PTEN GENE REDUCES HIV-1 TAT AND OPIATE-INDUCED NEUROTOXICITY. T.Y. Zhao, M.H. Adams, S.P. Zou, A.J. Bruce-Keller, N. El-Hage, K.F. Hauser & P.E. Knapp. Univ. KY, Lexington, KY.

Opiate abuse reportedly enhances progression of HIV-1 encephalitis. Striatal neurons are targets of HIV-1 proteins Tat and gp120, and Tat-induced neurotoxicity *in vitro* is amplified by morphine in a naloxone reversible manner. Exposure to Tat increases $[Ca^{++}]_i$ in striatal neurons and activates endonuclease-G and caspase-3. Since Tat activates multiple death pathways and Tat neurotoxicity is not attenuated by caspase inhibitors we targeted signaling pathways upstream of mitochondrial apoptotic events. Since several transcription factors identified on DNA arrays as having potential roles in Tat-morphine synergy are known to affect proliferation and survival through Akt/PKB phosphorylation, striatal neurons were transfected (Amaxa nucleofection) with siRNAs targeting PTEN, a negative regulator of pAkt. Tat(1-86) increased death of neurons transfected with control construct from 15% to 32% (72 hrs). PTEN silenced neurons were completely protected. Our findings identify PI3-kinase/Akt as a critical intermediary and possible therapeutic target for Tat/morphine-induced neurotoxicity. Support: P01 DA19398/DA15097.

TP-24

MORPHINE INHIBITS INTERFERON ALPHA EXPRESSION IN HUMAN HEPATOCYTES COUPLED WITH ENHANCED FULL-CYCLE HEPATITIS C VIRUS REPLICATION. Y. Li,[1] J.S. Peng,[2] C.Q. Wang,[3] T. Zhang,[1] Q. Wan,[1] Z. Chen,[1] and W.Z. Ho[1]; [1]Div. of Immunol. and Infect. Dis., The Children's Hosp. of Philadelphia, Dept. of Pediatrics, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104, [2]Wuhan Centers for Disease Control and Prevention, Wuhan, 430022, P. R. China, [3]The Children's Hosp. of Fudan Univ., Shanghai, 200032, P. R. China

Interferon (IFN) alpha plays a vital role in the host cell innate immunity against hepatitis C virus (HCV). We thus examined whether morphine, the active metabolite of heroin, has the ability to inhibit endogenous IFN alpha by human hepatic cells. We observed a significant decrease of endogenous IFN alpha mRNA expression in morphine-treated human hepatic cells in a dose-dependent fashion. Morphine also significantly inhibited secretion of IFN alpha protein from the hepatic cells as determined by ELISA. This morphine action on IFN alpha was abolished by the opioid receptor antagonists, naltrexone and beta funaltrexamine. The inhibition of endogenous IFN alpha by morphine was coupled with increase in full-cycle HCV replication in the hepatic cells. Morphine also compromised anti-HCV ability of recombinant IFN alpha in the hepatic cells. Investigation of the mechanism responsible for morphine-mediated inhibition of IFN alpha revealed that morphine had the ability to inhibit expression of IFN regulatory factor 5 (IRF-5), one of key transcriptional factors that initiate the cellular antiviral state. In addition, morphine suppressed expression of p38, an important signal-transducing molecule involved in IFN alpha-mediated anti-HCV activity. These findings that morphine compromises intracellular IFN alpha-mediated innate immunity against HCV provide a molecular mechanism by which HCV persists in the hepatic cells.

TP-25

CSF BIOMARKERS FOR HIV-1 ASSOCIATED DEMENTIA IN HISPANIC WOMEN. J. Pérez Laspiur[1], E. R. Anderson[1], P. Ciborowski[2], H. E. Gendelman[2], V. Wojna[1], J. Rodríguez-Orengo[1], E. Rodríguez[1], J. Anderson[2], and L. M. Meléndez-Guerrero[1]. [1]University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico; [2]University of Nebraska Medical Center, Omaha, NE

A proteomics platform was applied to the discovery of cerebrospinal fluid (CSF) biomarkers that affect the development of HIV-1 Associated Dementia (HAD) in Hispanic women. CSF was collected from 10 Hispanic women with HAD and from non-demented controls. Differentially expressed proteins were profiled by surface enhanced laser desorption ionization-time of flight (SELDI-TOF), reverse phase high performance liquid chromatography, one-dimensional electrophoresis, and tandem mass spectrometry. SELDI-TOF showed differentially expressed proteins, subsequently identified by mass spectrometry. Western blot analyses confirmed differences in SOD and catalase expression in CSF associated with HAD. Current studies will determine if macrophages contribute to the CSF redox imbalance during HIV induced cognitive disease.

TP-26

MORPHINE MODULATES TIGHT JUNCTION EXPRESSION IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS. S.D. Mahajan and M.P.N.Nair. Dept of Medicine, Division of Allergy, Immunology & Rheumatology, State University of New York at Buffalo, Buffalo General Hospital, 100 High street, Buffalo, NY14203

Tight junctions (TJ) are critical structural and functional elements of the blood brain barrier (BBB) and TJ disruption and breakdown of the BBB are key features of HIV-1 encephalopathy. TJ consist of claudins, occludins and junctional adhesion molecules (JAM). The role of opiates as a co-factor in the neuropathogenesis of HIV-1 is well established. We hypothesize that morphine alone and in combination with HIV-1 viral protein, tat, modulates the tight junction protein expression in BMVEC. Our results show that morphine (10^{-7}M to 10^{-9} M) and tat (50ng/ml) significantly increased JAM-2 gene expression by BMVEC, and morphine in combination with tat further increased JAM-2 expression, while morphine did not show any effect on ZO-1 or occludin. These results indicate that JAM-2 is critical for the barrier function of the BBB, and that the modulation of JAM-2 by morphine may contribute to increased trafficking of immunocompetent cells into the brain contributing to neuropathogenesis of HIV-1 disease.

TP-27

METHAMPHETAMINE AND HIV-1 TAT INTERACTIONS IN STRIATUM. [1]S. Theodore, [2]W.A. Cass and [1,2] W.F. Maragos. [1]Anatomy and Neurobiology and [2]Neurology, University of Kentucky, 40536.

HIV-1 infection with concurrent methamphetamine (MA) abuse results in enhanced neuronal damage. We demonstrated that exposure to both the HIV "virotoxin" Tat and MA enhanced the loss of striatal dopamine (DA). Neuroanatomical studies revealed extensive degeneration of DA

terminals in animals exposed to both Tat and MA compared to animals exposed to Tat or MA alone. Immunohistochemistry revealed increased activation of microglia and astrocytes in animals treated with Tat alone or Tat+MA. Tat treatment caused an early increase in striatal levels of TNF-alpha. In mice lacking TNF-alpha, Tat+MA neurotoxicity was attenuated. Cytokine array experiments revealed a prominent increase in monocyte chemotactic protein-1 (MCP-1), a downstream effector of TNF-alpha. Tat+MA neurotoxicity was attenuated in mice lacking the gene for MCP-1. The findings suggest that interaction of HIV-1 infection and concurrent drug abuse might aggravate neuroinflammatory changes resulting in subsequent release of cytotoxic mediators leading to enhanced DAergic neurotoxicity.

TP-28

VIRAL LOADS, CD4 & CD8 LEVELS DURING SIV INFECTION IN MORPHINE-DEPENDENT MACAQUES. Marcario JK[1*], Riazi M[1,2], Kenjali H[3], Adany I[3], Narayan O[3] & Cheney PD[1]. Molecular & Integrative Physiology[1], Hearing & Speech[2], Microbology, Molecular Genetics & Immunology[3], U. Kansas Medical Center, Kansas City, KS, USA.

To characterize the functional consequences of chronic morphine dependence in a SIVmac model of neuro-AIDS, 16 rhesus macaques were divided into 3 experimental groups: Group A (Morphine Only); Group B (SIV + Morphine); and Group C (SIV Only). Animals in Groups A and B were injected with 2.5 mg/Kg doses of morphine sulfate, 4x per day, for 59 weeks. Group C animals were sham injected with saline. Groups B and C were infected with neurovirulent strains (R71/E17) of SIVmac. All monkeys in Groups B and C were productively infected - viral RNA was recovered repeatedly in plasma and CSF. There was no significant difference in viral loads in plasma or CSF between Groups B and C, or the viral loads in plasma between the rapid (3/11) and slow (8/11) progressors. There was a significant difference ($p=0.0146$) in viral loads in CSF between rapid and slow progressors. (Sponsors: NDA12827 & HD02528)

TP-29

METHAMPHETAMINE MODULATES HIV-1 INFECTIVITY IN MONOCYTE DERIVED DENDRITIC CELLS. M.P.N. Nair, S. D. Mahajan, J. L. Reynolds, R. Aalinkeel, S. A. Schwartz, M.V. Bapardekar and D. E. Sykes. Dept of Medicine, Division of Allergy, Immunology & Rheumatology, State University of New York at Buffalo and Kaleida Health System, Buffalo General Hospital, 100 High street, Buffalo, NY 14203

The US is currently experiencing a grave epidemic of Meth use as a recreational drug. Recent studies show a high prevalence of HIV infection among Meth users. Dendritic cells (DCs) are the first line of defense against HIV-1 infections. Since the incidence of HIV-1 infection is significantly higher in Meth using patients, we hypothesize that Meth exarcebates HIV-1 infection of DC. Our results show that in vitro treatment of DC with Meth significantly upregulates HIV-1 infection as quantitated by LTR-RU5 gene expression by quantitative real time PCR, p24 Ag production by ELISA and cell infectivity as measured by MAGI cell assay. Further, the Meth induced upregulation of HIV infectivity is associated with upregulation of HIV-1 co-receptors, CCR5/CXCR4 and modulation of the HIV-1 virion associated MAP kinases. These studies report for the first time that Meth exacerbates HIV-1 infection.

TP-30

DOPAMINERGIC NEUROPROTECTION BY REGULATORY T CELLS IN A MOUSE MODEL OF PARKINSON'S DISEASE. A. Reynolds, C. Laurie, T. Anderson, J. Kipnis, H. E. Gendelman, R. L. Mosley; Center for Neurovirology and Neurodegenerative Disorders, Depts of Pharmacology and Experimental Neuroscience & Opthalmology and Visual Sciences, University of Nebraska Medical Center, Omaha, NE 68198

To assess the neuroprotective potential of CD4+CD25+ regulatory T cells (Treg) in a mouse model of PD, *in vitro* activated Treg and CD25+CD25- effector T cells (Teff) were adoptively transferred to mice treated with the dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Transfer of as few as 3.5 x 10^6 Treg reduced numbers of inflammatory (CD11b+) microglia by 2 days post-MPTP intoxication, and increased survival of tyrosine hydroxylase immunoreactive (TH+) dopaminergic neurons within the substantia nigra by day 7. No neuroprotective effect was demonstrated by the Teff population. These data support the use of immunomodulatory strategies that induce regulatory T cell-mediated immune responses to attenuate neuroinflammation and inhibit dopaminergic neurodegeneration associated with Parkinson's disease.

TP-31

METHAMPHETAMINE-INDUCED DIFFERENTIAL PROTEIN EXPRESSION BY MATURE DENDRITIC CELLS (MDC). J.L. Reynolds, S.D. Mahajan, S.A. Schwartz and M.P. Nair; Dept. Medicine, Div. of Allergy and Immunology, SUNY@Buffalo, Buffalo, NY 14203.

The US is currently experiencing an epidemic of methamphetamine (Meth) use as a recreational drug with high prevalence of HIV-1 infection. Although evidence of immune dysfunctions has been reported in Meth users, the molecular basis of the immunopathogenesis of HIV-1 infection in Meth users has not been delineated. Furthermore, the role of Meth on the expression of the proteome of mature dendritic cells (MDC) has not been elucidated. Herein we investigated Meth-induced differences in protein expression in MDC through difference gel electrophoresis (DIGE) combined with protein identification through HPLC-MS/MS. Meth-differentially expressed 20 proteins in MDC. Several of these differentially expressed proteins were further confirmed by western blot and their respective genes by real-time, quantitative PCR (Q-PCR) analysis. Identification of unique Meth-induced proteins may help to develop novel therapeutic strategies for drug targeting in Meth using HIV-1 infected patients.

TP-32

A PROTEOMIC FINGERPRINT OF BRAIN MICROVASCULAR ENDOTHELIAL CELLS (BMVEC) AND HIV-1 INFECTED MACROPHAGE SECRETIONS: A MODEL FOR BLOOD BRAIN BARRIER (BBB) DYSFUNCTION IN NEUROAIDS. M.Ricardo-Dukelow, P.Ciborowski, B. Morsey, Y. Persidsky, W. Rozek, I. Kadiu, H.E.Gendelman, G. Kanmogne. Dept. Pharm & Exp Neurosci. Univ. NE Med Ctr, Omaha, NE 68122.

BBB injury and transendothelial migration of HIV-infected monocyte-derived macrophages (MDM) underlies the neuropathogenesis of viral infection. We investigated the effects of HIV infected MDM and BMVEC using a proteomic platform of Surface Enhanced Laser Desorption Ionization Time of Flight (SELDI-TOF), 1D electrophoresis and tandem mass spectrometry. Using a transwell system BMVEC were co-cultured with HIV infected and noninfected MDM for 12 hours, SELDI-TOF analysis of secretomes showed peak differences at 10-75kDa between BMVEC co-cultured with HIV infected MDM compared to BMVEC co-cultured with uninfected MDM and untreated BMVEC control. Over 200 proteins were identified in secretomes of BMVEC co-cultured with HIV infected MDM and included structural, complement-associated, redox, heat shock and arachidonic acid proteins, and enzymes. 2D-DIGE Difference Gel Electrophoresis is being used to further facilitate identification of proteins secreted by BMVEC following contact with infected MDM and their role in HIV-induced BBB impairment.

TP-33

IMPAIRED CYTOKINE PRODUCTION AND SUPPRESSED LYMPHOCYTE PROLIFERATION ACTIVITY IN HCV-INFECTED COCAINE/HEROIN ("SPEEDBALL") USERS. E. Ríos-Olivares, L. M. Vilá, J. C. Reyes, J. W. Rodríguez, N.O. Pagán, A. Marrero, Z.M. Ríos-Orraca, N.M. Boukli, R.R. Robles. Department of Microbiology and Immunology and the Center for Addiction Studies, Universidad Central del Caribe School of Medicine, Bayamón, Puerto Rico 00960.

From a total population of 400 intravenous drug users, HCV+ "speedball" users (n=30) were randomly selected for cytokine (TNF-α, IL-1β, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-12) determination in plasma and lymphocyte cultures. In addition, in 49 HCV+ "speedball" users, lymphocyte proliferation was also measured. Compared with healthy individuals, except for IL-1β, all the other cytokines were significantly increased in plasma from both HCV+ and HCV- "speedball" users. Similarly, except for IL-10, all the other cytokines were augmented in PHA-stimulated PBMC culture fluid of HCV+ "speedball" users and overproduction of cytokines such as TNF-α, IL-1β, IFN-γ and IL-6, was consistently detected in supernatants when PBMC from HCV+ "speedball" users were stimulated with a biological response modifier. Furthermore, HCV+ "speedball" users showed a significant reduction in lymphoproliferative activity. Overall, the observed immunologic abnormalities could suggest impairment in the regulatory mechanism of the Th1-Th2 system in HCV+ "speedball" users. Supported by NIH/NIDA Grant # R24DA13335 and NIH/RCMI Grant # G12-RR03035.

TP-34

NICOTINE'S EFFECT ON HIV EXPRESSION IN HUMAN MICROGLIA. R.B. Rock, G. Gekker, S. Hu, W. Sheng, P.K. Peterson; Center for Infect. Dis. and Micro. Translational Res., Univ. MN, Minneapolis, MN 55455.

HIV-1 infection and nicotine addiction are global public health crises. In the central nervous system, HIV-1 causes a devastating neurodegenerative disease. It is well recognized that microglial cells play a pivotal role in the neuropathogenesis of HIV-1 and that drugs of abuse not only contribute to the spread of these agents but may facilitate their expression in microglial cells. However, little to nothing is known about the effect of nicotine on HIV infection. In this study, we

demonstrate the constitutive expression of nicotinic acetylcholine receptor mRNA in primary human microglial cells. Specifically, alpha-3, alpha-5, alpha-7 and beta-4 subunits were present, while alpha-4, beta-2 and beta-3 subunits were absent. Pretreatment of microglial cells with nicotine increased HIV-1 expression in a concentration-dependent manner, as measured by p24 antigen levels in culture supernatants. HIV pathway-specific microarrays were used to examine the underlying microglial gene response modulated by nicotine. The results of this study suggest that nicotine could be a cofactor in HIV-1 neuropathogenesis.

TP-35

EFFECT OF "SPEEDBALL" USE ON LYMPHOCYTE IMMUNO-PHENOTYPIC PROFILE IN HIV AND HCV INFECTED INDIVIDUALS. L.M. Vilá, E. Ríos-Olivares, J.C. Reyes, J.W. Rodríguez, A. Marrero, N.O. Pagán, and R.R. Robles. Department of Microbiology and Immunology and the Center for Addiction Studies, Universidad Central del Caribe School of Medicine, Bayamón, Puerto Rico 00960.

To our knowledge, no immunologic studies have been conducted to determine the impact of cocaine/heroin ("speedball") use. We determined whether lymphocyte subpopulations are altered in 324 "speedball" users, 17 heroin users, and 26 healthy individuals. The frequency of CD4+ T cells, CD8+ T cells, B cells, NK cells, and activated T cells (HLA-DR+ and CD38+) was examined by flow cytometry. "Speedball" users were more likely to have lower number of CD4+ T cells and NK cells, lower percentage of activated CD4+ T cells and higher percentage of activated CD8+ T cells than healthy controls. "Speedball" users were more likely to have lower count of CD4+ T cells and NK cells than heroin users. The lymphocyte abnormalities were significantly more profound in HIV+ than in HIV- "speedball" users. No significant differences were seen between HCV+ and HCV- "speedball" users. HIV-HCV- "speedball" users showed significant immunologic abnormalities as compared to controls. Overall, the observed abnormalities suggest that "speedball" use could increase susceptibility or exacerbate infectious diseases. Supported by NIH/NIDA Grant # R24DA13335 and NIH/RCMI Grant # G12-RR03035.

TP-36

LV/CB2-GFP, A LENTIVIRAL VECTOR FOR INVESTI-GATING THE EFFECTS OF CB2 RECEPTOR ON THE FUNCTION OF HUMAN MONOCYTES AND DENDRITIC CELLS. M.D. Roth, A. Harui, S. Beedanagari, R. Stripecke, D.P. Tashkin & S.M. Kiertscher. Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095.

A self-inactivating lentiviral vector was constructed to express human *CB2* under the control of a CMV promoter and green fluorescent protein (GFP) under the control of an internal ribosome entry site (LV/CB2-GFP vector). LV/CB2-GFP is pseudotyped in a vesicular stomatitis virus (VSV-G) capsid, allowing it to transduce a wide range of primary human cells and cell lines. At a concentration of 1.0 μg p24 antigen equivalents/ml, LV/CB2-GFP transduced >85% of renal 293T cells and produced high-level expression of both GFP (measured by flow cytometry) and CB2 (measured by semi-qantitative RT-PCR). Primary cultures of human monocytes will now be transduced with LV/CB2-GFP and CB2 over-expression evaluated for its impact on the differentiation and function of monocyte-derived dendritic cells, and their regulation by

cannabinoids. LV/CB2-GFP provides a novel approach for evaluating the biological consequences resulting from CB2 expression. Supported by NIDA grant DA03018.

TP-37

DADLE INHIBITS SDF-1alpha-INDUCED PHOS-PHORYLATION OF AKT IN HUMAN T-CELLS. N.A. Shahabi, K. McAllen, B.M. Sharp; Dept. of Pharmacology, Univ. TN, Memphis, TN

The activation of T-cell delta opioid receptors (DORs) has been shown to attenuate HIV-1 p24 antigen expression, which itself depends on binding and uptake of virus by the chemokine receptor CXCR4. Moreover, signaling through PI3K/phospho-Akt is required for HIV-1 replication in CD4+ T-cells. To determine if DOR agonists modulate CXCR4-dependent phosphorylation of Akt, we studied the effect of DADLE on Akt phosphorylation driven by SDF-1. Human peripheral blood T-cells were cultured for 48 h, rested in serum free medium, and then stimulated with SDF-1 10 nM for 5 min after pretreatment (1 or 3 h) with DADLE 10^{-6} M. SDF stimulated Akt phosphorylation by 5-fold. By 1 h, DADLE reduced this by 27%, and by 3 h, Akt phos-phorylation declined by 59% ($p=0.012$; $n=7$). By 1 h, DADLE alone had no effect on phospho-Akt. In addition, DADLE did not affect SDF-1-induced phosphorylation of ERKs 1 and 2. Thus, DORs can selectively modulate CXCR4 signaling, affecting phosphorylation of Akt but not ERKs. These findings suggest that DOR agonists may reduce HIV-1 p24 expression by diminishing the formation of phospho-Akt. Supported by DA-04196

TP-38

HUMAN NEURAL PRECURSOR CELLS EXPRESS FUNCTIONAL KAPPA OPIOID RECEPTORS. W.S. Sheng, S.Hu, H.T.Ni*, T.N.Rowen, G.Gekker, J.R. Lokensgard and P.K. Peterson; Center for Infectious Diseases & Microbiology Translational Research, Univ of Minnesota, and the *Stem Cell Dept., R&D Systems, Minneapolis, MN

Neural precursor cells (NPCs) play a key role in fetal brain development. Cellular and environmental factors are important for NPC proliferation and migration. Expression of mu and delta opioid receptors in rodent neuronal precursors and hippocampal progenitors has been reported by other investigators. In this study we demonstrated robust expression of kappa opioid receptors (KOR) in highly enriched (>95% nestin positive) human fetal brain-derived NPCs (n=20). We found that KOR ligands, U50,488 and dynorphin$_{1\text{-}17}$, but not dynorphin$_{2\text{-}17}$, stimulated proliferation and migration of NPCs in a concentration dependent manner. NPC proliferation stimulated by KOR ligands was maximal at 10^{-12} M and 10^{-14} M for U50,488 and dynorphin$_{1\text{-}17}$, respectively. A KOR-mediated mechanism was demonstrated by nor-BNI blockade. These findings suggest that activation of KOR impacts functions of NPCs that are relevant to brain development and repair.

TP-39

NEUROIMMUNOMODULATORY EFFECT OF MORPHINE IN MURINE VISCERAL LEISHMANIASIS. P. P. Singh and P. Singal; National Institute of Pharmaceutical Education and Research, S. A. S. Nagar, India.

Opioid modulation of host-resistance to infection is now well known. We investigated the effect of morphine on *Leishmania donovani* infection. Morphine, administered s.c. in *L. donovani*-infected

BALB/c mice, on both day 0 and day +15, exerted dose-dependent biphasic effect: low doses (1 mg/kg/day) suppressed ($p<0.05$) or even sterile-cleared (2 mg/kg/day) the infection, whereas, high doses (10 and 30 mg/kg/day) potentiated it. *In vitro,* low concentration ($1x10^{-9}$ and $1x10^{-11}$ M) morphine treatment of *L. donovani*-infected peritoneal macrophages (PM) endowed them with significant ($p<0.05$) leishmanicidal activity; paradoxically, high concentrations ($1x10^{-3}$ and $1x10^{-5}$ M) augmented parasite growth. Aminoguanidine significantly ($p<0.05$) blocked the morphine low dose/concentration-induced protective effect both *in vivo* and *in vitro*. These results may have implications in the efficacy assessment of new potential antileishmanial drugs and vaccines, and on the reactivation of latent VL, in areas where both opiate abuse and VL are co-existent.

TP-40

EFFECT OF ALCOHOL EXPOSURE ON MICROGLIA ACTIVATION. P.J. Syapin, M.H. Shearer, and R.C. Kennedy; Dept. Pharmacology and Neuroscience (PJS), and Dept. Microbiology and Immunology (MHS, RCK). Texas Tech Univ. Health Sci.Ctr., Lubbock, TX 79430.

Studies in rodents suggest that alcohol-induced brain damage may involve neuroinflammation. Both microglia and astrocytes have been implicated. In the present study we examined how *in vitro* exposure to ethanol modified the activation state of N9 microglial cells using flow cytometry, cytokine antibody arrays, and iNOS induction. Flow cytometry was used to also quantify microglial activation in C57Bl/6J mice made physically dependent on alcohol. Exposure of N9 cells to 44 mM ethanol for 12-48 h enhanced nitrite production and LPS-induced cell surface expression of CD11b and CD45. Effects on cytokine-activation varied according to the marker and cytokine used. LPS exposure also led to secretion of several cytokines and chemokines by N9 cells, which appeared further increased by ethanol exposure. Microglia isolated from brains of controls and ethanol-dependent adult mice also had increased CD11b expression, but CD45 did not appear changed. These data are consistent with an ethanol-enhanced responsiveness of activated murine microglia.

TP-41

K^+ AND CL^- CHANNELS CONTRIBUTE TO MICROGLIAL PRODUCTION OF REACTIVE OXYGEN SPECIES IN A LABORATORY MODEL OF PARKINSON'S DISEASE. Thomas, MP, Bass,TI, Reynolds,A, Glanzer,J, Gendelman H.E. Dept of Pharmacology & Experimental Neuroscience, Univ Nebraska Medical Center, Omaha, NE, 68198-5880.

Brain microglia affect the course of Parkinson's Disease (PD) by producing toxic products, including reactive oxygen species (ROS), a major contributor to oxidative stress. In attempts to find relationships between oxidative stress and microglial biology we developed a model of PD where aggregated nitrated alpha-synuclein (SYN; a major component of PD Lewy bodies) was used to stimulate ROS. The roles of membrane ionic currents were examined in a system reflecting microglial activation during SYN secretion from degenerating dopaminergic neurons. Chronic application of tumor necrosis factor-alpha (TNF-a) and acute phorbol myristate acetate served as a physiological control. Microglia expressed voltage-gated K^+ currents and stretch-activated Cl^- currents. Blockade of K^+ channels or Cl^- channels inhibited ROS production; however in SYN-activated microglia the effect of K^+ channel blockade was greater than in TNF-a stimulated cells. These results support a role for these ion channels in ROS production in models of PD, and suggest that channel species may play differential roles depending on the neuroinflammatory context.

TP-42

MACROPHAGES ATTENUATE NEURONAL EXCITABILITY: IMPLICATIONS FOR HIV-1-ASSOCIATED NEURONAL DYSFUNCTION. W. Wang and H. Xiong, Univ. of Nebraska Med Ctr, Omaha, NE

Macrophages (MPs) have been proposed to play an active role in HIV-associated neuropathology. We hypothesize that MPs alter neuronal excitability in HIV-1-infected brain and this alteration causes neuronal dysfunction. To test this hypothesis, we studied the influence of mouse monocyte-derived MPs (MDMs) on rat cortical neuronal physiology in MDM-neuronal co-cultures using whole-cell patch clamp techniques. When co-cultured with neurons, MDMs hyperpoloarized neuronal membrane and decreased membrane input resistance. MDM significantly decreased spontaneous spikes in comparison with controls. Spike firing in response to constant current depolarizing injection, action potential amplitude and afterhyperpolarization were significantly attenuated by MDMs. In addition, MDMs increased duration of APs and latency to spike firing. These results demonstrate that MDMs, when co-cultured with neuronal cells, alter neuronal passive membrane properties and reduce neuronal excitability. These alterations on neuronal physiology may result in neuronal dysfunction or injury as seen in HIV-1-infected brain. Supported by NIH grant NS41862.

ABSTRACTS FROM SYMPOSIUM SPEAKERS

(in alphabetical order)

SS-1

MECHANISMS OF NEUROAIDS-POTENTIAL PHARMACOLOGICAL TARGETS TO LIMIT CNS INFLAMMATION AND COMPROMISE. Joan W. Berman. Ph.D. Pathology Dept. Albert Einstein College of Medicine, Bronx, NY.

Encephalitis, cognitive impairment, and dementia associated with AIDS are characterized by leukocyte infiltration into the CNS, microglia activation, aberrant chemokine expression, blood-brain barrier (BBB) disruption, and eventual loss of neurons. Little is known about whether human immunodeficiency virus 1 (HIV-1) infection of leukocytes affects their ability to transmigrate across the BBB into the CNS parenchyma, and whether these HIV-infected cells alter BBB integrity. In addition, the mechanisms that eventually contribute to neuronal loss. We demonstrated that the chemokine CCL2, is a major factor in mediating the neuroinflammation associated with HIV CNS infection. This inflammation has been demonstrated to have damaging effects that result in cognitive impairment. There is no evidence of HIV infected neurons in individuals with NeuroAIDS. Thus this neuronal dropout seen in NeuroAIDS is likely due to the indirect effects of HIV infection of other CNS cells, through elaboration of inflammatory factors and neurotoxic viral proteins, including the viral transactivating protein tat. We and others demonstrated that tat induces apoptosis in human neurons and we have now characterized some of the mechanisms mediating neuronal tat toxicity. Interestingly, we have also demonstrated that, in the appropriate context, CCL2 protects neurons from tat induced apoptosis. The mechanisms that mediate these two apposing activities and the potential sites of therapeutic intervention will be discussed.

SS-2

COCAINE AND HIV-1 PROTEINS: HORMONAL MODULATION OF INTERACTIVE NEUROTOXICITY. R.M. Booze. Program in Behavioral Neuroscience, Univ of South Carolina, Columbia, SC, 29208.

The interactions between drugs of abuse (cocaine, methamphetamine) and HIV-1 neurotoxic proteins (Tat, gp120) are believed to play a major interactive role in accelerating the pathogenesis of HIV-associated brain pathology. Our studies demonstrate that simultaneous treatment of cultured neurons with low doses of Tat and cocaine significantly increases the neurotoxicity of Tat. Alterations in the dopamine transporter (DAT), may be one mechanism of synergistic toxicity of Tat and cocaine; selective neuroprotection was conferred by estrogen pretreatment. Our behavioral studies found that Tat microinjection into the nucleus accumbens core region blocked the subsequent effects of cocaine in enhancing locomotor activity. Thus, our data suggest the involvement of dopamine in the mechanism of synergistic toxicity of Tat and cocaine, and a potential role for estrogenic neuroprotection.

SS-3

A ROLE FOR CORTICOSTERONE IN IMPAIRED INTESTINAL IMMUNITY AND BARRIER FUNCTION IN A RODENT MODEL OF ACUTE ALCOHOL INTOXICATION AND BURN INJURY. M. A. Choudhry, X. Li and I. H. Chaudry. U of Alabama, Birmingham, AL 35294.

Our previous studies have shown that alcohol (EtOH) intoxication prior to burn injury exacerbates the suppression of intestinal T cell immunity and deteriorates intestinal barrier function. The loss of intestinal immunity/barrier function has been implicated in post-injury pathogenesis leading to sepsis and organ dysfunction in burn patients as well as in patients with a history of EtOH intoxication. Utilizing a rat model of acute EtOH intoxication and burn injury, we investigated the role of corticosterone (CORT) in impaired intestinal immunity and barrier function. Results from these studies suggest that EtOH intoxication prior to burn injury augments CORT release, which in turn suppresses intestinal T cell function by inhibiting mitogen activated protein kinase (i.e., p38 and ERK) pathway. Furthermore, the findings from these studies suggest that CORT does not directly alter the intestinal barrier function, rather it up-regulates interleukin-18 which then directly or indirectly contributes to impaired intestinal barrier function. (Support: NIAAA AA12901)

SS-4

A RAPID SIV MODEL IDENTIFIES NOVEL TREATMENT OF HIV CNS DISEASE. Janice Clements, Sheila Barber, Lucio Gama, Joseph Mankowski, and M. Christine Zink, Department of Comparative Medicine, JHU SOM, Balt.,MD 21205

Infection with HIV leads to the development of AIDS and in some individuals CNS disease. Although the incidence of HIV-associated CNS diseases has declined with the use of HAART, the prevalence continues to increase. Anti-retroviral drugs have variable penetration into the CNS; while virus replication may be suppressed in the periphery, the CNS serves as a reservoir for virus during HAART. To study treatment of HIV CNS disease in SIV-infected macaques, we developed a rapid, reproducible model of AIDS and encephalitis. The innate immune response in the brain initiated immediately after infection is a crucial factor in the control of acute SIV replication in CNS. However, control of virus replication in brain is lost during late stages of infection. We have evaluated minocycline treatment in this model to control inflammatory responses in the brain. Understanding the mechanisms by which minocycline decreases both virus replication and CNS lesions, has provided insights into novel treatment regimens for HIV CNS disease. Supported by grants from NIMH and NINDS.

SS-5

A NOVEL MECHANISM FOR IMMUNOSUPPRESSION: NEUROPEPTIDES ->REGULATORY T CELLS. Doina Ganea*, Elena Gonzalez-Rey#, Mario Delgado# *Temple University School of Medicine, Philadelphia, PA; #Instituto de Parasitologia y Biomedicina, Granada, Spain.

The neuropeptides vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) act as endogenous immunosuppressive agents through specific receptors on immune cells. Until recently, the mechanisms involved in the suppressive effect of VIP/PACAP consisted of macrophage/microglia deactivation primarily through the inhibition of NFkB, inhibition of hematopoiesis, and the shift in the Th1/Th2 balance in favor of Th2 responses. Recently, we

identified a novel mechanism for the immunosuppressive effect of neuropeptides, i.e. the generation of tolerogenic dendritic cells and subsequent recruitment of new antigen-specific regulatory T cells. Consequences of neuropeptide-generated tolerogenic dendritic cells are discussed in terms of models of autoimmune diseases such as experimental autoimmune encephalomyelitis (a model for human multiple sclerosis), rheumatoid arthritis, and in the suppression of graft-versus-host reaction during the raft-versus-tumor (leukemia) response.

SS-6

DOPAMINE RECEPTORS ARE NEUROCHEMICALLY ABNORMAL IN HIV ENCEPHALITIS. Benjamin B. Gelman MD PhD, U of Texas Medical Branch, Galveston, TX

Dysfunction of dopaminergic (DAergic) synapses can produce neurocognitive impairment. Neuroimaging data has suggested that tracer uptake in striatal DAergic synapses is abnormal in people with HIV-associated dementia (HAD). To determine if DAergic synapses are biochemically abnormal we undertook an analysis of synaptic protein in the striatum of decedents who had HIVE encephalitis (HIVE), which is the neuropathological substrate of HAD in many people. The concentration of pre- and postsynaptic protein markers of dopamine synapses were measured in immunoblots from six decedents with HIVE and HAD, and six uninfected controls. Striatal homogenates in the people with HIVE contained several abnormalities of DAergic synaptic proteins. The dopamine reuptake carrier (DAT), a presynaptic DAergic marker, was increased. Another Presynaptic protein, tyrosine hydroxylase (TH), was decreased reciprocally. The concentration of phosphorylated TH, a catalytically active form of TH, also was decreased. Postsynaptic membrane markers were significantly different. Dopamine receptor type 2 protein (D_2R) was decreased in striatum, while D_3R was increased reciprocally. Immunohistochemistry of brain samples suggested that the changes in striatal DAergic synapses occurred uniformly in striatum (versus being restricted focally to sites of HIVE pathology). Preliminary results suggest that abnormal DAergic synapses were more evident in specimens with a high concentration HIV pol mRNA. These initial biochemical findings suggest that striatal DAergic tone is increased, and that striatal synaptic transmission is likely to be changed physiologically in HIVE.

SS-7

CD16 IN MONOCYTES ARE ELEVATED IN A COHORT OF HISPANIC WOMEN CHARACTERIZED FOR HIV ASSOCIATED COGNITIVE IMPAIRMENT IN THE ERA OF HAART. Melendez-Guerrero, L.[1], Plaud-Valentin M.[1], Skolaski, R.[2], Kraiselburd E.[1], Gendelman H. [3], McArthur, J.[2], Nath, A.[2], and Wojna, V.[1] [1]University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico [2] Johns Hopkins University, Maryland, and [3]University of Nebraska Medical Center, Omaha, NE

Study was designed to determine the expression of CD14/CD16/CD69 in blood monocytes from an HIV positive cohort of Hispanic Women using HAART and characterized for cognitive function. Cognitive impairment was determined using the American Academy of Neurology HIV associated dementia criteria modified to include an asymptomatic cognitively impaired group (m-AAN). Blood samples collected from patients were evaluated for monocyte surface antigens by two-color flow cytometry analysis. CD14/CD16+ monocytes increased with the degree of cognitive impairment. There was a significant elevation of CD14/CD16+ monocytes in asymptomatics HIV+ compared to HIV + with normal cognition ($p<0.05$). Compared with previous studies in the pre- and post-HAART era, our results indicate that the CD14/CD16+ monocyte is activated in HIV+ early in the

asymptomatic stage. Findings suggest that the CD14/CD16+ monocyte activation in patients with asymptomatic cognitive impairment could be an early biomarker for the disease.

SS-8

SPINAL GLIA AND PROINFLAMMATORY CYTOKINES IN PAIN: IMPLICATIONS FOR INTERLEUKIN-10 IN PAIN CONTROL. Erin D. Milligan, Steve F. Maier, Linda R Watkins, Dept Psychology & Center for Neuroscience University of Colorado, Boulder, USA

Chronic pain caused by diverse etiologies is poorly understood and remains a major unresolved clinical problem. No currently available therapeutic effectively controls human chronic pain. This may be due to the fact that all of these drugs were developed to target neurons. In contrast, my research strongly implicates spinal cord glial cells and glial proinflammatory cytokines as major players in many forms of chronic pain. Targeting spinal cord glia and glial proinflammatory cytokines using the anti-inflammatory cytokine, interleukin-10, is proving to be a powerful new approach for pain control.

SS-9

INTERACTIONS BETWEEN SYSTEMIC INFLAMMATION AND BRAIN INFLAMMATION. V. Hugh Perry, CNS Inflammation Group, School of Biological Sciences, University of Southampton, Southampton SO16 7PX, UK

Systemic infections, or other systemic events that provoke an inflammatory response, lead to the local generation of cytokines and other inflammatory mediators. These molecules communicate with the brain to induce a spectrum of metabolic and behavioural changes that are now known collectively as "sickness behaviour". Cells of the mononuclear phagocyte lineage, the perivascular macrophages and the microglia, are believed to be important in the communication between systemic inflammation and the brain. Acute sickness behaviour is part of the body's defence against infection and does not lead to any permanent changes in the brain. However, in the diseased brain such as in persons with Alzheimer's disease, Parkinson's disease and prion disease the macrophages and microglia are no longer in their normal resting state but are activated. In animal models of chronic neurodegenerative diseases systemic challenge with inflammatory agents leads to exaggerated sickness behaviour, enhanced synthesis of pro-inflammatory molecules within the brain and acute neuronal degeneration. These studies and clinical studies suggest that systemic inflammation may play a significant role in acute behavioural changes and may accelerate the progression of these diseases.

SS-10

HIV-1 PATHOGENESIS STUDIED IN HUMANIZED MICE. S. Gorantla, H. Klasek, L. Walters, H. E. Gendelman, L. Poluektova; U of Nebraska Medical Center, Omaha, NE, 68198

Humanized mice can be used to address questions re-flective of HIV-1 infection and vaccine testing that could only be obtained by similar experiments in humans. The permanent reconstitution of mouse lymphoid tissue with human cells and their functional status are crucial for such models. Newborn Rag-2$^{-/-}$gamma$_c^{-/-}$ pups were re-constituted with human CD34$^+$ cells isolated from cord blood. We performed myeloablation by irradiation, with/ out busulfan s.c. injection, and tested graft status by res-ponses to human GM-CSF and HIV-1 infection. The pe-ripheral blood, spleen, bone marrow were examined for human lymphocyte markers by FACS up to 28 weeks. Collected

lymphoid and non-lymphoid tissues were examined by staining for human cellular and humoral immune responses and HIV-1p24. We achieved the highest level of engraftment by elimination of quiescent stem cells with busulfan in combination with irradiation. The HIV-1 pathology included enlargement of lymph nodes, viral cytopathic effects, reversed CD4: 8 ratios. Human cells in mice remain functional and responded to human -specific cytokine and HIV-1 infection. Funded by NIH P20RR15635, 2R37 NS36126 and 2R01 NS034239.

SS-11

POST-INJURY PAIN AND CYTOKINES: HUMAN AND ANIMAL STUDIES. Yehuda. Shavit; Department of Psychology, Hebrew University, Jerusalem 91905, ISRAEL

Postoperative pain is still of great concern for patients and physicians. We have recently shown in patients undergoing surgery, that the nature of perioperative pain management may have impact on the magnitude of immune alterations in the immediate postoperative period. Specifically, more effective pain management techniques resulted in diminished postoperative cognitive dysfunction, and were associated with attenuated unfavorable immune alterations, including cytokine alterations.

Using animal models of surgical procedures, we find that effective perioperative analgesia resulted in a faster recovery, and attenuated the surgery-induced activation of pituitary–adrenal axis and elevation of PGE2 in the amygdala. Using several animal models of tissue and nerve injury, we show that interleukin-1 plays a critical role in the development of postoperative hyperalgesia and allodynia following the injury.

These findings indicate bidirectional interactions between post-injury pain and proinflammatory cytokines that might have clinical implications.

SS-12

INTERVENTION WITH METHAMPHETAMINE USERS: THE EVIDENCE FOR MANAGEMENT OF DRUG USE AND INFECTIOUS DISEASE. Steve Shoptaw, Professor, Department of Family Medicine at UCLA

Methamphetamine is a potent, long-lasting psycho-stimulant that is currently the second most frequently used illicit drug (behind marijuana) involving 35 million individuals worldwide. Functional attributes that support use of the drug include: increased energy and focused attention, euphoria, brightened mood, and increased libido. These positive attributes allow users to work multiple shifts, to maintain a positive mood, and to facilitate social activities, including intense sexual episodes with multiple partners and often involving HIV-related transmission risk behaviors. Use of methamphetamine is popular in groups of working-class individuals, youth, and in urban gay and bisexual men in the U.S. With chronic use, however, neurobiological, social and work related consequences develop, frequently needing intervention. Interventions for methamphetamine users can range from community-wide prevention campaigns that focus on keeping those who are drug naïve from proceeding to regular use to formal treatment interventions that emphasize drug abstinence and relapse prevention. Effective interventions for reducing methamphetamine also reduce concomitant sexual HIV-transmission behaviors, a finding that provides support for including interventions that reduce methamphetamine use in a comprehensive strategy to prevent spread of infectious disease.

SS-13

THE NEUROIMMUNE JUNCTION IN THE HEALTHY SPLEEN AND IN INFLAMED SYNOVIAL TISSUE. Rainer H. Straub, Experimental Medicine, University of Regensberg, Germany

Sympathetic nerve endings contact immune cells in the spleen, particularly in areas of T lymphocytes and macrophages (building the neuro-immune junction). Neurotransmitters are released into the vicinity of nerve terminals and bind to specific postsynaptic receptors on the surface of these cells. This neuro-immune junction depends on microenvironmental factors such as infectious agents, and this conversation is needed to balance the function of both, the sympathetic nerve terminal and the immune system.

In the inflamed synovium in patients with rheumatoid arthritis, the sympathetic neuro-immune junction is largely destroyed due to a nearly complete loss of sympathetic nerve fibers. In contrast, sensory nerve fibers remain in the tissue and demonstrate an increased nerve fiber density in comparison to osteoarthritic patients. Loss of sympathetic nerve fibers is probably due to nerve repellent factors highly expressed in the inflamed tissue (by macrophages and other cells). This inflammation-induced reaction of sympathetic nerve fibers was evolutionarily conserved for the normal wound healing process. However, in rheumatoid arthritis the continuous repulsion of sympathetic nerve fibers is most probably an unwanted proinflammatory signal.

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