

ABSTRACT BOOK

11th SNIP CONFERENCE

CLEARWATER BEACH, FLORIDA April 6-10, 2005

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Plenary Lectures

P1:

STRESS AND NEUROENDOCRINE CORRELATES OF DRUG DEPENDENCE: NEUROIMMUNE CONSEQUENCES. Mary Jeanne Kreek; Rockefeller Univ.; New York, NY. (Abstract not available)

PAINFUL AND IMMUNOSUPPRESSIVE EFFECTS OF CROSS-TALK BY CHEMOKINE RECEPTORS. N. Zhang¹, T. Rogers², M. Caterina³ and J. J. Oppenheim^{1*}

¹Laboratory of Molecular Immunoregulation, NCI, NIH, Frederick, MD, ²Temple Univ. School of Medicine, Philadelphia, PA and ³John Hopkins Univ., Baltimore, MD.

We have observed that opioids heterologously desensitize chemokine receptors based on phosphorylation by calcium independent protein kinase (PKC). Conversely, chemokines induce desensitization and phosphorylation of opioid G protein coupled receptor (GiPCR) even more effectively by involving all the PKC's. This results in the inhibition of opioid induced 1) chemotaxis, 2) calcium flux, 3) enhanced cyclic antimicrobial peptides (cAMP) production and 4) central nervous system dependent analgesia. Pain perception was also enhanced by "sensitization" of the vanilloid (TRPV1) receptor by chemokines. This was dependent on coexpression of the TRPV1 and opioid receptors with chemokine receptors on neurons in the dorsal root ganglion of rats, and was also PKC mediated. Adenosine, which is a potent immunosuppressive metabolite, interacts with the A2a GiPCR, and desensitizes chemokine receptors, inhibiting in vitro chemotaxis and in vivo inflammatory cell recruitment. Thus, cross-talk between chemokine receptors and proximal receptors for neuropeptides, metabolites or noxious stimulants can markedly influence pathophysiological responses.

Symposium Session I

(Chairs: Jean Bidlack & Herman Friedman)

S1:

PAIN CONTROL BY NEUROIMMUNE INTERACTIONS. C. Stein; Dep. Anesthesiology, Charité – Campus Benjamin Franklin, Freie Universität Berlin, Germany

Opioid receptors are present and upregulated on peripheral sensory nerves, and opioid peptides are expressed in immune cells within peripheral inflamed tissue (*Nat Med.* 9:1003-8, 2003). Environmental stimuli (stress) and releasing agents (corticotropin releasing factor, cytokines) can liberate these peptides to elicit local analgesia, while suppression of the immune system abolishes these effects (*J Clin Invest.* 100:142-8, 1997). These findings have led to the concept that opioid peptides can be secreted from immunocytes, occupy opioid receptors on sensory nerves and produce analgesia by inhibiting the excitability of these nerves and/or the release of proinflammatory neuropeptides. Recent investigations have examined G-protein coupling in sensory neurons innervating injured tissue (*Mol Pharmacol.* 64:202-10, 2003; *J Pharmacol Exp Ther.* 308:712-8, 2004), subcellular pathways of opioid peptide processing and release in immune cells (*Endocrinology.* 145:1331-41, 2004) and adhesion molecules, chemokines and growth factors governing the migration of opioid containing immune cells to injured tissue (*Nat Med.* 4:1425-8, 1998; *J Neurosci.* 22:5588-96, 2002; *Anesthesiology.* 100:149-57, 2004; *Pain.*

108:67-75, 2004). Clinical studies have now shown that small doses of opioids (e.g. morphine) applied into the inflamed knee joint can not only produce long lasting pain relief but also decrease synovial inflammation (*Nat Med.* 9:1003-8, 2003; *Pain.* 83:525-32, 1999).

ROLE OF $\alpha 7$ -NICOTINIC ACETYLCHOLINE RECEPTORS IN T CELL FUNCTION.

S. Razani-Boroujerdi, T. Boyd, J. S. Nandi, M. I. Davila-Garcia, R. Langley, N. Mishra, J. C. Philippides and M. L. Sopori

Chronic exposure to cigarette smoke/nicotine impairs the immune and inflammatory responses, and causes T cell anergy. The immunosuppressive effects of nicotine are attenuated by the nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine; however the mechanism by which nicotine modulates T cell function is not clear. Neuronal nAChRs are ligand-gated ion channels that primarily promote Ca^{2+} influx into the cells and facilitate neurotransmission. However, nAChRs are also present on non-excitable cells, but their function is largely unknown. By several criteria, T cells express $\alpha 7$ -nAChRs that are essentially identical to neuronal $\alpha 7$ -nAChRs; however, these receptors do not form ligand gated ion channels and function entirely through second messenger systems. In T cells, nicotine modulates two distinct signaling pathways: 1. Nicotine increases $[Ca^{2+}]_i$ through the Src-like protein tyrosine kinase, Lck that requires the functional integrity of the T cell receptor (TCR). 2. Nicotine affects NF-kB activation through the MAP kinase pathway that is independent of TCR. Thus, T cells express $\alpha 7$ -nAChRs that are not ligand-gated Ca^{2+} channels. Moreover, nicotine-induced modulation of the T cell antigen receptor and MAP-kinase signaling pathways in T cells may affect the role of T cells in adaptive and innate immune responses, respectively.

CANNABINOID RECEPTORS IN THE BRAIN: A FUNCTIONAL RELEVANCE IN AN “IMMUNE PRIVILEGED” SITE?

G. A. Cabral* and F. Marciano-Cabral; Dept. Microbiology and Immunology, Virginia Commonwealth Univ., Richmond, VA 23298-0678.

Cannabinoids, including the major psychoactive component of marijuana Delta-9-tetrahydrocannabinol, exert immunomodulatory effects. Major targets of cannabinoids in the immune system are cells of macrophage lineage, including microglia that populate the brain. Microglia co-express CB1 and CB2 cannabinoid receptors in vitro. However, the CB2 is expressed differentially in relation to cell activation state, at levels exceeding those for the CB1, and maximally when cells are responsive and primed. These observations suggest a window of cell activation state-related activities for which these receptors are relevant functionally. Indeed, cannabinoid inhibition of inducible nitric oxide production by fully activated cells was linked to the CB1 while that of responsiveness to chemokines was linked to the CB2. Finally, inhibition of proinflammatory cytokines was linked neither to the CB1 or CB2 suggesting involvement of a third receptor. These observations are significant since recognition of similar events as operative in vivo should provide insight regarding risks associated with recreational or medicinal use of marijuana and yield fundamental data relevant to the selective targeting of cannabinoid receptors for ablating untoward inflammatory responses associated with various neuropathies.

Support: NIH awards DA015608, DA09789, and DA04381

NICOTINE, BRAIN PLASTICITY AND THE HPA AXIS.

B.M. Sharp; Dept. of Pharmacology, Univ. Tennessee, Memphis, TN 38163.

Nicotine, the principal psychotropic agent in cigarette smoke, has pleiotropic effects on the brain, mediated by nicotinic cholinergic receptors (nAChR). Its chronic effects have been

characterized using different approaches to drug administration. We have developed a rat model that permits chronic, 23 h/d access to self-administered nicotine. As expected, high affinity nAChRs were upregulated in multiple brain regions after chronic exposure. Effects on both highly abundant $\alpha 4$ subunit-containing and low abundance $\alpha 6$ -containing nAChRs were observed. Chronic exposure to nicotine also induced molecular and functional changes in the HPA axis. For example, the stress-induced secretion of ACTH and corticosterone were amplified. This was associated with alterations in the molecular phenotype of neuropeptides contained within the parvocellular region of the hypothalamic paraventricular nucleus (PVN), which regulates ACTH secretion. PVN vasopressin transcripts increased, whereas CRH diminished. In addition, the stress-induced secretion of PVN norepinephrine, which stimulates the release of CRH and vasopressin, was reduced. Thus, chronically self-administered nicotine induced changes in nAChR expression and in neuropeptide phenotype and function within the PVN and HPA axis.

Symposium Session II

(Chairs: Thomas Klein & Norbert Kaminski)

S2:

MORPHINE IMPAIRS HOST INNATE IMMUNE RESPONSE AND INCREASES SUSCEPTIBILITY TO *S. pneumoniae* LUNG INFECTION. Wang J, Barke RA, Charboneau R, Roy S. Dept of Pharmacology. Univ. of Minnesota. Minneapolis MN 55455

Chronic morphine use impairs host innate immune response and increases susceptibility to bacteria and virus. In this study, a novel mouse model of chronic morphine treatment followed by intranasal inoculation with *Streptococcus pneumoniae*, was used to investigate microbial events and host innate immune response. Chronic morphine treatment markedly delayed neutrophil recruitment, increased bacterial burden in the lung, spleen and blood with a subsequent increase in mortality. In morphine treated animals, prior to neutrophil recruitment, a significant decrease in TNF- α , IL-1, IL-6, and the chemokine MIP-2 was observed both in bronchoalveolar lavage (BAL) fluids and in lung tissue. Decrease in both Galectin-3 in the alveolar space and NF-kB levels of lung resident cells were also observed following morphine treatment. These results suggest that chronic morphine treatment in a *S. pneumoniae* infection model suppresses NF-kB induced transcriptional regulation of MIP-2 which together with Galectin 3 results in reduced migration of neutrophils thereby increasing susceptibility to *S. pneumoniae* infection. (Supported DA 12104, DA015349 and DA 11806)

MORPHINE'S EFFECTS ON INFLAMMATION AND METABOLITES IN THE BRAIN DURING ENDOTOXIN TOLERANCE. S. L. Chang*; Department of Biology, Seton Hall University, South Orange, NJ 07079

Using both short- and long-course LPS pretreatment regimens to induce endotoxin tolerance in rats, we observed that induction of TNF-alpha, IL-1beta and IL-6 still occurs in the brain in response to a subsequent challenge with LPS even when they are no longer induced in the periphery. We also measured *ex vivo* brain metabolites using ¹HMRS and found increased lactate levels in all measured brain regions, and decreased choline in the hypothalamus after repeated LPS treatment. We then studied the effects of morphine on the expression of an array of growth factors and cytokines in the hypothalamus of animals treated with either a short- or

long-course treatment regimen with LPS. Morphine treatment decreased the mRNA levels of these growth factors and cytokines in the hypothalamus of animals treated with the short-course LPS regimen, but increased the mRNA levels of these substances in the hypothalamus of animals treated with the long-course LPS regimen. Taken together, our studies suggest that, during endotoxin tolerance, an inflammatory response and an alteration in metabolites can occur in an animal's brain, and that exposure to morphine can have a biphasic effect on that inflammatory response [NIH DA 007058 & DA016149 (SLC)].

THERAPEUTIC IMMUNOMODULATORY ROLE OF MORPHINE. Pravin C. Singhal, Amit K. Dinda, Madhu Bhaskaran, and Nicholas Franki; Institute for Medical Research, North Shore Long Island Jewish Health System, New York

Background: Morphine has been reported to have a bimodal effect on cell growth. We studied whether; low-dose morphine (LDM) can modulate the high-dose morphine (HDM)-induced toxicity. **Methods:** We evaluated the dose response effect of morphine on murine macrophage cell growth and apoptosis in *in vitro* studies. We also studied the effect of morphine on macrophage TNF- α production, iNOS expression and P38MAPK phosphorylation. In *in vivo* studies, mice, in groups, received either normal saline (control); HDM, LDM, or LDM followed HDM (morphine priming, MP) twice daily for 10 consecutive days. **Results:** Low-dose morphine (LDM) promoted macrophage proliferation, whereas, HDM triggered macrophage apoptosis. Morphine priming (pre-incubation with low-dose morphine, MP) inhibited HDM-induced iNOS expression, P38 MAPK phosphorylation, TNF- α suppression and apoptosis. MP also attenuated the effects of HDM on macrophage capabilities of killing and containment of phagocytosed bacteria; interestingly, L-NAME (an inhibitor nitric oxide synthase) as well as SB202190 (selective inhibitor of P38 MAPK phosphorylation) blocked the rescuing effects of MP. All HDM mice showed a significant a peritoneal bacterial leak; none of the control and only two of 12 primed mice showed peritoneal bacterial leak and that was only minimal. HDM mice showed decreased macrophage migration into the peritoneal cavity; however, morphine priming inhibited this effect. *In vitro* studies, macrophages/bone marrow cells harvested from HDM mice not only showed enhanced apoptosis but also showed decreased migration. **Conclusions:** Morphine priming provides protection against HDM-induced degradation of the host defense barrier through preservation of macrophage function.

EFFECTS OF WITHDRAWAL FROM OPIOIDS ON IMMUNE RESPONSES AND INFECTION. T.K. Eisenstein*¹, P. Feng,^{1,2} J.J. Meissler, Jr.^{1,2}, and M.W. Adler^{1,2}. Center for Substance Abuse Research¹, Departs. of Microbiology and Immunology², and Pharmacology³, Temple University School of Medicine, Philadelphia, PA 19140.

We have investigated the effect of withdrawal from morphine on murine splenic immune responses and found that both abrupt and precipitated withdrawal result in profound immunosuppression, which is maximal at 24 to 48 hours after initiation of abstinence. We have asked the question as to whether the immunosuppression leads to enhanced sensitivity to microbial products and to infection. Our results show that mice in withdrawal are markedly sensitized to the lethal effects of Gram negative bacterial lipopolysaccharide, which correlates with an increase in tumor necrosis factor levels and nitric oxide in the blood. Inhibition of TNF- α is protective. Animals in withdrawal also have heightened susceptibility to oral and intraperitoneal infection with *Salmonella typhimurium*. Assessment of levels of proinflammatory cytokines reveals general upregulation upon challenge with LPS or infection,

with a selective exception, which may be of importance. These results raise the possibility of previously unexplored deleterious effects of withdrawal from opioids on resistance to infection and sepsis.

Supported by NIDA grants DA14223 and DA13429

Symposium Session III

(Chairs: Olimpia Meucci & Kamel Khalili)

S3:

DRUGS OF ABUSE AND NEUROPATHOGENESIS OF HIV INFECTION.: ROLE OF DC-SIGN AND IDO. Madhavan Nair*, Div. of Allergy, Immunology and Rheumatology, University at Buffalo, Buffalo, NY 14203.

Dendritic cells (DC) are the critical mediators of various immune responses and are the first line of defense against HIV infection. The recently discovered DC specific, CD4 independent HIV attachment receptor, DC-SIGN, and T cell suppressing factor, indolamine 2,3-dioxygenase, IDO are known to play a critical role in the immuno-neuropathogenesis of HIV infection. Our studies show that cocaine induces IDO and DC-SIGN expression by DC and increases the expression of DC-SIGN positive cells in HIV infected subjects. Further cocaine upregulates DC-SIGN and MMPs in brain microvascular endothelial cells supporting the hypothesis that cocaine causes membrane permeability facilitating endothelial transmigration of infected DC in to the CNS. Moreover, the combination of cocaine and specific HIV proteins work in synergy to enhance these neuropathogenic effects. The cocaine induced DC dysfunctions are mediated via suppression of p38 and JNK MAP kinases with a reciprocal upregulation of ERK MAP kinase. These studies suggest that DC-SIGN and IDO may serve as specific molecular markers facilitating neuropathogenesis of HIV infection.

ALCOHOL ABUSE AS CO-FACTOR IN HIV-1 BRAIN INFECTION: POTENTIAL MECHANISMS. Y. Persidsky*, UNMC, Omaha, NE 68198.

A number of neurodegenerative diseases (including HIV-1 encephalitis, HIVE) are associated with oxidative and inflammatory brain injury, and excessive alcohol use could be a co-factor in their progression. Given multiple confounding factors in human host, such processes can be dissected in relevant *in vitro* and *in vivo* models. Using *in vitro* blood brain barrier (BBB) model, we demonstrated that ethanol (EtOH or its metabolite, acetaldehyde) decreased BBB tightness (transendothelial resistance) that was reversed by EtOH or acetaldehyde withdrawal. EtOH activated myosin light chain (MLC) kinase (MLCK) leading to phosphorylation of MLC and tight junction proteins, and enhanced monocyte migration across BBB. MLCK inhibition, anti-oxidant treatment or suppression of EtOH metabolism in brain endothelium prevented these changes. Thus, EtOH may cause TJ dysfunction promoting leukocyte infiltration. Role of alcohol abuse in HIV-1 brain infection was tested in a small animal model for HIVE that reproduces acquired anti-viral immune responses (Poluektova et al., 2002). HIVE mice, chronically exposed to EtOH, demonstrated increased levels of viremia (HIV-1 p24), ineffective elimination of HIV-1 infected macrophages by cytotoxic lymphocytes in the brain, enhanced microglial reaction and prominent BBB damage. Our data proved that alcohol could be an exacerbating factor in HIV-1 CNS infection. Supported by: NIH R21AA013846.

CYTOKINES - A BIOLOGICAL INTERFACE BETWEEN COCAINE AND HIV. M.D. Roth*; Pulmonary and Critical Care Medicine, David Geffen Sch Med/UCLA, Los Angeles, CA 90095. (Abstract not available)

ARE CHEMOKINES THE THIRD MAJOR SYSTEM IN THE BRAIN? M. W. Adler*; Center for Substance Abuse Research, Temple Univ. Sch. of Medicine, Phila, PA 19140

Chemokines and their receptors are located throughout the brain in glia and neurons. Just as the neurotransmitters and neuropeptides in the brain are accepted as playing the major role in the functioning of the brain in maintaining homeostasis and reacting to perturbations of that homeostasis, we propose that the endogenous chemokine system in the brain, consisting of ligands and receptors, is a third major system of the brain. It is the third leg in the stool that supports brain function.

Among the potential therapeutic implications are an increase efficacy of opioids in inflammatory pain by blocking specific chemokine receptors; a decrease of certain unwanted side effects of opioids by chemokine desensitization of selected opioid pathways after determining neuronal localization of chemokine receptors; diminishing inflammatory conditions by using opioids to desensitize chemokine receptors; diminishing tolerance and potential for addiction by selective desensitization of certain reward pathways; altering HIV infectivity by altering chemokines involved in neuroAIDS.

The above hypothesis was developed and is being tested by collaborations with T. Rogers, L. Liu-Chen, T. K. Eisenstein, E. B. Geller, X. Chen, and K. Benamar. Supported by grants DA06650, DA13429, DA14230 and DA16544.

Symposium Session IV

(Chairs: Eugene Major & Phillip Peterson)

S4:

REGULATION OF VIRAL AND CELLULAR GENES IN HIV-1 INFECTION OF HUMAN MULTIPOTENTIAL CNS-DERIVED PROGENITOR CELLS DIRECTED TO VARIOUS LINEAGE PATHWAYS. Eugene O. Major, Diane Lawrence, Pankaj Seth* and Lynnae Schwartz. Laboratory of Molecular Medicine and Neuroscience, NINDS, NIH, Bethesda, MD, USA. *National Brain Research Center, Campus Manesar 122 050, Haryana, India.

HIV-1 is able to infect human fetal brain derived progenitor cells at low levels. However if infected cells are directed toward a glial phenotype, HIV-1 synthesis is greatly augmented while nearly completely turned off if directed toward a neuronal phenotype. The beta chemokine, monocyte chemoattractant protein 1 (MCP-1), shows similar increase in synthesis in response toward conditions that direct progenitor cell differentiation toward an astroglial phenotype. The underlying mechanisms that account for regulation of HIV-1 and MCP-1 appear to be, at least partially, at transcription of viral and cellular genes.

BUGS, DRUGS AND NEURAL PRECURSOR CELLS. P.K. Peterson* Department of Medicine, University of Minnesota, Minneapolis, MN 55455

Neural precursor cells (NPCs) are self-renewing, multipotent progenitors that give rise to neurons, astrocytes and oligodendrocytes. Because of their important role in brain patterning and memory formation, NPC cultures have been increasingly used as a model to study brain development, neurodegeneration and the effects of infectious and psychoactive agents on these processes. Using highly enriched human fetal brain-derived NPC cultures, we have shown that cytomegalovirus infection and stimulation with HIV-1 gp120 inhibit NPC proliferative activity. Moreover, gp120 inhibits the migratory activity of NPC towards SDF-1 due to an interaction of gp120 with CXCR4 on NPCs. Cocaine also inhibits NPC proliferation and migration toward SDF-1. Although cocaine by itself does not induce apoptosis, this drug enhances the apoptotic property of gp120. Studies of opioids have shown that NPCs constitutively express kappa-opioid receptor (KOR) mRNA and that ligands for KOR induce a NPC migratory response. KOR ligands also have been found to inhibit production of the chemokines by IL-1-stimulated NPCs. The results of our studies support the notion that NPC cultures can be a useful tool for studies of mechanisms whereby infectious and psychotropic agents impair brain development and repair.

THE CHEMOKINE CCL2 PLAYS A DUAL ROLE: A CHEMOTACTIC MEDIATOR OF NEUROINFLAMMATION AND A NEWLY DESCRIBED NEUROPROTECTIVE PROTEIN. HOW DO THE DIFFERENTIAL EFFECTS OF CCL2 CONTRIBUTE TO THE PATHOGENESIS OF NEUROAIDS? Joan W. Berman, Albert Einstein College of Medicine, Department of Pathology, Bronx, NY, USA

AIDS associated dementia is often characterized by chronic inflammation and increased chemokine expression, with infected macrophage infiltration resulting in the production of HIV products within the CNS, including tat and neurotoxins, which contribute to neuronal loss. In addition to its established role in leukocyte recruitment and activation, we identified a new role for the chemokines, CCL2 (MCP-1) in the CNS. We demonstrated that CCL2 protects neurons and glial cells from tat or NMDA-induced apoptosis. As has been previously reported by our laboratory and others, CCL2 is a potent chemotactic factor for uninfected leukocyte entry into the CNS. Using a tissue culture model of the human blood brain barrier (BBB), we have now demonstrated that HIV-1-infected leukocytes have an enhanced response to CCL2, resulting in significantly greater levels of transmigration across the BBB as compared to uninfected leukocytes. This enhanced transmigration was associated with BBB disruption that may be mediated by a reduction in tight junction protein (TJP) expression and induction of MMP-2 and MMP-9 in the endothelial cells and astrocytes that comprise the BBB, and by enhanced expression of the CCL2 receptor, CCR2, and TJP and connexins on the surface of HIV-infected leukocytes. All these changes were detected only when HIV-infected, as opposed to uninfected, leukocytes transmigrated in response to CCL2 and not to other chemokines including MIP-1 α or MIP-1 β .

CCL2-mediated leukocyte infiltration into the CNS during NeuroAIDS contributes to the loss of cognitive function characteristic of this disease. However, we have demonstrated that CCL2 also plays a neuroprotective role during tat and NMDA-induced neural and glial apoptosis. We showed that tat mediated apoptosis in mixed cultures of human neurons and astrocytes is a process that is LDL receptor related protein (LRP), nNOS and NMDA dependent. We demonstrated the formation of a new complex of proteins on the surface of NMDA receptor positive neurons that amplifies the process of tat-induced apoptosis. Tat treatment of enriched human neuronal cultures resulted in the formation of a neuronal complex comprised of tat, LRP, PSD-95, and NMDA receptors. PyK2 and nNOS were also recruited to this complex, suggesting

that tat uses the neuronal synaptic machinery to initiate apoptosis. We also demonstrated that CCL2 significantly reduced tat internalization and the formation of this tat-LRP-PSD-95-NMDA receptor complex, and inhibited apoptosis. We propose that the temporal and kinetic expression of CCL2 determines whether this chemokine functions predominately as a mediator of inflammation or as a neuroprotective protein. The balance between these two functions may play an important role in mediating the initial as well as ongoing response of the CNS to injury.

ASTROGLIA: A CENTRAL ROLE IN OPIOID-INDUCED CNS MATURATION AND HIV PATHOGENESIS. K.F. Hauser*, N. El-Hage, S. Buch, A. Nath, A.J. Bruce-Keller, and P.E. Knapp; Dept. Anat. & Neurobiol., Univ. Kentucky; Dept. Neurology, Johns Hopkins Univ.

The diversity of opioid peptides and receptors on neurons and glia strategically positions the opioid system to coordinate maturational and immune signals among diverse cell types and physiological systems. This is revealed by chronic opiate abuse, which disrupts normal maturation and alters the susceptibility of the CNS to disease. Mu opioid-receptor (MOR) expressing astroglia and glial precursors are directly affected by opioids during maturation. MOR activation inhibits astroglial precursor division without affecting viability; however, in the presence of HIV-1 Tat, opiates cause the death of glial precursors. By contrast, the response of mature astrocytes differs from precursors, and although opiates and HIV in combination are lethal to a small percentage of astrocytes, most of the effects are functional. Together, opiates and HIV synergistically disrupt astroglial function, while markedly increasing chemokine production. Chronic disruptions in astroglial function combined with losses in glial precursors may contribute to the enhanced CNS pathogenesis seen in HIV infected individuals who abuse opiates. Acknowledgements: NIH DA13559 and DA13728.

Symposium Session V

(Chairs: Avindra Nath & Howard Gendelman)

S5:

HIV-1, IMMUNE-MEDIATED SYNAPTIC ACTIVITY: PLAYING OUT OF BOUNDS.

H.A. Gelbard*, Center for Aging and Developmental Biology, Univ. Rochester Med. Cntr., Rochester, NY 14642

Compelling neuropathologic evidence suggests that neuronal cell death does not correlate with neurologic disease in HIV-1. Rather, evidence of dendritic degeneration, in the form of varicosities in brain tissue with the neuropathologic hallmarks of HIVE, suggests that synaptic dysfunction may underlie neurologic disease. We present evidence that this type of synaptic dysfunction, known as dendritic beading, occurs in animal models of neuroAIDS, and can be reproduced experimentally by immune mediators such as platelet activating factor (PAF), produced by antigenically activated brain-resident mononuclear phagocytes, with a concomitant failure of synaptic transmission. This is accompanied by caspase activation and mitochondrial dysfunction. Furthermore, dendritic beading is a reversible phenomenon that may be indicative of bioenergetic compromise that ultimately results in synaptic apoptosis with irreversible loss of synaptic transmission. These findings suggest discrete signaling pathways between the neural and immune systems that mediate this type of dysfunction and may be amenable to pharmacologic intervention (Supported by NIMH PO1 MH64570, MH56838, MH71176, NINDS PO1 NS31492).

MONOCYTE/MACROPHAGE TRAFFICKING IN HIV DEMENTIA COMPLEX. T.

Fischer-Smith¹, S. Coul², K. Rybicka¹, O. Haxhistasa¹, A. Adeniyi¹, R. Bonwetsch², S. Morgello³, K. Khalili¹, and J. Rappaport^{1*}. ¹Center for Neurovirology and Cancer Biology, Temple University, Philadelphia, PA. ²Drexel University College of Medicine, Philadelphia, PA. ³Mount Sinai School of Medicine, New York, NY.

Our studies investigated the mononuclear phagocyte (MP) population(s) in CNS tissues from patients with HIV encephalitis (HIVE) using markers distinguishing macrophages from microglia. Results demonstrate the perivascular macrophage as the major productive reservoir for HIV infection in the CNS. CNS invasion rather than proliferation appears to be the principal mechanism of MP accumulation in HIVE. Organ invasion of MPs in HIVE appears to be a generalized phenomenon; MP invasion outside the CNS may contribute to the long-lived reservoir of HIV infection. Furthermore, analysis of kidney specimens from patients with HIVE revealed features associated with HIV associated nephropathy. These immunohistochemical studies, together with results from viral-genetic analysis of HIV-1 V3 sequences from CNS, lymph node and bone marrow MPs, support the importance of MP activation and trafficking in the pathogenesis of HIVE. We propose that monocyte/macrophage activation, invasion and accumulation are influenced by virus induced cytokine dysregulation. The potential role of macrophage colony stimulatory factor (M-CSF) will be discussed.

NEUROPROTECTIVE AND ANTI-HIV ACTIVITY OF MINOCYCLINE. M.C. Zink*, P.M. Tarwater, J.E. Clements, S.A. Barber; Dept. Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, 21205.

The prevalence of HIV CNS disease has not decreased despite highly active antiretroviral therapy. Current antiretroviral therapeutics are expensive, require complex dosing regimens have significant side effects, including neurotoxicity, and few cross the blood-brain barrier. We examined the ability of minocycline, an antibiotic with potent anti-inflammatory and neuroprotective properties to protect against encephalitis and neurodegeneration using a rigorous SIV/macaque model of HIV CNS disease. Macaques were treated with 4 mg/kg/day of minocycline beginning during early asymptomatic infection and continuing until late infection. Minocycline-treated macaques had less severe encephalitis, reduced CNS expression of neuroinflammatory markers, less axonal degeneration and lower CNS virus replication than infected, untreated macaques. Further, minocycline suppressed replication of both HIV and SIV in cultured primary lymphocytes and macrophages via p38-dependent and p38-independent MAPK pathways, respectively. Minocycline is a safe, inexpensive, readily available antibiotic that should be investigated as an anti-HIV therapeutic.

MOLECULAR, FUNCTIONAL AND STRUCTURAL EFFECTS OF CHRONIC SIV INFECTION IN THE CNS. Howard Fox^{1*}, Michael Taffe¹, Steven Henriksen¹, Tricia Burdo¹,

Peter Gaskill¹, Cecilia Marcondes¹, Manisha Yadav¹, Ron Mervis², and Eleanor Roberts¹

¹Department of Neuropharmacology, Scripps Research Institute, La Jolla, CA 92037;

²NeuroStructural Research Lab, Tampa, FL 33612

The cognitive and motor disorders occurring in HIV-infected individuals result in significant morbidity and mortality, and remain a serious problem even in the era of highly active antiviral therapy. Although numerous potential neurotoxic molecules have been identified, the nature of the CNS disorder has remained elusive. Using SIV-infected rhesus monkeys, we have carried out

analyses of CNS parameters during different stages of the disease. Distinct as well as overlapping sets of genes are differentially regulated in acute infection, chronic infection without neurological symptoms, and during AIDS with CNS disease (neuroAIDS). A distinct pattern of altered gene expression is found in chronic infection corresponding to behavioral and neurostructural changes, indicating protective as well as pathological responses to the chronic viral-host interaction. As treatment is fortunately extending the lives of those with HIV, the chronic effects of infection can have profound effects on cellular processes and function in the brain. Supported by grants from the NIMH and NINDS.

Discussion Panel

(Chairs: Jag Khalsa & Robert Donahoe)

D1:

SUBSTANCE ABUSE AND HIV INFECTION: PROGRESSION AND RESISTANCE.

David Vlahov, Ph.D., New York Academy of Medicine.

The role of illicit drugs on the rate of HIV progression has been suggested by in vitro studies, which has stimulated investigation in observational cohort studies. Internationally, clinical epidemiologists have conducted multiple analyses to examine the effect of injection and non-injection drug use on HIV progression. Analyses have been directed at the following outcome measures: CD4 cell decline, T-cell homeostasis, serum immune activation markers, HIV viral load, and progression to AIDS, and neurologic manifestations during asymptomatic HIV infection. Before the introduction of highly active antiretroviral therapy (HAART), epidemiological evidence suggested a modest role of injection drug use on immunological progression of HIV infection; however the evidence is mixed since the introduction of HAART. Injection drug use has been associated with reduced access to antiretroviral therapy; therefore comparisons between drug users and other risk groups showed less effect overall. More recent studies with access addressed have shown highly active antiretroviral therapy is associated with dramatically improved survival in this population. However considerable challenges remain for adherence, resistance and relapse to high risk behaviors associated with initiation of potent antiretroviral therapy.

THE POSSIBLE EFFECT OF DRUG USE ON HIV DISEASE PROGRESSION: RESULTS FROM THE AMSTERDAM COHORT STUDY AMONG DRUG USERS.

Maria Prins, Municipal Health Service, Amsterdam, The Netherlands.

Several laboratory studies have suggested an unfavorable effect of opiate use on HIV disease progression. Our research group has evaluated the effect of drug use in the long-standing Amsterdam Cohort Study among drug users. In our first epidemiological study on this topic - *published in 1991*-, which we cross-sectional in design, we found that high frequencies of injecting were associated with lower T-cell reactivity in both HIV negative and HIV positive drug users. Subsequently, in a longitudinal design, HIV-infected injection drug users who reported a high frequency of borrowing used injection equipment had a less rapid progression to AIDS, compared with those who reported a low frequency. In a European study among drug users with a documented date of HIV seroconversion, we did not find evidence for an effect of drug use on disease progression. Among HIV-infected drug users from Amsterdam and Baltimore, we found that higher frequencies of borrowing resulted in a higher CD4 count already

present before seroconversion. In a recently published study, injection drug users with the highest pre-seroconversion drug-injecting frequencies showed slower CD4 T cell decline after HIV seroconversion than those who injected less. Use of mainly heroin in the HIV seroconversion interval resulted in a sharper decline until the first 6 months after seroconversion, but CD4+ values converged later on. An overview of all Amsterdam studies on the effect of drug use will be presented and the findings will be synthesized.

PROGNOSIS OF INJECTING DRUG USERS AFTER THE INTRODUCTION OF HAART AND SUBSTANCE ABUSE TREATMENTS. Roberto Muga*.Dept. Internal Medicine. Hospital Universitari Germans Trias i Pujol, Badalona, Spain.

In individuals with HIV/Aids a history of IDU remains a strong risk factor for worse clinical outcomes years after the introduction of HAART. Despite survival of IDUs from European Union has clearly improved there is an excess of deaths from external causes, liver disease and cancer among other non-Aids related conditions. With reduced mortality from AIDS, other chronic diseases that are common to HIV+ patients become relevant. HIV and hepatitis C virus co-infection is extremely frequent among IDUs. Co-infection has great public health consequences because it may lead to a large burden of end-stage liver disease (ESLD) among (ex-) IDUs. A faster progression to cirrhosis occurs in patients with hepatitis C that are co-infected with HIV. While co-infection does not appear to affect HIV viral suppression among those in HAART it may impair CD4+ T-cell recovery.

Late presenters IDUs with an AIDS defining condition in the era of HAART include candida, HIV encephalopathy, recurrent pneumonia, toxoplasmosis and TB. HAART use in IDUs has increased over time. However, delays in initiating HAART despite being indicated, discontinuations, active IDU or relapse into drug abuse, side effects of medications and psychosocial factors are involved in sub-optimal viral suppression and lack T-cell recovery. The Department of Internal Medicine at University Hospital Germans Trias i Pujol in Barcelona, provides general and subspecialty care for HIV/Aids. Analysis of clinical outcomes in IDUs admitted to detoxification or hospitalized and/or visited in the HIV/Aids unit since 1996 are crucial for describing trends over time.

Poster Session 1

Young Investigator Session

PW-1:

MODELING MONOCYTE MIGRATION AND REVERSE TRANS-ENDOTHELIAL IN THE BRAIN DURING HIV. T. H. Burdo* and H. S. Fox, Dept. of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, 92037.

Monocytes have been shown to be the principal targets for productive HIV-1 replication within the central nervous system. In addition, HIV-1 dementia has been shown to correlate with macrophage abundance in the brain. We have developed a novel transendothelial migration model that will allow us to test the ability of chemokines to alter monocyte trafficking. The migration and accumulation of peripheral blood mononuclear cells in the abluminal (i.e. tissue) side of an *in vitro* endothelial monolayer was investigated. The migrated cells were highly enriched for monocytes. We also investigated the reverse transmigration of monocytes from the abluminal to luminal side (i.e. circulation). The majority of infected macrophages in the brain are perivascular, thus the brain can act as a reservoir to seed the periphery. In the HIV-infected brain, molecules can be expressed that increase this reverse transmigration of these macrophages, leading to increased seeding of the periphery. Based on our microarray analyses that showed osteopontin, a potent chemoattractant protein, was upregulated in the brains of simian immunodeficiency virus encephalitis cases, we have compared the role of osteopontin in comparison to a classic fractalkine, in our transendothelial migration model. Although osteopontin does not act as a classic chemokine, we have shown that osteopontin increased the reverse transmigration of macrophages. Osteopontin may play an important role in the reseeding of the periphery during HIV-1 infection.

PW-2:

HIV-TAT AND OPIATE-INDUCED CHANGES IN ASTROCYTES PROMOTE CHEMOTAXIS OF MICROGLIA THROUGH THE RELEASE OF MCP-1 AND ALTERNATIVE CHEMOKINES. N. El-Hage, G. Wu, P. E. Knapp, A. J. Bruce-Keller, A. Nath and K.F. Hauser; University of Kentucky.

Opiate-induced increases in astroglial MCP-1 and RANTES in neuroAIDS were assessed. N9 microglial cell migration was significantly greater in conditioned medium (CM) from astrocytes exposed to morphine and/or Tat1-72 than in vehicle, opioid antagonist-, or mutant Tat- treated controls. Morphine and Tat in combination was markedly greater than either substance alone. CM from Tat and/or morphine-treated astroglia derived from CCL2(-/-) mice, or immunoneutralized with anti-MCP-1 MAbs, significantly N9 cell migration. Intrastriatal Tat injections caused increased numbers of glial fibrillary acidic protein immunoreactive astroglia and F4/80+ macrophages at 7 days post injection when combined with s.c. time-release morphine implants. Changes were not evident in mice injected with Tat alone, or in mice receiving morphine in combination with inactive (mutant) Tat or naltrexone. The findings indicate the importance of MCP-1 in mediating the response of microglia to Tat, and suggest that opiates exacerbate HIV by increasing astroglial-derived MCP-1 at focal sites of CNS infection.

PW-3:

MICROARRAY ANALYSIS OF HIV INFECTION IN HUMAN MONOCYTE DERIVED MACROPHAGE. N. Erdmann*, Y. Huang, S. Herek, W. Zhuang, J. Zheng; CNND and Dept. of Pharmacology Univ. NE Med. Ctr., Omaha, NE 68198-5880.

HIV-1 infects macrophage (MP) in the periphery as well as seeding the brain with infected MP that maintain a low level, productive infection in the CNS. In this study we aim to characterize the expression profile of infected MP in a productive infectious state using affymetrix microarray technology. Monocytes were collected via elutriation from healthy human donors and cultured with M-CSF for 7 days. Cultures were then infected with HIV-1 ADA virus and RNA was collected five days post-infection. Microarray analysis was conducted on HG U133A chips to evaluate ~13,000 genes from three unique, human donors in both control and infected conditions. Results were confirmed for selected genes of interest with Real-Time PCR, ELISA for secreted factors and western blot to evaluate protein levels. A pool of ~150 genes was identified with < .01 p value; sorting based upon fold change of >1-fold resulted in the identification of over 300 genes. Array data indicated increases in apoptotic control mRNAs such as XAF1 and TRAIL; cell cycle regulation alterations including CALM1 and CCND3; immune activation and upregulation of interferon response elements, as well as regulation of multiple transcription factors.

PW-4:

HIV-1 V3 SEQUENCES FROM CD16+ AND HIV-1 P24+ CELLS IN CNS AND VISCERAL TISSUES SUGGESTS MONONUCLEAR PHAGOCYTE TRAFFICKING IN HIVE. Tracy Fischer-Smith^{1*}, Sidney Croul^{1,2}, Katarzyna Rybicka¹, Robert Bonwetsch², Susan Morgello³, Kamel Khalili¹, and Jay Rappaport¹. ¹Center for Neurovirology and Cancer Biology (www.temple.edu/CNVCB), Temple University, Philadelphia, PA; ²Drexel University College of Medicine, Philadelphia, PA; ³Mount Sinai School of Medicine, New York, NY

In the studies presented here, we demonstrate two subsets of HIV-1 infected mononuclear phagocytes (MP) in the central nervous system (CNS) of patients with HIV encephalopathy (HIVE). In HIVE, accumulating perivascular macrophages are CD14+/CD16+/CD45+, invariably HIV-1 p24+ and appear to comprise multi-nucleated giant cells and nodular lesions. We also observed CD14-/CD16+/CD45-/HIV-1 p24+ ramified microglia in white matter. CD16 immunohistochemical analysis of MPs in visceral tissues from the same patients with HIVE also demonstrated an increase in CD16+ MPs, suggestive of systemic alterations in monocyte/macrophage trafficking. We then compared HIV-1 V3 sequences within CD16+ and HIV-1 p24+ cell populations. Using laser capture microdissection (LCM), CD16+ and HIV-1 p24+ cells were selected from formalin fixed paraffin embedded CNS, lymph node and bone marrow tissues from patients with HIVE. HIV-1 V3 sequences were obtained by PCR amplification of DNA extracted from the selected cells and compared by ClustalW alignment. Our results demonstrate a shared distribution of viral variants infecting MPs in the CNS, as well as macrophages outside of the CNS compartment. These data support the importance of trafficking of HIV-1 infected MPs into the CNS from the periphery in the pathogenesis of HIVE.

PW-5:

EFFICACY OF SIV INFECTION IN MACROPHAGES VARIES WITH SOURCE CELLS. PJ Gaskill, T Burdo, M Buchmeier and H Fox; Scripps Institute, La Jolla.

HIV is believed to enter the brains of HIV infected individuals by way of infected macrophages. Recent evidence that viral particles incorporate cell surface molecules and bud from different parts of the cell suggests that viruses generated from different cell types display different cellular proteins and thus interact differently with their target cells, through different attachment and entry mechanisms as well through variation in potential cellular pathogenicity. To examine how viral generation in various cell types affects the ability of virus to effectively infect macrophages and other target cells we grew different strains of SIV in both T-cells and macrophages and then cross-infected each of those cell types as well as coreceptor-dependent tissue culture target cell lines with homologous and heterologous virus. Our initial results indicate that viruses derived from macrophages are more efficient in establishing infection. Furthermore, distinct strain differences were found in the ability of macrophage-tropic viruses to induce cytopathic effects in CCR5-expressing target cells. We hypothesize that both viral and cell-specific host factors, the latter in the form of incorporated host proteins, affect viral infection and pathogenicity. Ongoing investigation of these host proteins may yield new therapeutic targets and provide a greater understanding of the unique processes of viral entry involved in NeuroAIDS.

PW-6:

COPOLYMER-1 REDUCES BRAIN INFLAMMATION IN MURINE HIV-1 ENCEPHALITIS. S.Gorantla*, L.Poluektova, L.Walters, H.Dou, H.E.Gendelman; Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE 68198.

HIV-1 infected immune competent mononuclear phagocytes (MP; macrophages and microglia) drive a paracrine inflammatory neurotoxic cascade leading to increased leukocyte migration and neuronal impairment in advanced viral infection. Cop-1 can deactivate microglia and protect neurons during neurodegenerative processes. We have now investigated whether Cop-1 could affect neuroinflammation and neuronal loss in a HIV-1 encephalitis (HIVE) model. Immunodeficient mice were reconstituted with human lymphocytes then immunized with Cop-1 loaded human dendritic cells. HIVE was induced by intracranial injection of HIV infected human macrophages. At day 7 following HIVE induction, brain tissue was collected and analyzed for lymphocyte infiltration/ inflammation, and cytokine secretion. Splenocytes were analyzed by ELISPOT to detect the immune responses to Cop-1. Cop-1 reduced lymphocyte infiltration, and tumor necrosis factor-alpha and inducible Nitric Oxide Synthase expression. Expression of the anti-inflammatory cytokine, interleukin-10 was increased. These data suggest that Cop-1 may be neuroprotective in murine HIVE. Further studies are underway to study the mechanisms of the Cop-1 anti-inflammatory and "putative" neuroprotective effects.

PW-7:

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

Certain physiological functions of opioids may be regulated through the activation of an opioid-like receptor (ORL1). Activation of ORL1 leads to the chemotaxis of certain immune

cells, cross-talk and desensitization of the mu-opioid receptor, and down-regulation of tyrosine hydroxylase, an enzyme activity associated with morphine use. The aim of the present work was to study the effects of N/OFQ administration on the pro-inflammatory chemokine response of cells of the immune system. Our results show that N/OFQ can suppress the expression of MCP-1 and RANTES protein in both unstimulated and LPS-activated monocytes. These effects were reversed in the presence of the ORL1 antagonist UFP-101, implicating the involvement of ORL1. Moreover, MCP-1 and RANTES mRNA expression is reduced in response to N/OFQ administration in monocyte-like cell lines. These data are in contrast to previous results involving mu-opioid receptor agonists, which induced the up-regulation of MCP-1 and RANTES. Our results suggest an anti-inflammatory role for the N/OFQ-ORL1 system, and may provide a natural opposing influence on the pro-inflammatory properties of certain other GPCR agonists. Supported by NIH grants DA-14230, DA-13249, DA-06650, and DA-16544.

PW-8:

MORPHINE WITHDRAWAL CONTRIBUTES TO T HELPER CELL DIFFERENTIATION BY BIAING CELLS TOWARD THE Th2 LINEAGE. J Kelschenbach and S Roy, Univ of Minnesota

The consequences that drug withdrawal has on immune functioning has only recently been appreciated, however given the wide variety of opiate analgesic use understanding the decrements to immune function that withdrawal from these drugs cause is of crucial importance. In the current studies, it was hypothesized that morphine withdrawal results in Th2 differentiation and subsequent immune dysfunction. To address this, mice were chronically treated with morphine followed by a 24 hour withdrawal period. Morphine withdrawal resulted in a decrease in IFN-gamma, the Th1 signature cytokine, whereas the Th2 cytokine, IL-4 was increased. Western blot and EMSA experiments revealed that morphine withdrawal induced Th2 differentiation was mediated through the classical Th2 transcription factors, Stat-6 and GATA-3. Additionally, the consequence of morphine withdrawal in the presence of an in vivo LPS stimulation prior to withdrawal was examined. It was found that the Th1 polarizing cytokine, IL-12, was significantly decreased, providing further support for the observation that withdrawal results in Th2 differentiation by possibly impacting the generation of an appropriate innate immune response which directs subsequent adaptive Th1/Th2 responses. Supported by T32DA07097, DA12104.

PW-9:

KAPPA – OPIOID INDUCED IL-7 RECEPTOR mRNA EXPRESSION IN R1.1 THYMOMA CELL LINE. M. Khimich*, J. Bidlack; Dept. of Pharmacology and Physiology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

It is known that opioid agonists can modulate the level of expression of cytokines and their receptors in immune cells and the overall effect of such modulation is almost exclusively immunosuppressive. In particular, the kappa-selective agonist U50,488 has been shown to decrease the level of expression of IL-7R on primary murine thymocytes. In the current study using RT-PCR technique we showed that U50,488 administration induces IL-7R alpha chain transcription in the R1.1 thymoma cell line, which is derived from very immature double negative mouse thymocytes. The effect is completely blocked by the kappa-selective antagonist nor-BNI. IL-7 is known to be the major survival factor in thymocyte development. Taking this into consideration, the data suggest that the effect of opioid administration may be

immunostimulatory and it may depend on the stage of T cell differentiation in thymus.
(Supported by grants K05-DA00360 and DA04355 from the National Institute on Drug Abuse).

PW-10:

MODULATION OF TUMOR GROWTH BY MORPHINE. L. Koodie*, S. Ramakrishnan, S. Roy. Dept of Pharmacology, U. of Minnesota. Minneapolis. MN55455

Angiogenesis is a critical factor for tumor growth and survival. We recently showed that chronic morphine treatment significantly inhibited tumor cell induced angiogenesis. To test if morphine treatment will also result in decreased tumor growth, nude mice were injected with Lewis lung carcinoma cells followed by either morphine or placebo treatment and tumor growth monitored for 21 days. Chronic morphine treatment significantly decreased tumor growth ($p < 0.007$) and tumor angiogenesis ($p < 0.05$) when compared to placebo animals. Since macrophage infiltration play an important role in tumor progression, we determined if morphine induced decrease in angiogenesis was a function of decreased macrophage infiltration. VEGF supplemented matrigel was implanted in WT and MORKO mice. Morphine suppressed VEGF induced angiogenesis ($p < 0.05$) and macrophage infiltration ($p < 0.05$). VEGF, a crucial factor in angiogenesis is regulated by HIF transcription factors. In vitro preliminary data suggest that morphine treatment is associated with decreased a) HRE binding b) HIF nuclear translocation and c) VEGF mRNA transcription in endothelial cells. Our results suggest that morphine suppresses immune cell infiltration and VEGF signaling and may be a therapeutically useful anti-angiogenic agent. (Supported by DA -12104)

PW-11:

CHOLINERGIC AGENTS ACT CENTRALLY TO MODULATE PERIPHERAL IMMUNITY THROUGH THE A.N.S. R. J. Langley*, J.S. Nandi and M.L. Soporì.

Immunology, LRRI, Albuquerque, NM. 87108.

The CNS plays a critical role in the neuroimmune communication, and cholinergic agents modulate the peripheral immune responses. However, the mechanism by which cholinergic agents regulate the immune system is not clear. To understand how cholinergic agents modulate the immune response, rats were exposed chronically to BBB-permeable (sarin, physostigmine) or BBB-impermeable (pyridostigmine, edrophonium) cholinergic agents. It was observed that peripheral administration (inhalation or subcutaneous treatment) of only sarin and physostigmine caused significant inhibition of the mitogen- and antigen-induced T cell proliferation and of anti-SRBC antibody production. However, ICV administration of both BBB-permeable and –impermeable cholinergics inhibited the immune responses. Moreover, pretreatment of rats with the ganglionic blocker chlorisondamine, abrogated the immunosuppressive effects of the cholinergic compounds irrespective of the exposure route. Our preliminary results suggest that nicotinic acetylcholine receptors might play a significant role in their immunosuppressive properties. This is the first demonstration that cholinergic compounds act centrally to suppress the immune system via the A.N.S.

PW-12:

DELTA-9-TETRAHYDROCANNABINOL (THC) SUPPRESSES IL-12P40 PRODUCTION IN DENDRITIC CELLS AND SUPPRESSES ANTIGEN SPECIFIC T CELL ACTIVATION. Lily T. Lu*, C. Newton, I. Perkins, H. Friedman, T. W. Klein. USF-HSC, Tampa, FL 33612.

Previously we showed that THC suppressed IL-12p40 production in murine bone marrow derived dendritic cells (DCs) infected with *Legionella pneumophila* (Lp) and that cannabinoid receptors were involved in this suppression. To further explore the role of receptors, DCs were pretreated with the Gi inhibitor, pertussis toxin, infected with Lp, and treated with THC. Pertussis toxin pretreatment attenuated the THC-induced suppression in a dose-dependent manner suggesting a role of a Gi-linked receptor. Regarding molecular mechanisms, the highly selective inhibitor (SB203580) of p38 MAP kinase, significantly reduced Lp-induced IL-12p40, indicating a requirement for p38 in IL-12p40 production. THC treatment enhanced phosphorylated p38 level within 10 min followed by a suppressive effect after 3 hrs. Together, these data suggested that THC suppresses IL-12p40 production in DCs through Gi signaling and p38 MAP kinase modulation. Regarding immune activation, THC treatment of Lp-infected DCs followed by co-culture with Lp-primed splenic CD3+ T cells was observed to suppress IFN-gamma production by the T cells. This suggested that THC impairs the ability of DCs to activate antigen specific T cells possibly through suppression of IL-12 by the DCs. More studies are needed to fully understand the mechanisms and consequences of THC-induced suppression of IL-12p40 production in dendritic cells. Supported by DA03646, DA10683 and AI45169.

PW-13:

MORPHINE REGULATION OF LPS AND HYPOXIA-INDUCED CHEMOKINE EXPRESSION IN MACROPHAGE. J.L. Martin, J. Wang, S. Roy; U Minnesota.

Recent reports that hypoxia produces pronounced inflammatory responses in mouse lung macrophages and that macrophage inflammatory protein 2 (MIP-2) is a useful model in investigating inflammation have led us to question the possible role morphine plays in regulating MIP-2 activity in the presence of lipopolysaccharide (LPS) or hypoxia using the mouse macrophage cell line RAW-264. Preliminary data from our laboratory shows a significant reduction of the inflammatory cytokine nuclear factor kappa B (NF-kB) in the presence of morphine and that morphine plays a modulatory role in macrophage expression of MIP-2 under a hypoxic state. With use of the MIP-2 inflammatory model, future directions exploring the mechanisms surrounding hypoxia, LPS, and morphine regulation in macrophages are underway. Experiments using such a model will enable us the address fundamental questions regarding chemokine and kinase pathway crosstalk, as well as, dissect the signals involved in sequestering an innate macrophage immune response. Data of these experiments are expected to be presented at the conference.

PW-14:

GAMMA DELTA/CD4 GAMMA DELTA /CD8 DUAL POSITIVE LYMPHOCYTES ARE SUSCEPTIBLE TO MORPHINE MODULATION. M.R. Olin*, T.W. Molitor. College of Veterinary Medicine. University of Minnesota, St. Paul MN.

Gamma delta can be divided into three subgroups, gamma delta TCR only or gamma delta expressed with either a CD4⁺ or CD8⁺. Previous studies reported that gamma delta lymphocytes

response to BCG vaccination is modulated by morphine administration. To extend our earlier examination on the effects of opiates on gamma delta T cells, we focused on gamma delta dual positive subpopulations. Pigs were placed in non-vaccinated, morphine-BCG vaccinated, or BCG vaccinated groups. Morphine was administered 10 d prior to BCG vaccination. Immune cells from various pig groups were isolated and analyzed for change in circulation populations, antigen directed IFN- γ production, and proliferation. It was observed that both gamma delta/CD8⁺ and gamma delta /CD4⁺ lymphocyte subpopulations increased in peripheral circulation and proliferated following BCG vaccination. Morphine administration suppressed these responses. Moreover, gamma delta/CD8⁺ subpopulation produced IFN-gamma following antigen stimulation. This cytokine production was also suppressed to morphine administration. In conclusion, morphine was able to modulate gamma delta dual positive subpopulation.

PW-15:

THE EFFECT OF OPIOID LIGANDS ON LPS-STIMULATED INTERLEUKIN-6 PRODUCTION IN A MOUSE MACROPHAGE-LIKE CELL LINE. A. L. Parkhill* and J. M. Bidlack; Dept. of Pharmacology and Physiology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

Lipopolysaccharide (LPS) treatment leads to the eventual transcription of proinflammatory cytokine genes, such as interleukin-6 (IL-6). Previous work showed that opioids modulated this immune response both *in vivo* and *in vitro*. In this study, P388D1 cells, a mouse macrophage-like cell line, were stimulated with LPS in the presence or absence of various opioid ligands. Both the kappa-selective agonist, U50,488, and the delta-selective agonist, SNC 80, significantly reduced LPS-stimulated IL-6 production as measured by ELISA. The U50,488 effect was mediated by the kappa opioid receptor, because nor-BNI, a kappa antagonist, blocked this inhibition. This reduction of IL-6 protein by U50,488 treatment may be due to decreases in IL-6 mRNA. RT-PCR experiments demonstrated that U50,488 treatment significantly reduced the LPS-mediated increase in IL-6 mRNA and that this effect was blocked by nor-BNI. The reduction of antigen-elicited proinflammatory cytokine production by opioids may contribute to the immunosuppressive effects of these compounds *in vivo*. (Supported by NIDA grants K05-DA00360 and DA04355).

PW-16:

COCAINE PHOSPHORYLATES EXTRACELLULAR SIGNAL RELATED KINASE (ERK) IN U373MG CELLS. J.L. Reynolds*, and M.P. Nair; Dept. Medicine. SUNY @ Buffalo, Buffalo, NY 14203.

Drugs of abuse act as co-factors in human immunodeficiency virus (HIV) infection and in the progression to HIV associated neuronal dysfunction. Recent evidence indicates that cocaine activates mitogen-activated protein kinases (MAPKs) to induce cellular changes. However, the effect of cocaine on MAPKs in astrocytes has yet to be determined. We hypothesize that cocaine alters signal transduction pathways in astrocytes leading to neuronal dysfunction in the infected subject. Treatment of U373MG cells with cocaine (10 nM) increased phosphorylation of extracellular signal related kinase (ERK) in a time dependent manner (maximum response 15 min) as determined by western blot analyses and FACS analyses. Furthermore, cocaine-induced phosphorylation of ERK occurred in a dose dependent manner (1 pM-1 μ M, 15 min) with a maximum response occurring at 1 μ M. However, no change in total ERK levels occurred.

Relative ERK mRNA expression increased in a time dependent manner following exposure to cocaine (10 nM), the maximum being at 4 hr. This study suggests that cocaine-induced modulation of neuroimmune pathogenesis of HIV infection may be mediated via dysregulation of MAPK. These studies may help to develop novel therapeutic strategies in drug using HIV infected subjects.

PW-17:

NEUROLOGICAL FUNCTION IN A PRIMATE MODEL OF DRUG ABUSE & NEURO-AIDS. Riazi, M^{1,2*}, Marcario, JK¹, Al-Hafez, B³, Samson, F¹, Bilgen, M^{1,3}, Brooks, WM^{1,3}, & Cheney, PD¹. Molecular & Integrative Physiology¹, Hearing & Speech², Hoglund Brain Imaging Ctr³, U. Kansas Med Ctr, Kansas City, KS 66160, USA.

To characterize the functional consequences of HIV-1 infection and opiate (morphine) dependence in a SIVmac (R71/E17) model of neuro-AIDS, the electrophysiological function of multiple brain systems was tested by recording cortical and spinal cord motor evoked potentials (EPs), auditory brainstem responses, somatosensory EPs, and visual EPs in 17 male, rhesus macaques. EP latencies were highly reproducible for all modalities across five control sessions. Two EP sessions were performed during the morphine dependency period - preliminary results do not show significant EP latency shifts. We are currently investigating the effects of chronic morphine exposure on post-inoculation EP latencies. Viral load, CD4+/CD8+ cell counts and white blood cell counts are obtained bi-weekly from blood samples. Viral RNA has been recovered from the blood plasma and CSF samples of all inoculated subjects (n=11). The effects of opiate dependence and combined opiate dependence and lentivirus infection on neurological integrity are ongoing. (Sponsored by NDA12827 and HD02528)

PW-18:

***Mycobacterium tuberculosis*-INDUCED CYTOKINE AND CHEMOKINE EXPRESSION BY HUMAN MICROGLIA AND ASTROCYTES: EFFECTS OF DEXAMETHASONE.** RB Rock*, S Hu, G Gekker, WS Sheng, B May, V Kapur, PK Peterson; Neuroimmunobiology Laboratory, Minneapolis Med. Res. Foundation, and Univ. of MN, Minneapolis, MN.

CNS tuberculosis (TB) accounts for 1-10% of all TB cases and carries a high mortality. To ameliorate morbidity and mortality, corticosteroids are often used as adjunctive therapy. However, the neuropathogenesis of *M. tuberculosis* (MTb) is incompletely understood. In this study, human microglia and astrocytes were infected with the MTb strain H37Rv and the ensuing cytokine/chemokine expression was examined with and without dexamethasone therapy. Uptake of H37Rv by microglia at 24 h occurred in 76% of microglia (4.2 per cell), versus 15% of astrocytes (1.3 per cell). Microglia challenged with MTb yielded robust amounts of CCL2, CCL5, CXCL8, CXCL10, TNF, IL-6 and IL-1beta by ELISA and RPA. Astrocytes yielded moderate amounts of CXCL10. Dexamethasone inhibited TNF, IL-6, CXCL8, CXCL10, and CCL2 expression from H37Rv-infected microglia. This study supports the concept that microglia play an important role in neuropathogenesis of CNS TB and suggests that the benefit of dexamethasone therapy could operate via modulation of cytokine/chemokine production by MTb-infected microglia.

PW-19:

MORPHINE-INDUCED DEGRADATION OF THE HOST DEFENSE BARRIER (DHDB): ROLE OF INTESTINAL MUCOSAL INJURY. Shilpa Sharma, Meera Sharma, Lia Frakanh, Madhu Bhaskaran, and Pravin C. Singhal, PGI, Chandigarh and LIJMC, New Hyde Park, NY

The effect of morphine on intestinal ulcer formation and on the DHDB was studied. Mice receiving morphine (MRM) showed mucosal ulcer formation in the ileum and in the upper 1/3 of the colon. In *in vitro* studies, morphine enhanced apoptosis of cultured human colonic cells (HCC). Nitric oxide synthase (NOS) inhibitors attenuated the proapoptotic effect of morphine. Moreover, morphine stimulated NO generation by HCCs. MRM also showed DHDB as well as injury to peritoneal macrophages. Although NOS inhibitors completely prevented morphine-induced intestinal ulcer formation it provided only a partial protection against the DHDB and peritoneal macrophage injury. Propranolol, an inhibitor of hemeoxygenase (HO) activity, did not inhibit the induction of intestinal ulcer formation in MRM; nevertheless, propranolol prevented DHDB as well as macrophage injury in MRM; whereas, hemin, an inducer of HO-1, exacerbated macrophage injury as well as the DHDB of MRM. These findings suggest that morphine-induced intestinal injury is mediated through the generation of nitric oxide. However, the DHDB correlates with macrophage injury but not with intestinal injury.

PW-20:

DELTA OPIOID RECEPTOR mRNA EXPRESSED IN BV-2, A MOUSE MICROGLIAL CELL LINE. S. Sumagin*, J.M. Bidlack; Dept. Pharmacology and Physiology, Univ. of Rochester, Rochester, NY 14642.

Microglia have a number of very important roles in neuro-inflammation. Studies show that microglial functions can be regulated by both kappa and mu opioid agonists, as well as morphine. While none of these data have been reported with delta agonists in microglia, similar results have been found using delta agonists in macrophages. Because there are, as of yet, no well-characterized human microglial cell lines, the BV-2 mouse microglial cell line has been used. Our hypothesis was that opioid receptors were present on the mouse microglial cell line BV-2. We used RT-PCR to detect mRNA levels for the three opioid receptors: mu, delta, and kappa. We stimulated BV-2 cells with LPS, interferon-gamma (IFN-g), and both LPS and IFN-g. Delta opioid receptor (DOR) mRNA was expressed in BV-2 cells in untreated cells and under all treatment conditions when cells were treated for 3, 6, 12, and 24 hr, but that 24-hr treatment with LPS decreased the expression of DOR mRNA. Neither mu nor kappa opioid receptor mRNA was detected under any conditions. The BV-2 mouse microglial cell line can be used as a model to study the effects of DOR interactions with microglia. (Supported by the grants K05-DA00360, DA04355, and T32 DA07232 from NIDA.)

PW-21:

THE EXPRESSION OF THE PRODYNORPHIN (PDYN) GENE IS DOWN-REGULATED BY ACTIVATION WITH LIPOPOLYSACCHARIDE (LPS) IN U937 CELLS. B. Sun* and J.M. Bidlack; Dept. Pharmacology and Physiology, Univ. Rochester, School of Medicine, Rochester, NY 14642.

Dynorphins are endogenous kappa opioid peptides widely distributed in the central nervous system. A number of recent reports have established their roles in modulating immunological functions. In this study, we investigated the expression of PDYN gene, which encodes the precursor of dynorphins, in a human macrophage cell line, U937 cells. We initially observed that PDYN mRNA was expressed at a detectable level, measured with a standard RT-PCR method in U937 cells, but not in Jurkat and Raji cells, which are human T and B lymphocytes, respectively. Further analyses with RT-PCR assays by using primers covering each exon of the PDYN gene showed that U937 cells expressed the adult brain type PDYN mRNA. Most interestingly, activation of U937 cells with LPS led to a decrease in PDYN mRNA levels. Results from transient transfection and promoter analysis suggest that this decrease is due to an inhibition of PDYN gene promoter activity. The precise pharmacological and molecular mechanisms responsible for this response, as well as the functions of dynorphin in the immune cells, are under investigation. (Supported by: NIH grants K05-DA00360 and DA04355.)

PW-22:

CONDITIONING OF HEROIN'S EFFECTS ON NITRIC OXIDE: ACQUISITION AND EXTINCTION. Jennifer L. Szczytkowski*, Donald T. Lysle; Dept. of Psychology, Univ. North Carolina, Chapel Hill, NC 27599

Research has shown that heroin induced alterations of inducible nitric oxide synthase (iNOS) can be conditioned to environmental stimuli associated with drug administration. iNOS is the enzyme responsible for the production of nitric oxide which is known to be involved in host defense. The present study was conducted to determine whether the conditioned effects of heroin follow fundamental principles of learning by testing whether the effects are susceptible to extinction. Extinction is the loss of responding that occurs after repeated exposure to the conditioning chambers without further administration of drug. Rats were given 5 sessions during which a 1 mg/kg subcutaneous injection of heroin was given before placement in the chambers. Conditioning was followed by ten days of extinction. Animals were re-exposed to the chamber and given subcutaneous injections of LPS to induce iNOS expression. Six hours after injection spleen, lung, and liver tissue were removed for analysis of iNOS mRNA by RT-PCR. Analyses indicate that extinction reversed the effect of heroin associated environmental stimuli on iNOS expression. These data are the first to demonstrate that the conditioning of heroin's immunomodulatory effects follows principles of learning. (Supported by DA13371)

PW-23:

MACROPHAGE ACTIVATION LEADS TO AN UPREGULATION OF KAPPA OPIOID RECEPTOR GENE EXPRESSION. C. Tipton* and J.M. Bidlack; Dept. of Pharmacology and Physiology, Univ. of Rochester, Rochester, NY 14642.

Several studies show the importance of the kappa opioid receptor (KOR) on macrophages in regulating cytokine release, phagocytosis, and HIV-1 expression. In the current study, the human macrophage-like cell line, U-937, was activated with lipopolysaccharide (LPS) and interferon-gamma (IFN γ), and KOR expression was measured via quantitative real time RT-PCR at time points from 2 hr to 36 hr after activation. KOR gene expression levels were found to increase up to ten fold upon macrophage activation, but only at time points past 12 hr. The resulting time course suggests an indirect mechanism of upregulation, possibly caused by autocrine/paracrine effects of inflammatory cytokine release. Past studies indicate that KOR

activation leads to a suppression of inflammatory cytokine production. Therefore, regulation of KOR expression by inflammatory cytokine release could serve as a negative feedback mechanism for inflammatory processes. This study will work to characterize the mechanisms underlying regulation of KOR expression during macrophage activation and the KOR-induced suppression of inflammatory cytokine production. (Supported by: NIH grants: K05-DA00360 and DA04355)

PW-24:

THE EFFECTS OF METHAMPHETAMINE AND FIV ON ASTROCYTES. K. Tran*, M Hadjiconstantinou, Dept. of Psychiatry and Pharmacology, Ohio State University, Columbus, OH 43210

There is a growing population of HIV-1 infected drug abusers and epidemiological evidence suggests that the psychostimulant drug methamphetamine (METH) is a risk factor for HIV Dementia (HIVD) however the mechanism(s) by which this occurs is not well defined. The feline immunodeficiency virus (FIV) is a lentivirus that causes a natural immunodeficiency syndrome including active encephalitis and neurological symptoms resembling HIVD. Astrocytes are immunocompetent resident cells of the brain and may participate in the FIV-induced neurotoxicity and/or act as reservoir for FIV infection. Our research demonstrates that METH enhances cell-mediated FIV infection of astrocytes as early as 24 h. Studies have shown that CXCR4 is an important co-receptor for FIV infection of astrocytes. To determine whether AMD3100 (antagonist to CXCR4) can prevent the enhanced cell-mediated FIV infection of astrocytes are still under investigation. To understand how METH facilitates the entry of lentiviruses into astrocytes, it is necessary that we first determine the effects of psychostimulant on astrocytes. These experiments were designed to explore the early effects of METH on astrocyte survival, proliferation and CXCR4 expression. Exposure of feline astrocyte cell line G355-5 to METH (1 microM) the concentration shown to facilitate the cell-mediated entry of FIV into astrocytes increased the cell viability and number of cell surface CXCR4 after 24 h. METH also increased CXCR4 mRNA expression at early time points (6 and 12 h). After the co-administration of METH and FIV we observed an increase in cell death compared to cell-mediated FIV infection of astrocytes alone. The effects of METH and FIV on cell death and similarity of these mechanisms to FIV-induced neurotoxicity are still under investigation. These results indicate that increased presentation of the CXCR4 receptors on the cell surface might contribute to METH-induced mechanism of the susceptibility of astrocytes to FIV infection and consequently cell death. This work is supported in part by NIDA grant RO1 DAO1381504.

PW-25:

MORPHINE WITHDRAWAL ENHANCES HEPATITIS C VIRUS EXPRESSION. Chuan-Qing Wang, Yuan Li, Steven D. Douglas, Xu Wang, and Wen-Zhe Ho; Division of Allergy & Immunology, Joseph Stokes, Jr. Research Institute at The Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

We previously demonstrated that morphine enhances hepatitis C Virus (HCV) replication in human hepatic cells. This study examined the impact of morphine withdrawal (MW), a recurring event during the course of opioid abuse, on HCV replication in human hepatic cells. MW enhanced viral RNA and protein expression in HCV replicon cells. Blocking opioid receptors by

naloxone after morphine cessation (precipitated withdrawal, PW) induced greater HCV RNA expression than MW. Investigation of the mechanism responsible for MW-mediated HCV enhancement showed that both MW and PW inhibited the expression of endogenous interferon alpha (IFN- α) in the hepatic cells. This down-regulation of intracellular IFN- α expression is due to the negative impact of MW or PW on IFN- α promoter activation and on the expression of IFN regulatory factor 7 (IRF-7), a key IFN- α enhancing factor. In addition, both MW and PW undermined the anti-HCV effect of recombinant IFN- α . These data indicate that morphine and MW may promote HCV disease by suppressing IFN- α -mediated intracellular innate immunity in the hepatic cells.

PW-26:

ROLE OF GRANZYME B IN ACTIVATED T CELL INDUCED NEUROTOXICITY. T. Wang*, R. Allie, P. Calabresi, K. Conant, N. Haughey, J. Steiner[#] and A. Nath; Dept. of Neurology, Johns Hopkins Univ., Baltimore, MD; [#]Guilford Pharm., Baltimore, MD.

Background: Multiple sclerosis (MS) involves focal demyelination and axonal loss associated with infiltrating T cells. The role of activated T-cells in causing neuronal injury remains unclear. Methods: T-cells from a healthy donor were activated by anti-CD3 and -CD28 for 3 days. Culture supernatants (sups) were incubated (1:10 dilution) with human fetal or rat neurons. Cell damage was monitored by trypan blue exclusion at 48 hrs; or mitochondrial membrane potential (MMP) by TMRM at 3 hrs. Results: Activated T cell sups-induced neuronal death ($P<0.05$). Immunodepletion with anti-granzyme B(GrB) antisera reduced the toxicity ($P<0.05$). Recombinant GrB(1nM) also caused neurotoxicity ($P<0.05$) which was attenuated by G_i protein inhibitor pertussis toxin and caspase inhibitor Z-VAD-fmk ($P<0.05$). GrB also induced a drop of MMP in neurons. Antioxidants (Vitamin E, selegiline) or neuroimmunophilin ligands(GPI compounds) all attenuated GrB-induced neurotoxicity ($P<0.05$). Conclusion: GrB mediates T-cell induced neurodegeneration via activation of G_i coupled receptors, mitochondrial dysfunction and caspase activation. Antioxidants and GPI compounds may be of therapeutic benefit in MS.

PW-27:

EXPRESSION OF PROINFLAMMATORY CYTOKINES IN THE BRAIN DURING ENDOTOXIN TOLERANCE. H. Zhou*, J. Beltran, R. Chen, L. Malellari, and S. L. Chang; Dept. of Biol., Seton Hall Univ., South Orange, NJ 07079

Exposure to a sublethal dose of an endotoxin decreases the animal's responsiveness to a subsequent challenge with the same substance, which is termed endotoxin tolerance. In this study, we used both short- and long-course pretreatment regimens with lipopolysaccharide (LPS) to induce endotoxin tolerance in adult male Sprague-Dawley rats, and examined the expression of tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 in the brain, and serum corticosterone level in these endotoxin-tolerant rats. In response to a challenge dose of LPS, the animals pretreated with short-course LPS exhibited augmented expression of all three cytokines in the brain, and similar serum corticosterone level compared with animals pretreated with saline. In response to a challenge dose of LPS, the animals pretreated with long-course LPS exhibited similar levels of TNF-alpha, IL-1beta, decreased level of IL-6 in the brain, and lower serum level of corticosterone compared with animals pretreated with saline, suggesting that

repeated activation of the hypothalamus-pituitary (HPA) axis may desensitize the HPA axis. Our data suggest that expression of proinflammatory cytokines in the brain during endotoxin tolerance may be mediated via corticosterone. [NIH DA 007058 & DA016149 (SLC)].

PW-28:

GENOMIC ANALYSIS OF MONONUCLEAR PHAGOCYTE HETEROGENEITY. J.G. Glanzer*, Y. Enose, P. Ciborowski, H.E. Gendelman Center for Neurovirology and Neurodegenerative Disorders and the Departments of Pharmacology and Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE 68198-5880.

Mononuclear phagocyte (MP) heterogeneity is believed affected by tissue-specific environmental cues. Here we tested whether molecular signatures exist within MP subpopulations (microglia, splenic macrophages and monocytes derived from the bone marrow) by isolating pure populations of cells and cultivating them under identical laboratory conditions. The genetic linkages of spatial MP subtypes were investigated by microarray platforms. All MP populations displayed a high degree of similarity, with spleen and bone marrow cells showing the highest correlation. Those genes related to migration, neuroprotection, and immune response were significantly differentially expressed in microglia and included MMP-3, MMP-12, RGS1 and Arginase I. The results suggest that specific sets of molecular fingerprints exist between MP populations. These may contribute to the role of microglia in brain homeostasis or other MP-specific subtype that affect specific functional outcomes in health and disease.

PW-29:

EVALUATION STRATEGIES FOR EDUCATIONAL PROGRAMS ON NERVOUS SYSTEM MEDICATED HEALTH CONSEQUENCES. M.K. Demetrikopoulos*, K. Mu, and A.M. Zardetto-Smith; Institute for Biomedical Philosophy, Atlanta GA, 30340: Univ of Nebraska at Omaha, and Creighton University, Omaha, NE

Teaching students about health consequences of drugs, alcohol & stress provides an avenue to explore organ systems and their functioning. Standards-based physiology can be studied along with the detrimental effects of these compounds and the mechanisms of addiction. To ensure the development of a successful educational program, it is critical to employ an effective evaluation strategy. A panel of formative and summative assessment instruments assess project implementation, content knowledge, process knowledge, performance, and attitudes. Baseline measurements and Beta testing of a customized assessment panel allow for early evaluation and adjustment of ongoing projects. Instruments designed to specifically address attitudes toward science and technology as well as to address biomedical ethics issues are also important tools for evaluating program effectiveness. Exploration of the relationship of changes in content knowledge to attitudes can also yield information helpful in guiding programmatic development. Effective evaluation strategies allow programs to disseminate best practices.

Poster Session 2 General Session

PT-1:

MODELING HIV-1 INFECTION OF BONE MARROW PROGENITOR CELLS. A. Alexaki*, B.P. Irish, M.R. Nonnemacher and B. Wigdahl. Department of Microbiology and Immunology, and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, USA.

Human immunodeficiency virus type 1 (HIV-1) infection of monocytes/macrophages is involved in the pathologic events associated with the acquired immunodeficiency syndrome (AIDS) and HIV-1 dementia (HIVD). Interestingly, CD34⁺/CD38⁻ progenitor cells within the bone marrow are resistant to HIV-1 infection, possibly due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5, which upon differentiation are upregulated, increasing susceptibility to infection. The CD34⁺/CD38⁺ TF-1 cell line was selected as a model to study HIV-1 infection during the differentiation process of hematopoietic progenitor cells. TF-1 cells were treated with a number of metabolic activators including PMA, conditioned media from PMA-treated cells, as well as cytokines such as GM-CSF, M-CSF, IL-1-beta, TNF-alpha, IL-4 and their maturation was monitored through their surface marker expression by flow cytometry. Interestingly, combined treatment with GM-CSF, IL-1beta, and TNF-alpha leads to CXCR4 upregulation and preservation of CD4 expression. Moreover, transient transfection analysis demonstrated that HIV-1 LTR activity was significantly increased following treatments that promoted differentiation.

PT-2:

THC INHIBITS PRODUCTION OF NITRIC OXIDE BY HUMAN MACROPHAGES; INVOLVEMENT OF TLR-2 AND NF-KAPPA-B SIGNALING. G.C. Baldwin, S. Uh, K.M. Whittaker, S.M. Kiertscher, D.P. Tashkin, & M.D. Roth*; Heme-Onc & Pulmonary Medicine, David Geffen School of Medicine/UCLA, Los Angeles, Ca 90095.

Alveolar macrophages (AM) from the lungs of marijuana (MJ) smokers are impaired in their ability to phagocytose and kill bacteria, effects related to their inability to upregulate *i*NOS mRNA or produce nitric oxide (NO). To characterize the mechanisms involved, we set-up an adaptable *in vitro* assay in which human monocyte-derived macrophages (MACS) are stimulated by *S. aureus*. Exposure of these cells to THC leads to a dose-dependent impairment in killing and NO production. The role of TLR signaling was investigated by comparing *S. aureus* (a TLR-2 activator) and LPS (a TLR-4 activator) as NO inducers. MACS only produced NO when exposed to *S. aureus* and pretreatment with anti-TLR-2 blocked NO production. Stimulation with *S. aureus* was also associated with a time-dependent phosphorylation of p38 MAPK and I-kappaB-alpha. These experiments suggest that stimulation of TLR-2, rather than TLR-4, as well as signaling through NF-kappa-B, may be required for stimulating NO production from human MACS and that this antibacterial response pathway is inhibited by THC. Supported by NIDA

PT-3:

COCAINE POTENTIATES HIV-1 TAT NEUROTOXICITY VIA THE DOPAMINE TRANSPORTER. R.M. Booze*, M. Y. Aksenov, M.V. Aksenova, S.B. Harrod, C.F. Mactutus, D R. Wallace; Dept Pharmacology, OSU-CHS, Tulsa, OK and Dept Psychology, Behavioral Neuroscience Program, Univ. of South Carolina Columbia, SC 29208.

Recent imaging studies have indicated that the dopamine transporter (DAT) levels in the basal ganglia are reduced in patients with HIV-induced cognitive and motor disorders (Wang et al., 2004). The neurological consequences of cocaine abuse and HIV are particularly severe. We examined whether cocaine combined with HIV-1 proteins (Tat) exerts synergistic toxicity via DAT. First, using cell culture techniques, we found that the selective DAT inhibitor, GBR-12909, is able to mimic the effects of cocaine and enhance Tat toxicity, implicating an important role for DAT in cocaine toxicity. We next examined the ability of Tat to alter dopamine uptake using rat striatal synaptosomes. We found that Tat blocked the uptake of [³H]-dopamine into synaptosomes. In vivo experiments confirmed the ability of Tat to interfere with the actions of cocaine in the nucleus accumbens. Collectively, our results implicate DAT as a mediator of combined cocaine and Tat toxicity, and loss of DAT may produce cognitive and motor dysfunction. Supported by DA013137, DA014401, HD043680

PT-4:

PHARMACOLOGICAL MODULATION OF IMMUNO-GLOBULIN A (IgA) SECRETION IN INTESTINAL MUCOSA. Y. Xie, M. Lyte[‡] and D.R. Brown*; Dept. Vet. Biomed. Sci., U. Minnesota, St. Paul, MN 55108 and [‡]Dept. Surgery, Mpls., Med. Res. Fndn./Hennepin Cty. Med. Ctr., Minneapolis, MN 55404

Secretory IgA is the major immunoglobulin class present on all mucosal surfaces that provides specific immune defense against microbial pathogens. We tested the hypothesis that IgA secretion in ileal and colonic mucosae is modulated by enteric neurotransmitters. Porcine gut mucosa explants were mounted in Ussing chambers; luminal IgA immunoreactivity was measured before and after contraluminal drug addition. The cholinergic agonist carbachol (10 μ M) rapidly increased luminal IgA secretion by approx. 9- and 2-fold in ileum and colon; its effects were prevented by the muscarinic antagonist atropine. In ileal explants, VIP (0.1 μ M) doubled IgA secretion; agonists at *delta*-opioid and cannabinoid receptors or the axonal conduction blocker saxitoxin had no effect. Norepinephrine (10 μ M) increased ileal and colonic IgA secretion by 4- and 3-fold; its effects were prevented by the *alpha*-adrenoceptor blocker phentolamine. Adrenergic, cholinergic, and VIPergic nerves projecting to the gut lamina propria may act to phasically modulate IgA release. Supported by NIH grants DA-10200 and AI-44918.

PT-5:

CROSS-DESENSITIZATION BETWEEN MU, DELTA OR KAPPA OPIOID RECEPTORS AND THE CHEMOKINE FRACTALKINE RECEPTOR CX3CL1 IN RATS. X.-H. Chen*, E. B. Geller, M.S. Deitz, T. J. Rogers and M. W. Adler; Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140

We have reported that there is cross-desensitization between the mu, delta or kappa opioid receptors and either the chemokine RANTES/CCL5 receptor(s) or SDF-1/CXCL12 receptor in the regulation of antinociception in rats. Fractalkine, a chemokine that exclusively binds CX3CL1 receptors, has been reported to interact with morphine in the spinal cord on

antinociception. The purpose of the present study was to see if cross-desensitization between the chemokine fractalkine/CX3CL1 receptor and mu, delta or kappa opioid receptors occurs in the periaqueductal grey (PAG) of adult male S-D rats. The cold water tail-flick test was used to measure antinociception. The results showed that intra-PAG injection of 100 ng fractalkine/CX3CL1 30 min before administration of 400 ng DAMGO, 100 ng DPDPE or 20 µg dynorphin significantly reduced the antinociception induced by each of these peptides. These results demonstrate that activation of the CX3CL1 receptor causes cross-desensitization of mu, delta and kappa opioid receptors in rats. (Supported by NIDA Grants DA 06650, DA 13429, DA 11130 & DA 14230).

PT-6:

ANGIOTENSIN II INDUCES MACROPHAGE APOPTOSIS: ROLE OF OXIDATIVE STRESS. A.K.Dinda*, R.S.Bhat, K.Reddy, M.Bhaskaran, A. Mongia, N.Franki, I.Hatsukari, and P.C.Singhal, K.Reddy, P.C.Singhal. Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY

Angiotensin II (ANG II) has been shown to have direct biological effects on a variety of cells. In the present study, we evaluated the effects of ANG II on macrophage injury and the involved molecular mechanism. Apoptosis of murine macrophages and human monocytes was evaluated. ANG II promoted apoptosis of macrophages in a dose dependent manner. This effect of ANG II was inhibited by antioxidants and was associated with reactive oxygen species (ROS) production. Anti-TGF-β antibody inhibited the ANG II-induced macrophage ROS/NO generation as well as apoptosis. BAPTA (Ca chelator) inhibited ANG II-induced macrophage superoxide generation as well as apoptosis. On the other hand, thapsigargin (Ca agonist) stimulated macrophage apoptosis under basal as well as ANG II stimulated states. ANG II receptor type I antagonist not only attenuated the proapoptotic effect of ANG II, but also inhibited macrophage superoxide and nitric oxide production. We conclude that ANG II promotes oxidative stress in macrophages contributes to their injury.

PT-7:

COCAINE MEDIATED APOPTOSIS OF HUMAN ENDOTHELIAL CELLS: ROLE OF ANGIOTENSIN AND NITRIC OXIDE. A.K.Dinda*, K.Reddy, P.C.Singhal. Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY

Cocaine is known to modulate vascular functions including spasm of small vessels resulting in ischemic injury. The present study evaluated the interactive role of angiotensin II (ANG II) with nitric oxide (NO) and reactive oxygen species (ROS) in cocaine-mediated cultured human endothelial (hEC) injury. Cocaine caused increased apoptosis of hEC in a dose dependent manner. Pretreatment of cells with angiotensin II receptor type 1 (AT1) blocker reduced apoptotic rate by 40%. The AT2 blockade did not show significant effect. NO donors like S-nitroso-N-acetylpenicillamine (SNAP) and DETA-NONOate were effective in reducing cocaine induced hEC apoptosis. N(omega)-nitro-L-arginine methyl ester (L-NAME) a NO synthase inhibitor enhanced cocaine mediated apoptosis. NADPH inhibitor DPI as well as antioxidant ascorbic acid partially inhibited apoptosis. Cocaine treatment of hEC decreased Bcl2 and increased Bax expression. These findings suggest the possibility of reduced NO production by hEC as well as synergistic interaction between ANGII and ROS in cocaine mediated endothelial injury.

PT-8:

MICE WITHDRAWN FROM MORPHINE ARE SEPTIC. P. Feng*^{1,2}, A. Truant^{2,4}, J.J. Meissler, Jr.^{1,2}, M.W. Adler^{1,3}, and T.K. Eisenstein^{1,2}. ¹Center for Substance Abuse Research, Depts. of ²Microbiol. & Immunol., ³Pharmacol., and ⁴Pathol. Temple Univ. Med. Sch., Phila, PA 19140.

The effect of withdrawal from morphine on the leakage of endogenous bacteria from the intestinal tract was examined. Mice were made opioid dependent by implantation of slow-release morphine pellets. Controls received placebo pellets. Bacterial colonization was assessed 24 h post withdrawal by plating tissue homogenates or peritoneal fluid onto blood agar plates. Bacterial species were identified from pure cultures using a semi-automated microbial identification system (Biomerieux Vitek System) in the Clinical Laboratory at Temple University Hospital. It was found that morphine withdrawn mice had enteric bacteria detected in the Peyer's patches (4/5), mesenteric lymph nodes (4/5), spleens (4/10), livers (6/10) and peritoneal cavities (8/10). In contrast, in placebo withdrawn mice, only one positive culture was observed in Peyer's patches and one in the liver. The most frequently detected organism was *Enterococcus faecium*, followed by *Klebsiella pneumoniae*. Both are part of the normal gastrointestinal flora. The presence of these organisms at sites beyond the intestine strongly indicates an abnormal leakage of microbes through the intestinal mucosal barrier. We conclude that morphine withdrawal alters gastrointestinal permeability and predisposes mice to bacterial sepsis. Supported by NIDA grants DA14223 and DA13429.

PT-9:

VIRUS-SPECIFIC IMMUNE RESPONSE IMBALANCE IN HIV ASSOCIATED LEUKOENCEPHALOPATHIES. *¹P.Ferrante, ¹S. Delbue, ¹M. Saresella, ¹E. Colombo, ¹F.R. Guerini, ¹M.Valli, ¹I. Marventano, ¹R. Mancuso, ²G. Sotgiu, ²R. Maserati.*¹ Don C.Gnocchi Foundation, IRCCS, Milan, ²Infectious Disease Inst Univ. Pavia, Italy.

We are performing a longitudinal survey to investigate AIDS related leukoencephalopathies, including PML and JC virus (JCV) negative leukoencephalopathy (NDLE). HIV+ HAART treated patients are subjected to MRI examination, CSF was studied for the presence of JCV and all the human herpesviruses. HIV and JCV viral loads and MCP-1 were measured in CSF. PBMC production of TNF α , IFN γ , IL-2 and MCP-1, previous and after JCV HLA-restricted peptides stimulation was performed by flow cytometry. 22 MRI+ (8 PML, 12 NDLE, 2 OND), 41 MRI- (30 asymptomatic, 11 OND) were studied with 27 healthy controls. Plasma, but not CSF, HIV load was higher ($p < 0.05$) in PML than in NDLE. MCP-1 and HIV RNA in CSF were positively correlated in the three groups (PML: $r = 0.11$ $p = 0.89$; NDLE: $r = 0.39$ $p = 0.19$; OND: $r = 0.26$ $p = 0.39$). No viruses were found in NDLE CSF, whereas VZV and EBV were found in CSF of 2 OND MRI+. IFN γ was overproduced ($p < 0.05$) in CD4+ cells of NDLE and PML. The JCV specific immune response and increased cytotoxic activity suggests that JCV, with an immune mediated mechanism, is relevant in NDLE pathogenesis.

PT-10:

CB2 RECEPTOR-MEDIATED INHIBITION OF HIV-1 EXPRESSION IN HUMAN MICROGLIAL CELL CULTURES. G. Gekker*, S. Hu, G. Cabral, B. R. Martin and P.K. Peterson; Dept. Medicine, University of Minnesota, Minneapolis, MN 55455; Virginia Commonwealth University, Richmond, VA 23298, USA

CB2 cannabinoid receptors have been identified on macrophages and T lymphocytes. Recently, we have shown that WIN 55,212-2, a ligand for CB1 and CB2 receptors, inhibits HIV-1 expression in human microglial cells, the resident macrophages of the brain, and in activated CD4+ lymphocytes. In this study, we tested the hypothesis that WIN 55,212 suppresses HIV-1 expression in microglia via a CB2 receptor-related mechanism. Using highly enriched human fetal microglial cell cultures, we compared the HIV-1 inhibitory activities of four recently synthesized CB2 receptor agonists (0-2137, 0-1661, 0-1826, 0-3223) to WIN 55,212 and CP 55,940, another CB1/CB2 receptor agonist. At 10^{-6} M, the highest concentration tested, all of the ligands inhibited significantly viral expression (% inhibition vs control [mean \pm SD, n=3-7 experiments]: WIN 55,212 50 ± 4 ; CP 55,940 39 ± 4 ; 0-2137 17 ± 6 ; 0-1661 17 ± 9 ; 0-1826 13 ± 6 ; 0-3223 15 ± 9). The anti-HIV-1 activities of WIN 55,212-2 and CP 55,940 were blocked significantly by pretreatment of microglia with the CB2 receptor antagonist SR 144,528. In more limited studies, similar findings were obtained when CD4+ lymphocytes were used as viral targets. These findings support the hypothesis that suppression of HIV-1 expression in microglial cells is mediated, at least in part, through CB2 receptors.

PT-11:

ROLE OF ASTROCYTE-CD38 EXPRESSION IN HIV-1 DEMENTIA. M. Deshpande, C-H Shao, L. Wu, K. Bidasee and A. Ghorpade* Univ. of NE Med. Center, Omaha, NE.

HIV-1-associated dementia (HAD) is an important complication of HIV-1 infection. Reactive astrogliosis is a key feature of HAD and our data showed that activated astrocytes induce neuronal injury. The investigations of the mechanisms involved in astrocyte-mediated neurotoxicity are ongoing. Using gene arrays we found that astrocyte activation with the HAD-relevant pro-inflammatory cytokine, IL-1beta, led to CD38 upregulation. Real-time PCR further validated these results. Since CD38 acts as an ADP-ribosyl cyclase, CD38 upregulation likely elevates cyclic ADP-ribose. This in turn, would elevate intracellular calcium levels and enhance glutamate release that may cause excitotoxic neuronal injury. IL-1beta treatment of astrocytes significantly reduced the activity of ryanodine receptor calcium-release channels as assessed by radioligand binding assays. Western blot analyses indicated that IL-1-beta treatment decreases sarco(endo)plasmic reticulum calcium ATPase (SERCA2a) expression in astrocytes. We propose that the mechanism by which CD38 upregulation in astrocytes leads to neurotoxicity is through disruption of intracellular calcium cycling. Furthermore, astrocyte CD38 upregulation in astrocytes may have important implications in neuroinflammation and neurodegeneration.

PT-12:

A USER DEFINED MICROPARTICLE BASED MULTIPLEX ASSAY SYSTEM. Scott Howard*, Richard Fuerstenberg, Tina Ba Dour; R&D Systems, Inc. Minneapolis, MN 55413

Microparticle based multiplex assays are being increasingly employed as a low cost, time saving alternative to single analyte ELISAs. These assays allow users to simultaneously quantify multiple analytes in a single small sample. With increasing use, the demand for expanded assay panels becomes greater, sometimes resulting in a lack of availability of specific assays. To help close this gap, we have developed a user defined multiplex assay system for use on the Luminex[®] LX-100[™] analyzer. The system utilizes goat anti-mouse (GAM) antibodies coated onto ten distinct microparticle regions. Analyte specific mouse monoclonal antibodies are bound by the GAM, forming a solid phase that serves as the basis for a microparticle based assay. Using

analyte specific biotinylated detection antibodies, users can construct multiplex assays of their own design. Using commercially available antibodies, we have demonstrated the utility of this system to detect human IL-1beta, IL-6, IP-10, MCP-1, RANTES and TNF-alpha simultaneously. With minimal optimization, we compared the basic performance of the GAM based multiplex to that of optimized, validated assays with very favorable results including measurement of natural proteins in serum, plasma and cell culture media.

PT-13:

VIRAL MARKERS PREDICTIVE OF HIV-1 CNS DISEASE. B. Irish*, M. Nonnemacher, F. Krebs, and B. Wigdahl. Department of Microbiology and Immunology, and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, USA.

Studies have suggested that LTR sequence variation plays an important role in HIV-1 replication and CNS tropism. However until recently, direct examination of LTR sequences with respect to disease progression has failed to reveal any significant association. We have examined sequence variation at C/EBP sites I and II and Sp sites I, II, and III in peripheral blood (PB)-derived LTRs from HIV-1-infected patients with increasing degrees of disease severity. The 3T configuration of C/EBP site I (C to T change at position 3) and 5T configuration of Sp site III (C to T) were the only variants examined that were found in low frequencies in PB-derived LTRs from patients at early stages of HIV-1 disease, but were found at increasing frequencies in patients at later stages of disease. Sequence variation at these sites was also examined in LTRs derived from brain tissues of patients with and without HIVD. The 3T C/EBP site I was found in 25% of brain-derived LTRs from patients diagnosed with HIVD but not found in patients without HIVD. These results suggest that the 3T C/EBP site I, and possibly the 5T Sp site III could be utilized to predict the likelihood of HIV-1-infected individual's developing HIVD.

PT-14:

ETHANOL INDUCES APOPTOSIS IN HUMAN COLON EPITHELIAL CELLS. P.K. Jalal*, J. T. Matthew, P.C. Singhal. Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY.

Alcoholics are prone to infections. In previous studies, ethanol has been reported to promote translocation of bacteria through the gut. However, in these studies, the direct effect of ethanol was not studied. Therefore, we evaluated the direct effect of ethanol on apoptosis of human colonic epithelial cells. Equal number of cultured human colon cells (HCC) were incubated in media containing either buffer or variable concentrations of ethanol (5 to 125 mM) for 24 hours (n=4). In parallel experiments, cells were incubated with ethanol (25 mM) for variable time period (n=3). To determine the role of nitric oxide (NO), cells were pretreated with a NO synthase inhibitor, L-NAME, in the presence or absence of ethanol for 24 h (n=3). At the end of the incubation period, cells were assayed for apoptosis by staining with H33342 and propidium iodide. Ethanol induced HCC apoptosis in a dose- and time-dependant manner. L-NAME partially inhibited the proapoptotic effect of ethanol. The present study provides a basis for hypothesis that ethanol-induced breach of intestinal barrier may be the direct effect of ethanol.

PT-15:

TAT INDUCES TRANSCRIPTION FACTOR ACTIVITY IN ASTROCYTES: INTERACTION WITH OPIATES. Zou S-P, Adams MH, Zhao T-Y, Nath A¹, El-Hage N, Hauser KF, Bruce-Keller AJ and Knapp PE*. Dept. Anat. & Neurobiol., Univ. KY and ¹Dept. Neurol., The Johns Hopkins University.

Opiates can act synergistically with the HIV protein Tat to destabilize astrocyte function. Previous work from our labs has shown that Tat alone drives changes in $[Ca^{2+}]_i$, ROS production, and secretion of inflammatory chemo/cytokines, and that combined exposure to Tat+morphine enhances these responses. To identify signaling pathways involved we used TransSignal™ Protein/DNA Arrays (Panomics) to screen the activity of 153 transcription factors. Murine astrocytes were exposed for 4 or 24 hrs to Tat, morphine, or Tat+morphine. Nuclear extracts were probed with biotinylated DNA-binding oligonucleotides. Transcription factor-DNA complexes were hybridized to arrays with consensus-binding sequences for multiple transcription factors, and detected with chemiluminescence. Most factors were unchanged or depressed by morphine alone at 4 and 24 hrs. Tat alone, or Tat+morphine upregulated a number of families, prominent among which were AP-1/NFAT, forkhead factors (FKHR, FOXO4, Freac), GATA, ATF/CREB, and Rel/NFkB. Many of these modulate pathways involved in inflammation and cell survival/death, with downstream effects through PI3-kinase/Akt or MAPK.

PT-16:

MORPHINE MEDIATED ACCELERATION OF SIV/SHIV-INDUCED AIDS IN RHESUS MACAQUES. R Kumar, C. Torres, Y Yamamura, S Staprans, E Kraiselburd and A Kumar*; Ponce School of Medicine, Ponce, PR 00732, UPR-MSC, San Juan, PR and Emory Vaccine Center, Atlanta, GA.

Six morphine-dependent rhesus macaques along with 3 controls were inoculated with mixture of SHIV and SIV. The Macaques in both morphine and control groups showed precipitous CD4 loss after infection but 1/6 morphine-dependent and all 3 control animals showed significant CD4 recovery. Furthermore the CD4 loss in morphine-dependent animals was generally more pronounced than that in control animals. All three viruses replicated in the systemic compartment of control and morphine macaques, but only SIV crossed the blood brain barrier in control macaques and SIV and SHIVKU in morphine macaques. The peak viral load in both groups was comparable but the same was significantly higher in morphine-dependent macaques after week-6, post infection. Viral load in the CSF of morphine macaques represented two different sub groups; one group of 3 animals with higher viral load in CSF of morphine-dependent animals and another group of 3 animals with low viral loads in CSF as also seen in all 3 control animals. The 3 animals with higher viral load in CSF succumbed to SIV/SHIV-induced AIDS and euthanized at week 18, 19 and 20, respectively with other alive at week 48 after infection.

PT-17:

CHARACTERIZATION OF DOWNSTREAM C/EBP BINDING SITES WITHIN THE CLADE B HIV-1 LTR. Y. Liu*, M.R. Nonnemacher, K. Alexaki and B. Wigdahl. Department of Microbiology and Immunology, and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA, USA.

Previous studies have demonstrated that two functional CCAAT enhancer binding protein

(C/EBP) binding sites upstream of the TATA box are required for efficient viral replication in cells of the monocyte/macrophage lineage; however, no studies have been reported concerning downstream C/EBP binding sites (DS) within the HIV-1 LTR. Analyses of 228 clade B LTR sequences revealed three putative C/EBP binding sites within the downstream LTR. However, electrophoretic mobility shift (EMS) analyses revealed that only one of the three (DS3, +158 – +172) was able to bind C/EBP family members. Interestingly, this downstream site is highly conserved across various clades of HIV-1, suggesting that it may represent a functionally important cis-acting element. Variants within DS3 are very rare, with only four different variants identified, 7A, 3C, 1G4A8A and 2T8A. Compared with the clade B consensus downstream binding site, EMS showed that the 3C variant has a similar relative affinity; 7A has a much weaker relative affinity, while 1G4A8A and 2T8A showed no affinity for C/EBP. Future studies will be aimed at elucidating the functional significance of these sites in cells of myeloid origin.

PT-18:

BEHAVIORAL PERFORMANCE IN A MACAQUE MODEL OF CHRONIC MORPHINE DEPENDENCE & NEURO-AIDS. Marcario JK* & Cheney PD. Molecular & Integrat Physiol, U. Kansas Med Ctr, Kansas City, KS 66160.

To characterize the functional consequences of chronic morphine dependence in a SIV model of neuro-AIDS, 17 rhesus macaques were behaviorally trained to perform 3 different tasks: 1) motor skill (MS), 2) forelimb tremor (FT), and 3) progressive ratio (PR). These animals were then divided into 3 experimental groups: Group A (Morphine Alone); Group B (SIV + Morphine); and Group C (SIV Alone). Animals in Groups A and B were injected with increasing doses of morphine sulfate over 25 weeks until the target dose (2.5 mg/Kg) was achieved. Group C animals were sham injected with saline. After collecting data during the morphine-only dependency period, animals in Groups B and C were infected with neurovirulent strains of SIVmac (R71/E17). Viral RNA has been recovered from the plasma and CSF of all monkeys in Groups B and C. Cortisol levels, CD4+/CD8+ cell counts, and white blood cell counts were also measured. Force accelerometers were used to collect data on general activity and sleep patterns. We have observed morphine-associated declines in the behavioral performance of most (82%) monkeys in groups A and B. Behavioral testing during SIV disease progression is on-going (Sponsors: DA12827 & HD02528)

PT-19:

COCAINE MEDIATES VASCULAR SMOOTH MUSCLE CELL APOPTOSIS VIA ROS AND NITRIC OXIDE. S.K.Maulik*, A. K. Dinda, K. Reddy, P.C. Singhal. Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY & Dept. of Pharmacology, All India Institute of Medical Sciences, Delhi, India.

Cocaine abuse is associated with a wide spectrum of cardiovascular diseases. However the mechanism of cocaine associated vascular injury is not well defined. We studied the effect of cocaine on cultured human vascular smooth muscle cell (hVSMC) injury. Cocaine triggered apoptosis of hVSMC in a dose dependent manner. Pretreatment of cells with Losartan, an angiotensin II (ANG II) receptor type 1 (AT1) blocker, reduced hVSMC apoptotic rate by 50%. However, blockade of AT2 receptors did not show any effect on cocaine-induced hVSMC apoptosis. Nitric oxide (NO) donors (S-nitroso-N-acetylcysteine, SNAP and DETA-

NONOate) further enhanced proapoptotic effect of cocaine, whereas, N(omega)-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, inhibited cocaine-induced hVSMC apoptosis. Antioxidants (DPI and ascorbic acid) partially inhibited cocaine-mediated apoptosis. These findings suggest that ANG II plays a role in cocaine-induced hVSMC apoptosis. This effect of ANG II seems to mediate through the generation of ROS and NO by hVSMCs.

PT-20:

DELTA-9-TETRAHYDROCANNABINOL (THC) INDUCES THE NOTCH LIGAND, JAGGED1, IN DENDRITIC CELLS (DCs). Cathy Newton, Izabella Perkins, Herman Friedman, Thomas W. Klein, USF College of Medicine, Tampa, FL 33612

Legionella pneumophila infection causes in mice increased blood levels of IL-12 and IFN gamma followed by antigen-specific production of IFN gamma and specific anti-*L. pneumophila* IgG_{2a} antibodies (Th1 response). Treatment with THC prior to *L. pneumophila* infection results in a biasing from Th1 responses to Th2 responses characterized by decreased IFN gamma and IL-12 production and increased IL-4 and IgG₁ antibodies. Treatment with cannabinoid receptor (CBR) antagonists prior to THC and utilizing CBR gene deficient mice showed an attenuation of the THC effect on Th1 biasing, indicating a receptor-mediated response. Dendritic cells (DCs) are major antigen presenting cells and regulate the differentiation of either Th1 or Th2 CD4+ lineages by varying the production of different biasing factors. One of these factors is Jagged1, a member of the Notch ligand family, which induces Th2 responses by increasing IL-4 and GATA3 in CD4+ cells. We hypothesized that THC was biasing toward Th2 by increasing Jagged1. THC treatment induced Jagged1 mRNA in mouse DC cultures and in separate studies IL-4 protein and GATA3 mRNA were shown to be increased in spleen cells from THC-treated mice. These studies suggest that THC treatment causes Th2 immunity by increasing the activity of the Notch pathway in both DC and CD4+ cells and future studies will examine the link between CBR signaling and Jagged1 ligand activation. Supported by DA03646, DA10683, and AI45169.

PT-21:

NICOTINE MODULATES CYTOKINE PRODUCTION IN *Chlamydia pneumoniae* INFECTED HUMAN PERIPHERAL BLOOD CELLS. Y. Mamata, A. Hakki, Y. Yamamoto, C. Newton, T.W. Klein, *S. Pross, and H. Friedman

Nicotine, the addictive component of tobacco, has been shown to have immunomodulatory effects including alteration of proinflammatory cytokine production by immune cells. This study focuses on the effects of nicotine on infection by *Chlamydia pneumoniae* (Cpn), a ubiquitous intracellular pathogen that causes acute and chronic inflammatory diseases including pulmonary infections, and is thought to impact on arthritis and atherosclerosis. The effect of nicotine on TGF-beta 1, IL-10, IL-12 and TNF-alpha production in Cpn-infected human peripheral blood mononuclear cells was assessed by ELISA. The results showed that Cpn infection increased the production of IL-10, IL-12, and TNF-alpha. Nicotine treatment of the Cpn-infected cells resulted in an up-regulation of IL-10 production, but not of TNF-alpha or IL-12. Nicotine treatment resulted in a down-regulation of TGF-beta 1 production that was greater than the decrease seen with either nicotine or Cpn treatment individually. Since nicotine use and Cpn infections are both prevalent, a combined exposure would not be uncommon, warranting further investigation into the roles of these agents on chronic inflammatory diseases.

PT-22:

CYTOKINE PRODUCTION BY BONE MARROW DERIVED DENDRITIC CELLS STIMULATED WITH MICROBIAL PRODUCTS MODULATED BY CATECHIN.

James Rogers*, Izabella Perkins, Alberto van Olphen, Nicholas Burdash, Thomas W. Klein and Herman Friedman; Depart. Medical Microbiology and Immunology, Univ. South Florida, Tampa, FL 33612

Monocytic cells are essential for innate immunity, especially against intracellular opportunistic bacteria like *Legionella pneumophila*. Cytokine production by immune cells, including phagocytic macrophages and dendritic cells (DCs), activate Th1 helper cells important in resistance to infectious organisms. The primary polyphenol in green tea extract is the catechin epigallocatechin gallate (EGCG). The present study showed that bone marrow derived murine DCs, stimulated with microbial products, including LPS from Gram negative bacteria, the cell wall component MDP from Gram positive bacteria, as well as infected with Legionella, produced the Th1 helper cell activating cytokine IL-12 as well as the proinflammatory cytokine TNF-alpha, determined by ELISA assay. Treatment of the stimulated cells with EGCG inhibited in a dose dependent manner IL-12 production but, in contrast, enhanced production of TNF-alpha. Therefore, production of these important cytokines by dendritic cells stimulated with microbial products was markedly affected by EGCG, a ubiquitous small molecular weight polyphenol.

PT-23:

THE EFFECTS OF HEROIN ON iNOS EXPRESSION INVOLVE A DOPAMINERGIC MECHANISM. T.B. Saurer*, K.A. Carrigan, S.G. James, and D.T. Lysle; Dept. of Psychology, Univ. North Carolina, Chapel Hill, NC 27599

Administration of opioids such as heroin and morphine produce immunosuppressive effects on a number of immune parameters. Recently, we have shown that morphine-induced reductions in splenic natural killer cell activity are mediated by dopamine receptors in the nucleus accumbens, as intra-accumbens administration of the D1 receptor antagonist SCH23390 blocks morphine's suppressive effect. The aim of the present study was to determine whether SCH23390 would also prevent the effect of heroin on inducible nitric oxide synthase (iNOS) expression. Male Lewis rats were injected with SCH23390 (0, .005, .05, .5 mg/kg) 30 min prior to heroin (1.0 mg/kg) or saline in conjunction with an injection of LPS. Rats were sacrificed 6 h later and spleen, lung, and liver tissues were removed for determination of iNOS expression using real-time RT-PCR. Analyses showed a significant reduction in iNOS expression in heroin-treated animals relative to saline-treated animals, and this reduction was prevented by SCH23390. This study shows that a dopamine receptor mechanism is also involved in heroin-induced immune alterations and suggests that the modulation of central dopamine signaling may be a common mechanism involved in opioid-induced immunomodulation.

PT-24:

GENE EXPRESSION IN HUMAN NEURONS IN CULTURE AND BRAIN. USE OF DIFFERING MICROARRAY AND BIOINFORMATICS TECHNOLOGIES.

P Shapshak*¹, K Goodkin¹, R Duncan², A Nath³, J Turchan³, W Davis⁴, P Kanguane⁵, EM Duran¹, F Ziegler¹, A Minagar⁶, R Seth⁷, & T Kazic⁷. ¹Dept. Psychiatry, ²Epi, U of Miami, Miami, FL. ³Neurol, Johns Hopkins U, Baltimore, MD. ⁴Stat Sci, Baylor U, Waco, TX. ⁵Natn'l

U, Singapore, ⁶Neurol, Louisiana State U, Shreveport, LA. ⁷U of Missouri, Columbia, MO.

We report human neuron gene expression in culture and brain (after laser capture microdissection [LCM]) using differing Microarray (Affymetrix & Amersham) & Bioinformatics technologies (e.g. Spotfire, SAS). Neurons were treated with cocaine & HIV-1 proteins tat & envelope. We are also investigating gene expression in neurons LCM'd from patients with/without HAD, HIVE, and drug abuse. One-way ANOVA showed 35 genes significant across neuron treatment groups. Factorial ANOVA showed correlations including 30 with tat, 17 cocaine, 10 env, & 6 tatxenv. Different genes are identified using diverse culture, tissue type (brain), and Bioinformatics analyses. We are building pathways and a public database for Dementia, called Agora/Artemis. We acknowledge the Four National NeuroAIDS Tissue Consortium Brain Banks and NIDA /NIMH /NINDS /NIGMS for support.

PT-25:

TNF-ALPHA AND IL-1BETA-INDUCED CHEMOKINE PRODUCTION BY HUMAN NEURAL PRECURSOR CELLS. W.S. Sheng*, S. Hu, H.T. Ni[§], T.N. Rowen, J.R.

Lokensgard and P.K. Peterson; Department of Medicine, University of Minnesota Medical School and the [§]Stem Cell Group, R&D Systems, Inc. Minneapolis, Minnesota.

Recent studies have shown that proinflammatory cytokines damage rodent neural precursor cells (NPCs), a source of self-renewing multipotent cells that play an important role in the developing as well as adult brain. In this study, human NPCs (>90% nestin- and >80% CD133-positive) obtained from 6-8-week-old fetal brain specimens stimulated with TNF- α and IL-1 β produced abundant amounts of the chemokines CCL2 and CXCL10. Receptors for TNF- α mRNA were constitutively expressed. However, only TNFR1 was involved in TNF- α stimulated chemokine production demonstrated by antibodies blocking study. Both TNF- α and IL-1 β treatment induced p38 mitogen activated protein kinase (MAPK) phosphorylation, and SB202190, an inhibitor of p38 MAPK, blocked cytokine-induced chemokine production. Thus, this study demonstrated that NPCs constitutively express receptors for proinflammatory cytokines which when activated trigger via a p38 MAPK signaling pathway production of two chemokines, CCL2 and CXCL10, which are involved in infectious and inflammatory diseases of the brain.

PT-26:

THE POTENTIAL ROLE FOR CREM AND ICER IN MORPHINE INDUCED SUPPRESSION OF IL-2 SYNTHESIS IN STIMULATED T CELLS. Jinghua Wang,

Roderick A. Barke, Richard Charboneau, Sabita Roy Depts of Pharmacology and Surgery, University of Minnesota

Chronic morphine treatment suppresses IL-2 transcription and synthesis in CD3 and CD28 stimulated T cells. Morphine up-regulated CREM, and down-regulated p-CREB expression 2 hours after CD3 and CD28 stimulation. Transfection of an anti-sense CREM plasmid into T cells blocked the expression and binding of CREM to IL-2 promoter and rescued inhibition of IL-2 production induced by morphine. Mutation of the CRE binding site in the IL-2 promoter abolished morphine's inhibitory effect when compared to WT constructs. In addition, as assessed by chromatin immunoprecipitation analysis, morphine increased CREM binding and inhibited the acetylation of histones. Our data suggest that upon T cell activation, morphine increases CREM, and CREM competes with phosphorylated CREB for binding at the -180 site of the IL-2 promoter. This results in a decrease in histone acetylation and down-regulation of IL-2

production. Alternatively, morphine treatment can result in an increase of inducible cAMP early repressor (ICER) expression. ICER competes with p-CREB binding to the cAMP responsive elements (CREs), which then leads to uncoupling of CBP/p300, and thereby abrogate early stages of transcription of IL-2. Both mechanisms will be clarified in these studies. Support: NIH RO-1 DA 12104 and KO2 DA015349, and P50 DA 11806.

PT-27:

SELECTIVE T CELL DYSFUNCTION IN AN ANIMAL MODEL OF HUMAN DRUG ABUSE: CNS CIRCUITRY AND NEUROENDOCRINE MECHANISMS. R. J. Weber^{1*} and T. J. Martin² University of Illinois College of Medicine at Peoria, Peoria, IL, USA and Wake Forest University, Winston-Salem, NC, USA.

Advances in the neurobiology of addictive substances have shown that neuronal plasticity plays a key role in establishing and maintaining drug self-administration. Our previous work in a rat model of human drug abuse, demonstrated that exposure to relatively low doses of self-administered (SA) heroin resulted in the selective suppression of splenic T lymphocyte proliferative responses without altering other parameters of immunocompetence. Furthermore, T-cell suppression correlated with a two-fold increase in plasma adrenocorticotropin hormone, and a three-fold increase in corticosterone. Results from this study suggested mechanistic differences in immunosuppression induced by acute non-contingent experimenter-delivered vs intermittent SA opiates. Autoradiographic analysis showed significant increases in mu opioid receptor-G-protein coupling efficiency in the rostral medial thalamus and the anterior hypothalamus. We hypothesize that in SA animals more effective opioid action in the anterior hypothalamus followed by increases in ACTH and corticosterone subserves the suppression of T lymphocyte function observed. Supported by NIDA/NIH DA12095.

PT-28:

COCAINE-INDUCED ALTERATIONS IN GENE EXPRESSION IN MICROGLIA CELLS. Yuferov, V.*¹, Nielsen D.A.¹, Hu, S.², Peterson, P.K.², and Kreek, M.J.¹ The Rockefeller University, New York¹; University of Minnesota Medical School, Minneapolis²

Cocaine has been shown to alter a number of microglial cell functions. Alterations in gene expression in primary human microglia by cocaine (10^{-6} and 10^{-8} M) for 1, 8 and 24 h, and by interferon-gamma for 8h were analyzed using U133A arrays (Affymetrix). Data was analyzed using Gene Spring 7 software. Genes with 1.4-fold change displaying significance by ANOVA T-test were considered differentially regulated. Cocaine at 10^{-6} M up-regulated 62 genes and down-regulated 65 genes. At 24 h, 156 genes were up-regulated by 10^{-8} M cocaine. These included toll-like receptor 4, its ligand Hsp70, kinase TBK1, the retinoic acid receptor, RARRES1, and the glutamate transporter, SLCA3. A different set of genes were regulated by interferon. IFN treatment resulted in increase expression of 501 genes and a decrease of 782 genes. IFN-activated genes included chemokines and MHC II genes, while IFN receptor 1, cytokine interleukin-10, and ERK-1 genes were down-regulated. Twenty-five genes were inversely regulated by INF as compared to cocaine treatment. Thus, cocaine affects gene expression in microglia which could alter their functional properties. NIH-NIDA DA-P60-05130, DA-00049, and 12848 to M.J.K.; DA09924 to P.K.P.

PT-29:

CELLULAR IAP1 REGULATES APOPTOSIS INDUCED BY TRAIL IN HUMAN NEURAL PROGENITOR CELLS. H. Peng*, Y. Huang, Z. Duan, D. Xu and J. Zheng; Dept. Pharm and CNND, Univ. NE Med Center, Omaha, NE 68198.

The underlying mechanisms regulating neural progenitor cell (NPC) survival during disease states are unknown. Previously, we demonstrated upregulation of TNF-Related Apoptosis Inducing Ligand (TRAIL) on HIV infected and immune activated macrophages. We have also shown TRAIL triggers neuronal cell death by binding TRAIL death receptors. In this study we hypothesized that TRAIL mediates apoptosis in human NPC, therefore altering neurogenesis during HIV-1 associated dementia (HAD). We showed that TRAIL-R2 is highly expressed on NPC, yet TRAIL induced only nominal apoptosis (5-10%). We found mRNA and protein for caspase 8, an important element in the TRAIL mediated death pathway, to be minimally expressed in human NPC. Inhibitor of apoptosis proteins (IAP), such as c-IAP1 and Survivin are highly expressed. The transcription inhibitor, Actinomycin-D, sensitized the cells to TRAIL-induced apoptosis in human NPC. Further, inhibition of c-IAP1 expression by siRNA increased TRAIL mediated caspase-3 activation and apoptosis. Thus, the inhibitory apoptotic protein, c-IAP1 protected NPC against TRAIL-induced apoptosis and suppressed activation of caspase-3. These mechanisms illustrate NPC resistance to pathological agents, such as TRAIL, during HAD and other possible disease states. (Supported by NIH R01 NS 41858-01, P20 RR15635-01 and 1 P01 NS043985).

THE END