13th SNIP CONFERENCE – April 11-14, 2007

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TP-33 **NICOTINE ALTERS THE GENE PROFILE OF HIV-1 INFECTED HUMAN MICROGLIA.** R.B. Rock, G. Gekker, S. Hu, R.N. Aravalli, P.K. Peterson; Center for Inf. Dis. & Micro. Trans. Res., U of MN, Minneapolis, MN.

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POSTER SESSION 1

- Young Investigator Session -

WP–1:

CANNABINOIDS INDUCE ANTIBODY CLASS SWITCHING IN CULTURES OF MOUSE PURIFIED B LYMPHOCYTES. M. Agudelo, T. Sherwood, L. Nong, I. Perkins, C. Newton, H. Friedman, TW Klein; Molecular Medicine, Univ. South Florida, Tampa, 33612.

Cannabinoid treatment increases Th2 activity and previous reports showed B cells express abundant CB_2 receptors and suggested cannabinoids play a critical role in B cell activation and maturation. Previously we reported evidence of Th2 biasing and class switching in cannabinoid treated and antigen challenged mice and we now explore the possibility that cannabinoids directly influence B cell antibody class switching. Mouse splenic B cells were purified by negative selection and cultured with IL4/anti-CD40 in the presence or absence of the nonselective agonist, CP55,940, or the CB_1 selective agonist, methanandamide, and analyzed at different days by flow cytometry for surface expression of either IgM or IgE. Cells treated with CP55,940 showed a concentration-dependent increase in expression of IgE by day 5 in culture; methanandamide had no effect. In addition, CP55,940 induced an increase in secreted IgE analyzed by ELISA in culture supernatants. These results suggest that the cannabinoids bias toward antibody immunity by directly inducing B cell class switching from IgM to IgE and that CB_2 receptors on B cells are involved. Supported by NIDA grants DA03646 and DA19824.

WP-2

IDENTIFICATION OF FUNCTIONAL MU-OPIOID RECEPTOR-1 ON THE TF-1 BONE MARROW PROGENITOR CELL LINE. A. Banerjee, V. Pirrone, A. Alexaki, B. Wigdahl, M. Nonnemacher; Department of Microbiology & Immunology, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, USA

$CD34^+/CD38^-$ progenitor cells within the bone marrow are refractile to HIV-1 infection, probably due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5. We have previously shown that the human $CD34^+/CD38^+$ TF-1 erythromyeloid progenitor cell line can be utilized as a model to study how differentiation of hematopoietic progenitor cells effects the levels of the HIV-1 receptor, co-receptors and HIV-1 susceptibility. Given these observations, we have initiated studies to identify the presence of the mu opioid receptor on the TF-1 bone marrow progenitor cell line and to determine the functional relevance of these receptors in altering the phenotypic markers of monocyte/macrophage lineage commitment, HIV-1 co-receptor expression, susceptibility to HIV-1 infection, and HIV-1 replication during growth factor- and cytokine-induced TF-1 progenitor cell differentiation.

WP-3

GTPgammaS INCORPORATION IN THE RAT BRAIN: A STUDY ON MU-OPIOID RECEPTORS AND CXCR4. S. Burbassi, V. Aloyo, N. Chaijale,K. Simansky, and O. Meucci; Dept. Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102.

The overall goal of our research is to establish whether opioids regulate the activity of CXCR4 (one of the major HIV co-receptors) in the brain. Here, the [35S]GTPgammaS "binding" assay was used as an indication of G-protein activation by CXCL12, the natural CXCR4 ligand, or by MOR agonists. Brain slices or homogenates from Holtzman rats of different ages (2-7 days old pups and adult animals), were treated with CXCL12 (1-100 nM), DAMGO (0.1-10 μM) or Morphine (1 and 10 μM) and then processed for the assay. Our initial results show stimulation of MOR and CXCR4 in several brain areas, including cortex and hippocampus; this effect is dose-dependent and the magnitude of response varies among different brain regions; also, as expected, younger animals were more sensitive to CXCL12 stimulation than adult animals. Our findings suggest a similar pattern of expression for MOR and CXCR4 in the brain, supporting the possibility of an interaction between the two GPCRs in vivo. This might be relevant to the role of opiates in HIV neuropathogenesis.

WP-4

EVALUATION OF MONOCYTE/MACROPHAGE MATURATION UNDER DIFFERENT CULTURE CONDITIONS. C.M. Buckner, P.J. Gaskill and J.W. Berman; Dept. Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

In human immunodeficiency virus (HIV) infection, monocyte/macrophage trafficking across the blood brain barrier (BBB) is involved in the establishment of viral reservoirs within the central nervous system (CNS), which is an important contributor to neurological disorders that are prominent during HIV pathogenesis. We showed that the chemokine CCL2 is uniquely involved in the enhanced entry of HIV infected peripheral blood mononuclear cells (PBMC) into the CNS parenchyma, resulting in subsequent BBB damage. In order to determine the contribution of monocytes/macrophages during transmigration across the BBB, optimal culture conditions for the survival and maturation of these cells were established in this study. The effects of different culture conditions on phenotypic markers of maturation in monocytes/macrophages isolated from PBMC were examined by flow cytometry using antibodies to CD14, CD71 and CD49c. In addition, the effect of HIV infection on these phenotypic markers was examined. These studies should contribute to our understanding of monocyte/macrophage differentiation and function in the context of NeuroAIDS.

WP-5

ALTERED STRIATAL DOPAMINE TRANSMISSION IN RATS EXPOSED TO THE HIV-1 TAT PROTEIN AND COCAINE. M.J. Ferris, D. Frederick-Duus, J. Fadel, C.F. Mactutus, and R.M. Booze; Dept. of Psychology, Behav. Neurosci. Prog., and Dept. of Pharm., Physio., and Neurosci., Univ. of South Carolina School of Medicine, Columbia, SC 29208.

Subcortical dopamine (DA) systems have been shown to be vulnerable in HIV-infected patients, and multiple models have demonstrated HIV-1 Tat-induced toxicity of dopamine neurons. Microdialysis techniques were used to investigate Tat-induced alterations in DA response in rats exposed to daily administration (10 mg/kg, i.p.) of cocaine (Coc) or saline (Sal). Across 3

microdialysis sessions, Coc-induced DA efflux for rats receiving both Tat and Coc was significantly reduced relative to rats receiving vehicle control (Veh) and Coc. Potassium-evoked DA efflux was lowest for rats in the Tat/Coc group, followed by higher levels for Tat/Sal and Veh/Sal. Rats in the Veh/Coc group had the highest levels of potassium-evoked DA efflux. These results indicate the possibility of Coc-induced neurochemical (cross-) sensitization that is blocked in the context of Tat. Furthermore, Tat and Coc act synergistically to deplete both Coc and potassium evoked DA efflux.

WP-6

NEUROAIDS: HIV-1 PROTEIN-INDUCED TOXICITY IN A RODENT MODEL. S. Fitting, R.M. Booze and C.F. Mactutus; Dept. of Psychology, Behav. Neurosci. Prog., Univ. of South Carolina, Columbia, SC 29208.

Tat and gp120 are proposed to be likely agents of the observed neuronal loss in the brains of pediatric AIDS patients. To determine the role of Tat and gp120 at different levels of analyses, we conducted various *in vivo* studies to characterize the potential deficits induced by these two HIV-1 proteins. Rats were bilaterally injected into the hippocampus with Tat and/or gp120 on postnatal day (P)1 and tested as young and adult rats. (1) A longitudinal behavioral study examining intrahippocampal Tat injection (50μg) indicated adverse effects on sensorimotor gating at P30, P60 and P90. (2) A pharmacological gp120 dose-response study (1.29, 12.9, or 129ng) indicated a significant long-term (P270) reduction in response inhibition as a function of gp120 dose. Use of the dopamine (DA) agonist, apomorphine, revealed long-lasting alterations in the integrity of the DAergic system following neonatal gp120 exposure. (3) A stereological design-based Tat/gp120 study (25μg/150ng), currently in progress, will define the cell loss in subdivisions of the hippocampus. (Supported by DA013137, DA014401 and HD043680).

WP-7

MU-OPIOID RECEPTOR REGULATION OF RANTES THROUGH TGF-BETA. C. Happel, M.A. Kutzler and T.J. Rogers, Fels Institute for Cancer Research and Molecular Biology and Dept. of Pharmacology. Temple University, Philadelphia, PA 19140.

Opioids have been shown to have diverse effects on cells of the immune system. The mu-opioid receptor (MOR) has been shown to possess immunomodulatory activity as well as the ability to regulate cytokine production important for host defense and the inflammatory response. Previous results have shown the MOR selective agonist DAMGO has the capacity to increase the expression of the pro-inflammatory chemokines; MCP-1, RANTES and IP-10 in PBMCs. Preliminary data has shown that MOR induction of RANTES is regulated through an intermediate protein, TGF-beta. PBMCs treated with DAMGO result in an increase in RANTES RNA and protein but this effect is abolished in the presence of an anti-TGF-beta antibody. DAMGO is also able to increase TGF-beta expression and regulate a number of transcription factors. These data suggest a pro-inflammatory role for MOR and a novel role for TGF-beta as a regulator of pro-inflammatory chemokines. Supported by NIH grants T32 DA07237, DA14230, DA16544, DA13429, DA06650.

WP-8

MAST CELL DEPENDENT NEUTROPHIL CHEMOTAXIS INDUCED BY CANNABINOIDS IS MEDITATED BY CB1 AND CB2 RECEPTORS. Venkatesh L. Hegde, Shweta Hegde, Mitzi Nagarkatti, Prakash Nagarkatti. Dept. Pathology, Microbiology & Immunology, Univ.South Carolina, Columbia, SC.

Cannabinoids possess significant immunomodulatory properties. We observed that i.p. administration of wild type BL/6 mice with delta-9-tetrahydrocannabinol (THC) resulted in dramatic accumulation of cells ($9\pm3.5x10^6$ *vs* Veh, $1.8\pm0.6x10^6$) in the peritoneal cavity. FACS analysis showed that >80% of them were granulocytes (Gr) expressing Gr-1 and a neutrophil-specific marker. Chronic nasal challenge with THC resulted in Gr accumulation in lungs. Similar results in C3H mice ruled out contaminating LPS. Mast cell-deficient (W/Wv) mice did not show significant Gr infiltration upon THC injection. Mast cells derived from murine bone marrow cells expressed both CB1 and CB2 mRNA. CB1 and CB2 select agonists (JWH 133 & ACEA) induced Gr infiltration *in vivo*. Moreover, pretreatment with CB1 or CB2 select antagonists resulted in partial blocking, and a combination completely blocked THC-induced Gr infiltration. In conclusion, THC can activate tissue mast cells *in vivo* through CB1 and CB2 receptors leading to dramatic neutrophil-specific chemotaxis.

WP-9

CXCL12 STIMULATES PRODUCTION OF CX3CL1 BY CORTICAL NEURONS. R. Hippensteel, S. Shimizu, and O. Meucci; Dept. Pharmacology and Physiology, Drexel Univ. College of Medicine, Philadelphia PA, 19102.

CX3CL1 (Fractalkine) is a predominant chemokine in neurons, which acts as a membrane bound protein or can be cleaved by metalloproteases and act on other cells. In vitro and in vivo studies suggest that CX3CL1 may have neuroprotective effects. The aim of this study was to determine whether neuronal production of CX3CL1 is regulated by other chemokines, specifically by CXCL12 (SDF-1) that is also known for its critical roles in neuronal survival. We are using pathway-specific DNA microarrays, RT-PCR, ELISA, and Western blot to study the effect of CXCL12 (20nM; 18-24 hrs) on neuronal CX3CL1 expression/production. Our data show that CX3CL1 is present in both immature and differentiated neurons, though its extracellular content increases with time in culture. Furthermore, treatment of neuronal cultures with CXCL12 stimulates expression (likely in a p53-dependent manner) and cleavage of CX3CL1. The latter effect is independent of glia, but sensitive to metalloproteases inhibitors. These findings uncover novel interactions between different groups of chemokines in the CNS that may regulate their function.

WP-10

GLATIRAMER ACETATE-INDUCED NEUROPROTECTION IN MURINE HIV-1 ENCEPHALITIS. S. Gorantla, J. Lui, H. Sneller, H. Dou, L. Poluektova, A. Holguin, H.E. Gendelman. Dept. of Pharmacology and Experimental Neuroscience, Univ. of Nebraska Med. Ctr., Omaha, NE 68198-5880.

Virus-infected and immune competent mononuclear phagocytes (MP: perivascular macrophages and microglia) drive the neuropathogenesis of human immunodeficiency virus type one (HIV-1) infection. Modulation of the phenotype of MP from neuro-destructive to neuroprotective remains a primary therapeutic goal for disease. As Glatiramer Acetate (GA) has long been known to elicit

neuroprotective responses in a variety of inflammatory and degenerative disorders of the central nervous system we tested whether GA immunization could affect an HIV-1 infected MP destructive phenotype. We now demonstrate that HIV-1 replication is inhibited by GA and that GA-stimulated virus-infected MP protect against chemical neuronal injury. In vivo studies using a murine model of HIV-1 showed that GA elicits anti-inflammatory and neuroprotective innate immune responses animals. These data demonstrate that GA can modulate innate immunity and improve neurological outcomes for HIVE.

WP-11

MONOCYTE DIFFERENTIATION: LINKS TO PRODUCTIVE HIV-1 REPLICATION. I. Kadiu-Kieken[1], M. Ricardo-Dukelow[1], J. Schlautman[1], P. Ciborowski[1] and H. E. Gendelman[1]; Dept. of Pharmacology and Experimental Neuroscience [1] , Univ. of Nebraska Med. Ctr., Omaha, NE 68198-5880.

Cellular processes leading to increased susceptibility to HIV-1 infection of monocytes during macrophage differentiation remain incompletely defined. SELDI-TOF, Differential Gel Electrophoresis (DIGE), and LC-MS/MS were used to identify the proteome of human monocytes during differentiation into macrophages. Culture fluids and cell lysates were analyzed at days 0 and 7 in culture. Proteomic analysis identified 703 intracellular proteins to be either up (333) or downregulated (373) 2-10 fold from monocytes to macrophages. Importantly, transcription/translation related factors (Tu transcription factor; tRNA ribosyltransferase), redox proteins (cAMP-dependent protein kinase, vacuolar ATPAse), structural (syntaxin, fllotilin 1, maturase), and chaperone proteins (heat shock protein 70, 90) some, but not all, known to affect the HIV-1 life cycle were significantly modified during differentiation. Defining the phenotypic changes that affect the susceptibility of monocytes to HIV-1 infection may lead to new avenues for therapeutics.

WP-12

HCV NS5A PROTEIN COLLABORATES WITH HIV TAT PROTEIN TO ENHANCE HIV REPLICATION AND TRANSCRIPTION. Lei Kang, Ying Zhu and Jianguo Wu; State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China.

To analyze the effects of HCV on the replication of HIV, all 10 genes of HCV were cloned and co-transfected, respectively, with pNL4-3 R-E- (a plasmid bearing the whole genome of HIV) into Hela cells. Results showed that HCV NS5A protein significantly up-regulated HIV replication. Further studies demonstrated that the Tat protein and an intact LTR with TAR of HIV were involved in the enhancement of HIV replication regulated by NS5A. In addition, we demonstrated that Tat could relocate NS5A from the ER membrane to the nuclei and was required for the enhancement of HIV LTR activation regulated by NS5A. Thus, our results suggested that HCV NS5A protein collaborated with HIV Tat protein to enhance HIV replication and transcription. Detailed mechanism involved in the regulation of HIV replication and transcription by NS5A and Tat is currently under investigation.

WP-13

MODULATION OF IL-7 RECEPTOR EXPRESSION BY KAPPA OPIOID AGONISTS IN EL4.IL-2 AND R1.1 CELL LINES AND IN PRIMARY MURINE THYMOCYTES. M. Khimich and J.M. Bidlack; Dept. of Pharmacology and Physiology, Univ. Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

Endogenous stimulation of the kappa opioid receptor (KOR) provides a positive signal for T-cell maturation. However, U50,488 decreased the level of expression of IL-7 receptor (IL-7R) on primary thymocytes. The IL-7R plays a critical role in T-cell development. To further elucidate the role of the KOR in thymocyte development, we investigated the effect of U50,488 administration on the transcription of IL-7R alpha chain in the EL4.IL-2 and R1.1 thymoma cell lines corresponding to stages I and IV of thymocyte development, respectively. As measured by RT-PCR, U50,488 had opposite effects on these cell lines. It decreased IL-7R mRNA expression in EL4.IL-2 cells, and increased IL-7R mRNA level in the R1.1. Analysis of IL-7R expression on primary murine thymocytes revealed that U50,488 decreased the IL-7R expression during stages I and II of thymocyte development and slightly increased IL-7R level during stage IV. These findings suggest that the effect of a kappa opioid agonist may depend on the stage of thymocyte differentiation.

WP-14

ChIP OF TAT: INTERACTION WITH CELLULAR PROMOTERS. A. J. Luers and J. W. Berman; Dept. of Pathology, Albert Einstein College of Medicine. Bronx, NY 10461.

The Human Immunodeficiency Virus (HIV) protein Tat has pleiotropic activities, including transactivation of viral genome replication. Tat is secreted from infected cells, released from dead cells, and enters neighboring cells. Tat treatment causes upregulation of cellular genes associated with inflammation and chemotaxis; however, little is known about this mechanism. Tat also interacts with host cell chromatin affecting host gene expression. Studies in humans and macaques indicate that cognitive impairment correlates with Central Nervous System (CNS) inflammation**.** We hypothesize that Tat interacts with cellular proteins and chromatin directly, affecting transcription of host cell genes responsible for inflammation and chemotaxis of cells of the monocytic lineage into the CNS. To examine this, we are using chromatin immunoprecipitation (ChIP) to identify host cell genes with which Tat directly interacts. We have also successfully ChIPped the viral LTR with anti-Tat antibody. We have also sequenced the LTR of HIV_{ADA} to facilitate comparison of Tat's activity at the LTR with its activity at host cell promoters.

WP-15

SMALL-MOLECULE HIV-1 GP120 INHIBITORS TO PREVENT HIV-1 ENTRY. Min Luo[1,2], Junming Quan[1], Zhengshuang Xu[1], Xiaolei Yin[1,2], Zhigang Lu[1], Mingxiao Ding[1,2], Kehu Yuan[1], Hongkui Deng[1,2], [1]Laboratory of Chemical Genomics, The Shenzhen Graduate School of Peking University, Shenzhen, 518055; [2]College of Life Sciences, Peking University, Beijing 100871, China.

The interaction between HIV-1 gp120 and the CD4 receptor is highly specific and conservative, presenting a great opportunity for targeting entry with small molecule inhibitors. Here, we report a group of small molecules, which can bind to HIV-1 gp120 and inhibit the HIV-1 entry into cells. Particularly, we found that a small molecule can dock to the CD4 binding site and decrease the binding affinity of CD4 with gp120 not through a directly competitive manner, but mainly through

an allosteric way, which may increase the energy of CD4 binding form of gp120. This identified small molecule may represent a prototype of a new class of HIV-1 entry inhibitors.

WP-16

INHIBITION OF THE E2F1 TARGET CDC2 PREVENTS HIVgp120-INDUCED NEUROTOXICITY. J.L. Nicolai, M.Z. Khan, S. Shimizu and O. Meucci Dept. Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA 19102.

The chemokine receptor CXCR4 is involved in HIV infection and contributes to HIV neuropathology in a direct and indirect manner. Indeed, abnormal activation of CXCR4 alters neuronal and glial signaling causing neuronal apoptosis. However, the transduction pathways leading to neuronal injury and death are still being investigated. Previous work from our group showed that dysregulation of the CDK/Rb/E2F pathway is involved in the neurotoxicity induced by X4-using HIV gp120s, and that CXCL12 - the endogenous CXCR4 ligand - normally modulates this pathway promoting neuronal survival. This study aims to establish the role of the cyclin dependent kinase, Cdc2, in the gp120-induced neuronal death. Cdc2 is a direct E2F1 target that activates the pro-apoptotic protein bad. Here, we have used shRNA and dominant negative Cdc2 mutants to knock-down expression of Cdc2 mRNA and/or inhibit cdc2 activity in primary rat cortical neurons. Our findings indicate that cdc2 function is responsible for gp120 neurotoxicity in these cultures.

WP-17

MORPHINE MODULATES BOTH MANNOSE AND FC RECEPTOR MEDIATED PHAGOCYTOSIS. J. Ninkovic and S. Roy. Department of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

The effects of opiates on immune system have only recently been explored. However, given the prevalent use of opiates, understanding the effects and mechanism of the immune impairment is of crucial importance. To gain insight into how chronic morphine use modifies susceptibility to opportunistic infection in vitro, we have explored its impact on phagocytosis and bactericidal effect. Our results show that chronic morphine treatment in vitro modulates both mannose and Fc receptor mediated phagocytosis in murine alveolar macrophages (CRL-2019). Using fluorescence microscopy and fluorometry, following morphine treatment we show decreased internalization of both opsonized FITC tagged *S. aureus* bacterial particles as well as live GFP tagged *E. coli*. Time-course of phagocytosis shows that at later time-points, morphine groups retained higher amount of viable internalized bacteria indicating morphine's influence on decreased bactericidal effect of *E. coli*. Ongoing studies will further elucidate the mechanism of morphine induced decrease in internalization and the role of key initiators of phagocytosis such as Cdc42, Rac and Rho kinase.

WP-18

MORPHINE INDUCES CHAOS IN SPLENOCYTE TRAFFICKING. M.R. Olin, S. Oh, R. Charboneau, S. Roy J. Ohlfest, T.W. Molitor, and P.K. Peterson. Departments of Medicine and Pharmacology. University of Minnesota.

While opiate-mediated effects on the immune system is a subject of major interest, an important gap in knowledge remains regarding their influence on the migratory patterns of cells of the immune system. The present study was designed to assess the effect of morphine dependence on

splenocyte homing into the spleen and the central nervous system (CNS) following intraventricular (icv) administration of IFN-gamma. Splenocyte trafficking was measured using a novel bioluminescent imaging technique. Splenocytes from transgenic luciferin-positive mice were adoptively transferred via tail vein to determine trafficking patterns in morphine- versus placebo-pelleted animals. Morphine dependent mice had markedly altered splenocyte trafficking patterns into splenic and extrasplenic tissues, including the CNS. Following icv IFN-gamma, morphine dependent mice had significantly increased splenocyte infiltration into the CNS. The results of this study suggest that morphine dependence markedly disrupts trafficking patterns of splenocytes in normal mice as well as in mice with neuroinflammation.

WP-19

SYNUCLEIN MICROGLIAL ACTIVATION AND ITS FUNCTIONAL LINKS TO PARKINSON'S DISEASE. A. Reynolds, et al.; Center for Neurovirology and Neurodegenerative Disorders, Depts of Pharmacology and Experimental Neuroscience & Biochemistry, Univ. of Nebraska Medical Center, Omaha, NE 68198 and Dept of Biochemistry, Univ. of Nebraska-Lincoln, Lincoln, Nebraska 68588.

Modulation of microglial function following engagement with alpha-synuclein (a-syn) released from Lewy bodies of affected dopaminergic neurons may play a prominent role in neurodegeneration during Parkinson's disease (PD). Indeed, exposure to a-syn generated a microglial signature consisting of nuclear factor-kappa B activation and changes in the proteome and secretome coincident with an inflammatory phenotype resulting in dopaminergic neurotoxicity. However, over-time this activation profile evolved into a phenotype characterized by up-regulation of redox-associated proteins and metabolite ratios reflective of a trophic cell. Preliminary analysis of the substantia nigra and basal ganglia from PD cases suggests similar processes are active in PD. The data provide evidence for an undefined role for microglia in PD.

WP-20

SIV EVOLUTION IN MORPHINE-DEPENDENT MACAQUES: TISSUE COMPARTMENTALIZATION. Vanessa Rivera-Amill[a], Richard J. Noel, Jr.[a], Yashira García[a], and Anil Kumar[b].[a]AIDS Research Program, Ponce School of Medicine, Ponce, PR 00732, USA; [b]Department of Pharmacology, School of Pharmacy, University of Missouri, Kansas City, KS 64108, USA.

Previous studies in our laboratory have revealed that a fraction of morphine-dependent SIV-infected macaques progress rapidly to AIDS in the absence of specific immune responses whereas other morphine-dependent and control animals survived for much longer. The main purpose of this project was to characterize the contribution of morphine on SIV evolution and disease progression. Morphine-dependent animals exhibited a higher percentage of diversity in both compartments within envelope variable region 4 when compared to controls. We also found a direct correlation in V4 evolution and rapid disease progression. These results indicate that morphine dependence plays a role in the pathogenesis of SIV/SHIV infection and *env* evolution providing some evidence of the contribution of morphine in both the pathogenesis of SIV/SHIV infection and *env* evolution. Work is currently underway to determine whether this evolution is tissue-compartmentalized.

WP-21

DAMGO REGULATES NEURONAL CXCR4 VIA A GLIA INDEPENDENT MECHANISM INVOLVING CXCR4 PHOSPHORYLATION. R. Sengupta, S. Burbassi, S. Shimizu and O. Meucci; Dept. Pharmacol & Physiol. Drexel University Coll. Med., Philadelphia, PA.

The chemokine CXCL12 and its receptor CXCR4 are implicated in CNS physiology and pathology. Recent studies in our laboratory have shown that synthetic and endogenous mu-opioid agonists impair the pro-survival action of CXCL12/CXCR4 in primary neurons, This study aims to determine the role of glia (if any) in the opioid inhibition of CXCR4, and the effect of mu-opioid receptor activation on post translational changes (primarily phosphorylation) of CXCR4. Our data so far demonstrate that the mu opioid agonist DAMGO blocks the activation of neuroprotective pathways (Erk and Akt) in neurons cultured in the presence of glia (i.e. bilaminar co-culture system) as well as in isolated primary neurons. On the other hand DAMGO did not affect CXCR4-induced responses in astrocytes. In addition, DAMGO prevents the phosphorylation of CXCR4 caused by CXCL12 in neurons. These findings suggest that glia is not required for the inhibitory action of opioids on neuronal CXCR4 and suggest a novel molecular pathway modulating chemokine signaling in the brain.

WP-22

MORPHINE ENHACES MODULATING EFFECTS OF RENAL TUBULAR CELLS ON T CELL FUNCTION. Priyanka Singh[1,2] MD, Helena Schmidtmayerova[1],PhD and Pravin C. Singhal[1,2] MD; [1]Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, NY [2]Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.

Ligation of PD-L1 (programmed death receptor ligand –1) with its specific receptor PD-1 (programmed death receptor-1) has been shown to negatively regulate T cell receptor signaling resulting in decreased T cell proliferation. Renal tubular epithelial cells (TEC), which function as APCs, constitutively express PD-L1. In this study we show that morphine up regulates PD-L1 expression on HK-2 cells (human TEC line) in a dose dependent manner. Interaction of morphine treated HK-2 cells with HIV-1-infected and control T cells resulted in decreased T cell proliferation. The population of CD4+T cells dropped significantly after interaction with HK-2 cells, and the effect was more pronounced in HIV-1-infected CD4+T cells and in T cells interacting with morphine treated HK-2 cells.HIV-1 infected CD4+T cells express higher level of PD-1,suggesting involvement of PD-1: PD-L1 pathway. Altogether, our results indicate that tubular cells may preferentially deplete CD4+T cells especially those infected with HIV-1 and the effect is augmented by morphine.

WP-23

DEC-205 MEDIATED INTERNALIZATION OF HIV-1 RESULTS IN SILENT INFECTION. Priyanka Singh[1,2] MD, Helena Schmidtmayerova[1], PhD and Pravin C. Singhal[1,2] MD; [1]Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, Manhasset, NY [2]Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.

HIV-1 infection of renal cells has been suggested to contribute to the development of HIV-associated nephropathy (HIVAN). However, renal cells have been reported to lack receptors for HIV-1 entry. In this study we show that renal tubular cells do not express CD4, CCR5, CXCR4, DC-

SIGN and MMR, but express the C-type lectin DEC-205. Interaction of HIV-1 with DEC-205 resulted in the internalization of the virus and establishment of a nonproductive infection. HIV-1-specific strong stop DNA was detected in the infected tubular cells during the follow up period and the virus could be rescued by co-cultivation with macrophages and T cells. HIV-1 entry could be blocked by pre-treatment of tubular cells with anti-DEC-205 specific antibody. Moreover, transfection of DEC-205 in cells lacking the DEC-205 receptors rendered them susceptible to HIV-1 infection. These findings suggest that DEC-205 acts as a HIV-1 receptor mediating internalization of the virus into renal tubular cells, from which the virus can be rescued and disseminated by encountering cells.

WP-24

COCAINE UPREGULATES MCP-1 EXPRESSION IN ASTROCYTES: IMPLICATIONS FOR AIDS-ASSOCIATED DEMENTIA. S.Sukumaran[†], N.Dhillon[†], R.Williams[†], YTsai[†], S.Callen[†], S. Dhillon*, O.Narayan* & S. Buch[†]. Dept of Mol. & Int. Physiol[†], Univ. of KS Med Ctr, Kansas City, KS.

Cocaine has wide-ranging effects on the immune and neuroendocrine systems with up-regulation of cytokines & chemokines. Astrocytic foot processes form a major cellular component of the BBB restricting the passage of inflammatory cells to CNS. The present study was aimed at testing the hypothesis that treatment of astrocytes with cocaine could lead to dysregulation of the chemokine, MCP-1, which in turn, could result in increased monocytic infiltration in CNS of HIV-infected individuals. The experimental approach involved assessing the effect of cocaine on expression of MCP-1 RNA & protein in the astrocytic cell line, U251 using semi-quantitative RTPCR, Real-time RT-PCR & immunostaining. We also explored whether cocaine effect could be further modulated by the viral gp120. Our initial findings demonstrated that both cocaine & gp120 enhanced the expression of MCP-1 via expression of egr-1. These findings will have implications in AIDS dementia as increased MCP-1 could amplify the leukocyte migration in the CNS.

WP-25

CONDITIONING OF HEROIN'S EFFECTS ON NITRIC OXIDE: THE BASOLATERAL AMYGDALA. Jennifer L. Szczytkowski, Donald T. Lysle; Dept. of Psychology, University of North Carolina, Chapel Hill, NC 27599.

Recent studies in our laboratory have shown that associative learning plays a critical role in the conditioned effects of heroin on nitric oxide synthesis. Little is known about the brain areas that mediate these effects; however, the basolateral amygdala (BLA) has been implicated in the formation of stimulus-reward associations within models of drug abuse. The present study investigates the role of the BLA in the conditioned effects of heroin on nitric oxide. Rats were given five conditioning trials in which they received an injection of heroin immediately upon placement into a conditioning chamber. Rats were then re-exposed to the conditioning chamber ten days later without further drug administration. Prior to re-exposure, rats received intra-BLA microinfusions of a mixture of the GABA agonists, muscimol and baclofen. Analyses using real-time RT PCR indicate that inactivation of the BLA blocked the effect of heroin associated environmental stimuli on iNOS expression. This study is important because it is the first to demonstrate that heroin's conditioned effects on nitric oxide are mediated through the BLA. (Supported by DA13371).

WP-26

SIGNALING PATHWAYS INVOLVED IN CCL2 PRODUCTION IN GLIA. Wendy L. Thompson and Linda J. Van Eldik; Dept. of Cell and Molecular Biology, Northwestern Univ., Chicago IL 60611.

In Alzheimer's disease (AD) reactive glia are found surrounding amyloid plaques and near areas of neurodegeneration. The activated glia can produce proinflammatory mediators (PM) that may activate neighboring glia as well as damage neurons, propagating a neuroinflammatory cycle. The migration of glia to plaques likely increases the production of PM thus contributing to subsequent neuronal dysfunction. Therefore, blocking migration of glia to plaques may decrease neuroinflammation and damage to neurons. While the identity of these chemotactic proteins is unknown, recent evidence suggests that chemokines may be involved in AD. The chemokine CCL2 has been found near amyloid deposits in the AD brain, is upregulated in the cerebrospinal fluid of AD patients, and has been found elevated in serum from mild AD patients. We are interested in elucidating the signal transduction pathways that result in CCL2 production in glia activated by inflammatory stimuli such as A-beta. Understanding the mechanisms leading to CCL2 production may help identify potential molecular targets for blocking neuroinflammation.

WP-27

KAPPA OPIOID RECEPTOR ACTIVATION INHIBITS LIPOPOLYSACCHARIDE-INDUCED TNF-ALPHA PRODUCTION IN THE U-937 HUMAN MONOCYTE CELL LINE. C.M. Tipton and J.M. Bidlack. University of Rochester School of Medicine, Rochester, NY.

The kappa opioid receptor (KOR) has been linked to the regulation of immune function in a variety of cell types. In the current study, the U-937 human monocyte cell line was used to analyze the relationship between KOR activation and TNF-alpha production. KOR activation by each of the agonists, U50,488, U69, 593, and enadoline inhibited LPS-induced TNF-alpha mRNA expression, and was blocked by the kappa-selective antagonist, nor-BNI. Luciferase-reporter assays were used to show that KOR activation led to a decrease in NF-kappaB activity, providing insight into a possible mechanism for the inhibition of TNF-alpha production. In addition, U-937 cells were analyzed for expression of the KOR, and mRNA expression levels were measured while the cells were in an activated state and at rest. U-937 cells were activated with lipopolysaccharide for 3 to 24 hr, and KOR mRNA expression was measured using quantitative real time RT-PCR. KOR mRNA was found to increase after 12-hr stimulation with LPS, suggesting upregulation through an indirect mechanism, possibly involving pro-inflammatory cytokines/chemokines.

WP-28

METHAMPHETAMINES AND HIV IMPAIR ADULT HIPPOCAMPAL NEUROGENESIS: PROTECTION BY ANTIOXIDANTS. A. Venkatesan, M. Lee, N. Haughey, J. L. Cadet, and A. Nath. Dept of Neurology, Johns Hopkins Hospital, Baltimore, MD 21287.

Maintenance of normal cognition throughout adulthood may occur, in part, through neurogenesis, the formation of new neurons from neural progenitor cells (NPCs) in the hippocampal dentate gyrus (DG). We hypothesized that METH and HIV may disrupt DG neurogenesis, thereby impairing cognitive function.

We administered bromodeoxyuridine (BrdU) to METH-treated and Tat transgenic mice in order to elucidate effects on neurogenesis. Acute METH exposure caused a 25% reduction in BrdU+

proliferating DG NPCs ($p<0.01$), while Tat transgenic mice exhibited a 50% decrease ($p<0.001$). *In vitro*, METH, but not Tat, caused a reduction in NPC proliferation and survival that correlated with the production of oxidative and nitrosative stress. Importantly, antioxidants protected NPCs from the effects of METH.

In conclusion, METH directly impairs NPC proliferation and survival, while HIV Tat likely exerts its effects indirectly. We identify the oxidative stress pathway as a novel therapeutic target to protect NPCs from METH.

WP-29

ALCOHOL-MEDIATED SYNERGISTIC UPREGULATION of PRO-INFLAMMATORY CYTOKINES INDUCED BY HIV TAT86. A.S.Verma, R.J.Noel, Jr, A. Nath and A. Kumar. Division of Pharmacoloy, School of Pharmacy, University of Missouri, Kansas City, MO 64108; Ponce School of Medicine; and Department of Neurology, John Hopkins University, Baltimore, MD 21287.

Chronic alcohol abuse has been implicated in HIV pathogenesis, especially with Neuro-AIDS. In this study, we used SVGA, a human astrocytes cells transiently transfected with HIV-tat86 plasmid and treated every 8 hours upto 3 days with 25, 50 and 100 mM alcohol. The IL-8 and IL-6 mRNA and protein ($p<0.05$) were synergistically up-regulated post 72 hrs treatment of alcohol, while we have observed a non-significant changes in the expression of MCP-1. Alcohol treatment enhances the apoptosis significantly ($p<0.02$) compared to control treated cells, when Annexin V positive cells were selected using flow-cytometery. Our results suggests that intracellular expression of tat86 gene induces pro-inflammatory cytokines in response to alcohol, which in turn may be a contributing factor for exacerbating Neuro-AIDS conditions in a HIV-infected individual. Further studies on the role of NF-kappa-B is under progess.

WP-30

ACTIVATION OF AMPA RECEPTORS ON HUMAN NEURAL PROGENITOR CELLS. N.P. Whitney, H. Peng, N. Erdmann, J. Zheng; Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE 68198.

Neural progenitor cells (NPC) are capable of proliferating and differentiating into new neurons through neurogenesis. The stimulation of neurogenesis would be vital to the recovery from neurodegenerative disorders, such as HIV associated dementia (HAD). Previously, we demonstrated that increased production of glutamate from HIV infected macrophages leads to neuronal injury. On the other hand, we propose that glutamate may mediate neurogenesis through processes resulting from the activation of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors. In this study we use human NPC as a model to investigate if the activation of AMPA receptors results in calcium influx, which activates pathways linked to proliferation, migration, differentiation, and survival of NPC. Calcium influx due to AMPA receptor activation was measured, and our data suggests that calcium influx in NPC occurs through AMPA receptors mediated mechanisms. This result demonstrated that AMPA receptors are functional on human NPC, and the stimulation of these receptors by glutamate may stimulate neurogenesis.

WP-31

METHAMPHETAMINE ENHANCES HEPATITIS C VIRUS REPLICATION IN HUMAN HEPATIC CELLS. L. Ye, J-S. Peng, X. Wang. 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.

Very little is known about the possible interactions of hepatitis C virus (HCV) infection and methamphetamine (METH), a highly abused psychostimulant and a known risk factor for HIV infection. This study examined whether METH has the ability to inhibit endogenous alpha interferon expression, and facilitate HCV replication in human hepatic cells. METH inhibited intrahepatic alpha interferon expression, which was associated with the increase in HCV replication in the hepatic cells. METH also compromised the anti-HCV effect of recombinant alpha interferon. Further investigation of mechanism responsible for the METH action revealed that METH was able to inhibit expression of IFN regulatory factor 5 (IRF5), a key transcriptional factor that initiates the cellular antiviral state. These *in vitro* findings show that METH compromises intrahepatic alpha interferon- mediated innate immunity against HCV, suggesting that METH may have a cofactor role in facilitating HCV persistence in human hepatocytes.

WP-32

ETHANOL INDUCED HYPOTHERMIA: EVIDENCE FOR THE INVOLVEMENT OF NEUROPEPTIDE Y. C.J. Zammit, S. Ijames, T. Thiele, and D. T. Lysle, Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC.

Ethanol consistently produces a profound immediate reduction in body temperature with a slow return to baseline level. However, surprisingly little is known about the mechanisms of ethanol induced hypothermia and the processes involved in that effect. Neuropeptide Y (NPY) is involved in a wide range actions concerning energy expenditure that make it a candidate for involvement in the thermoregulatory effects of ethanol. The present research shows that NPY knockout mice display a significantly greater reduction of core body temperature in response to ethanol compared to wild-type mice. This effect was shown in both low and high doses of ethanol and was independent of the behavioral effects of ethanol. These findings indicate that NPY serves an important function in controlling ethanol induced hypothermia. To further these findings, additional testing is being conducted to determine the type of NPY receptor involved in the effect. More specifically, the role of the Y_1 receptor is being examined using the Y_1 receptor antagonist (BIBP 3226) and Y_1 knockout mice. (Supported by DA13371, AA013573 and AA015148,and DoD R054214)

POSTER SESSION 2

- General Session -

TP-1

HIV-1 TAT TOXICITY IS ATTENUATED BY 17 BETA-ESTRADIOL IN RAT FETAL CORTICAL CELL CULTURE. S. Adams, M. Aksenova, R.M. Booze; Dept. of Psychology, Univ. of South Carolina, Columbia, SC 29208.

The HIV-1 protein Tat is thought to play a vital role in the pathogenesis of HAD. Cocaine appears to enhance the neurotoxic potential of HIV-1 proteins. Prior research indicates that cocaine induced neurotoxicity may be linked to the activation of apoptotic caspase cascades . Also, studies have found that 17 beta-estradiol prevented the synergistic neurotoxicity induced by HIV-1 viral proteins and cocaine. In rat fetal cortical cell cultures Tat-treatment induced significant increases in cell death as determined by initiation of early apoptotic events. These results were enhanced by cocaine. The addition of 17 beta-estradiol prevented this synergistic neurotoxicity of Tat and cocaine. These results suggest that the interaction between cocaine and HIV proteins may produce a more severe toxicity that may account for the rapid progression of HAD among drug abusing HIV infected persons. Additionally, estrogenic compounds may prove beneficial in alleviating HAD pathogenesis. Supported by: DA11337, DA09160, DA84401, HD43680.

TP-2

BDNF MODULATES EXPRESSION OF CHEMOKINE RECEPTORS IN THE BRAIN. F. Ahmed, L. Tessarollo and I. Mocchetti; Georgetown University Medical Center, Washington DC 20057, and Neural Development Group, NCI, Frederick, MD 21702.

CXCR4 and CCR5 play a key role in the neuropathogenesis of AIDS dementia (ADC). Thus, new insight into the expression of CXCR4 in the central nervous system may help develop therapeutic compounds against ADC. The neurotrophin BDNF is known to reduce gp120 toxicity. The scope of this work is to establish whether CXCR4 expression is modulated by BDNF in human cell lines and *in vivo*. We show that BDNF induces a time-dependent reduction of membrane-associated CXCR4 in SH-SY5Y cells and rescues consequently these neurons from gp120 toxicity. To examine whether BDNF modulates CXCR4 expression *in vivo*, RT-PCR and immunohistochemistry were used to determine CXCR4 and CCR5 mRNA and protein levels, respectively, in the brain of BDNF heterozygous mice and wild type littermates (WT). BDNF +/- mice exhibited an increase in CXCR4 mRNA and protein levels compared to WT in the cortex, hippocampus and striatum. Our data indicate that BDNF may modulate the expression of chemokine receptors implicated in HIV infection in adult brain.

TP-3

THE SELECTIVE BLOCKER OF DOPAMINE UPTAKE GBR 12909 ENHANCES HIV-1 TAT TOXICITY. M.Y.Aksenov, M.V.Aksenova, J.M.Silvers, C.F.Mactutus and R.M.Booze; Program in Behavioral Neuroscience, USC, Columbia, SC, 29208.

Cocaine is a risk factor for neurological complications in HIV infection. Although not been neurotoxic by itself, cocaine exacerbates HIV-associated brain pathology. We demonstrated that the selective blocker of dopamine uptake, GBR 12909, but not the blockers of serotonine

(sertraline) or norepinephrine (nisoxetine), was able to mimic cocaine-mediated enhancement of Tat toxicity in rat fetal brain primary cell cultures. The effect of GBR 12909 is consistent with the specific binding of its analog [3H]GBR 12935. However, another dopamine uptake blocker, WIN 35,428, did not affect Tat toxicity, either in hippocampal cultures where the binding of this ligand is low or in midbrain cultures, which exhibit significantly higher specific binding of [3H] WIN 35,428. Our results suggest that indirect effects of cocaine, rather then the direct blockade of monoamine transporter proteins, may underlie the ability of this drug to enhance neurotoxicity of HIV-1 Tat. Supported by DA11337, HD043680; DA13137.

TP-4

ACCELERATED NEURO-AGEING IN HAART TREATED HIV INFECTED SUBJECTS. Anthony IC, Dingwall T, Norby K, Carnie F, Simmonds P & Bell JE. Neuropathology, University of Edinburgh, Scotland.

Despite the introduction of HAART many minor neurocognitive problems persist in infected subjects. We have examined frontal lobe and hippocampus for evidence of early neurodegeneration in both pre-HAART and post-HAART subjects, using a panel of neurodegenerative markers and correlating results with the presence of neuroinflammation. Our data shows excessive hyperphosphorylated Tau but not beta amyloid or alpha synuclein in HAART treated subjects. In conjunction with this finding we have observed elevated neuroinflammation in the form of microglial activation, which shows a degree of correlation with the presence of hyperphosphorylated Tau. Hyperphosphorylated Tau is found at high levels in subjects with Alzheimer's disease and also accumulates in the brain at a lower level with increasing age. Our data suggests an accelerated deposition of Tau in individuals who received HAART therapy. This may predispose such individuals to early neurocognitive decline and may become a significant problem in an ageing HIV population.

TP-5

INHIBITION OF TOLL-LIKE RECEPTOR 2 SIGNALING IN PRIMARY MURINE MICROGLIA. R.N. Aravalli, S. Hu, G. Gekker, and J.R. Lokensgard; Department of Medicine, University of Minnesota Medical School, Minneapolis, MN 55455.

Microglia respond to herpes simplex virus (HSV)-1 by producing proinflammatory cytokines and chemokines. Following this inflammatory burst, they undergo apoptotic cell death. We have recently demonstrated that both virus-induced immune mediator production and apoptosis were mediated through Toll-like receptor (TLR) 2 signaling. Based upon these findings, we hypothesized that inhibition of TLR2 signaling may serve as a means to alleviate excessive neuroinflammation. In the present study, we have cloned the ORFs of four vaccinia virus proteins which disrupt TLR signaling, and overexpressed them in both primary murine microglial cells and in a cell line stably expressing murine TLR2. Using an NF-kappaB-driven luciferase reporter gene assay, we show that, upon stimulation with HSV and *Listeria monocytogenes,* all four viral proteins inhibited TLR2 signaling, with different specificities in murine microglia and in the TLR2 expressing cell line. This report is the first to demonstrate endogenous inhibition of TLR signaling in primary microglial cells.

TP-6

MODELING THE SYNERGISTIC RELATIONSHIP BETWEEN COCAINE AND HIV IN THE HUPBL-NOD-SCID/IL-2R-GAMMA-NULL MOUSE. G.C. Baldwin, S.M. Kiertscher, K.M. Whittaker, and M.D. Roth; Dept. of Medicine, UCLA, Los Angeles, CA 90095-1690.

Epidemiologic and *in vitro* studies suggest that cocaine abuse may have an impact on the pathogenesis of HIV independent of needle sharing and high-risk sexual behavior. *In vitro* results from our group suggest that cocaine influences the immune response to HIV by impacting human dendritic cells (DC) and subsequent modulation of DC-activated T cell responses. We have hypothesized that these effects may lead to enhanced HIV infection *in vivo*. To test our hypothesis, we created mouse/human chimeras using NOD-SCID/IL2rgamma^{null} mice as graft recipients for human lymphocytes (PBL). Using this model, we have recovered functional human T cells, have found that subsequent implantation of human DC results in their trafficking to the spleen, and, have shown that chimeric animals are susceptible to HIV infection, resulting in a decrease in recovered CD4+ human cells. These preliminary findings provide us with an experimental platform to examine the synergistic relationship between cocaine, T-cell activation and HIV replication *in vivo*. Supported by NIDA grant DA03018.

TP-6a

EXPRESSION OF THE MU OPIOID RECEPTOR IS ENHANCED IN THE BRAIN OF HIV-1 TRANSGENIC RATS. J.A. Beltran[a], K.F. Hauser[b], G. Wu[b] and S.L. Chang[a]; [a]Dept. of Biology, Seton Hall Univ., S. Orange, NJ 07079; [b]Dept. Anatomy and Neurobiology, Univ. Kentucky, Lexington, KY 40536.

We have previously reported that the level of the mu opioid receptor (MOR) mRNA was significantly higher in the peritoneal macrophages of the HIV-1 transgenic (HIVTg) rats compared to control rats. Our anti-gp120 immunoneutralization studies also suggested that circulating gp120 contributes to increased MOR expression by macrophages in HIVTg rats. Using real-time RT-PCR, we have further shown that the MOR mRNA in the central nervous system (CNS) including striatum, thalamus, hypothalamus and spinal cord of the HIVTg rats is higher than that of the control rats. Using immunocytochemical co-localization to enumerate the proportion of F4/80-immunoreactive CNS macrophages/microglia ± MOR in the striatum of HIVTg and control rats, we found significant increases in the proportion of F4/80-immunoreactive brain macrophages/microglia both with and without MOR in HIVTg rats. Thus, CNS macrophages were increased in HIVTg rats. Moreover, heightened levels of MOR expression in the striatum appear to be caused, in part, by increases in the proportion of MOR immunoreactive macrophages/microglia. [DA007058, DA016149, DA019836 to SLC and DA019398 to KFH].

TP-7

PSYCHOSTIMULANTS ALTER *SALMONELLA TYPHIMURIUM* DT104 INTERNALIZATION IN PORCINE PEYER'S PATCHES (PPs). D.R. Brown and L.D. Price. Univ. of Minnesota, Dept. of Vet. Biomed. Sci., St. Paul, MN 55108.

PPs in the small intestine are a target for infection by pathogenic viruses and bacteria, including *Salmonella*. They are highly innervated by catecholaminergic nerve fibers. We hypothesized that exogenous catecholamine neurotransmitter substances, as well as the sympathomimetic drugs methamphetamine and cocaine, alter microbial invasion of PPs. Jejunal PPs from

pigs were mounted in Ussing chambers. *S*. Typhimurium DT104 in mid-log growth phase contacted the luminal surface of PPs for 60 min. Internalized bacteria were determined with a gentamycin resistance assay. Treatment with norepinephrine, dopamine or either psychostimulant (30 μM, contraluminal addition) reduced *Salmonella* recovery from PPs. Norepinephrine also decreased *Salmonella* internalization into monolayers of a porcine primary enterocyte cell line (IPEC J2), but the other drugs had no effect. Sympathomimetic drugs may alter early enteropathogen invasion of PPs through interactions with enteric catecholaminergic nerves. Funded by NIH/NIDA R01 DA-10200.

TP-8

NEURO-IMMUNE INTERACTIONS IN PEYER'S PATCHES (PPs): HISTOCHEMICAL EVIDENCE FOR OPIOID MODULATION. L. Vulchanova, M.A. Casey and D.R. Brown. Univ. of Minnesota, Dept. of Vet. Biomed. Sci., St. Paul, MN 55108.

PPs are the inductive site for mucosal adaptive immunity in the intestinal tract and are highly innervated by enteric neurons. We addressed the hypotheses that (1) the PP innervation may serve an immunomodulatory role, and (2) PP nerve fibers express *delta*-opioid receptors (DOR). Using laser scanning confocal microscopy, porcine jejunal PP at volumes > 100 μm in thick transverse sections and > 200 μm in whole-mount preparations were imaged, yielding a three-dimensional perspective of the neuronal network superimposed on PP follicles. Nerve fibers expressing substance P/calcitonin gene-related peptide were seen in intimate association with suprafollicular immunocytes in PP domes labeled with either CD3 (T cells), IgA or IgM (B cells), or MHC class II (presumptive dendritic cells). A subset of substance P fibers, which appeared in close proximity to MHC class II-positive cells, co-expressed DOR. DOR may modulate aspects of neuro-immune cross-talk and sensory neurotransmission in PP domes. Funded by R01 DA-10200 and K01 DA017236.

TP-9

INTERFERON-GAMMA (IFN)-INDUCED TRYPTOPHAN (TRP) CATABOLISM IN ALZHEIMER DISEASE (AD): INDOLEAMINE 2,3-DIOXYGENASE (IDO) AND NEOPTERIN (NEO) IN ASTROCYTES. Cano OD[1,4,5], Boasso A[1], Pardo CA[2], Fuchs D[3], Rugeles MT[4], Lopera FJ[5], Hardy AW[1], Shearer GM[1]. [1]EIB, NCI, NIH. Bethesda, MD, USA. [2]Johns Hopkins Univ., Baltimore, MD, USA. [3]Innsbruck Medical Univ., Innsbruck, Austria. [4]Immunovirology and [5]Neuroscience, Univ. of Antioquia, Medellin, Colombia.

AD is a cause of dementia. IDO catalyzes TRP into kynurenine (KYN). IFN induces IDO in different cell types and is associated with NEO production, a marker of immune activation. We found increased IDO and IFN mRNA in brains of AD patients compared to controls. Histopathology showed IDO protein expression in AD brains. IFN induced IDO mRNA in human astrocyte cultures, and supernatants contained decreased TRP and increased KYN and NEO. Our results suggest that: IFN stimulation induces IDO activity in astrocytes during AD; which may be a source of increased NEO found in CSF of AD. Inflammation associated with IFN production resulting in neurotoxic products of the KYN pathway may contribute to neurodegeneration in AD, suggesting therapeutic intervention aimed at blocking IDO.

TP-10

MORPHINE INDUCES PDL-1 EXPRESSION IN CD14+ cells AND G2/M ARREST OF CD3+ THYMOCYTES. A.N. Chaudhary, P.Singh, H. Vashistha, H. Schmidmayerova, and P.C. Singhal. Immunology and Inflammation Center, Feinstein Institute for Medical Research, Manhasset, NY.

Morphine has been shown to decrease thymic weight and apoptosis of lymphocytes and macrophages in *in vitro* studies. In this study, we examined the role of programmed death ligand-1 (PDL-1) in the modulation of thymic cell population. Mice in groups of 4 were administered either normal saline or morphine for consecutive 3 or 6 days. Subsequently, mice were sacrificed and thymic cells were harvested. Thymic cells were stained with directly labeled mouse monoclonal antibodies specific for CD14, CD3, PDL-1, PD-1 and corresponding isotype controls. Staining was analyzed by flow cytometry. In addition, cells labeled with anti-CD3 antibodies were stained with PI for cell cycle analysis.

CD14+ cells harvested from each mice at 3d, showed 2- to 8-fold increase in PDL-1 expression, whereas CD3+ cells did not show any difference in PD-1 expression. At day six, more than 40% of cells harvested from morphine treated mice showed G2/M arrest as compared to control mice. These results raise a possibility that CD14+ cells expressing PD-L1 are involved in the modulation of the cell growth of CD3+ thymic cells.

TP-11

ANTINOCICEPTION INDUCED BY WIN 55212-2 IN RATS WAS DOSE-DEPENDENTLY REDUCED BY A CB1 ANTAGONIST AND THE CHEMOKINE CCL5/RANTES. X. Chen, E. B. Geller, T. J. Rogers and M. W. Adler; Center for Substance Abuse Research, Temple Univ Sch of Med, Phila, PA.

We previously reported that the chemokine CXCL12/SDF-1alpha, but not fractalkine, reduced the antinociception induced by the cannabinoid (CB) agonist WIN 55212-2 (WIN). In the present experiments, the CB1 antagonist SR 141716A and the chemokine CCL5/RANTES were tested for their effect on antinociception induced by WIN. Rats were given an injection of CCL5/RANTES into the periaqueductal grey (PAG), a primary pain center in the brain, or a subcutaneous (s.c.) injection of SR141716A, 30 min before a s.c. injection of WIN or a vehicle alone (10% Cremophor). The results show that (1) SR 141716A (1, 5 or 10 mg/kg, s.c.) dose-dependently reduced WIN (1 mg/kg, s.c.) antinociception, indicating CB1 receptor involvement; (2) Pretreatment with CCL5/RANTES (1, 10 or 100 ng, PAG) 30 min prior also dose-dependently reduced the WIN-induced antinociception, showing that the chemokine CCL5/RANTES also antagonized the CB1 receptor-mediated antinociception in rats. These findings extend our previous observations demonstrating an interaction between the receptors for the cannabinoid WIN and the chemokine receptor CXCR4. (Supported by NIDA Grants DA 06650,13429, 11130 & 14230)

TP-12

COMPLEMENT FACTOR 3 (C3) REGULATION BY HIV-1 VIRAL PROTEIN R (Vpr). P. K. Datta, T. Morlu, B. E. Sawaya and J. Rappaport; Dept. of Neuroscience, Temple Univ, Philadelphia, PA 19122.

HIV/SIV infection is known to induce the synthesis of C3 in macrophages and brain and contribute to the pathogenesis of NeuroAIDS. Since, Vpr is one of the HIV-1 encoded proteins that play an

important role in viral pathogenesis, and inhibition of complement synthesis and activation may represent a putative therapeutic approach, we analyzed the mechanism of Vpr-mediated C3 expression. Our studies utilizing C3 promoter-Luciferase (-1.1 kb) construct demonstrate that Vpr induces C3 promoter in promonocytic cells and astrocytes. This Vpr-mediated transactivation involves transcription factors, C/EBP and NFkappaB since overexpression of the inhibitory isoform of C/EBP-beta, liver inhibitory protein (LIP) and the dominant negative IkappaBalpha mutant attenuates Vpr-mediated C3 promoter activation respectively. Furthermore, over-expression of IkappaB alpha mutant attenuates Vpr mediated transactivation of the minimal promoter construct (-199 bp) of C3 harboring the C/EBP binding site. These results suggest that there is a interaction between C/EBP and NFkappaB in C3 regulation by Vpr.

TP-13

ETHANOL ALTERS CHEMOKINE EXPRESSION IN HUMAN ASTROGLIAL CELLS. D.J. Buck[1], N. Saffarian[1], P.J. Syapin[2] and R.L. Davis[1]; [1]Dept. of Pharmacology and Physiology, Okla. St. Univ. Ctr. for Health Sci., Tulsa, OK 74107; [2]Dept. of Pharmacology and Neuroscience, Texas Tech Univ. HSC, Lubbock, TX 79430.

We have characterized the in vitro effects of chronic ethanol and ethanol withdrawal on human astroglial chemokine expression and astroglial induced microglial chemotaxis. Ethanol (25mM) enhanced proinflammatory induced CXCL10 mRNA expression; yet, 50mM ethanol was inhibitory. CXCL10 protein expression was also inhibited of 50mM ethanol. Withdrawal (72h) from chronic ethanol potentiated LPS+IL-1 induced CXCL10 protein expression, but not TNF+IL-1+IFN induced expression. Astroglia constitutively released factor(s) which induced microglial chemotaxis. Serum deprivation (48h) of astroglia prior to the 24h stimulation period enhanced astroglial induced microglial chemotaxis. 50 mM ethanol did not alter astroglial-induced microglial chemotaxis (effects of 25mM ethanol remain to be determined). These findings may provide insights into the mechanisms of ethanol-induced brain pathologies.

TP-14

HIV-1 GP120 & COCAINE AFFECT BLOOD-BRAIN BARRIER PERMEABILITY. N. Dhillon*, R. Williams*, F. Peng*, K. Kim[#] & S. Buch*. Department of Physiology* & Microbiology[#], Kansas Univ. Med. Center, KS-66160.

Approximately 20% of AIDS patients develop neurological deficits severe enough to be diagnosed as dementia. Breakdown of blood brain barrier (BBB) is known to play an important role in the development of HIV-1-associated dementia (HAD). Cocaine, often abused by HIV-infected patients, has also been suggested to disrupt the BBB. We thus hypothesized that cocaine could synergize with HIV gp120 to increase BBB permeability and affect the progression of HAD. Both gp120 (100ng/ml) & cocaine (1microM) together increased the permeability of human brain micro vascular endothelial cells (HBMECs) to fluorescein-dextran without affecting HBMEC viability compared to cells treated with either agent alone. Exposure of HBMECs to gp120 & cocaine resulted in destruction of tight junction proteins as early as 30 min. Gp120 & cocaine also reorganized the cytoskeleton and induced stress fiber formation. This suggests that cocaine can potentially contribute as a co-factor in HIV-1 neuropathogenesis.

TP-15

BIDIRECTIONAL HETEROLOGOUS DESENSITIZATION BETWEEN THE KAPPA OPIOID RECEPTOR (KOR) AND CXCR4. M. J. Finley, M.W. Adler, and T. J. Rogers, Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140.

Previously, cross desensitization of CCR5 has been shown to occur following mu and delta opioid receptor activation. Cross desensitization of KOR and CXCR4 is of interest because both receptors are considered to be relatively resistant to desensitization. Here, we show KOR desensitization of CXCR4 following treatment with U50,488H, a KOR-selective agonist, as measured by Ca^{2+} mobilization. Additionally, we find the cross-talk between these receptors is bi-directional. Internalization has been evaluated by flow cytometry and does not account entirely for the changes reflected in Ca^{2+} mobilization. Interestingly, KOR activation by U50 is also responsible for decreased CXCR4 expression at the mRNA level as evaluated by real-time PCR and luciferase reporter assays. These data give promise to the use of KOR agonists as a therapeutic tool for the treatment of HIV-1 infection or certain inflammatory diseases. Supported by NIH grants T32 DA07237, DA14230, DA16544, P30 DA13429, and DA-06650.

TP-16

P53 IMPACTS THE PATTERN OF MICROGLIA ACTIVATION. S. Jayadev, S. Myers, W. Guo, A. Case, J. To, N. Nesser and G. A. Garden, Dept. of Neurology, Univ. of Washington, Seattle, WA 98195.

Microglia, like macrophages, can adopt classical (M1) or alternative (M2) patterns of activation that lead to toxic or trophic actions respectively. The HIV/gp120 coat protein causes M1 activation in microglia leading to TNF-alpha dependent neurotoxicity in mixed cerebrocortical cell culture. Microglia from p53 deficient mice fail to transmit the toxicity of gp120 to neurons. To determine why p53 is required for gp120 induced neuronal injury, whole mouse genome expression arrays were performed using mRNA extracted from cultured microglia generated from strain matched p53 containing and p53 deficient neonatal mice. We observed that p53 has a dramatic effect on the regulation of gene expression in microglia. We also observed that p53 deficient microglia have increased expression of many genes associated with the M2 pattern of activation and a blunted M1 cytokine response following exposure to interferon-gamma. Taken together, these findings suggest that p53 is involved in promoting M1 activation and in the absence of p53, microglia default to an M2 activation pattern.

TP-17

DOPAMINE EXPOSURE AFFECTS HIV REPLICATION IN MACROPHAGES. P.J. Gaskill and J. W. Berman; Dept. of Pathology, Albert Einstein College of Medicine, Bronx, New York, 10461.

Among human immunodeficiency virus (HIV)-infected individuals the effects of HIV infection in the CNS are strongly potentiated by intravenous drugs like cocaine and methamphetamine that affect the dopaminergic system. Correlative studies demonstrate higher rates of HIV encephalitis, dementia and other neurocognitive disorders among HIV-infected drug users, but the mechanisms leading to this increased pathology are still not well understood. As macrophage lineage cells are the primary targets of HIV infection in the CNS, we investigated the effects of dopamine on HIV infection in this cell population by treatment or pretreatment of monocyte-derived macrophages (MDM) with

different concentrations of dopamine during HIV infection. Our preliminary data suggest that continual treatment with dopamine increases HIV replication in MDM. We are continuing our investigation of the mechanisms of this effect in HIV infected MDM by examining DA mediated changes in gene expression and changes in DA transporter and receptor regulation.

TP-18

DISSOCIATION OF ANALGESIC AND HORMONAL RESPONSES TO STRESS USING OPIOID RECEPTOR KNOCKOUT MICE. Claire Gavériaux-Ruff , Candice Contet, and Brigitte L. Kieffer Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, BP 10142, 67404 Illkirch Cedex, France.

Exposure to stress triggers hormonal and behavioral responses. The opioid system was described to mediate analgesia induced by mild stressors and to modulate the activation of the hypothalamic-pituitary-adrenal axis. Our study assessed the contribution of opioid receptors in stress-induced analgesia, ACTH and corticosterone release by a genetic approach. We performed a parallel analysis of mice deficient in mu, delta or kappa opioid receptors, as well as of triple opioid receptor knockout mice, following exposure to a mild stress (3-minute swim at 32°C). In wild-type mice, stress elicited an increase in jumping latency on the hot plate. This analgesic response was reversed both by naloxone and by the triple mutation, and decreased in mu and delta opioid receptor knockout females. In addition, stress produced an increase in ACTH and corticosterone plasma levels. This endocrine response remained unchanged in all mutant strains. This implies that opioidergic brain circuits mediating analgesic and hormonal responses to stress can be dissociated.

TP-19

CHRONIC CXCL10 ALTERS ERK1/2, CREB and NF-kB LEVELS IN HIPPOCAMPAL NEURONAL CELL CULTURE. H. Bajova, T.E. Nelson and D. L. Gruol: MIND, The Scripps Res. Inst., La Jolla, CA 92037, USA.

The chemokine CXCL10 is elevated in the CNS of individuals infected with HIV. CNS neurons express receptors for CXCL10 and may be affected by the elevated levels. In the current study we investigated neuronal signaling pathways as potential targets of the elevated CXCL10 using rodent hippocampal cultures chronically exposed to CXCL10. Western blot analyses show that chronic CXCL10 increased the levels of phosphorylated ERK1/2 as well as the phosphorylated form of transcriptional factors CREB and NF-kB. Alterations were also observed in total protein levels for ERK1/2, CREB and NF-kB. Bcl-2, an anti-apoptotic protein whose expression can be regulated by a pathway involving ERK1/2, CREB and NF-kB was increased in the CXCL10 treated cultures. Consistent with this result, levels of neuron specific enolase and the glial protein GFAP were also increased. These results implicate a role for ERK1/2, CREB and NF-kB in neuronal effects of CXCL10 and suggest that chronic CXCL10 may have a neuroprotective role during certain neuroinflammatory conditions. Supported by MH63712 and MH62261.

TP-20

DOPAMINE INDUCES NEUROTOXICITY THROUGH DOPAMINE RECEPTOR 1-MEDIATED MECHANISAM. S. Hu, W.S. Sheng, J.R. Lokensgard and P.K. Peterson. Dept. Medicine, Univ. of Minnesota Medical School, Minneapolis, MN.

Dopamine (DA), a major neurotransmitter in the central nervous system, can be auto-oxidized and metabolized to products that can induce neurotoxicity. In the current study, primary human cortical neuronal cultures were treated with DA (30-100 μM). After 48 to72h treatment, neurotoxic effects of DA were observed as determined by MTT assay and Tunel staining for apoptosis. The apoptotic effect of DA was mediated through a dopamine receptor (DR) 1 mechanism demonstrated by pretreatment with a DR1 antagonist. By using an 8-isoprostane EIA assay, we also found that DA-induced neurotoxicity was associated with lipid peroxidation. Using dopaminergic neuronal cultures differentiated from neural precursor cells (NPCs), we also found that DA treatment (100 μM, 48h) decreased DA transporter activity. The results of this study indicate that DA can play a role in the neural damage which is the hallmark of several neurodegenerative diseases.

TP-21

COCAINE MODULATES DC DIFFERENTIATION AND THE TH1/TH2 CHARACTER-ISTICS OF ACTIVATED T CELLS. S.M. Kiertscher, A. Harui, G.C. Baldwin, and M.D. Roth; Dept. of Medicine, UCLA, Los Angeles, CA 90095-1690.

Human monocytes exposed to cocaine during their differentiation into DC express decreased CD86, but increased CD83 and CXCR4, compared to control DC. Cocaine also decreased the expression of the Th1-related genes IFN-gamma, IL-12, IL-15, IL-2, GM-CSF, 4-1BB and STAT4. When used to stimulate allogeneic T cells in a MLR assay, cocaine-exposed DC induced normal levels of proliferation as measured by CFSE. However, the proliferating T cells expressed less CD25, and produced a Th2 cytokine profile, in contrast to the strongly Th1-biased response induced by control DC. Using the MLR model, we found that interaction with T cells induced infection of DC by HIV, and that the percentage of infected DC was increased by cocaine exposure. T cells stimulated by cocaine-exposed DC also showed enhanced frequency and magnitude of HIV infection. Our results suggest that cocaine may influence the immune response to HIV by impacting human DC and the Th1/Th2 characteristics of DC-activated T cells. NIDA # DA08254.

TP-22

VULNERABILITY OF OLIGODENDROGLIA TO HIV-1 TAT: INTERACTIONS WITH MORPHINE. K.F. Hauser, V.V. Adjan, Y.K. Hahn, S.P. Zou, A.J. Bruce-Keller, A. Nath*, & P.E. Knapp. Univ. KY, Lexington, KY & *Johns Hopkins Univ., Baltimore MD.

Opiate abuse reportedly furthers the progression of HIV-1 encephalitis. Co-exposure to opiates augments HIV-1 Tat neurotoxicity in vitro, and also enhances astroglial $[Ca^{++}]_i$ destabilization and cytokine production. We have begun to examine Tat effects on oligodendrocytes (OLs) and potential interactions with opiates. In vivo studies utilized transgenic mice expressing Tat_{1-86} regulated by a doxycycline inducible GFAP promoter. Although western blots of Tat transgenic mice detected no change in MBP levels in CNS, other structural and apoptotic indices were abnormal. Total process length per OL was significantly increased by 7d Tat exposure, as measured by Neuro-Lucida tracing in Golgi-Kopsch impregnated OLs. This may be due to loss of small, immature OLs since initial findings suggest that OL progenitors are quite sensitive to Tat. After only 2d of Tat induction, APC^{+}

OLs showed a significant upregulation of both active caspase-3 and TUNEL staining. All measures showed additive interaction with morphine and were typically naltrexone reversible. Collectively, our data show that OLs *in situ* are sensitive to Tat +/- morphine, and may provide a basis for some myelin changes observed in HIV patients. Support: P01 DA19398.

TP-23

INDUCTION OF CANNABINOID AND OPIOID RECEPTORS AFTER T CELL ACTIVATION. J. Kraus, C. Börner, A. Bedini and V. Höllt; Dept. of Pharmacology and Toxicology, Magdeburg University, 39120 Magdeburg, Germany.

Expression of type 1 cannabinoid- and mu opioid-receptors (CB1 and MOR, respectively) in resting T lymphocytes is either absent (MOR), or only at very low levels (CB1). Here, we show that activation of Jurkat T cells with CD3/28 induces transcription of MOR de novo and that of CB1 about 30 fold. Using decoy oligonucleotides directed against transcription factors, which typically mediate gene induction in activated T cells, we demonstrate that both genes are trans-activated by AP-1, NFkappaB and NFAT. Binding sites for AP-1 (nt's -1434, -2388) and NFkappaB (nt's -207, -557, -2174) on the human MOR gene promoter were previously identified by our group. Out of 5 putative NFAT sites, two were shown to trans-activate the human MOR gene, which are located at nt's -486 and -1070. In transient transfection assays these sites confered responsiveness to PMA and ionomycin, which typically activate NFAT. Together, these data provide structural bases for neuro-immune interactions between cannabinoids/opioids and T lymphocytes.

TP-24

DYSREGULATED INTERFERON-GAMMA RESPONSES DURING LETHAL CYTOMEGALOVIRUS BRAIN INFECTION OF IL-10-DEFICIENT MICE. M.C-J. Cheeran, S. Hu, J.M. Palmquist, G. Gekker, and J.R. Lokensgard. University of Minnesota, Minneapolis, MN.

MCMV brain infection induces chemokine production which precedes the infiltration of $CD3^+$ lymphocytes. We hypothesized that an absence of anti-inflammatory cytokines would result in sustained proinflammatory neuroimmune responses. Direct *icv* injection into IL-10 KO mice produced an unexpected result: while wild-type animals cleared MCMV, the infection was 90% lethal in IL-10 KOs. Identical infection of IL-4 KOs did not produce lethal disease. Infected brain tissue from both wild-type and IL-10 KO animals was assessed for cytokine and chemokine levels, as well as viral gene expression. The data show vastly elevated levels of IFN-gamma, CXCL9, and CXCL10, as well as IL-6 in brain homogenates obtained from IL-10 KO animals. However, MCMV viral load, gB mRNA levels, and the titers of infectious virus were similar in both IL-10 KO and wild-type animals. These data demonstrate that MCMV brain infection of IL-10-deficient mice causes lethal disease, which occurs in the presence of a dysregulated IFN-mediated neuroimmune response.

TP-25

MORPHINE ENHANCES PD-L1 EXPRESSION IN CD14 +VE BONE MARROW CELLS. L. Luan, A.N. Chaudhary, H. Patni, P. Singh, H. Schmidmayerova, and P.C. Singhal. Immunology and Inflammation Center, Feinstein Institute for Medical Research. Manhasset, NY.

Opiate addicts have been reported to have lymphopenia. Various investigators have shown the suppression of bone marrow lymphocyte progenitor cell (LPC) growth in animal experimental

studies. We examined the effect of morphine on PD-L1 expression and G2/M arrest in bone marrow LPCs.

Mice in groups of 4 were administered either normal saline or morphine for consecutive 3 or 6 days. Subsequently, mice were sacrificed and bone marrow cells were harvested. Cells were labeled with mouse monoclonal antibodies for CD14, CD3, PD-L1, PD-1, and respective isotype antibodies for FACS analysis. In addition, cells labeled with anti-CD3 antibody, were also stained with PI for cell cycle analysis.

CD14 +ve cells harvested from each mice at 3 –days, showed 2- to 4-fold increase in PD-L1 expression; whereas, CD3 +ve cells showed no difference in PD-1 expression. However, expression of PD-L1 expression decreased more or less to control level by six days. It appears that morphine stimulated- CD14 +ve-PD-L1 expressing cells have a potential to modulate cell growth pattern of PD-1 expressing LPCs.

TP-26

HUMAN *MU* OPIOID RECEPTOR-1 EXPRESSION IN SK-N-SH CELLS AFTER IL-1BETA TREATMENT. S. Mohan, R. L. Davis and C. W. Stevens; Dept. Pharmacology and Physiology, Oklahoma State University – Center for Health Sciences, Tulsa, OK

The proinflammatory cytokine, IL-1beta, produces anti-analgesic effects after exogenous administration. Opioids, like morphine are the most potent clinically used analgesics. Studies have shown that opioids markedly modulate immune cell functions. Therefore, our aim is to characterize the interaction between IL-1beta and morphine on the expression of human *mu* opioid receptor type 1 (hMOR1) in SK-N-SH neuroblastoma cells. SK-N-SH cells also expression IL-1 receptor (IL-1r) as shown through RT-PCR. We report from initial studies, administration of IL-1beta inhibited the expression of hMOR1. Similarly, administration of morphine reduced the expression of hMOR1. These findings suggest that IL-1beta and other proinflammatory cytokines might be involved in the efficiency of opioid analgesia. Changes in hMOR1 gene regulation by IL-1beta and other cytokines is to be explored in detail at the cellular level, providing an insight into the signals that drive hMOR1 transcription.

TP-27

NORADRENERGIC MODULATION OF SYSTEMIC RESPONSE TO INFECTION. KW Mathis, P Greiffeinstein, C Vande Stouwe, PE Molina. Dept. of Physiology, LSUHSC, New Orleans, LA 70112.

Previous studies from our laboratory have demonstrated a central role for the activation of the sympathetic nervous system in the control of pro-inflammatory responses following hemorrhagic shock. Whether similar inhibitory control of pro-inflammatory responses is involved in the response to infection is unclear. This study examined the modulatory role of tissue norepinephrine on host responses to cecal ligation and puncture (CLP). Male Sprague-Dawley rats underwent systemic chemical sympathectomy (SNSx; 6-hydroxydopamine, i.p., 50 mg/kg/d X 3d) prior to laparatomy and cecal ligation and puncture. Time-matched control animals were injected with vehicle (ascorbic acid, i.p.) and subjected to laparatomy only (sham). Mortality averaged 27% in CLP animals and this was increased to 41% in SNSx + CLP animals. CLP produced an 18% (P=0.001) reduction in rectal temperature at 6 h in control and SNSx animals. Rectal temperature 24 h post-CLP was similar in all experimental groups (37±0.1 °C). Both mean arterial blood pressure (MABP) and heart rate (HR) were similar in all experimental groups at 6 h post-CLP (115±3 mmHg and 399±11

bpm). Neither CLP nor SNSx alone altered MABP 24 h post-CLP, however SNSx + CLP animals had significantly lower MABP at 24 h (13%; P=0.005) than CLP and than control animals. At 24 h post-CLP HR was increased in CLP (37%; P=0.000), SNSx and SNSx+CLP animals (24%; P=0.010). CLP increased expression of spleen TNF (74%; P=0.007), IL-1 (342%; P=0.000), IL-6 (47%; P=0.002) and IL-10 (190%; P=0.000) at 24 h post-CLP in surviving animals. SNSx blunted the CLP-induced rise in spleen TNF and IL-10, without altering the CLP-induced increases in spleen IL-1 & IL-6. A similar pattern was noted in cytokine levels in peritoneal lavage fluid (PL). SNSx blunted the CLP-induced rise in PL TNF (35%; NS), IL-6 (49%; NS), and IL-10 (61%; P=0.014). These results show vital noradrenergic modulation of host response to infection. SNSx impaired hemodynamic responses and increased mortality from peritoneal infection. Supported by DOD PR-054196, NIAAA-AA7577, and DA020419-01A1.

TP-28

EFFECT OF MORPHINE ON SIV CONCENTRATIONS IN BRAIN OF MACAQUES. J.K. Marcario, O. Narayan, T. Yankee, M. Mayo, M. Riazi, and P.D. Cheney; Univ. of Kansas Medical Center, Kansas City, Kansas 66160.

To characterize the consequences of chronic morphine dependence in a SIVmac model of neuro-AIDS, 11 rhesus macaques were divided into 2 experimental groups: VM (SIV + Morphine, n=6); and V (SIV Only, n=5). Group VM animals were given 2.5 mg/Kg of morphine sulfate, 4x per day. Group V animals were sham injected with saline. Both groups were infected with SIVmac (R71/E17) 26 weeks into morphine administration, and were followed for 31 weeks. Two V animals and 2 VM animals died with extremely high virus burdens in the CNS. The main predictor of viral encephalitis was extremely high viral RNA titers in the CSF. Of the remaining animals, none of the VM animals (n=4) and all of the V animals (n=4) developed ELISPOT responses to the virus. Examination of tissues from these animals showed higher virus concentrations in the brains of the VM compared to the V animals, suggesting that loss of CMI responses caused by morphine contributed to the accumulation of virus in different regions of the brains of these animals.

TP-29

SIV EVOLUTION IN MACAQUE MODEL OF DRUG ADDICTION AND AIDS: DIFFERENT IMPORTANCE OF ACCESSORY GENES ON PROGRESSION. R.J. Noel Jr., V. Rivera-Amil, R. Kumar, A. Toro, L. Maldonado, G. Chompre, Z. Marrero, and A. Kumar; Ponce School of Medicine Ponce, PR 00716

The impact of morphine abuse on progression of HIV infection is controversial - either accelerating disease or to providing protection to the user. We used a rhesus macaque model of drug abuse and AIDS to address this controversy. We found very rapid progression and death by 20 weeks post infection in 50% of our morphine addicted macaques. We examined the evolution of three accessory genes, *tat*, *vpr*, and *nef*, in blood and cerebrospinal fluid to determine if evolution of these important pathogenic genes was related to the different disease progression rates. We found an inverse correlation between two of these genes (*tat* and *vpr*) and progression in both plasma and CSF. Although it is widely appreciated for its importance in pathogenesis, we found no correlation between *nef* evolution and rapid progression. This indicates that although all three genes are critically important for *in vivo* pathogenesis of primate lentiviruses, they may have different levels of impact on very rapid pathogenesis during drug abuse.

TP-30

HIV-1 NEUROPATHOGENESIS STUDIES IN HUMANIZED NOD/SCID-IL-1R GAMMA CHAIN NULL MICE (HU-NSG). L.Y. Poluektova, H. Sneller, C.L. Gebhart, H.E. Gendelman and S. Gorantla; Dept. Pharmacology and Experimental Neuroscience, Univ. Nebraska Med. Cntr., Omaha, NE 68198-5880.

A rodent model for the studies of HIV-1 neuropathogenesis was developed by reconstitution of newborn irradiated NSG mice with human hematopoietic stem cells from umbilical cord blood. In 12 hu-NSG animals development of human lymphoid tissue and migration of macrophages into the mouse brain was shown. HIV-1 infection with macrophage-tropic viruses lead to the development of chronic disease with lymphadenopathy and sustained viral replication in 6 mice. At 3 months after infection human HIV-1p24+ macrophages were demonstrated in the meninges and perivascular spaces. Depletion of CD8+ cells 3 weeks after established infection by cM-T807 antibodies increased viral load by 0.7 log and induced meningitis, human lymphocyte brain infiltration, formation of perivascular cuffs and the presence of HIV-1p24+ cells with microglial-like morphology.

TP-31

EFFECT OF METHAMPHETAMINE ABUSE IN ANIMAL MODEL OF HIVE. R. Potula[1], B. Morsey[1], D. Heilman[1], M. R. Brodie, Y. Persidsky[1, 2]. [1]Dept Pharmacol. Exp Neurosci, [2] Dept. Pathol. Microbiol, UNMC, Omaha, NE 68198-5215, USA

Methamphetamine (METH), a sympothemimic stimulant affects central nervous system (CNS) and is thought to play a role in modulation of immune responsiveness. Emerging body of evidence supports the idea that METH abuse causes immune dysfunction and thus, may be implicated as a cofactor in the immunopathogenesis of HIV-1 infection. However, whether the synergistic effect of chronic METH abuse and HIV-1 on systemic and anti-viral immunity in the CNS in HIV-1 encephalitis (HIVE) exists, is unknown. Using a chronic METH-HIVE small animal model we sought the effects of METH and HIV-1 infection on peripheral adaptive immune responses (including virus-specific cytotoxic T lymphocytes), control of HIV-1 replication in the CNS, clearance of virus-infected brain macrophages, neuroinflammation and neuronal injury. Preliminary results suggest that METH and HIV-1 CNS infection have synergistic effects on neuroinflammatory responses and neurotoxicity. METH-HIVE small animal model will address the mechanisms thus enabling therapeutic interventions ameliorating the combined effects of METH and HIV-1 on immune system and CNS.

TP-32

MU AND DELTA RECEPTORs INTERACT IN THE MODULATION OF HUMAN MACROPHAGE PHAGOCYTOSIS. I. Morales-Cardona and F. Renaud; Biology Department, University of Puerto Rico, Río Piedras Campus, San Juan, PR 00931.

Previous work had shown that opioids inhibit Fc-mediated phagocytosis by human monocyte-derived macrophages (MDM). In this work we wished to identify the opioid receptors involved in this inhibition by using receptor selective agonists. DAMGO (mu) and DSLET (delta) showed similar effects in terms of potency and Imax (30%), but U50,488 (kappa) had only marginal effects. The effect of DAMGO was reduced to about half in the presence of the mu antagonist CTOP; but surprisingly, a similar effect was also observed in the presence of the delta antagonist naltridol.

Similarly, the effect of DSLET was reduced to about half in the presence of naltridol, but also in the presence of CTOP. Furthermore, when both antagonists were combined, the effect of both DAMGO and DSLET was reduced by 90%. When the effect of CTOP was titrated in the presence of a single concentration of DAMGO or DSLET an almost identical IC50 was detected. These data suggest that mu and delta receptors inhibit phagocytosis in MDM by a functional association.

TP-33

NICOTINE ALTERS THE GENE PROFILE OF HIV-1 INFECTED HUMAN MICROGLIA. R.B. Rock, G. Gekker, S. Hu, R.N. Aravalli, P.K. Peterson; Center for Inf. Dis. & Micro. Trans. Res., U of MN, Minneapolis, MN.

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 this agent but may facilitate viral expression in microglial cells. Previously we showed that pretreatment of microglial cells with nicotine increased HIV-1 expression in a concentration-dependent manner, as measured by p24 antigen levels in culture supernatants. In this study, we demonstrate that nicotine robustly alters the gene profile of HIV-1- infected microglia as identified by microarray analysis specific for cellular pathways associated with HIV-1. Among the genes that are upregulated in response to nicotine are TGF-beta1, IL-4, CX3CL1, CCR2, and CXCR6. Among the genes that are down-regulated in response to nicotine are IL-8, IL-10, TNF, CXCR4, and CCL2. The profound alteration of the gene profile of HIV-1-infected microglia by nicotine suggests that nicotine may be a cofactor in HIV-1 neuropathogenesis.

TP-34

NOVEL HOST DEFENSE MECHANISMS IN THE NEUROPATHOGENESIS OF HIV INFECTION. J. Rumbaugh, M. Bachani, T. Malpica, and A. Nath; Dept. of Neurol., Johns Hopkins Univ., Baltimore, MD 21287.

We have discovered two novel host defense mechanisms, which prevent excitotoxicity in the setting of HIV infection. We treated human fetal neuronal cultures with recombinant Tat, select matrix metalloproteinases (MMPs), antisera to Tat, and NMDA, individually or in various combinations. Surprisingly, we found the combination of Tat and MMPs produced significant attenuation of neurotoxicity, as measured by cell death and mitochondrial potential. Using protein electrophoresis techniques, we found that MMP-1 can degrade Tat, an effect blocked by MMP inhibitors. MMP-2 and -9 also prevented Tat-toxicity but did not degrade Tat. The degradation by MMP-1 was specific for Tat and not other viral proteins. Interestingly, monoclonal antibodies against the C- or N-terminal of Tat not only prevented Tat-toxicity, but the Tat-antisera complexes also attenuated NMDA-mediated excitotoxicity. This effect was specific for NMDA and was not seen for kainate. Further characterization of these MMP and antibody mediated defense mechanisms will be important in developing new therapeutic strategies for HIV dementia, and may also be operative in other viral encephalitides.

TP-35

STROMAL CELL-DERIVED FACTOR 1-ALPHA (SDF)-INDUCED CHEMOTAXIS IN h-T-CELLS BECOMES PI3K/AKT INDEPENDENT: ROLE OF PKC-THETA. Nahid A. Shahabi, Kathy McAllen, Burt M. Sharp; Dept. Pharmacology, Univ. Tennessee, Memphis, TN 38163.

SDF is the exclusive ligand for the chemokine receptor CXCR4. This receptor plays a pivotal role in immune responses, the pathogenesis of infection, such as HIV, and cellular trafficking. However, the molecular mechanisms regulating SDF-driven cell migration are not well defined. In this study, we determined the role of Akt and PKC-theta in SDF-induced T-cell migration in fresh vs cultured T-cells. Purified h-T-cells (fresh vs 48h in media, unstimulated or activated/anti-CD3+CD28) were used. Western blots showed that SDF induced phospho (p)-Akt (Thr308 and Ser473) in fresh cells and p-PKC-theta in 48h unstimulated cells. LY294002 (PI3k inhibitor) reduced SDF-induced chemotaxis in fresh cells by 51%, whereas it minimally affected chemotaxis in 48h-unstimulated or activated cells. However, a specific PKC theta inhibitor, pseudosubstrate for PKC-theta, reduced chemotaxis in 48h unstimulated T-cells by 70%. Thus, chemotaxis becomes independent of PI3K/Akt signaling in human T-cells cultured for 48h. Under these conditions, PKC-theta is phosphorylated by SDF, and chemotaxis becomes largely PKC-theta-dependent.

TP-36

DOPAMINE INHIBITS NITRIC OXIDE PRODUCTION BY HUMAN ASTROCYTES: INVOVLEMENT OF HEME OXYGENASE-1. W.S. Sheng, S. Hu, N. Penner, J.R. Lokensgard and P.K. Peterson. Dept. Medicine, Univ. of Minnesota Medical School, Minneapolis, MN.

Little is known about the effects of dopamine (DA) on free radical production by astrocytes. We studied the effects of this neurotransmitter on nitric oxide (NO) generation by interleukin (IL)-1 stimulated human astrocytes. Homogeneous cultures of fetal astrocytes were found to express DA receptor mRNA (D1, D2, and D5), and DA potently suppressed, in a concentration-dependent manner, generation of NO (assessed by Griess reagent) from these cells when stimulated with IL-1. The antioxidant/anti-inflammatory enzyme heme oxygenase (HO)-1 has been shown to downregulate NO production in other cell culture systems. We found that hemin, which is known to upregulate HO-1, also suppressed NO release from IL-1-stimulated astrocytes. To investigate whether HO-1 is involved in the mechanism whereby DA inhibits NO production by IL-1-stimulated astrocytes, cells were treated with the HO-1 inhibitor tin protoporphyrin (SnPP) prior to DA exposure. This treatment blocked DA-induced suppression of NO production. These findings expand understanding regarding the effects of DA on brain cells other than neurons and could be relevant to neuropathogenesis of certain neuroinflammatory and neurodegenerative diseases.

TP-37

NEW THERAPEUTICS DEVELOPMENT FOR HIV-MEDIATED NEURODEGEN-ERATION. Joseph P. Steiner, Daniella Asch, Norman Haughey, Justin McArthur and Avindra Nath, Department of Neurology, Johns Hopkins University, Baltimore, MD.

The goal of this study is to screen and identify neuroprotective compounds relevant to HIV-Dementia and drug abuse. We have mimicked the co-morbid effects of drugs of abuse on neurotoxic HIV-1 Tat and gp120 in in vitro assays by combining cocaine and/or morphine with Tat and gp120. We have screened more than 2000 compounds that included FDA approved drugs for protective

efficacy against oxidative stress-mediated neurodegeneration and identified selective serotonin reuptake inhibitors (SSRIs) as potential neuroprotectants. Numerous SSRIs were then extensively evaluated as protectants against neurotoxicity as measured by changes in mitochondrial potential, neuronal cell death and induction of nitric oxide synthase elicited by HIV Tat and gp120 in the presence of cocaine and/or morphine. While many SSRIs demonstrate neuroprotective actions, we find that paroxetine is potently neuroprotective against these toxins. Therefore, SSRIs such as paroxetine, may provide an adjunctive neuroprotective therapy to treat HIV patients with HIV neuropathy and dementia.

TP-38

MORPHINE MODULATION OF ALVEOLAR MACROPHAGES INCREASES PULMONARY *S. pneumoniae* INFECTION. Jinghua Wang, Roderick A. Barke, Richard Charboneau, Sabita Roy. Depts. of Pharmacology and Surgery, Univ. of Minnesota, VAMC.

Resident alveolar macrophages and respiratory epithelium constitute the first line of defense against invading pneumococci. Our study showed that increased mortality, bacterial outgrowth and dissemination seen in morphine-treated mice were abolished by depleting the alveolar macrophages in vivo with liposomal clodronate. This suggests that alveolar macrophages may be a critical cell population involved in morphine induced compromise of innate immunity. Using an in vitro alveolar macrophage and lung epithelial cell infection model, we found that significant MIP-2 release was detected in alveolar macrophages, but not in lung epithelial cells after 4 h of cell infection. Morphine reduced MIP-2 release in pneumococci stimulated alveolar macrophages. Moreover, morphine inhibited pneumococci induced NF-kappaB-dependent gene transcription in alveolar macrophages. Morphine impaired TLR9-MyD88-NF-kappaB signaling triggered by pneumococci in resident macrophages, leading to a decreased innate immune response during the early stages of infection. Support by DA112104, T32 07097.

TP-39

NATURAL KILLER CELL INHIBITS HIV REPLICATION IN CHRONICALLY INFECTED IMMUNE CELLS. T. Zhang, X. Wang, Y. Li, Y-J. Wang, S. Douglas, W-Z. Ho; Div. of Immunol., The Children's Hosp. of Phila, Dept. of Pediatr., Univ. of Penn. Sch. of Med., Phila., PA 19104.

Natural killer (NK) cells are a crucial component of the host innate immune system. We investigated the noncytolytic anti-HIV activity of NK cells in chronically HIV-infected immune cells. Supernatants collected from NK cell cultures (both primary NK cells and NK cell lines, YTS and NK 92) inhibited HIV activation in PBMCs from HIV-infected subjects. NK supernatants (NK SN) also suppressed TNF-alpha-induced HIV activation in chronically infected cell lines (U1 and ACH-2 cells). The antibody to IFN-gamma blocked NK SN-mediated anti-HIV effect, while the antibodies to CC-chemokines had no impact on NK SN-mediated HIV inhibition in U1 and ACH-2 cells. Investigation of mechanism(s) responsible for the NK action showed that NK SN inhibited TNF-alpha-mediated activation of HIV-long-terminal repeat (LTR), and upregulated the expression of STAT-1 and phosphorylated P38 mitogen-activated protein kinase (MAPK). The P38 MAPK inhibitor (SB 203580) blocked NK SN-mediated HIV inhibition. These data provide compelling evidence that NK cells have a critical role in controlling HIV activation in the reservoirs.

TP-40

BEHAVIORAL CHARACTERIZATION OF HIV-1 TRANSGENIC RATS. K.M. Webb[1], S. Fitting[2], C.F. Mactutus[2] and R.M. Booze[2]; 1 – Univ. of South Carolina School of Medicine, Dept. of Pharmacology, Physiology and Neuroscience 2 - Dept. of Psychology, Univ. of South Carolina, Columbia SC 29208.

HIV-Associated Dementia (HAD) impairments are a collection of cognitive, motor and behavioral dysfunctions that affect 10-15% of HIV-positive individuals. The current studies examined behavioral changes in the HIV-1 transgenic rat, a model of HAD. An early sign of HAD progression is an alteration in the Auditory Brainstem Response, which is correlated to the Acoustic Startle Reflex (ASR) in rats. Transgenic rats displayed significantly greater response amplitudes, but no alteration in response latency. Locomotor Activity was measured to indicate motivation to explore environment. Compared to wildtype and control animals, transgenic rats displayed significantly less exploratory locomotion, less total activity and rearing behaviors. Transgenics also were less active in center of the testing apparatus, demonstrating decreased exploration and possibly apathy towards exploration. These results suggest possible cognitive deficits in this model. Additional studies are needed to validate the HIV-1 transgenic rat as a model of HAD. Supported by DA11337, DA09160, DA84401, HD43680.

TP-41

HIV-1 gp120 INHIBITS LONG-TERM POTENTIATION VIA PRESYNAPTIC MECHANISMS. Y. J. Zhou, J. Dong and H. Xiong Dept. Pharmacol. & Exp. Neurosci., Univ. of Nebraska Med. Ctr, Omaha, NE 68198-5880.

Shed off from virions and/or secreted from virus-infected mononuclear phagocytes (MP), HIV-1gp120 has the potential to diffuse and interact directly or indirectly with surrounding and distant neural cells and synapses. Our previous study demonstrated that gp120 inhibited long-term potentiation (LTP) in the CA1 area of rat hippocampus (Dong and Xiong, J. Neurosci Res. 83:489-498, 2006). To further investigate the site(s) of action, we studied effects of gp120 on both spontaneous and electrically evoked mini excitatory postsynaptic currents (mEPSCs) recorded in the CA1 pyramidal cells in rat hippocampal slices. Bath application of gp120 (200pM) significantly reduced spontaneous mEPSC frequency without change on mEPSC amplitude. Quantal analysis revealed that gp120 reduced the mean quantal size. Heat-inactivated gp120 failed to produce these changes. Taken together, these results indicate that gp120 inhibits LTP through presynatic mechanisms. The alteration of LTP by gp120 may underlie the pathogenesis of HIV-associated dementia. Supported by NIH grant NS41862.

ABSTRACTS FROM SYMPOSIUM SPEAKERS

SS-1

REGULATION OF THE EXPRESSION OF KAPPA AND DELTA OPIOID RECEPTORS ON MACROPHAGES, MONOCYTES, AND MICROGLIA. J.M. Bidlack; Dept. of Pharmacology and Physiology, University of Rochester, Rochester, NY 14642.

Biochemical, molecular, and functional studies have demonstrated the presence of opioid receptors on cells from the immune system. The kappa opioid receptor (KOR) is expressed predominantly on thymocytes, macrophages, microglia, and T cells. Kappa opioids attenuated LPS-induced TNF-alpha and IL-6 levels in the U-937 and P388D1 monocyte cell lines, respectively. These effects were blocked by the kappa-selective antagonist nor-BNI. The adult brain-type prodynorphin gene is expressed in U-937cells. LPS stimulation of U-937 cells lead to a decrease in prodynorphin gene expression and an increase in the KOR mRNA levels. Dynorphin may play an autocrine role on monocytes. The mouse BV-2 microglial cell line expresses the delta opioid receptor (DOR) under basal conditions. Interferon-gamma, TNF-alpha, and IL-6 decreased DOR mRNA expression. In contrast to the KOR on U-937 cells, activation of BV-2 cells with LPS resulted in a decrease in DOR expression. Collectively, opioid receptors are expressed on certain types of immune cells and opioids regulate the expression of many cytokines by direct effects on immune cells.

SS-2

SOCIAL STRESS IN SIMIAN AIDS: CAUSES AND CONSEQUENCES FOR IMMUNE FUNCTION AND DISEASE PROGRESSION. J.P. Capitanio, California National Primate Research Center, University of California, Davis, CA 95616.

Since early in the AIDS epidemic, stress has been thought to influence rate of disease progression, and studies using the SIV/rhesus macaque model have supported a link between stress and survival. Not all individuals respond to a stressor in the same way, however. Given that recent research has indicated that events early in infection are especially important for the establishment of the disease, we examined how two host factors, the personality dimension “Sociability” and genotype for the serotonin transporter promoter, affected early indicators of disease in animals subjected to social stress. Results show that three measures of early immune function are associated with viral load, which is itself a strong predictor of survival. Further, correlations between personality, behavior, plasma cortisol concentrations, and expression of interferon-stimulated genes were all significantly related – but only for animals experiencing stressful social circumstances. Understanding of mechanisms by which individual-level factors can affect disease-related immune function could provide novel avenues for treatments that could supplement standard anti-retroviral therapy.

SS-3

THEILER’S VIRUS/INFECTIOUS SEIZURE MODEL. J.E. Libbey[1], M.C.P. Smith[1], T. Tanaka[1], N.J. Kirkman[1], K.S. Wilcox[2], H.S. White[2] and R.S. Fujinami[1], Depts. of Neurology[1] and Pharmacology & Toxiciology[2], University of Utah, Salt Lake City, UT 84132.

We have established a novel virus infection-induced seizure model in C57BL/6 mice. C57BL/6 mice are susceptible to seizures induced by Theiler's murine encephalomyelitis virus infection. Approximately 50% of C57BL/6 mice develop transient seizures that are not associated with fever. Motor function and coordination are impaired in seized mice. Pyramidal neuron loss and transforming growth factor-beta expression correlate with seizure activity which is inversely correlated with inflammation in the hippocampus. The characterization of this model will enable the investigation of viral and innate immune contributions in the central nervous system to the development of seizure disorders in humans.

SS-4

EFFECTS OF DRUG ABUSE IN AN INDUCIBLE HIV-1 TAT TRANSGENIC MOUSE. K.F. Hauser, N. El-Hage, S. Buch, K.A. Kelps, Y.K. Hahn, S.P. Zou, A. Chauhan*, A. Nath*, A.J. Bruce-Keller & P.E. Knapp. Univ. KY, Lexington, KY & *Johns Hopkins Univ., Baltimore, MD.

Opiate abuse reportedly can accelerate HIV-1 encephalitis (HIVE). Transgenic (tg) mice expressing Tat_{1-86} regulated by a doxycycline inducible GFAP promoter were used to assess opiate-HIV interactions in the striatum. Tat induction (2 d) significantly increased GFAP+ (immunoreactive) astroglia and F4/80+ macrophages/microglia. Morphine (s.c. implants) further increased the Tat-induced glial activation and the proportion of nitrotyrosine+ macrophages. Morphine's effects were reversed by naltrexone. At 2 d, morphine also exacerbated the number of active caspase-3+ neurons and oligodendroglia, suggesting wide-spread glial and neuronal injury. Although neuron numbers appeared stable, we found dendritic pruning in Golgi-impregnated medium spiny neurons following 7 d morphine or Tat-induction, suggesting neuronal injury without death. Our findings using HIV-1 Tat tg mice suggest that Tat *per se* contributes to HIVE, and imply that opiates exacerbate inflammation and injury through interactions with Tat. Support: DA19398, DA18633.

SS-5

OPIOIDS, HCV AND NEUROPATHOGENESIS. Wenzhe Ho Division of Allergy and Immunology, The Children's Hospital of Philadelphia, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

As many as 2.4 million Americans are heroin users who are a high-risk group for HCV infection. Although it is known that both opioids and HCV are involved in neurological dysfunction, their roles in the neuropathogenesis remain to be determined. We hypothesize that morphine alone and in the combination with HCV modulate intraneuronal innate immunity, particularly type I IFN signaling pathway that has a critical role in the intracellular immunity and neuroprotection in the CNS. Our data showed that morphine inhibits IFN-γ expression and enhances HCV replication. HCV has the ability to infect both macrophages and neuronal cells *in vitro*, although the infectivity was low. Similar to human immune cells, the neuronal cells also express IFN-γ IFN expression, however, was inhibited by morphine and/or HCV. Since IFN-γ not only has a key role in host innate immunity against viral infections but also involves in the CNS functions, our data indicate that opioids and/or HCV impair the innate immune protection for the neuronal cells, which constitutes a plausible mechanism for the CNS disorders often seen among heroin users infected with HCV. Support: NIDA12815 and NIDA 16022

SS-6

REGULATION OF OPIOID RECEPTORS BY CYTOKINES. V. Höllt, C. Börner and J. Kraus, Dept. of Pharmacology & Toxicology, Univ. of Magdeburg, Leipzigerstr. 44, D-39120 Magdeburg, Germany.

Mu opioid receptors (MOPs) are expressed in neuronal and immune cells. We showed that IL-4, IL-6 and TNF-alpha upregulate MOP transcription in neuronal cells, and induce de novo transcription of the gene in various immune effector cells, such as B and T lymphocytes, monocytes, granulocytes, endothelial cells and dendritic cells. In unstimulated immune cells MORs are not expressed. Transcriptional induction of the human MOR gene by IL-4, IL-6 and TNF-alpha is mediated by the transcription factors STAT6, STAT1/3 and NF-kappaB, respectively. Recent findings from our laboratory demonstrated that cytokines also inhibit MOR transcription. Thus, IFN-gamma inhibits constitutive transcription of the MOP gene in SH SY5Y cells and IL-4-evoked transcription of the gene in Jurkat T cells. MOPs are also induced after antigen activation in T-cells. MOPs are localized in the cell membrane, and internalized after opioid binding, similar to neuronal cells. In the T cells, opioids mediate various immunomodulatory effects, including inhibitory effects on T cell receptor signaling such as activation of MAPK and LAT.

SS-7

INTERLEUKIN-2 SUPPRESSION BY 2-ARACHIDONYL GLYCEROL OCCURS INDEPENDENTLY OF CANNABINOID RECEPTORS 1 AND 2: EVIDENCE OF AN INHIBITORY ROLE FOR PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR. C.E. Rockwell, N.T. Snider, J.T. Thompson, J.P. Vanden Heuvel, and N.E. Kaminski; Department of Pharmacology & Toxicology and the Center for Integrative Toxicology, Michigan State University, East Lansing, MI 48824.

2-Arachidonyl glycerol (2-AG) is an endogenous arachidonic acid derivative, which binds cannabinoid receptors, CB1 and CB2, hence termed an endocannabinoid. 2-AG also modulates a variety of immunological responses, including expression of the autocrine/paracrine T cell growth factor, interleukin (IL)-2. The objective of the present studies was to determine the mechanism responsible for IL-2 suppression by 2-AG. Due to the labile properties of 2-AG, a non-hydrolyzable analogue of 2-AG, 2-AG ether, was also employed. Both 2-AG and 2-AG ether suppressed IL-2 expression independently of CB1 and CB2, as demonstrated in leukocytes derived from CB1/CB2 null mice. Moreover, we demonstrated that both 2-AG and 2-AG ether treatment activated peroxisome proliferator activated receptor γ (PPARγ), as evidenced by forced differentiation of 3T3-L1 cells into adipocytes, induction of aP2 mRNA levels, and activation of a PPARγ-specific luciferase reporter in transiently transfected 3T3-L1 cells. Consequently, the putative role of PPARγ in IL-2 suppression by 2-AG and 2-AG ether was examined in Jurkat T cells. Concordant with PPARγ involvement, the PPARγ-specific antagonist, T0070907, blocked 2-AG and 2-AG ether-mediated IL-2 suppression. Likewise, 2-AG suppressed the transcriptional activity of two transcription factors crucial for IL-2 expression, NFAT and NFκB, in the absence but not in the presence of T0070907. 2-AG treatment also induced PPARγ binding to PPAR response element. Collectively, the aforementioned studies identify PPARγ as a novel intracellular target of 2-AG, which mediates the suppression of IL-2 by 2-AG in a manner that is independent of CB1 and/or CB2. (supported in part by NIH Grant DA12740 and DA15276)

SS-8

VACCINATION FOR DRUG ADDICTION. Thomas Kosten MD, Baylor College of Medicine.

Antibody therapy through active vaccination or passive transfer of monoclonal antibodies is designed to prevent drugs of abuse from entering the central nervous system. Anti-drug antibodies reduce rush, euphoria, and drug distribution to the brain primarily by the binding capacity of the antibody, as well as through a pharmacokinetic antagonism, which reduces the amount of drug in the brain, the rate of clearance across the blood brain barrier, and the volume of drug distribution. Because the antibodies remain in the circulatory system, they have no apparent central nervous system side effects. Active immunization with drug-protein conjugate vaccines has been tested for cocaine, heroin, methamphetamine and nicotine in animals, with one cocaine and three nicotine vaccines in phase 2 human trials. Passive immunization with high affinity monoclonal antibodies has been tested for cocaine, methamphetamine, nicotine and phencyclidine in preclinical animal models. Antibodies have two immediate clinical applications in drug abuse treatment: to treat drug overdose and to reduce relapse to drug use in addicted patients. Their major therapeutic benefits over conventional small molecule agonists and antagonists are specificity of the therapies, lack of addiction liability, minimal side effects, and long-lasting protection against drug use. Immunotherapies can also be combined with other anti-addiction medications and enhance behavioral therapies. Current immunotherapies already show efficacy, but improved antigen design and antibody engineering promise highly specific and rapidly developed treatments for both existing and future addictions.

SS-9

ALZHEIMER'S DISEASE AND NEUROINFLAMMATION. Gary Landreth, Department of Neurosciences, Case Western Reserve University, Cleveland, OH 44106

Alzheimer's disease is characterized by the accumulation and deposition of fibrillar forms of beta amyloid (fAβ) within the brain. AD has a significant inflammatory component owing to the presence of abundant, activated microglia associated with amyloid plaques. The activated microglia elaborate a wide variety of inflammatory molecules that contribute to disease progression and neuronal death. We have focused our attention on the mechanisms through which microglia detect and respond to fibrillar forms of Aβ. Microglia bind fAβ through an ensemble of receptors that form a functional complex leading to the activation of downstream proinflammatory signaling cascades. Importantly, exposure to fAβ leads to the stimulation of phagocytosis and ingestion of amyloid peptides by the microglia. However, this process is suppressed by the proinflammatory mileau in the AD brain, leading to the accumulation of fAβ and its subsequent deposition. We have explored the efficacy of anti-inflammatory drugs in suppressing microglial activation and their impact on disease pathogenesis. These studies support the development of therapies that target microglia-mediated inflammatory processes.

SS-10

WINDOW TO THE BRAIN: DETERMINING T CELL SPECIFICITY AND CONTROL OF VIRAL PERSISTENCE. M.B.A. Oldstone; Viral-Immunobiology Laboratory, Molecular and Integrative Neurosciences Department and Department of Infectology, The Scripps Research Institute, La Jolla, CA 92037.

Viral diseases of the CNS and elsewhere have been known since antiquity while the primary role

of T lymphocytes and their molecules in either preventing or controlling virus infections understood for only over 30 years. Recently the ability to specifically localize, quantitate and observe in real time the migration of and interaction of T cells and their molecules with infected targets and identify the molecule(s) that lead to a persistent infection has occurred. This presentation will document first in real time, the step-by-step mechanism how virus-specific T cells interact with and lyse viral infected cells. Second, it will display novel technology to analyze trafficking and location of virus-specific T cells and associated effector molecules at the interface of virus-infected cell interaction. Third, we will identify a host molecule that abrogates T cell function thereby allowing viruses to persist and use of a therapeutic strategy that restores T cell function and control of infection.

SS-11

ENHANCED DNA VACCINE POTENCY AND IMMUNE PHENOTYPE TARGETING HIV-1. David Weiner, University of Pennsylvania.

Infectious diseases including HIV take an enormous toll impacting both life expectancy as well as life quality around the globe. DNA vaccines are a developing technology for the induction of broad immunity against infectious agents including HIV. Since the early 1990's these vaccines have been tested in thousands of individuals. These early clinical studies have established that while safe, the potency of this technology as a stand alone platform for induction of protective immunity is less effective than desired. We demonstrate in nonhuman primate models that through the use of improved molecular adjuvants, improved insert design and physical delivery, significant improvement in the potency of the DNA platform can be achieved. We have reported that the immune responses induced by DNA vaccines can be enhanced in by co-delivering molecular adjuvant encoding plasmids as part of the vaccine formulations expressing specific immune modulators such as defined cytokine genes and immune trafficking molecules. Creative design of molecular adjuvants allows fine tailoring of the vaccine induced immune response in a manner previously not achievable by a systemic vaccine platform. Specifically, this strategy allows the control of migratory immune populations adjuvanting both the magnitude as well as phenotype of the immune response. Combined with improved physical delivery this platform appears highly attractive for further testing in the HIV arena. Some of these new vaccine prototypes have recently entered clinical examination. The implications of these studies for HIV vaccine development will be discussed.

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