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# Poster Presentations

## P.1

### Identification of a cell entry receptor for wild type measles virus on human neuronal cells

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Subacute sclerosing panencephalitis and measles inclusion body encephalitis are two neurological complications of measles virus (MV) infection. Neurons and oligodendrocytes are infected and to a lesser extent astrocytes in both conditions. However, the molecules which allow virus entry into these cell types are unknown. The known cell entry receptor for both vaccine and wild type (WT) MV strains, signalling lymphocyte activation molecule (SLAM or CDw150) is specific to T and B cells, monocytes as well as activated dendritic cells. Although human cofactor CD46 has been shown to be expressed at a low level on a subset of neurons, on oligodendrocytes and astrocytes it is not a receptor for wild type (WT) MV strains. This indicates that another receptor(s) is present on neural cells and is required for WT virus infection. The NT2 cell line, derived from a human teratocarcinoma, exhibits properties characteristic of a committed human neuronal precursor at an early stage of development and can be differentiated to produce mature, CD46 negative neurons (NT2-N) with retinoic acid. We have found that these neuronal cells as well as the human epithelial cell derived neuroblastoma cell line, SHSY5Y, which is also CD46 negative, can be infected with the WT Dublin strain of MV. Both cell lines express well characterised neuronal markers and therefore provide suitable systems to investigate cell entry receptor(s) for WT MV. Using a combined novel approach we have prepared an antibody library against SHSY5Y cells and have selected a monoclonal antibody (Mab) which blocks infection of these cells by WT MV. The putative receptor which this antibody recognises on SHSY5Y cells is currently being characterised. In addition, the effect of the Mab on WT MV infection in NT2-N cells and other human cell types as well as on veterinary morbillivirus infection in appropriate cell lines is being investigated.

## P.2

### Infection of human neuronal cell lines by wild type, rodent adapted and vaccine strains of measles virus

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Measles virus (MV) is a highly contagious human pathogen and the etiological agent of subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis. Post mortem examination of SSPE brain tissue demonstrated that MV antigen and RNA were present in neurons and neuronal processes as well as oligodendrocytes, microglia and to a lesser degree in astrocytes and endothelial cells. Although human cofactor CD46 has been shown to be expressed at a low level on a subset of neurons it is a receptor for vaccine but not wild type (WT) MV strains. Furthermore, signalling lymphocyte activation molecule (SLAM) which has been identified as a receptor for both vaccine and WT strains of MV is not detected on CNS cells. To examine virus entry in human neuronal cells we used the WT Dublin-3267 MV strain to inoculate the human neuroblastoma cell line SHSY5Y as well as NT2 cells during different stages of differentiation. The latter are derived from a human teratocarcinoma and exhibit properties characteristic of a committed human neuronal precursor at an early stage of development and can be differentiated using retinoic acid to mature neurons. WT MV was capable of infecting SHSY5Y cells and fully differentiated NT2 neuronal cells, both of which were CD46 negative and MAP-2 positive. However, unlike vaccine and rodent brain adapted strains of MV the WT virus did not infect NT2 cells which were CD46 positive and MAP-2 negative during the early stages of differentiation. The results therefore suggest that an unknown MV receptor(s) is present on mature human neuronal cells, which allows WT MV infection.

## P.3

### Spleen necrosis virus (SNV) based vectors could efficiently transfer small inhibitory RNA (siRNA) against the chemokine receptor CCR5 into the human brain cells to block HIV-1 entry

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Human immunodeficiency virus type 1 (HIV-1) associated dementia (HAD) remains a major problem among HIV-1-infected individuals even in the era of highly active antiretroviral therapy (HAART). Limited penetration of antiretrovirals through the blood-brain barrier (BBB), a protective sheath of the brain is considered a major obstacle in overcoming residual HIV-1 in the brain. The BBB constituted by brain microvascular endothelial cells (MVECs), besides protecting the brain is also conduit for viral entry into the central nervous system (CNS). These cells are devoid of CD4, a major receptor for HIV-1, however, they express various chemokine receptors such as CCR5, CXCR4 and CCR3. We hypothesized that down-modulation of CCR5 expression could reduce the passage of viral entry into the brain MVECs. Based on this hypothesis, we used small inhibitory RNA (siRNA) to reduce the expression of CCR5 on human brain MVECs followed by their infectivity with various laboratory adapted and primary isolates of HIV-1. A series of spleen necrosis virus (SNV)-based retroviral transfer vectors capable of expressing short hairpin RNA (shRNA) against the chemokine receptors CCR5 were constructed. For positive controls, lentiviral based vector pCMV-GIN-Neo, expressing microRNA adapted shRNA against CCR5 (shRNAmir) and Promega Corporation's GeneClip$^{TM}$ U1 Hairpin System expressing shRNA against CCR5 were used. The ability of these constructs to down-modulate the expression of CCR5 was tested in primary isolated human brain MVECS. The results showed that all the three constructs are capable of reducing CCR5 expression up to 80% of innate levels, while cells transfected with control vector maintained normal levels of CCR5. To determine if down regulation of CCR5 co-receptor inhibits viral replication, human brain MVECs transfected with siRNA were challenged with R5-tropic (ADA) strains of HIV-1. Substantial reduction in viral infection as determined by HIV p24 ELISA and Real Time PCR analyses of the HIV-1 gag copy numbers was seen in siRNA transfected MVECs challenged with R5-tropic HIV-1 when compared with control cells. Besides the primary human brain MVECS, we also tested these constructs in astrocytes, CD14+ monocyte derived macrophages and peripheral blood mononuclear cells (PBMCs) from HIV-1-seronegative donors. Our preliminary data suggest that shRNA against CCR5 delivered through SNV-based vectors are highly selective and inhibits the expression of CCR5 co-receptor in these primary cells which is further associated with protection from HIV-1 infection. The results of this study suggest that delivery of siRNA into the human brain by SNV based vectors could be used in the design of therapeutics against HIV-1-associated neurodegenerative disorders.

## P.4

### Dual lentivirus infection potentiates neuroinflammation and neurodegeneration

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Objective: Herein we investigated the neuropathogenic outcomes and underlying mechanisms of dual lentivirus infections with genetically distinct viral strains as co-infections or super-infections.

Design: Experimental Feline Immunodeficiency Virus (FIV) infections were performed using cultured feline cells and an in vivo model of AIDS neuropathogenesis.

Methods: Dual infections were carried out with two FIV strains (FIV-Ch and FIV-PPR) as co-passaged or superinfected viruses ex vivo in feline monocyte-derived-macrophages and in vivo in an established feline model with subsequent analyses of outcomes using real time RT-PCR, neuropathological features, quantitation of viral load and measures of neurobehavioral performance.

Results: Dual infections caused greater IL-1beta, TNFalpha and IDO expression and neurotoxic properties in infected feline macrophages. Co-infection and sequential FIV super-infection in vivo also induced greater IL-1beta, TNFalpha and IDO expression in the basal ganglia (BG) and frontal cortex (CTX), compared to the mono- and mock-infection groups, although viral loads were similar in mono- and dual-infected animals. Immunoblot analyses disclosed lower synaptophysin immunoreactivity resulting from FIV super-infection and co-infection in the CTX. Both cholinergic and GABAergic neuronal injury were evident in the in CTX of animals with dual FIV infection. With increased astrocytic and microglial activation and neuronal loss in dual FIV-infected brains, immunohistochemical analysis also revealed elevated detection of cleaved caspase-3, concurrent with dysmorphic changes in neurons and associated neurobehavioural abnormalities.

Conclusions: These findings highlight the adverse biological effects of dual lentivirus infections in super-infection and co-infection models for which the underlying pathogenic mechanisms represent an escalation in neuroinflammation and ensuing neurodegeneration.

## P.5

### Induction of proinflammatory cytokines by human T cell leukemia virus type 1 Tax protein as determined by multiplexed cytokine protein array analyses of human monocyte-derived dendritic cells

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Human T cell leukemia virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by a hyperstimulated immune response including elevated levels of inflammatory cytokines/chemokines, and the oligoclonal expansion of virus-specific CD8+ T cells in the cerebrospinal fluid. Studies have shown that the HTLV-1 transactivator protein Tax is available for immune recognition by antigen presenting cells (APCs) such as dendritic cells (DCs). DCs are relevant to the pathogenesis of HAM/TSP since the presentation of Tax peptides by activated DCs to naïve CD8+ T cells may play an important role in the induction of a Tax-specific immune responses observed in HAM/TSP. In this study, a human cytokine protein array was used to study the secretion of cytokines by monocyte-derived DCs (MDDCs) exposed to Tax. Of the 16 cytokines analyzed, 6 cytokines were secreted in significantly high amounts (~2-fold) including Th1 cytokines (IFN-gamma, IL-12, and TNF-alpha) and C-C chemokines (Eotaxin, MCP-1, and MCP-3). Selected cytokines were further examined at two concentrations of Tax and two time periods. Furthermore, a transient exposure to Tax did not result in any cytokine production when examined at three different time points after exposure, indicating that the prolonged presence of Tax was required for its activity. Finally, inhibition of the NF-kappaB signaling pathway by specific inhibitors abrogated Tax-mediated cytokine secretion. Collectively, these investigations suggest that Tax-induced cytokine secretion from MDDCs may be an important factor in the cellular activation and tissue damage observed in HAM/TSP.

## P.6

### Analysis of the DrexelMed HIV/AIDS cohort for envelope markers

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Microglial cells and perivascular macrophages support most productive HIV-1 replication within the brain, although both have low CD4 levels making macrophage tropism and preferential CCR5 utilization features of CNS-derived viruses/envelopes. Sequence analysis has demonstrated both viral evolution within the CNS and compartmentalization of sequences between brain and peripheral tissues, suggesting the potential for adaptation to replication in the brain environment. Previous studies of human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) have identified the 3T configuration of CCAAT enhancer binding protein C/EBP site I (C-to-T change at nucleotide position 3) and 5T configuration of Sp site III (C-to-T change at nucleotide position 5) to correlate with peripheral blood disease progression and HIV-associated dementia (HAD). The 3T/5T-containing LTR may be representative of an LTR genotype that is preferentially retained, because of specific functional properties involved in disease pathogenesis, in the PB, CNS, and perhaps other compartments as HIV-1-associated disease severity increases suggesting that it may be useful as a predictive viral marker. In addition, we propose certain envelope sequences are co-selected with this LTR, which play a key role in cell types infected within the peripheral blood and. Herein, we present an analysis of the DrexelMed HIV/AIDS cohort for the presence of specific configurations of HIV-1 LTR transcription factor binding sites in association with specific envelope genotypes. Future studies will address functional properties of these viral genetic signatures.

## P.7

### Phosphorylation of pRb is differentially regulated in neurons in HIV-Associated Dementia

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HIV Associated Dementia (HAD) is characterized by microgliosis, astrogliosis, neuronal loss and dendritic damage. While the mechanisms of neuronal death in HAD remain only partially defined, neuronal damage has been linked to soluble factors released by HIV infected, and non-infected, activated macrophages/microglia in the brain. The inflammatory infiltrate secretes both neurotoxic and neuroprotective factors. Phosphorylation of pRb has been shown to be required in in vitro models of neuronal apoptosis including trophic factor withdrawal, beta-amyloid treatment and oxidative stress. To define the differential effects of neurotoxic and neuroprotective factors on pRb phosphorylation in HAD, we used an in vitro model where we treated primary rat neuroglial cultures with supernatants from human monocyte-derived-macrophages infected with a neurovirulent strain of HIV-1 (HIV-M/M). We assessed pRb phosphorylation on characterized sites and the interaction of these phospho-pRb isoforms with cell cycle proteins. Using dilutions of HIV-M/M that produced 25% neuronal loss by 4 hours or 50% neuronal loss by 20 hours, we observed an early increase in p-pRbSer249/Thr252, and p-pRbSer795 by western blotting (WB), and a late increase in p-pRbSer780 by HIV-M/M by both WB and immunofluorescence (IF), similar to results obtained in cultures treated with Rantes. BDNF led to a delayed increase in these p-pRb epitopes. We observed similar increases by WB and IFA in the mid-frontal cortices of autopsied brain tissue of HIV(+) individuals. We did not observe an increase in p-pRbSer608 or p-pRbThr821 levels with either HIV-M/M, BDNF or Rantes by WB in vivo. Interestingly, p- pRbSer807/811,

which was observed in the cytoplasm of untreated neurons by IF, was detected in the nuclei of neurons in HIV M/M treated cultures, while its protein levels were not increased, as assessed by WB. p-pRbSer807/811 is a known epitope for cdk5. By co-immunoprecipitation, we observed a loss of interaction of p- pRbSer807/811 with cdk5 when cultures were exposed to HIV-M/M, whereas it was not disrupted in response to BDNF, Rantes or NGF treatment. Thus, we hypothesize that phosphorylation of pRb is differentially regulated in neurons in response to neurotrophic factors and the binding between p-pRbSer807/811 and cdk5 might be a determining factor in neuronal viability.

## P.8

### IL-1beta-induced differentiation of a human bone marrow promonocytic progenitor cell line and susceptibility to HIV-1

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The role of the bone marrow compartment in the pathologic events associated with AIDS and HIV-1 dementia (HIVD) has not been elucidated. Interestingly, CD34+/CD38- progenitor cells within the bone marrow are refractile to HIV-1 infection, possibly due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5, which upon differentiation are upregulated, potentially increasing susceptibility to infection. The CD34+/CD38+ TF-1 bone marrow progenitor 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-1beta, TNF-alpha, IL-4 and their maturation was monitored through surface marker expression by flow cytometry. Interestingly, IL-1beta, alone or in combination with TNF-alpha leads to CXCR4 and CCR5 upregulation and preservation of CD4 expression while differentiating TF-1 cells, thereby providing a window of opportunity for HIV-1 infection to occur. Moreover, transient and stable transfection analysis demonstrated that the HIV-1 LTR activity was significantly increased following treatment of TF-1 cells with IL-1beta and conditioned media. Importantly, IL-beta treatment led to a large increase in p24 production in TF-1 cells infected with the CCR5 using virus BaL and to a modest increase in cells infected with the CXCR4 using virus IIIB. These results indicate that progenitor cell differentiation leads to upregulation of HIV-1 co-receptor expression and enhancement of the LTR activity, contributing to increased HIV-1 susceptibility and productive replication.

## P.9

### Assessment of leukocyte movement in encephalitic brains of SIV infected macaques suggests bidirectional trafficking across the blood brain barrier

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Cell movement is essential for immune system function. While much is known about trafficking of leukocytes between blood and peripheral tissues, relatively little is known about leukocyte trafficking to, and possibly from, the brain. Examination of these issues in the brain is made more difficult by the relative inability to safely collect repeated samples. In vitro and immediately ex vivo models can be used to address many issues but the relevance of the observations to the biology in vivo can be difficult to assess. In an effort to address this problem we have developed a standardized method for analyzing direction of movement of immune system cells from static confocal images by applying simple algorithms based on cell morphology. Immunofluorescence staining for nonmuscle myosin II heavy chain A (MyH9) was performed in suspension. This molecule was examined because of its activation during cell movement. Specifically, MyH9 is involved in cell polarization, cell motility, contraction of the actin-myosin network in the uropods of T-cells. In addition, the actin networks of cells were analyzed by staining with Phalloidin, a molecule that labels F-actin in mobile cells as well as vessels. Visualizing the distribution of F-actin is important to understand directional cell movement because it is highly localized in the uropods suggesting involvement in the contraction of actin-myosin networks at the rear of the cell. The staining of these molecules was further examined by analyzing the morphology of these polarized cells with the hypothesis that cells in motion have an elongated morphology, as compared to static cells with round morphology, from which a mathematical determination of direction of movement can be made in three dimensions by drawing a vector from the center of mass of the cytoplasm to the center of mass of the nucleus while implementing simple algorithms of the distance formula and the point-slope method. The resulting mathematical assessment of direction of movement of cells in 3 dimensions, was firstly validated by in vitro live cell microscopy studies, and then applied to CD3+ lymphocytes and CD68+ and CD163+ monocytes/macrophages found in brain parenchyma and perivascular space. Such analysis revealed bidirectional movement of leukocytes across the BBB in cases of SIV encephalitis.

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## P.10

### Differences in cerebral blood flow (CBF) and cerebral metabolic rate of oxygen consumption (CMRO2) in HIV+ patients compared to seronegative controls using quantitative functional magnetic resonance imaging (qfMRI)

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Introduction: qfMRI has been used to study the basic physiology of both cortical and subcortical regions. Simultaneous acquisition of the blood oxygen level dependent (BOLD) and CBF responses during functional activation and mild hypercapnia experiments allows for non-invasive determination of CMRO2. We used qfMRI to determine if physiological responses within the basal ganglia to a simple fingertapping task were affected by HIV.

Methods: 16 seronegative healthy controls and 16 HIV+ subjects were studied at 3 T using QUIPSSII/PICORE ASL technique with a dual echo spiral k-space acquisition of CBF and BOLD data. All subjects initially received two trials of a 5% CO2 gas mixture for 3 minutes with 4 minutes between CO2 challenges. Mild hypercapnia was used to manipulate CBF changes independent of CMRO2. During four subsequent activation scans, subjects viewed numbers in the center of the screen flickering at 2Hz corresponding to finger taps on a 4-button box. Each functional scan consisted of 4 trials using a block-design stimulus (20 sec on, 60 sec off). A region-of-interest was drawn for each subject corresponding to the basal ganglia. This region was chosen as HIV often leads to pathological changes within this subcortical area. From this region trial-averaged CBF and BOLD responses were obtained for the functional activation paradigm and for mild hypercapnia. CMRO2 values were subsequently calculated using a standard equation. Paired t-tests were performed between the two groups for both measured (CBF and BOLD) and calculated (CMRO2) values with p values significant if $p < 0.05$.

Results: Both CBF and BOLD responses were reproducibly observed within the basal ganglia for functional stimulation and hypercapnia in HIV+ subjects and seronegative controls. Changes in CBF, BOLD and CMRO2 for functional activation were significantly greater within HIV+ subjects compared to seronegative controls. There was no correlation between nadir CD4, current CD4, or viral load and qfMRI measures within HIV+ subjects.

Conclusions: qfMRI may act as a non-invasive surrogate biomarker for assessing effects of HIV in the brain.

## P.11

### Metabolic syndrome risk factors are elevated in HIV+ patients with previous stroke

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Introduction: Components of the metabolic syndrome including hypertriglycerides (TRG), increased body mass index (BMI), hypertension (HTN), and elevated serum glucose (GLU) and uric acid (UA) levels have become common in HIV+ patients since introduction of combination antiretroviral therapy (cART). These factors can increase the risk of stroke. We compared the prevalence of these risk factors (TRG, HTN, GLU, BMI, and UA) in a confirmed cohort of HIV+ patients with strokes (cases) to age and sex matched HIV+ subjects with no history of stroke (controls).

Methods: A retrospective chart and database analysis was performed of over 2,500 HIV+ individuals followed at the HIV Neurobehavioral Research Center (HNRC) at the University of California San Diego (UCSD). Cases were selected based on ICD -9 codes consistent with stroke (342 and 430–438). From this group 21 cases were identified. Each case was reviewed by a board certified neurologist (B.A.) to ensure stroke etiology was most likely due to HIV and not other factors (i.e. current stimulant use, progressive multifocal leukoencephalopathy, toxoplasmosis, etc.). Each case was rated as definite, probable, or unlikely according to focal findings on neurological examination, acute onset of neurological disturbances, laboratory data, and neuroimaging abnormalities. From this set, 12 definite cases were identified. For each case five matched HIV+ controls were identified using the following criteria: age (± 4 years), sex, number of HNRC visits, and year of stroke (± 4 years). Laboratory values and mean arterial pressure (2/3 systolic pressure and 1/3 diastolic pressure) were obtained at the outpatient visit closest to the date of confirmed stroke for each case. Pair-wise student t-tests with a Sidak correction for multiple comparisons were performed between cases and controls for each risk factors associated with metabolic syndrome.

Results: Mean TRG, random serum GLU, and serum UA were significantly elevated within cases ($p = 0.03$) compared to controls. However, no significant differences were seen for other risk factors including BMI, MAP, CD4 count, plasma HIV RNA, hemoglobin, cholesterol, or current cART regimens between the two groups.

Conclusions: Three components of the metabolic syndrome (hyper-triglyceridemia, -glycmeia, and –uricemia) but not CD4 or plasma HIV RNA, appear to be associated with increased stroke risk in HIV infected patients, almost all of who were on antiretroviral therapy.

## P.12

### Biomarkers and neuropsychological deficit pattern in clinically asymptomatic HIV-positive patients

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Objective: Human immunodeficiency virus (HIV) provokes different forms of brain disease, like virus associated dementia (HAD) and its precursors. Neuropsychological test batteries can identify deficits in these patients, but to date there is no pattern identified, allowing to determine the prognosis of an individual patient. Biomarkers for disease progression, like CD4+-cell count and plasma viral load in systemic HIV disease, are lacking as well in virus associated brain disease.

Methods: 33 male homo-/bisexual men without clinically overt neurological deficits were included in a prospective study. Patients were examined neurologically, neuropsychologically as well as immunologically by determining cerebrospinal fluid cytokine levels using a solid-phase protein array in order to evaluate the role of defined cytokines as biomarkers for HIV-induced brain disease.

Results: Patients were comparable with respect to age, CD4+-cell count and neuropsychological test performance. TH2 reaction like interleukin-1, 4 and 10 was upregulated in treated AIDS-patients, whereas IL-10 was significantly down-regulated in therapy-naïve non-AIDS patients (CDC A1+2, B1+2), who showed a bad performance in the digit symbol test and in parameters of fine motor speed. Untreated late stage patients (CDC A3, B3 and C1-3) had a poor performance in the AIDS dementia scale. The longer patients were known to be infected, the higher were the levels of CXCL16, GM-CSF and TGF-beta1.

Conclusions: The study showed a supportive value of CSF-cytokine analysis in neuropsychologically impaired patients. Solid-phase protein arrays are a useful method to analyse cytokine patterns in the CSF of HIV-carriers. Thus, neuropsychological testing and CSF-cytokine-analysis may be useful tools for monitoring HIV associated brain disease.

## P.13

### Cell associated heparan sulfate proteoglycans are involved in HIV-1 attachment and entry into primary human astrocytes

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Astrocytes represent a major cellular component in the brain and appear to significantly contribute to HIV-1-mediated neuropathogenesis. Human primary astrocytes do not express CD4 but still bind HIV-1 gp120 through alternative receptor(s). To date several candidate receptor proteins have been described, including the mannose receptor and the chemokine receptor D6. We and others have recently demonstrated that cell-associated proteoglycans serve as major HIV-1 receptors on CD4-negative CNS target cells, namely brain microvascular endothelial cells. In the current study, we examined whether proteoglycans also act as HIV-1 receptors on normal primary human astrocytes (obtained from ScienCell Research Laboratories). First, we characterized the expression of specific proteoglycans in astrocytes via RT-PCR and Western blot analysis. Our preliminary data indicate strong expression of syndecan-1, a cell-surface transmembrane heparan-sulfate proteoglycan (HSPG), recently shown to efficiently mediate attachment and entry of HIV-1 in human macrophages and in primary genital epithelial cells through a CD4-independent mechanism. Enzymatic digestion of cell-surface heparan-sulfate and chondroitin-sulfate side-chains with specific lyases (heparitinase III and chondroitinase ABC, 10U/ml respectively) significantly inhibited HIV-1 (X4-tropic strain NL4-3) attachment onto human astrocytes, as indicated by the amount of p24 in cell lysates via ELISA. Moreover, our data from quantitative proviral DNA (HIV-1 gag) real-time PCR analysis in astrocytes pretreated with lyases and subsequently incubated with NL4-3 indicate that cell-associated proteoglycans facilitate efficient entry of HIV-1 into human astrocytes. The molecular mechanisms by which proteoglycans promote CD4-independent viral entry, infection, and spread in the CNS and its cellular components remain an area of active investigation. Understanding the infection of the human brain by HIV-1 is crucial in targeting this potential viral reservoir site.

## P.14

### The IFN-induced expression of APOBEC3G in human blood brain barrier exerts a potent intrinsic immunity to block HIV-1 entry to CNS

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In the human genome the apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC)3 gene has expanded into a tandem array of genes termed APOBEC3A-H. Several members of this family have been found to have potent activity against human immunodeficiency virus type 1 (HIV-1). The expression and distribution of APOBEC family members in the Central Nervous System (CNS) is currently unknown. In this study, we have shown that APOBEC-3B/3C/3F and -3G are expressed in the major component cells of CNS, namely differentiated post-mitotic mature neurons, primary human astrocytes, and primary human brain microvascular endothelial cells (MVECS).

Moreover, we have demonstrated that both interferon-alpha (IFN-a) and IFN-gamma (IFN-g) significantly enhance the expression of APOBEC-3G/3F and drastically inhibit HIV-1 replication in primary human brain MVECS, the major component of blood brain barrier (BBB). As the viral inhibition can be neutralized by APOBEC3G-specific short interfering RNA (siRNA), APOBEC3G plays a key role to mediate the anti-HIV-1 activity of IFN-alpha and/or IFN-gamma. Our findings suggest that, in addition to the restriction at viral entrance level, the restriction from APOBEC3 family could account for the low-level replication of HIV-1 in MVECS. Given the marked response to IFN stimulation in MVECS, the manipulation of IFN-APOBEC3 signaling pathway could be a potent therapeutic strategy to prevent HIV invasion to CNS.

## P.15

### Predictors of progression from HIV-associated minor cognitive-motor disorder to HIV-dementia

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Background. HIV-associated neurocognitive disorders, such as minor cognitive-motor disorder (MCMD) and dementia, are common and seriously debilitating illnesses. The exact pathogenesis of such disorders remains unclear, but the use of highly active antiretroviral therapy (HAART) can improve clinical deficits and potentially reverse neuronal damage. The objective of this study was to determine the ability of neurocognitive tests to predict the progression from HIV-associated minor cognitive-motor disorder (MCMD) to HIV-associated dementia.

Methods. The Northeast AIDS Dementia (NEAD) Consortium cohort study enrolled HIV positive patients (n = 406) in four academic centers with a CD4 cell count <200 cells/uL, or <300 cells/uL with evidence of cognitive impairment. Individuals with MCMD (as defined by the Memorial Sloan Kettering (MSK) stage = 0.5) at baseline and who had completed neurocognitive assessment, and who were seen 12 months after baseline were included in this analysis (n = 96). Progression to HIV-associated dementia at 12 months after baseline, as defined by the MSK scale score greater than or equal to 1. We examined the ability of a variety of neurocognitive assessment z-scores (adjusted for age and education) and other patient characteristics at baseline to predict progression to HIV-dementia through logistic regression and ROC curve analysis.

Results. A logistic regression model intended to predict progression to HIV-dementia from MCMD was developed using baseline age (OR = 5.93, 95% CI: 1.42 to 24.73, p = 0.015), Rey Delayed Recall Z-Score (OR = 0.29, 95% CI: 0.13 to 0.65, p = 0.003), depression as defined by the Beck Depression Inventory ≥16 (OR = 0.21, 95% CI: 0.04 to 1.01, p = 0.052), Karnofsky Performance Score (OR = 0.90, 95% CI: 0.84 to 0.97, p = 0.004), and the Rey Complex Figure Immediate Recall Z-Score (OR = 0.57, 95% CI: 0.31 to 1.05, p = 0.072). A receiving operator characteristic (ROC) curve analysis was performed, and the logistic regression model predicted progression from MCMD to dementia with an area under the curve (AUC) of 0.87; at a probability cutpoint of 0.25, the model had a sensitivity of 80.0%, a specificity of 80.3%, and a positive predictive value of 51.6%.

Conclusions. This logistic regression model may have clinical utility in predicting which patients will progress from MCMD to dementia; the model may therefore be able to guide HAART regimen modifications to prevent progression to more severe neurocognitive impairment.

## P.16

### Biomarkers for temporal change of cognitive status in patients infected with HIV

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The neurological manifestations of HIV-infection are considerably different since the advent of highly active antiretroviral therapy. In general, the degree of neurological impairment is less severe, and many people infected with HIV-1 develop a chronic condition of minor cognitive motor impairment. With antiretroviral therapy, there can be bidirectional transitions in cognitive status, and the onset of dementia is no longer a predictor of mortality. In this new era of HIV-dementia, many of the biomarkers that previously associated with dementia are now of limited utility, and new ways of classifying patients, and identifying biomarkers are beginning to emerge. One suggestion has been to categorize patients according temporal changes in cognitive status. This approach has the advantage of potentially identifying biomarkers that are associated with-, and those can predict neurological disease. In this study we sought to identify biomarkers in CSF that could predict the onset of dementia and markers that were associated with dementia in patients infected with HIV-1. HIV-infected patients from the North Eastern AIDS Dementia (NEAD) cohort were grouped based on changes in cognitive status over a 1-year time period. Sphingolipid, sterol, triglyceride, antioxidant, pro-oxidant, and lipid peroxidation levels were quantified from CSF. We found that increased

levels of the antioxidant vitamin E, triglyceride C52, and iron predicted the onset or worsening of dementia. Increased levels of sphingomyelin were associated with an inactive dementia. Increased ceramide, with the accumulation of lipid peroxidation products were associated with an actively progressing dementia. These findings suggest that early in the course of HIV-dementia, there is an up-regulation of endogenous antioxidant defenses in brain. The exhaustion of these endogenous neuroprotective mechanisms is followed by increased concentrations of sphingomyelin, and moderate neuronal dysfunction that is clinically apparent as an inactive dementia. In an active dementia, ceramide, and reactive aldehyde levels are elevated, consistent with an ongoing degenerative condition. Thus, an increase in endogenous antioxidant defenses may predict, while disruptions in lipid balance are associated with dementia in patients infected with HIV-1.

## P.17

### Critical review on a possible role of Borna disease virus infection in neuropsychiatric disorders

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Introduction: After the first description of BDV specific serum antibodies in depressed patients in 1985 a large number of seroepidemiological studies on BDV antibodies in patients with neuropsychiatric disorders have been published, leading however to controversy. A critical review seems appropriate concerning a possible impact of BDV infections in humans.
Method: Published results are discussed regarding methodological aspects, contradicted and replicated findings and compared to standards in other CNS infections including our considerations, investigations and methods - based on the results of extended own studies in large neuropsychiatric and in various human control samples, and of investigations of horses with acute and chronic BDV infections.
Results: Considering exclusively certified methods and confirmed findings and the established gold standard in neurological medicine, a relatively clear picture arises. BDV infection seems to share many aspects with other neurotropic virus infections: 1. The pathogenicity appears low, probably below 10% of infected humans. Nevertheless, epidemiological studies on the prevalence BDV serum antibodies suggest that BDV infection may be responsible for or contributed to pathogenesis of psychiatric disorders in 1 – 3% of psychiatric hospitalized patients. The specificity of the human BDV antibodes in serum and CSF was confirmed by micro array analysis. 2. The sensitivity of methods changes with the time of infection: in acute infection, only during a short time period of few days virus is detectable in CSF but not in blood. Later, chronic infection or chronic autoimmune reaction can develop, detected indirectly by demonstration of specific intrathecal immune reactions or directly by investigation of the brain (possible in animals, but rarely in humans). Such intrathecal BDV specific immune reaction was found in few human cases with affective or schizophrenic spectrum disorders, indicating a causal relationship to BDV infection.
Conclusion: Prevalence and specificity of serum and CSF antibodies suggest that BDV or a related agent can infect humans. But up to now the proof is lacking that BDV infection can be pathogenic for humans, though CSF findings indicate that in rare cases of affective or schizophrenic spectrum psychoses BDV was presumably the causal agent. Epidemiological findings may suggest that BDV infection could be of some relevance or even causal for psychiatric disorders in 1–3% of hospitalized psychiatric patients.

## P.18

### Immunophenotypic diversity of brain macrophages: implications for neuroAIDS

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Infection of humans with HIV or macaques with SIV often leads to an encephalitis (HIVE or SIVE) characterized by perivascular accumulations of macrophages and multinucleated giant cells in brain, and many of which are productively infected. It is assumed that the development of SIVE/HIVE is associated with increased migration (and retention) of circulating monocytes into the brain. The immigration of these monocytes into the brain would facilitate neuroinvasion and provide additional cells for infection. Direct assessment of this hypothesis has been frustrated by the lack of specific markers that can easily differentiate among perivascular macrophages, recently immigrated monocytes, and parenchymal microglia. Recently, expression of CD14 and CD45 in conjunction with CD11b has been used to differentiate parenchymal microglia (CD11b+CD14–CD45–) from perivascular macrophages (CD11b+CD14+CD45+) and define the latter as the primary cell type productively infected in the CNS. It was clear, however, that the population of SIV-infected cells that shared expression of CD11b, CD14 and CD45 was heterogeneous. This suggested that we might be looking at several populations of cells including those that had recently immigrated from the blood. To assess this further and try to define the immunophenotype of additional populations of brain macrophages, including those that has recently entered the CNS, we performed multilabel confocal microscopy combined with in situ hybridzation (ISH) for SIV for a variety of cell markers which are known to be expressed by macrophage population. The markers used in this study included CD11b, CD45, myeloid histocyte antigen (clone MAC387), myeloid related proteins 8 (MRP8), CD163, LN5, CD68 and HAM56.

Major observations include the following: 1) Common macrophage markers such as HAM56 and CD68 colocalized nearly completely and are seen primarily within SIVE lesions. Many such cells are also infected by SIV; 2) CD163 largely colocalized with CD68 and HAM56 within SIVE lesions. Many of these cells were infected with SIV. CD163 was also expressed in cells morphologically compatible with microglia throughout the CNS of animals with SIVE and others encephalitides. No such labeling was seen in normal animals. None of the CD163+ cells outside of classic SIVE lesions were SIV positive; 3) LN5 are expressed in SIV infected cells with or without colocalization of CD68 and CD163 in the SIVE lesions, whereas uninfected cells also expressed LN5 around SIVE lesions; and 4) MAC387 and MRP8 primarily labeled cells within vessels or immediately adjacent to vessels, including those in SIVE lesions. However, none of these cells were SIV+ nor CCR5 by ISH or IHC, respectively. This data indicates that the cellular infiltrates of monocyte/macrophages in SIVE are heterogeneous. The presence of MAC387+ and MRP8+ cells not infected by SIV could represent either recently recruited monocyte/macrophages or a population of macrophages resistant to infection. Lastly, CD163 appears to label "activated" microglia as no labeling of parenchymal cells which was seen in normal animals, while in animals with encephalitis CD163 labeling was extensive.

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## P.19

### Search for molecular markers of HIV neuropathogenesis by gene expression profiling of brain tissues.

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Neurodegeneration and cognitive impairment caused by HIV-1 infection are common complications of AIDS. Introduction of highly active antiretroviral therapy reduced the incidence of HIV-1-associated dementia with active viral replication in brain (HAD with encephalitis, or HIVE) but so far has had limited effect on the high frequency of milder neurological disorders. The latter alter include HAD without encephalitis and Minor Cognitive and Motor Disorder (MCMD), both of which can present with no or minimal histopathological changes in the brain. The prevalence of MCMD can reach 30-35 percent in patient cohorts followed in the U.S.A., regardless of their antiviral treatment status. Clearly there is a need for better understanding and treatment of these milder CNS manifestations of HIV.

We are trying to identify molecular markers of HIV infection in the CNS that can supplement or serve in lieu of brain histopathology for characterization and design of better treatments of HIV associated neurological disorders. For the study presented here, we selected archived, snap-frozen brain tissues from 14 HIV-positive individuals who were consented participants in an ongoing prospective organ donation program run by the Manhattan HIV Brain Bank (MHBB), a member of the National NeuroAIDS Tissue Consortium. All the tissues were from the anterior frontal lobe and for each patient they were sub-dissected into separate white (WM) and grey (GM) matter samples while still frozen. As controls, we selected similar sets of brain tissues as above from 6 HIV-negative individuals who died without neurological symptoms and had no pathological changes. Overall, this study included 40 brain samples from 20 subjects: 6 controls, 6 HAD with HIVE (HAD/HIVE), and 8 with HIV-associated cognitive impairment without HIVE: 4 HAD, 1 who had fluctuated between HAD and MCMD in their lifetime, and 3 MCMD. For the purpose of some microarray and statistical analyses, MCMD and HAD without HIVE were treated as a single brain-symptomatic category (HAD/MCMD) totaling 8 subjects. The 40 subdissected brain samples were further subdivided into smaller adjoining fragments without thawing; for each pair of adjoining WM and GM fragments one was tested for HIV RNA and DNA burdens by real time PCR and the other was subjected to gene expression profiling on a Affymetrix U133Plus2 oligonucleotide microarray platform. We found that HIV burdens in brains from HIVE patients were highly variable. HIV DNA burdens in brains from HAD/MCMD subjects were either very low or undetectable and none of these patients had detectable viral RNA. Like in HAD/HIVE samples, there was a limited correlation between brain virus burdens and peripheral virus. Next we compared gene expression profiles obtained from GM and WM of HAD/HIVE and HAD/MCMD groups of patients. Microarray results for the most relevant genes were confirmed by Real Time PCR and changes in some gene products were confirmed by immunohistochemistry in brain sections and Western blotting in tissue extracts. The initial analyses indicate that both HAD/HIVE and HAD/MCMD brain tissues exhibit extensive, although for the most part different, changes in their cellular gene expression profiles. In HAD/MCMD, molecular changes in the brain were independent of virus burdens in the tissue. Full results will be presented. The approach described here may facilitate identification of cellular pathways or individual genes important in HIV mediated neuropathogenesis, with or without active viral replication in brain.

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## P.20

### Varicella zoster virus induced apoptosis in cultured cells proceeds through the intrinsic pathway

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Primary infection by varicella zoster virus (VZV) usually results in chickenpox (varicella), after which virus becomes latent in multiple ganglia along the entire human neuraxis. Virus reactivation decades later produces shingles (zoster). Previous studies have shown that VZV induces apoptosis in non-neuronal cells, but not in neurons. The cascade of apoptosis proceeds through intrinsic (mitochondrial) or extrinsic (death receptor) pathways. To elucidate the pathway utilized by VZV, we used Western blot analysis to compare VZV-infected and uninfected MeWo cell lysates for the presence of various pro-apoptotic proteins (caspases) and anti-apoptotic proteins at 24, 40, 48, 64 and 72 hours post infection. Caspases are a group of cysteine-rich proteases that are sequentially cleaved to active forms that induce apoptosis. Active forms of caspases 9, 3, 7 and 6 as well as the cleaved fragments of the DNA degrading protein PARP were increased in VZV-infected cells as compared to uninfected cells beginning 40-48 hours post infection. A reduction in the anti-apoptotic protein Bcl-2 was also seen. Levels of caspase 8, an extrinsic pathway marker, did not change during the course of infection. Apoptosis produced by VZV utilizes the intrinsic (mitochondrial) pathway in non-neuronal cells.

## P.21

### Searching for novel therapeutics with anti-JCV activity among known bioactive drugs and experimental compounds

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Progressive multifocal leukoencephalitis (PML) is a rare but frequently fatal disease caused by uncontrolled replication of JC polyomavirus in the brain of some immunocompromised individuals. Currently, no effective anti-viral treatment has been found despite a number of clinical trials. The best option in the management of PML is offered by reconstitution of patients' own immunity either via HAART therapy in HIV+ individuals or via reduction of an immunosuppressive treatment regimen in pharmaceutically immunosuppressed individuals. Since effective restoration of the immune response is not always possible or timely enough, finding an effective anti-JCV therapy is highly desirable. As a first step in the identification of such therapy, we undertook screening of a collection of ~1500 approved drugs and biologically active molecules, for anti-JCV activity. We looked for drugs that effectively inhibited active virus production of a hybrid JC virus Mad1/SVEdelta in SVG-A cells, a JC virus susceptible human glial cell line. We identified a number of different drugs and compounds with significant anti-JCV activity at micromolar concentrations without any significant toxicity to the cells. Active compounds form several structurally distinct classes with well-defined structure-activity relationship. Reported pharmacokinetic values promise to achieve comparable plasma concentrations for some of the drugs we identified. Implications of these findings for further steps in identification of human PML therapy will be discussed.

## P.22

### Redox-based regulation of macrophage inflammation by opiates and HIV Tat

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HIV-1 patients who abuse opiate-based drugs have increased rates of HIV dementia, which may be related to brain inflammation. To better understand how opiates could derange HIV-related brain inflammation, the effects of morphine and HIV-Tat on free radical production and redox-based inflammatory signaling was measured in primary macrophages and microglia. Data show that administration of Tat to either macrophages or microglia stimulated the production of free radicals, which were key to the subsequent production and release of cytokines. However, co-administration of morphine with Tat significantly increased Tat-induced free radical production and intracellular oxidation, while significantly decreasing cytokine release. Finally, data show that the ability of free radicals to control inflammatory signaling may be based on oxidative alterations to the multicatalytic proteasome and/or on differential recruitment of free radical–producing enzymes to lipid raft signaling platforms. Together, these findings suggest that free radical production in monocytic cells could be a key mechanism controling cytokine release, and that this mechanism is subject to regulation by opiates. Supported by RO1 NS046267, P20 RR15592, and P01 DA19398.

## P.23

### HIV and cocaine interplay: It is all in the had

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HIV-1 infection commonly leads to serious HIV-1-associated neurological disorders, such as HIV-1-associated encephalopathy and dementia. Intravenous drug use (IVDU) and HIV infections are two linked global health crises since needle sharing is a well recognized mode of HIV transmission. Cocaine, often

abused by HIV-infected patients, has been suggested to worsen the HIV-associated dementia via unknown mechanisms. Since brain is the target organ for both cocaine and HIV, the objective of the present study was to explore the effects of cocaine on virus replication in macrophages, the target cells for the virus in the CNS. Cocaine markedly enhanced virus production in Simian Human Immunodeficiency Virus (SHIV)-infected monocyte derived macrophages (MDMs) and in U1 cells, a chronically infected promonocytic cell line as monitored by ELISA and immunocytochemistry. Cocaine treatment also resulted in the activation of NF-$\kappa$B and transcriptional activation of the HIV-LTR gag-GFP. Analyses of chemokines in cocaine-treated macrophages by Realtime RT-PCR and Luminex assays suggested increased expression of the chemokines, CXCL10 – CCR2 and the cytokine, IL-10, all of which are known to promote HIV replication in MDMs. In addition to enhancing cytokine/chemokine expression, cocaine also caused an up-regulation of the macrophage activation marker, HLA-DR in MDMs. The synergistic effect of cocaine on virus replication and its enhancement of host activation markers suggest that cocaine functions at multiple pathways to accelerate HAD.

## P.24

### PDGF synergistically enhances IFN-? induced expression of CXCL10 in blood-derived macrophages: implications for HIV-dementia

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There is increasing cumulative evidence that activated mononuclear phagocytes (macrophages/microglia) releasing inflammatory mediators in the CNS are a better correlate of HIV associated dementia (HAD) than the actual viral load in brain. Earlier studies on Simian Human Immunodeficiency Virus (SHIV)/rhesus macaque model of NeuroAIDS confirmed that pathological changes in brains of macaques with encephalitis were associated with upregulation of platelet-derived growth factor and the chemokine, CXCL10. Since complex interplay of inflammatory mediators released by macrophages often leads to the induction of neurotoxins in HAD, we hypothesized that PDGF could interact with IFN-? to modulate the expression of CXCL10, in these primary virus target cells. While PDGF alone had no effect on induction of CXCL10 in human macrophages, in conjunction with IFN-?, it significantly augmented the expression of CXCL10 RNA & protein through transcriptional and post-transcriptional mechanisms. Signaling molecules such as JAK and STATs, PI-3 kinase (PI3K), MAP kinases and NF-$\kappa$B were found to play a role in the synergistic induction of CXCL10. Furthermore, PDGF via its activation of p38 MAP kinase was able to increase the stability of IFN-?-induced CXCL10 mRNA. Understanding the mechanisms involved in the synergistic upregulation of CXCL10 could aid in the development of therapeutic modalities for HAD.

## P.25

### Inhibition of CXCR4-mediated G protein stimulation in the cortex and the hippocampus of morphine-treated animals

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The chemokine CXCL12/SDF-1a and its specific receptor (CXCR4) have been increasingly studied in the CNS due to their critical roles in neuronal patterning and survival. CXCR4 is also one of the major HIV-1 co-receptors and is involved in HIV neuropathogenesis. Recent evidence suggests that drugs of abuse, including opiates, can facilitate progression to neuroAIDS. The goal of this study is to establish whether in vivo morphine treatment alters CXCR4 coupling to G proteins. This hypothesis is based on previous studies suggesting an interaction between CXCR4 and mu-opioid receptors in cultured neurons and brain slices. The [35S]GTPgammaS incorporation assay was used to determine CXCL12-induced stimulation of GTP "binding" in various brain areas. Rat pups (8-14 days old) were used for these experiments since they showed similar regional distribution of G protein stimulation in response to CXCL12 and MOR agonists. Animals were treated with Morphine for short (1-24 hrs) or relatively long (3-5 days) time periods and GTPgammaS autoradiography was performed at different times. Brain sections from vehicle and morphine-treated animals were incubated with saline or CXCL12 (1-100nM) in the presence or absence of the CXCR4 antagonist AMD3100 (100ng/ml). The data show a reduction in CXCR4 coupling to G proteins after morphine pre-treatment in several brain areas, including cortex and hippocampus. These findings support the hypothesis that opioids may alter CXCR4 function and are supported by our studies with cultured cortical neurons. We propose that impairment of CXCR4 by opiates contribute to the neurological deficits associated with HIV infection. (Supported by NIH grants DA15014 and DA19808 to OM, and DK67648 to KJS).

## P.26

### The intrathecal B cell repertoire in subacute sclerosing panencephalitis brain shares most features with the plasma cell repertoire

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A prominent immunologic feature of subacute sclerosing panencephalitis (SSPE), a chronic fatal encephalitis caused by measles virus, is the presence of increased IgG and bands of oligoclonal IgG in brain and CSF that are directed against measles virus. To develop strategies and techniques to analyze oligoclonal IgG in chronic inflammatory CNS diseases of unknown etiology, we previously demonstrated that antibodies from most CD38+ plasma cells in SSPE brain are directed against measles virus. Herein, we analyzed the repertoire of CD20+ B cells in the same brain, to compare the antibody response in the two groups of cells.

B lymphocytes were identified in frozen sections of postmortem SSPE brain by immunostaining for CD20, and individual cells were microdissected with a nondestructive infrared laser. cDNA was synthesized from unfractionated cell lysates, and sequences of specific IgG heavy/light chain pairs expressed by each B cell were determined by nested RT-PCR amplification. A repertoire of 51 cells from a single SSPE brain revealed features of antigen-driven selection and affinity maturation in both the overrepresented clonal populations as well as in cells encountered only once in the repertoire. Seven of the nine clonally expanded sequences from B cells were also found in the plasma cell repertoire, with virtually identical patterns of mutation from germline sequences. Most nonclonal sequences in the B cell and plasma cell repertoires did not overlap, and may reflect the limited number of sequences analyzed in each repertoire. Overall, IgGs expressed by B-lymphocytes and plasma cells in SSPE share many clonal features and specificities. These techniques can be applied to other chronic inflammatory CNS diseases of unknown cause, such as multiple sclerosis, CNS sarcoid and Behcet's disease, to determine the disease-relevant targets of the immune response.

## P.27

### Dopamine inhibits claudin 5 and caveolin 1 expression in human brain microvascular endothelial cells and HIV induces the novel expression of D2 dopamine receptors in activated human T lymphocytes: possible role in T lymphocyte transmigration across the blood brain barrier

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Although highly active antiretroviral therapy (HAART) has proven very effective in suppressing human immunodeficiency 1 (HIV) replication and viral-mediated dysregulation of the immune system, central nervous system (CNS) complications of HIV infection persist. The prevalence of cognitive, motor and behavioral abnormalities has increased in the HAART era as infected individuals live longer on antiretroviral therapies. HIV enters the CNS early after infection and significant numbers of infected individuals develop a wide spectrum of CNS abnormalities. Although neurons are not productively infected by HIV, neuronal damage results from secondary effects of HIV infection of macrophages and microglia, and from subsequent inflammatory cascades initiated and propagated within the brain. The entry of HIV into the CNS is thought to occur by the transmigration of infected monocytes across the blood brain barrier (BBB). Thus leukocyte transmigration is postulated to play an important role in the neurological pathogenesis of HIV infection. CNS damage elicited by HIV infection is compounded by addictive drugs. Evidence suggests that in HIV infected individuals who are also drug abusers, accelerated and more severe neurocognitive dysfunction occurs when compared to non drug abusing infected populations. In some studies, increased localization of lymphocytes, including CD8+ T lymphocytes, to perivascular and parenchymal regions of the CNS has been detected in tissue sections from presymptomatic HIV infected drug abusers when compared to uninfected drug abusers or non drug using HIV infected individuals. This chronic T lymphocyte infiltration may contribute to enhanced neurological damage in infected drug abusers. Drugs of abuse share common stimulatory effects on dopaminergic pathways, which contribute to their rewarding properties. The addictive and reinforcing effects of many drugs, including cocaine and methamphetamine, have been attributed to elevated synaptic levels of dopamine. In studies with simian immunodeficiency virus (SIV)-infected monkeys, increased CNS levels of dopamine were associated with enhanced numbers of perivascular and parenchymal lymphocytes. In order to determine the effects of dopamine on T lymphocyte transmigration across the BBB, we first treated human brain microvascular endothelial cells (BMVEC) with dopamine. Our studies show that BMVEC express D1, D2, D3 and D4 dopamine receptors and that dopamine treatment (20 umol for 6 hr) decreases the expression of caveolin 1 and the tight junction protein (TJP) claudin 5 in BMVEC. Activated (PHA and IL-2) human T lymphocytes express D1, D3 and D4 dopamine receptors and HIV infection induces the unique, additional expression of the D2 dopamine receptor in this cell type. These data suggest that elevated levels of dopamine in HIV infected drug abusers may alter the BBB by modulating the expression of TJPs in BMVEC. Dopamine also inhibits caveolin 1, which has been shown to contribute to TJ disruption in epithelial cells, as well as to changes in transcellular versus paracellular T lymphocyte transmigration across EC. In addition, the unique induction of D2 dopamine receptor expression in HIV infected T lymphocytes may contribute to changes in T lymphocyte transmigration across the BBB in response to chemokines expressed in the brain, in particular CXCL12 (SDF-1). Thus, dopamine may alter the BBB and exacerbate T lymphocyte infiltration into the CNS, resulting in a chronic inflammatory T lymphocyte response that contributes to BBB disruption, HIV entry and infection of parenchymal cells, and neuronal damage.

## P.28

### Efficient expression of sTNFR-Fc in stably transduced mononuclear phagocytes and neuronal cells: implications for neuroAIDS therapeutics

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Human immunodeficiency virus type (HIV-1) infection and immune activation of mononuclear phagocytes (MP; perivascular macrophages and microglia) is directly linked to the neuropathogenesis of HIV-1 infection. Indeed, viral and cellular products secreted from MP affect neuronal viability and cognitive function in disease. Amongst these factors, tumor necrosis factor-? (TNF-?) stands as one that elicits paracrine and autocrine production of a variety of inflammatory factors and neurotoxicity. Our works center on blocking TNF-? through the delivery of a soluble TNF receptor (sTNFR)-Fc fusion protein as a decoy for cytokine binding. To explore this as a potential approach for neuronal protection, a HIV-1-based lentiviral vector containing an expression cassette for sTNFR-Fc was constructed. To facilitate detection of transduced cells, cells co-expressed the green fluorescent protein (GFP) using an IRES element. High-titer vector stocks were used to transduce rodent bone marrow derived macrophages (BMM), human microglial (CHME-5) and neuroblastoma (HTB-11) cells. At a multiplicity of infection of 10, approximately > 50% of the cells were GFP positive and increased to nearly 100% when cells were transduced a second time with the vector. Transduced cells showed no morphological alteration as compared to control cells, and no loss in viability was detected upon MTT assay. Secretion of sTNFR-Fc from these transduced cells was demonstrated using Western blotting. High, stable level of sTNFR-Fc was detected in the media of all the transduced cell cultures by ELISA at 116.8 to 520.0 ng/mL. The expressed sTNFR-Fc was stable in cell culture medium, and the biological activity of this fusion protein was confirmed by in vitro protein binding assay using recombinant TNF-? and neuroprotection experiments using primary neurons exposed to recombinant HIV-1-Tat. BMM were shown to directly penetrate areas of diseased brain with active HIV-1 encephalitis in a murine model of human disease. These results establish the feasibility of using lentiviral vectors to express sTNFR-Fc as a potential novel therapeutic intervention for Neuro-AIDS.

## P.29

### JCV infection of human brain microvascular endothelial cells and B lymphocytes: Potential mechanisms for JCV transmigration across the blood-brain barrier

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Background: The mechanism(s) of the transmigration of human polyomavirus JC (JCV) across the blood-brain barrier (BBB) remains unclear. Hypothesis: We hypothesized that JCV crosses the BBB by infecting human brain microvascular endothelial (HBMVE) cells and B lymphocytes. Materials and Methods: To test this hypothesis, we compared the replication kinetics of JCV in HBMVE cells and B lymphocytes, and monitored the transmigration of cell-free JCV across an in-vitro BBB model. Results: Our data, based on the expression of JCV early and late genomes, mRNA transcripts and protein, conclusively demonstrated that JCV productively infects HBMVE cells and in-vitro, approximately 4% of JCV crosses the BBB. On the other hand, when B cells were infected with JCV, the JCV genome copy number decreased dramatically from day 0 to day 15 post-inoculation, and early and late JCV mRNA transcripts and early T antigen protein, were not detected in JCV-infected B lymphocytes. Moreover, immunofluorescence microscopy data indicated that the number of JCV-infected B cells decreased from 10% on day 0 to 0% on day 15 post-infection suggesting that JCV infection of B lymphocytes is non-productive. However, JCV-infected B cells were capable of transmitting JCV infection to PHFG cells and the DNase protection assay confirmed that the JCV virions remained intact inside the B cells for several days. Conclusions: Efficient replication of JCV in HBMVE cells, the critical component of BBB, suggests that JCV may cross the BBB by infecting HBMVE cells and non-productively infected B cells may also serve as a vehicle to transport JCV across the BBB.

## P.30

### Cytomegalovirus infection and interferon gamma modulate MHC class I expression on neural stem cells

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Cytomegalovirus (CMV) is the leading cause of congenital brain abnormalities in children and ventriculoencephalitis in AIDS patients. Many of the pathologies associated with CMV brain infection are seen predominantly in the periventricular region, which is known to harbor neural stem cells (NSCs). In the present study, using an adult murine model for CMV brain infection, we demonstrated that nestin-positive NSCs in the subventricular zone were positive for the

virus. This infection was further characterized using monolayer NSC cultures. NSCs supported productive murine CMV (MCMV) replication; demonstrating 3-4 Log increases in viral titers and peak gB expression at 3 days postinfection (d p.i). In addition, MCMV infection induced a robust infiltration of peripheral immunocytes into the brain at 5 d p.i. RNA expression analysis of FACS sorted brain leukocyte populations demonstrated interferon (IFN)-gamma expression in CD45(hi)CD11b(dim) cells. To further characterize the effects of neuroinflammation and viral infection on NSCs, MHC class I expression and cell proliferation characteristics were studied. IFN-gamma and tumor necrosis factor (TNF)-alpha increased MHC I expression on NSCs. On the other hand, viral infection inhibited IFN-gamma-induced MHC I expression. Furthermore, IFN-gamma, but not TNF-alpha, IL-1beta, or IL-10, suppressed NSC proliferation in vitro. Extracellular release of lactate dehydrogenase (LDH) was not altered in IFN-gamma treated cultures suggesting that this cytokine may inhibit cell division. It can be concluded from these studies that NSCs are susceptible to MCMV infection and that inflammatory mediators, such as IFN-gamma, alter stem cell function.

## P.31

### Death induction of human immunodeficiency virus-infected macrophages using anti-PI3K/Akt pathway agents

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The interaction of Human Immunodeficiency Virus Type 1 (HIV-1) with CD4+ T lymphocytes is well-studied, and typically results in virally-induced cytolysis. In contrast, relatively little is known concerning the interplay between HIV-1 and macrophage/microglia in terms of cell fate. A recent report suggested that, counter-intuitively, HIV-1 infection may extend the lifespan of these cells. Indeed, we have found that expression of a single virus-encoded protein, HIV-1 Tat, as well as HIV-1 infection, results in a powerful cytoprotective effect in a microglial cell line. Previous mechanistic studies in our lab showed that the pro-survival effects of intracellular Tat could be attributed to activation of the PI-3-Kinase (PI-3-K)/Akt pathway. Further dissection of this mechanism revealed that both HIV-1 infection as well as expression of HIV-1 Tat significantly reduced levels of PTEN, a negative regulator of this pathway. As a result, we also observed greatly increased Akt kinase activity following transduction of macrophage with an HIV-1 vector. Consistent with this, immunoprecipitation studies revealed that following binding of intracellular HIV-1 Tat to p53, the ability of PTEN to bind to p53 was drastically reduced. Since the binding of Tat to p53 has been shown to occur through the basic domain of Tat, we constructed a Tat basic domain mutant. Interestingly, this mutant had elevated levels of PTEN and lacked the cytoprotective effect exerted by HIV-1 Tat. Based on these findings, a model of possible regulatory circuits that intracellular Tat and HIV-1 infection engage during this cytoprotective event in macrophage and microglia was envisioned. To attempt to antagonize this regulatory circuit, we tested the effect of PI3K and specific Akt inhibitors on viral replication and the survival of HIV-1 infected macrophages. Inhibitors of PI3K and Akt kinase, including miltefosine and perifosine which are currently being tested in clinical trials, were able to drastically reduce HIV-1 production in macrophages, due to the increased incidence of cell death in HIV-1 infected macrophages exposed to inhibitor. In conclusion, we propose that the expression of Tat may enable HIV-1 infected macrophages to serve as long-living viral reservoirs, leading to persistent infection and HIV-1 production.

## P.32

### Mechanisms of herpes simplex virus type 1 (HSV-1) infection of mice brain organotypic cultures

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Background: HSV-1 is an important cause of severe morbidity and mortality, including encephalitis. The mechanisms of HSV-1 penetration into the brain and its predilection to infect certain neuronal regions are unknown. We previously reported that HSV-1 has a restricted and specific pattern of infection of mice brain in organotypic cultures and that the neonate brain is more permissive than the mature adult brain for infection (Braun et al, J. Gen. Virol. 2006). We have used this system to follow and characterize HSV-1 infection, the role of the extracellular matrix (ECM), and the mechanism of viral access into the infected cell. Materials and Methods: Following brain tissue extraction from neonate mice, the tissues were treated with either collagenase, hyaluronidase, heparin, heparinase, acyclovir or 6 different agents that interfere with endocytosis and infected the mice brain tissue slices with HSV-1 mutants expressing reporter genes. Pattern and extent of HSV-1 infection was characterized and measured. Infection with adenovirus was used as control. Results; Collagenase, an enzyme responsible for collagen degradation did not affect the pattern and extent of HSV-1 infection of brain tissue slices (compared to skin slices infected with HSV-1 that were affected), but hyaluronidase did. Heparinase abolished infection, as did pre incubation of HSV-1 with heparin.

Pre-treatment of the brain slices with acyclovir did not prevent infection and reporter gene expression. Using the agents that interfere with endocytosis we were able to demonstrate that infection takes place mainly via membrane fusion. Conclusions: Brain ECM plays a major role in brain infection in this system. HSV-1 infection is mediated, at least in part via haparan sulphate receptors and internalization of HSV-1 is achieved mainly by membrane fusion.

## P.33

### Subclinical reactivation and shed of infectious VZV in saliva of astranaunts

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## P.34

### Comparison of different HIV-1 isolates of clades B and C on macrophage-mediated inflammatory factor, glutamate production and neurotoxicity

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HIV-1 clade C is currently responsible for more than 50% of new infections and is becoming the most commonly transmitted subtype worldwide. While HIV-1-associated dementia (HAD) continues to be the major neuropathological manifestation of AIDS among clade B-infected individuals in the US and Europe, a low incidence of HAD has been reported in countries like India and sub-Saharan Africa, where clade C is prevalent. Potential differences between clade B and clade C regarding neurovirulence and macrophage-mediated pathogenesis are yet to be investigated. Differential regulation of proinflammatory factors and neurotoxins (such as glutamate) by macrophages infected by different clades/strains may differentially induce neuronal injury and dysfunction, consequently altering neuropathogenesis. Human monocyte-derived macrophages (MDM) were infected for 4, 7, 14, and 21 days using the same TCID50 by 3 laboratory clade B strains, 4 primary clade B strains derived from HIV-1 infected brain, choroid plexus or spleen, and 2 pairs of clade C isolates from HIV-1 infected mothers (M) and infants (I). Viral infection was monitored by reverse transcriptase (RT) activity and characterized by proinflammatory factors MIP-1beta, MCP-1 and TNF-alpha production as determined by ELISA. The concentration of extracellular glutamate was measured in MDM supernatants by RP-HPLC. Primary rat cortical neurons treated with conditioned media from different viral strain-infected MDM were used to assess macrophage-mediated neurotoxicity. Laboratory strains (clade B) and 3 primary clade B strains (A-00-086, G0048 and D02-2562) reached highest levels of viral infection 7–14 days after infection, while clade C strains reached highest levels of viral infection at 21 days post-infection. MIP-1beta and MCP-1 production was associated with HIV-1 infection as determined by RT. The highest glutamate production and neurotoxicity induced by different viral strains in infected MDM ranked as: laboratory strains > primary clade B strains> primary clade C strains. Correlation analysis with all strains tested suggests a correlation between RT, glutamate production, and neurotoxicity induced by HIV-1 infected MDM.

## P.35

### HIV infection inhibits GM-CSF-induced microglial proliferation

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It is well known that infection by HIV dysregulates cell physiology, but little information is available on the consequences of HIV infection in primary macrophages and microglia. We examined the relationship between cell proliferation and HIV infection in primary cultures of microglia and in human CNS. In cultures infected with HIV (ADA and BaL), granulocyte macrophage colony-stimulating factor (GM-CSF)-mediated cell proliferation was reduced in productively infected (p24+) cells as compared to p24− cells. The reduction was observed with both Ki67 and BrdU labeling, suggesting a G1/S block. The reduction was insignificant when microglia were infected with a Vpr-mutant virus. In human CNS, proliferating (Ki67+) cells were rare but were increased in the HIV+ and HIV encephalitis (HIVE) groups compared to the HIV− group. We found a positive correlation between GM-CSF immunoreactivity and Ki67 counts, implicating GM-CSF as a growth factor in human CNS. The relationship between total macrophage (CD68+) proliferation and infected macrophage (p24+) proliferation was assessed in HIVE by double labeling. While 1.2% of total CD68+ cells were Ki67+, only 0.5% of

HIV p24+ cells were Ki67+ ($p < 0.05$). Furthermore, staining for CD45RB (as opposed to CD68) facilitated the identification of Ki67+ microglia, indicating that CD68 could underestimate proliferating microglia. We conclude that while there is increased expression of GM-CSF and increased cell proliferation in the CNS of HIV-seropositive individuals, cell proliferation in the productively infected population is actually suppressed. These data suggest that there might be a viral gain in the suppressed host cell proliferation.

## P.36

### Dynamics of NF-kB activation by HIV-1 Tat and CD40L in microglial cells

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HIV-1-associated dementia (HAD) occurs in a significant number of HIV-1 infected individuals, in part, due to the inflammatory response to viral proteins in the central nervous system. We have shown that host factors, such as soluble CD40 ligand (sCD40L) that are found to be elevated in the cerebrospinal fluid and plasma of HAD patients, synergize with HIV-1 encoded Tat to activate microglial cells in an NF-kB-dependent manner. Here we demonstrate that, besides proteolysis of the inhibitory molecules IkB-alpha, the activation of NF-kB by Tat in microglial cells is associated with de novo synthesis of RelB, and processing of p100 (as defined by generation of the processing product p52), followed by translocation of RelB and p52 into the nucleus. However, under these conditions, neither RelB, nor p52, exhibited DNA binding activity at a cis-acting NF-kB responsive element. We further show that the simultaneous exposure to Tat and CD40L promotes DNA binding activity of p52, but not of RelB. Finally, ectopic expression of processing-deficient mutant p100, as well as RelB-specific short hairpin RNA (shRNA), revealed that the biogenesis of p52 and RelB in Tat plus CD40L-treated microglia is crucial for the synthesis of TNF-alpha. Taken together, these studies highlight the mechanism by which viral proteins and host factors (like Tat and CD40L) work in concert to activate NF-kB in microglial cells and explain how this process is dynamically controlled.

## P.37

### HIV-1 Tat inhibits NGF-induced Egr-1 transcriptional activity and consequent p35 expression in human neuronal cells, resulting in apoptosis

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In the context of neuroAIDS, the HIV-1 transactivator protein Tat and the host neurotrophin NGF are important regulators of neuronal cell survival and are involved in the modulation of the cell cycle, differentiation, cytoskeleton dysfunction and apoptosis. We reported recently that Tat dysregulates the NGF-signaling pathway, including suppression of Egr-1, a key pleiotropic mediator of the expression of genes involved in cell growth. NGF induces the expression of Egr-1, which in turn induces p35, an activator of CDK5 by binding to its three Egr-1 binding sites in the promoter region. Here, we investigate the impact of HIV-1 Tat on the NGF-induced Egr-1 transcriptional activity in SK-N-MC neuroblastoma cells. Our data from Western-blot analysis show that Egr-1 and p35 protein levels are elevated in NGF-treated SK-N-MC cells, but not in Tat-expressing cells. We have assessed functional interactions between Tat and Egr-1 transcription factors and demonstrated that whereas Tat itself has no strong effect on the p35 promoter, it weakly synergizes with Egr-1 to induce expression of a p35 promoter-driven reporter in the absence of NGF. Our results from reporter assays revealed inhibition of NGF-induced Egr-1 activity in NGF-treated cells. NGF treatment of SK-N-MC cells increases the binding of Egr-1 to the 232-bp element in the p35 gene promoter in ChIP assays, whereas expression of Tat protein reduces binding to p35 promoter. Tat also inhibits binding of Egr1 to Egr1-binding oligonucleotide driven from p35 promoter in electrophoretic mobility shift assay (EMSA). Our results from GST-pull down assays demonstrated that Tat physically interacts with Egr-1 through its central part and, thus, interferes with its NGF-induced activation of p35. Deletion of the basic amino acid region of Tat results in the loss of its binding ability to Egr-1, and can no longer affect Egr-1-mediated transactivation of the p35 promoter. These data suggest that in NGF-treated Tat-expressing cells, Egr-1 loses its ability to up-regulate p35 gene transcription and activate Cdk5/p35 complex, and this can contribute to the dysregulation of NGF signaling in neuronal cells. Cells expressing Tat produce less phosphorylated neurofilament NF-H, a substrate of the neuron-specific p35/Cdk5. This reduction was correlated with a decreased level of Cdk5 and low kinase activity of the p35/Cdk5, complex which plays a critical role in neuronal cell differentiation and survival. Tat had an apoptotic effect in neuronal cells in the presence of NGF, as shown by the increased level of the pro-apoptotic proteins Bax and Bad, Comet assay and DNA fragmentation assay. Tat also increased the level of the mitochondrial porin in NGF-treated cells, which plays an important role in apoptosis by recruiting pro-apoptotic proteins of the Bcl2 family to mitochondria. NGF treatment of Tat-expressing cells resulted in the increase of cleaved active caspase-3 level and induction of caspase-3 activation in the caspase-3 GLO assay. Altogether these data demonstrate that Tat dysregulates p35/Cdk5 signaling, inhibits the phosphorylation of the neuroskeletal protein

NF-H, activates pro-apoptotic proteins and caspase-3 cleavage, resulting in neuronal cell death.

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## P.38

### Possible role of Puralpha in neurovascular dysfunction in Alzheimer's disease through the inhibition of Meox2 expression

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Alzheimer's disease is the major cause of dementia in the elderly population. The exact causes of Alzheimer's disease are unknown, and it is likely that the etiology of the disease is heterogeneous. Neurovascular dysfunction is an important feature of Alzheimer's disease and could have a major impact on the pathogenesis of a chronic neurodegenerative condition. Mesenchyme Homeobox 2 (Meox2) regulates the transcription of genes necessary for many vascular cell processes, tube formation and differentiation of vascular smooth muscle cells and cardiomyocytes. Genes required for the cytoskeleton (SM alpha-actin), cell cycle (Cyclins, p21) and cell adhesion (integrins, uPA/uPAR, and Ephrins/Eph) are all downstream targets of Meox2. Meox2 is downregulated in the endothelial cells from blood vessels in Alzheimer's disease patient's brains. Decreased Meox2 levels reduced neurovascular angiogenesis in the Meox+/- mice. Low levels of expression of Meox2 may have an important role in neurovascular dysfunction in Alzheimer's disease resulting from the reduction of brain capillary density and vessel regression. The link between decreased Meox2 expression and impaired neurovascular functions in Alzheimer's disease makes meox2 homeobox gene an attractive target for the treatment of the disease. To analyze the factors which may play a role in the downregulation of Meox2 is important and will help to develop novel treatments for diseases that result from improper vessel function. Puralpha, a multifunctional protein, implicated in a variety of biological events, including transcription, replication and cell cycle, negatively regulates smooth muscle alpha-actin and beta-myosin gene expression in fibroblast and vascular smooth muscle cells, suggesting that it may play an important role in neurovascular formation. A possible link between low expression of Meox2 and impaired neurovascular functions in Alzheimer's disease, control of smooth muscle actin gene transcription by Meox2 and inhibitory regulation of SM alpha-actin by Puralpha, provided a rationale to analyze possible role of Puralpha in Meox2 expression, neurovascular dysfunction and Alzheimer's disease. Microarray assay from the cells stably expressing nuclear Puralpha demonstrated greater than 9 fold downregulation of meox2 gene. Inhibition of the level of expression of Meox2 protein was confirmed by Western blot analysis of cells expressing Puralpha. Expression of Meox2 was also low in mouse brain extracts, where a high level of Puralpha was present. In fibroblast cells expressing Puralpha a weak staining of Meox2 was observed. Study of the meox2 promoter and gene structure revealed multiple Puralpha GC-rich binding sites in the promoter region, including the 5′-UTR. Interestingly, antisence meox2 DNA contains eleven (GGT) repeats, known as a classical Puralpha-binding domain. Results from in vitro transcription/translation studies demonstrated that Puralpha blocked Meox2 translation. Altogether, in this study we have demonstrated that Puralpha inhibited the expression of Meox2. This observation, suggests that Puralpha may play an important role in neurovascular dysfunction in Alzheimer's disease through the inhibition of Meox2 expression, providing new insights for possible therapeutic targets in the treatment of Alzheimer's disease and a better undersatanding of this disorder.

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## P.39

### Inflammation and neurogenesis in an experimental model of Japanese Encephalitis

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Most of the CNS infections and the brain disorders that we encounter are associated with CNS inflammation and neurodegeneration. In fact, neuroinflammation has become the hallmark of most of the CNS infection by neurotropic viruses (e.g. HIV, JEV etc.) Here we demonstrate how following Japanese encephalitis infection there is robust microglial activation and a major upheaval in the level of the pro-inflammatory mediators (the cytokines, chemokines and the reactive oxygen and nitrogen species), which represents the profound neuroinflammatory condition. These various mediators have been shown to be detrimental for the neuronal survival and eventually bring about neuronal death. Interestingly, the enhancement in levels of these pro-inflammatory mediators was mainly observed in the adult neurogenic regions of the brain, the subventricular zone and the dentate gyrus of the hippocampus. Apart from the mature neurons being the target of JEV, it was also investigated whether cognitive deficits that have been observed in the survivors of JE can be attributed to the deficits in neurogenesis and malformation of new neurons from the neural stem/progenitors in these neurogenic regions. In vivo studies have clearly demonstrated that there is a decrease in the proliferating neuronal progenitor pool as well as in the immature neuron formation.

Neurospheres cultured from P7/P8 mouse pups were used to assess the impaired neurogenesis pattern in JE infected animals as compared to control ones. A vivid decrease in the proliferative ability of the progenitor cells were noticed in terms of the generation potential of the spheres isolated from JE infected animals. Furthermore, neurospheres cultured in presence of conditioned media obtained in JE-activated microglia show arrested growth and distorted morphology. Contradictorily, in vitro JE infection to the spheres did not render a decrease in the progenitor population as indicated by the percentage of nestin positive population. Moreover, no direct apoptotic death of the neuronal progenitor cells was also observed after in vitro JE infection, probably suggesting that inflammation following JEV has a key role in determining the fate of the neuronal stem/progenitor proliferation and differentiation.

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## P.40

### Molecular characterization of JC virus genomic region encoding Viral Protein 1 outer loops and evaluation of JCV viral load in CSF of patients with different forms of PML

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After the introduction of Highly Active Antiretroviral Therapy (HAART), the prognosis of Progressive Multifocal Leukoencephalopathy (PML) has changed, becoming variable, and thus reliable and easy-to-use markers of PML clinical evolution are needed. The purpose of our study was to determine whether or not specific markers, such as amino acid substitutions in the genomic region encoding Viral Protein 1 (VP1) outer loops and viral load of JC Virus (JCV), isolated from Cerebrospinal Fluid (CSF) of patients with different forms of PML, could be associated with the progression of the disease.

In the CSF collected from 21, both HIV+ and HIV-, PML patients (9 Fast Progressors-FP- and 12 Slow Progressors-SP), JCV viral load was evaluated by means of specific RealTime PCR. In addition, JCV VP1 outer loops were amplified in CSF collected from 8 patients and in urine collected from 10 controls, cloned, sequenced and translated using the ExPASy software.

JCV viral load was significantly higher ($p < 0.001$) in CSF of FP PML patients (5.51 log copies/ml) than in CSF of SP PML patients (4.14 log copies/ml). All the JCV strains isolated from CSF of SP PML patients showed specific amino acid mutations at four defined hot spots in the VP1 genomic region encoding the outer loops (amino acid positions 75, 117, 128, 167). No significant difference from Archetype strains were found in the VP1 loops isolated from the urine.

Taken together, the results confirm that high CSF JCV viral load is predictive of poor outcome of PML patients, whereas some specific polymorphisms of VP1 loops are associated with longer survival of patients. Among slow progressors, mutation at Arg-75, recently suggested to be involved in cell recognition and resulting in non-viable virus, is highly frequent. Thus we could hypothesize that the alteration at the defined hot spots determines a decrease in the viral activity with the consequence of a slow progression of the disease.

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## P.41

### Expression of JCV T-antigen in glioblastoma multiforme. Nuclear translocation of IRS-1 and alteration in homologous recombination dependent DNA repair

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Despite significant advances in surgical techniques and development of new chemotherapeutic agents, Glioblastoma Multiforme (GBM), the most aggressive and frequent of brain tumors, is always associated with poor prognosis. One of the hallmarks of glioblastoma is genomic instability, which inevitably leads to the development of new cellular adaptations. Therefore, it is possible that basic DNA repair mechanisms responsible for the maintenance of genomic integrity could be impaired in GBMs. We have previously reported detection of the early oncoprotein of human polyomavirus JC, large T-Antigen, in the nuclei of neoplastic cells in 25 of 76 glioblastoma cases examined; and the involvement of JCV T-Antigen in the inhibition of homologous recombination DNA repair (HRR), which seem to require the presence of the cellular protein, insulin receptor substrate 1 (IRS-1). Our ongoing studies demonstrate an active IGF-IR-IRS-1 signaling system in both JCV T-Antigen positive and negative glioblastoma clinical samples. This included a strong membrane associated immunolabeling of a total, as well as tyrosine phosphorylated (pY1316) IGF-IR, and the accumulation of cytosolic IRS-1 in comparison to healthy brain tissue. Interestingly, we have detected a strong nuclear IRS-1 in approximately 30% of neoplastic cells in clinical samples of GBM, which were also expressing JCV T-Antigen. Double labeling studies

demonstrated nuclear co-localization between IRS-1 and JCV T-Antigen. In addition, immunolabeling with the antibody, which detects the phosphorylated form of histone H2AX (?H2AX, a marker of DNA damage) showed a much stronger immunolabeling in the nuclei of JCV T-Antigen positive glioblastoma cells, as well as co-localization between ?H2AX and nuclear IRS-1, and between nuclear IRS-1 and the major enzymatic component of HRR, Rad51. In summary, our findings indicate the involvement of JCV T-Antigen – IRS-1 signaling axis in homologous recombination of actively growing glioblastomas, a critical mechanism for the fidelity of DNA repair during DNA replication, which could provide a novel target for the treatment of these highly aggressive cerebral tumors.

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## P.42

### Unique features of measles encephalitis in the romanian HIV cohort

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Background: Subacute measles encephalitis was usually described as a rare complication of measles in severely immuno-supressed patients. During two consecutive measles outbreaks in Romania, we observed a specific neurologic clinical picture, characterized by myoclonus, occurring in HIV infected children. We decided to study the new clinical entity, that we called SMME, based on epidemiologic, clinical, laboratory and postmortem pathologic data in HIV infected patients followed at the "Victor Babes" Hospital in Bucharest, Romania.

Methods: We included in our study 34 HIV infected children and adolescents: 22 from the 1997-1998 and 12 from the 2006-2007 measles epidemics. The inclusion criteria were: a previous measles episode or presumed contact with measles virus, clinical features of myoclonus associated with motor deficits and stable mental status at onset, followed by rapid progress to death. The definitive diagnosis was based on immunocytochemistry for measles virus in paraffin embedded brain autopsy tissues.

Results: The average time from exposure to measles or the onset of clinical disease to SMME was 89 days (range 42-300). Clinical measles diagnosis was made in 12 patients (35%), 5 with atypical features. The median CD4 cell count was 44. The onset of SMME was sudden in the majority of patients with unilateral continuous myoclonus and preserved mental status. Myoclonic jerks expended then rapidly, followed by severe motor deficit, altered mental status and coma. The mean survival time from the onset of SMME was 19 days (5-66). Serology for measles was positive in 28 of 34 patients. Neuroimaging revealed unilateral focal areas of high T2 and FLAIR signal. At autopsy, immunohistochemistry for measles was positive in all cases studied.

Conclusions: The key clinical feature in our study was myoclonus and it occurred between 2 and 9 months post-exposure to the measles virus, usually in the absence of clinical signs of acute measles disease. This is particularly critical in the HIV infected population in limited resources settings. In the HIV infected cohort we have studied, 60% of the patients did not have protective antibodies to measles, despite the fact that vaccination is mandatory in Romania. The absence of protective levels in immunosupressed patients indicates the need for aggressive prophylactic therapies during measles epidemics. This is particularly critical in the HIV infected population in limited resources settings.

## P.43

### Inhibition of synaptic proteins by HIV-1 Tat involves the activity of microRNA

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## P.44

### Use of protease inhibitors does not independently contribute to distal polyneuropathy in HIV infection

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Background. A recent analysis (Pettersen et al, 2006) found that exposure to HIV protease inhibitors (PI), an important component of modern combination antiretroviral (ARV) therapy (ART), was associated with an increased likelihood of distal polyneuropathy (DPN) in patients with HIV infection. In dorsal root ganglion sensory neurons, PI exposure resulted in significant neurite retraction and process loss, suggesting neuronal toxicity. If confirmed, these findings

could prove to be a substantial limiting factor in the long-term use of protease inhibitors in HIV-infected patients.

Methods. We evaluated current and past exposure to PIs as a risk factor for sensory neuropathy in 1159 HIV-infected individuals enrolled in a large, NIH-funded, multicenter, cohort study. All subjects underwent a complete neurological exam to determine whether signs of DPN were present, including diminished ability to sense vibration on bilateral great toes, decreased ability to discriminate between sharp and dull, and absent or weakened ankle reflexes. Subjects were grouped into 5 categories according to past and current exposure to any ARV and to PIs into the following categories: Group 1 - past ARV use/no PIs (n = 64), Group 2 - past ARV and PI use (n = 107), Group 3 - currently on ARVs/never used PIs (n = 170), Group 4 - currently on ARVs/past PI use (n = 147), Group 5 - currently on ARVs and PIs (n = 507). The odds of having DPN were calculated for each group relative to a sixth group of subjects who were ARV naïve (n = 164). Concomitant risk factors were evaluated in multivariate models, including age, exposure to dideoxynucleoside reverse transcriptase inhibitors (d-drugs) and markers of HIV disease progression (CD4 nadir, plasma viral load, and duration of HIV infection).

Results. Relative to individuals never exposed to ARVs the odds for having DPN were significantly higher for all groups except group 1. Subjects currently on PIs or who had been exposed to PIs were more likely to have DPN relative to ARV naïve subjects (OR [95% CI] Group 1: 1.4 [0.8-2.5], Group 2: 2.2 [1.4-3.7], Group 3: 2.8 [1.8-4.4], Group 4: 3.8 [2.4-6.1], Group 5: 4.0 [2.8-5.9]). However, in a multivariate model accounting for other significant concomitant risks, no group was more likely to have DPN compared to ARV naïve subjects (adjusted OR [95% CI] Group 1: 0.6 [0.2-1.5], Group 2: 0.9 [0.4-2.1], Group 3: 1.4 [0.7-2.7], Group 4: 1.1 [0.5-2.3], Group 5: 1.6 [0.9-2.7]).

Conclusions. Evaluation of confounding risk factors for neuropathy in HIV infection suggests that the independent risk attributable to PI exposure, if any, is small. This risk must be weighed against the important role of PIs in modern ART regimens.

## P.45

### Decreased CSF HIV load in patients with subacute myoclonic measles encephalitis

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Background: It has been previously reported that the plasma HIV load drops significantly during acute measles infection. However, there are no data on cerebrospinal fluid (CSF) HIV RNA in patients with acute measles and neurological complications of measles. We planned to measure HIV RNA levels in the CSF and plasma in a group of Romanian adolescents diagnosed with subacute myoclonic measles encephalitis (SMME).

Methods: We studied 12 HIV positive, severely immunosupressed patients, diagnosed with SMME. The neurologic symptoms consistent with SMME started after a median period of 80 days (range 40-304) post exposure to the measles virus. CD4 cell counts and HIV plasma and CSF viral load were measured at the time when neurological complications started.

Results: All 12 patients had severe immune suppression; at the time of SMME diagnosis median CD4 cell count was 18 (range 1-231). Eleven patients were on HAART at the debut of neurologic symptoms, 7 of them with virological failure. Analysis of paired plasma-CSF samples at the time of SMME diagnosis showed that the median HIV RNA load in the CSF was 344 copies/ml (range 112-779) while the plasma median level was 34440 copies/ml (range 178-1000000). All patients had preserved blood-brain barrier. Clinical diagnosis of measles was established in 6 patients, half of them with uncommon clinical presentations. Dynamic measurements of plasma HIV RNA available in 4 patients revealed a temporary ten-fold decrease at the time of measles diagnosis.

Conclusions: The strikingly low CSF HIV RNA levels in patients with SMME suggest an inhibitory effect of measles on HIV replication in the central nervous system. We propose that a significant drop in the HIV CSF burden in severely immunosupressed patients may indicate the presence of a serious opportunistic brain pathology, SMME, that can affect a large population in limited resource settings.

## P.46

### Evidence that morphine intensifies oxidative and synaptic damage in gp120-transgenic mice

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Nearly 30% of injection drug users are infected with the human immunodeficiency virus (HIV), and nearly half of the HIV seropositive women in the United States have contracted the infection via drug use. Although opioids are not generally neurotoxic, opioids can synergize with toxic agents, including HIV-proteins, to exacerbate neuronal damage. In this study we sought to determine if morphine exacerbates neuronal damage in mice transgenic for gp120. Morphine,

or placebo tablets were implanted subcutaneously into gp120- and non-transgenic mice. Levels of antioxidants, reactive oxygen species, sphingolipids, phospholipids and synaptic proteins were measured from cortical tissue on days 4, and 7 after implant to determine the effects of chronic morphine administration, and withdrawal from morphine. On day 4, morphine levels in plasma were 2.7–3.2 ng/ml. Morphine levels were undetectable on day 7, and were not detected in animals administered placebo. Gp120-, and non-transgenic mice administered placebo had similar levels of cellular oxidation, but sphingomyelins were decreased and ceramides increased in gp120- compared with non-transgenic mice. Levels of the pre-synaptic protein PSD-95 were lower in gp120- compared with non-transgenic mice. After 4 days of morphine, there was evidence of oxidative stress in gp120-, and non-transgenic mice. In non-transgenic mice administered morphine there were trends toward decreased sphingomyelin, and increased ceramide in rodents. In gp120-transgenic mice administered morphine, sphingomyelin levels were increased, and ceramide levels decreased compared to placebo. During drug withdrawal, redox, sphingomyelin, and ceramide levels normalized to a greater extent in non-transgenic compared with gp120-transgenic mice. PSD95 was decreased in gp120-transgeic mice compared to all other groups of animals. These findings suggest that the additional perturbation of gp120 promotes synaptic damage during morphine withdrawal.

## P.47

### Functional dysregulation of dopamine transmission and the dopamine transporter associated with the HIV-1 Tat protein and repeated cocaine administration

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Injection drug use, including the use of cocaine, is a leading risk factor in the transmission of Human Immunodeficiency Virus (HIV). Injection drug use is responsible for one-third of all HIV-infections in the United States. Furthermore, cocaine abuse and HIV are known to alter brain dopamine (DA) systems. To date, little research has been aimed at directly investigating the interaction of cocaine-associated DA system neuroplasticity with HIV-induced DA system toxicity in the context of in vivo DA transmission. In vivo brain microdialysis is a technique that is ideally suited for direct monitoring of neurotransmitter alterations.

Thus, a series of experiments using traditional, reverse, and zero-net-flux microdialysis were designed to elucidate HIV-1 Tat-induced alterations in DA transmission and the DA transporter (DAT), both alone and in the presence of repeated cocaine administration. In experiment one, a 33% reduction in potassium-evoked DA overflow was observed in Tat-treated rats relative to vehicle-control. In experiment two, a significant reduction in DA efflux to cocaine challenge (10 mg/kg i.p.) was demonstrated in chronic cocaine + acute Tat treated animals relative to cocaine + vehicle treated animals. Experiment three demonstrated alterations in DAT function in cocaine + vehicle treated animals and cocaine + Tat treated animals relative to saline + vehicle treated animals.

Together, these experiments replicate the plasticity associated with DA transmission and DAT function upon repeated cocaine administration. Furthermore, these experiments demonstrate that DA transmission and DAT function in the HIV-positive cocaine abusing population may be more severely compromised than the HIV-positive non-drug abusing population.

## P.48

### High affinity binding of Vpr to the HIV-1 LTR at C/EBP site I is correlated with HAD

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Human immunodeficiency virus type 1 (HIV-1) viral protein R (Vpr) is a virion-associated protein that transactivates the HIV-1 long terminal repeat (LTR). The electrophoretic mobility shift assay has been utilized to demonstrate the direct binding of purified Vpr (strain pNL4-3) to HIV-1 LTR sequences that span the adjacent CCAAT/enhancer binding protein (C/EBP) site I, NF-kappa B site II and ATF/CREB binding site (nt -95 to -130, relative to the start of transcription). Binding between HIV-1 Vpr and the LTR C/EBP site II (nt -167 to -175) has also been observed. A total of 94.7% of LTRs derived from peripheral blood displayed high relative Vpr binding affinity at C/EBP site I, while only 5.3% exhibited a low relative Vpr binding affinity phenotype. All LTRs derived from peripheral blood exhibited a high relative Vpr binding phenotype at C/EBP site II. These results suggest a preference for the maintenance of two cis-acting elements with high affinity for Vpr within LTRs derived from peripheral blood in late stage disease and during development of HIVD. Additional studies have also demonstrated that naturally occurring sequence variation within C/EBP site I and II that correlates with disease progression can dramatically alter the relative affinity of Vpr for these cis-acting elements. Further studies have also suggested a competitive interaction between Vpr and C/EBP factors for binding sites I and II. These studies indicate that Vpr may regulate the

interaction of members of the C/EBP transcription factor family with the viral LTR.

## P.49

### CD163/CD16 co-expression by circulating monocytes/macrophages in HIV-1 infection: potential role in the pathogenesis of HIV CNS disease

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The accumulation of macrophages in the CNS of patients with HIV encephalopathy (HIVE) provides strong evidence for the role of increased monocyte/macrophage trafficking in its pathogenesis. CD163, a monocyte/macrophage specific scavenger receptor for hemoglobin-haptoglobin complex, is reported to be expressed in the CNS by perivascular macrophages but not by resident microglia in human brain. Recently, we utilized this disparity in CD163 expression by brain macrophages to further characterize the MPs in the CNS in HIVE. CD163 expression was seen by cells that accumulate within perivascular cuffs as well as by parenchymal cells with microglial morphology in HIVE, suggesting that the numerous parenchymal microglia seen in HIVE are derived from cells that have recently emigrated from the peripheral blood into the CNS compartment. Moreover, these cells are largely $CD16^+$and appear to be the sole source of productive HIV-1 infection in the CNS. In a separate study, flow cytometry analyses of PBMC from HIV infected volunteers showed a positive correlation between $CD163^+/CD16^+$ monocytes and viral load that was not observed between $CD4^+$ absolute T cell counts and viral load. Even more significant was our observation of a strong inverse correlation between $CD163^+/CD16^+$ monocytes and $CD4^+$ absolute number of T cells when T cell counts drop below 450. Alterations in blood monocyte homeostasis may contribute to AIDS pathogenesis and trafficking of monocytes into the CNS and patients who develop HIVE. In these individuals, the $CD163^+/CD16^+$ subset may contribute to the development of cognitive dysfunction by bringing virus into the CNS as well as through secreted factors and by-products associated with their activation status.

## P.50

### Developmental neurotoxicity of HIV-1 proteins in the rat hippocampal formation: A design-based stereological study

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Design-based stereology was used to examine the role of neonatal intrahippocampal injections of HIV-1 proteins, gp120 and Tat, on neuroanatomy of the hippocampus in adult rats. Postnatal day (P)1-treated Sprague-Dawley rats were bilaterally injected with vehicle (VEH) (0.5$\mu$l sterile buffer), gp120 100ng, Tat 25$\mu$g or combined gp120+Tat (100ng+25$\mu$g). Using Nissl-stain, estimated total neuron number were quantified in five subdivisions of the rat hippocampus [granual layer (GL), hilus of the dentate gyrus (DGH), cornu ammonis fields (CA)2/3, CA1, and subiculum (SUB)], and glial number (astrocytes and oligodendrocytes) in two subdivisions (DGH and Sub). Overall, results demonstrated a significant reduction of neuron number in the CA2/3 subfield by Tat and gp120, [$F(1, 16) = 7.6$, $p < 0.05$ and $F(1, 16) = 7.2$, $p < 0.05$, respectively] and a significant reduction in the DGH by Tat only [$F(1, 16) = 5.4$, $p < 0.05$]. There was a corresponding significant increase in glial numbers with gp120 and Tat demonstrating differential effects. For astrocytes a significant gp120xTat interaction was noted in the DGH [$F(1, 16) = 32.7$, $p < 0.01$] and supported by a significant Tat effect [$F(1, 16) = 10.4$, $p < .01$] but no effect for gp120. For oligodendrocytes in the DGH and astrocytes in the SUB a significant effect was noted only for Tat [$F(1, 16) = 12.1$, $p < .01$ and $F(1, 16) = 9.1$, $p < .01$]. Collectively, results suggest differential effects of the HIV-1 proteins, gp120 and Tat, on the total number of neurons, as well as on the expression of glia cells. Potential relationships to behavioral observations in the animals will be examined. (Support by DA013137, DA014401, HD043680).

## P.51

### Use of the DrexelMed HIV/AIDS cohort to identify viral markers predictive of disease progression and neuroAIDS

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The long terminal repeat (LTR) regulates human immunodeficiency virus type 1 (HIV-1) viral gene expression via interactions with multiple viral and host transcriptional control factors. Previous studies have examined sequence variation at CCAAT enhancer binding protein (C/EBP) sites I and II, and Sp sites I, II, and III in peripheral blood (PB)-derived LTRs from HIV-1-infected patients representing 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 change at position 5) were variants found in low frequencies in PB-derived LTRs derived from patients at early stages of HIV-1 disease, and at relatively high frequencies in patients in late stage disease. Sequence variation at these sites was also examined in LTRs derived from various brain compartments of patients with and without HIV-associated dementia. The 3T C/EBP site I and 5T Sp site III were identified in brain-derived LTRs from patients diagnosed with HIVD, but was absent in patients without dementia. To assess whether these or other correlative or predictive viral markers could be identified in a larger patient population in the era of highly active antiretroviral therapy (HAART), HIV-1-positive patients from the Drexel University College of Medicine HIV/AIDS Clinic have been enrolled in a longitudinal study to examine the change in prevalence of specific viral genetic markers. Herein, an initial analysis of the DrexelMed HIV/AIDS cohort for the presence of specific configurations of HIV-1 LTR transcription factor binding sites is presented.

## P.52

### Macrophages and dopamine: a role in NeuroAIDS

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Human Immunodeficiency Virus (HIV) infection of the central nervous system (CNS) can result in a variety of neuropathological complications, including minor cognitive motor disorder and dementia, which affect a significant number of HIV positive individuals. One of the major pathological hallmarks of these complications is trafficking and accumulation of monocyte derived macrophages (MDM) in the CNS. HIV induced neurological complications are compounded by dopaminergic drugs, such as cocaine and methamphetamine, that synergistically increase the pathology of HIV infection in the CNS. Recent studies have shown that increased extracellular dopamine expression enhances viral expression and neuropathology in SIV infected macaques, but the mechanisms for this enhancement remain unclear. As MDM are the principal targets for HIV in the CNS, it is important to examine the role of these cells in this enhanced neuropathogenesis. We hypothesize that MDM express receptors for dopamine and that dopamine therefore modulates the functions of both infected and uninfected macrophages in the CNS. In addition, we suggest that HIV infection dysregulates macrophage function, resulting in changes in CNS dopamine levels, altering the susceptibility of macrophages to HIV infection and enhancing neuropathogenesis. Our preliminary data indicate that MDM express dopamine receptors 1 and 2, the dopamine transporter and tyrosine hydroxylase. We also show that the expression of these proteins in MDM is altered by HIV infection, and that the presence of dopamine during initial infection enhances HIV infection levels in MDM.

## P.53

### National NeuroAIDS tissue consortium

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NIH established the multi-site NNTC in 1998 to provide neuroAIDS researchers with high quality brain and fluid samples from well-characterized HIV-infected patients. In meeting this goal, more than 2873 tissues specimens have been shipped for 206 requests submitted by 124 different investigators.

The NNTC has become an invaluable resource that uniquely expands the field of translational NeuroAIDS research by making available to both new and established investigators neurologically focused tissues linked to extensive antemortem clinical data. Nervous and systemic tissue, fluids, and/or associated databases are available to qualified investigators worldwide at no charge upon approval of their application. Consortium-wide protocols are adhered to in the preparation of specimens as well as in the collection and archiving of associated clinical data. The NNTC collects, stores and distributes specimens from the nervous system (brain, spinal cord, peripheral nerve, CSF), muscle, liver, blood and other tissues from HIV-infected individuals. Linked neuropsychological (e.g., cognitive testing), neuromedical (e.g., neurological deficits, history of ART use), neuropathological and laboratory (e.g., viral loads, CD4 counts) data are also available.

Since the formation of the NNTC in 1998, significant changes have occurred in HIV treatment strategies and research methods that have an effect on future NNTC initiatives. Potent antiretroviral treatment regimens have resulted in the growth of a surviving longitudinal cohort for which extensive clinical data is available. The NNTC is adapting to these changes by evaluating its methods and resources to ensure it remains an effective ongoing resource to the research community. The evolving NNTC cohort now presents a unique opportunity more epidemiological studies examining the interactions between aging and HIV and factors that predict long-term survival. In addition correlating neurological progression with CNS pathology, immunology, and virology at autopsy remain a core potential of our resource. We describe the NNTC's ongoing efforts and update of progress made in the collecting tissues, research usage, longitudinal cohort analysis, and resource generated publications. The

website for the NNTC, including investigator information and cohort description, is www.nntc.org.

## P.54

### Dysregulation of TGF-beta signaling in virus-induced neurologic disease: implications for the function of both regulatory and effector T cells

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Recent studies from our group have demonstrated expression of CD4+CD25+ regulatory T cell (Treg)-specific Foxp3 was significantly reduced in patients with human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) compared to Tregs from healthy donors. This was associated with an inability of Tregs from HAM/TSP patients to suppress proliferation of healthy donor CD4+CD25- T cells. Furthermore, expression of HTLV-I Tax into healthy donor CD4+CD25+ T cells resulted in down-regulation of Foxp3 expression and loss of suppressive function. The present study was aimed at determining the mechanisms underlying the loss of Foxp3 expression and lack of suppressor function in Tregs from HAM/TSP patients. Our evidence suggests that HTLV-I Tax inhibits Foxp3 expression in human CD4+CD25+ T cells through transactivation of the CREB signaling pathway. As HTLV-I Tax has been reported to block TGF-beta/Smad signaling through its ability to functionally interact with members of the CREB pathway, and that TGF-beta signaling has been previously demonstrated to play a critical role in the induction and maintenance of Foxp3 expression, we investigated this potential link between Tax, TGF-beta, and Foxp3. Herein we report a defect in the TGF-beta/Smad signaling pathway in patients with HAM/TSP, including aberrant expression of the type II TGF-beta receptor in CD4+CD25+ as well as CD4+CD25- T cells, which was associated with dysregulation of Treg and effector T cell function. This evidence demonstrates a virus-induced breakdown of immunological tolerance at the level of both regulatory and effector T cells.

## P.55

### Characterization of the IGF-I receptor–mediated growth survival and differentiation responses of neural progenitors

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The contribution of insulin-like growth factor I receptor (IGF-IR) in neuronal survival is well documented. The question remains however how the signal from activated IGF-IR affects growth and differentiation of neural progenitors. We have addressed this question by utilizing three-dimensional cultures of neural progenitors (neurospheres) isolated from the brain of mouse embryos with targeted disruption of the IGF-IR gene (IGF-IR(-/-), and from age-matching non-transgenic littermates. There are several reasons why we have decided to use this model: (i) it will enable evaluation of the IGF-I -mediated effects on neuronal outgrowth in the presence of three-dimensional interactions between neurons, astrocytes, and oligodendrocytes; (ii) it will allow cell signaling studies in the context of cell cycle progression, ROS accumulation, and cell survival in actively proliferating neural progenitors (nestin positive); and in neural progenitors stimulated towards astrocytic, oligodendrocytic or neuronal differentiation; and (iii) it will make possible the distinction between insulin receptor-mediated and IGF-IR-mediated signaling responses. Our results indicate that secondary neurospheres kept for 5 days in the medium supporting cell proliferation, consist of cells that are mostly nestin positive, indicating neural progenitor phenotype. These proliferating neurospheres also contain a small population of cells that are both nestin and GFAP positive, indicating early glial differentiation. These double-labeled cells were localized at the periphery of the neurosphere. Interestingly, we have found also a small number of cells, which were labeled with betaIII tubulin antibody, indicating neuronal differentiation. These few cells were nestin negative and showed early stages of neuronal morphology. Following attachment and differentiation of secondary neurospheres, we have performed single and double immunocytofluorescent labeling to detect cellular markers expressed during differentiation of nestin positive neural progenitors. Five days after the induction of differentiation, three morphologically distinct cell populations were detected. These cells were subsequently distinguished by immunolabeling with anti betaIII tubulin antibody, an early neuronal marker, anti-GFAP antibody, astrocytic marker, and anti-GalC antibody, oligodendrocytic marker. We have also started to evaluate potential differences between neurospheres from IGF-IR(-/-) embryos and those from age matched embryos from non-transgenic littermates. Our results indicate that the percentage of cells replicating DNA (BrdU incorporation) is significantly lower in neurospheres from the IGF-IR(-/-) embryos than in neurospheres isolated from non-transgenic littermates. This significantly lower rate of DNA replication was observed despite of the fact that IGF-IR(-/-) neurospheres contained about 20% more nestin positive cells and less betaIII tubulin and GFAP positive cells, again in comparison to neurospheres from non-transfenic littermates. These data indicate that the absence of IGF-IR slows down the rate of cell proliferation and decreases the percentage of progenitors, which differentiate towards neuronal and astrocytic lineages.

## P.56

### Molecular bases of JCV T-antigen–mediated transformation of neuronal progenitors—involvement of the IGF-IR – Survivin signaling axis

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The contribution of insulin-like growth factor I receptor (IGF-IR) in polyomavirus T-antigen-mediated cellular transformation is well documented. In addition, both IGF-IR and JCV T-antigen are suspected to play a role in the transformation of neuronal progenitors from the cerebellar granular layer – a critical step in the development of medulloblastoma. Therefore, we investigated how IGF-IR supports cellular transformation by JCV T-antigen. We have addressed this question by utilizing three-dimensional cultures of neural progenitors (neurospheres) isolated from mouse embryos with targeted disruption of the IGF-IR gene (IGF-IR(-/-), and from age-matching non-transgenic littermates. Our results indicate that neurospheres kept in culture conditions supporting cell proliferation, maintain nestin positive neural progenitors phenotype for several generations. Following differentiation of secondary neurospheres, we have detected three distinct cell populations, which were betaIII tubulin (early neuronal marker), GFAP (astrocytic marker), and GalC (oligodendrocytic marker) positive, independently from the presence or absence of the IGF-IR. Nestin positive neurospheres were subsequently transfected with JCV T-antigen, and tested for cell cycle distribution, and protein levels. Our results demonstrate that: (i) more than 50% of IGF-IR(+/+) neurospheres were positive for JCV T-antigen 48 hours after nucleoporation –mediated gene delivery, while only 7% of IGF-IR(-/-) neurospheres showed the expression of this viral oncoprotein. (ii) In IGF-IR(+/+) neurospheres the expression of JCV T-antigen shifted the cells from G1 towards S–G2M phase of the cell cycle, which significantly increased the rate of cell proliferation. In contrast, vast majority of JCV T-antigen positive IGF-IR(-/-) neuronal progenitors were TUNEL positive. (iii) Western blot analyses demonstrated that anti-apoptotic protein, survivin, which expression has been linked with cell proliferation, was downregulated about 10-fold in IGF-IR(-/-) in comparison to IGF-IR(+/+) neuronal progenitors. Interestingly, JCV T-antigen failed to increase survivin protein level in the absence of IGF-IR. In contrast, (iv) JCV T-antigen expressed in differentiated IGF-IR(+/+) neurospheres elevated expression of survivin to the levels detected during cell proliferation. Our results indicate that one of the mechanisms which could explain necessity of the IGF-IR in JCV T-antigen–mediated cellular transformation is reactivation of survivin, which according to our results requires IGF-IR.

## P.57

### Bimolecular fluorescence complementation (BiFC) is a sensitive and quantitative method for studying the interaction between HIV-1 Gag and Annexin 2

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Bimolecular fluorescence complementation (BiFC) is a technique for studying protein-protein interactions in which fragments of a fluorescent protein, which by themselves do not fluoresce, are fused to proteins of interest. When the fusion proteins are coexpressed in cells, their binding brings the fluorescent protein fragments close enough to form a complex and fluoresce. Because this assay does not require cell lysis or antibody staining, BiFC enables the visualization of protein-protein interactions in living cells in their proper intracellular environment (Kerppola, 2006). This assay has thus been used to study interactions that would be difficult to investigate with more traditional methods, such as weak and short-lived interactions as well as interactions that require certain intracellular components, such as membranes (e.g. Nyfeler et al., 2005). The interaction between the HIV-1 polyprotein Gag and the cellular protein Annexin 2 occurs near CD63-positive compartments in macrophages and is important for proper viral assembly and budding and the formation of infectious virions (Ryzhova et al., 2006). As HIV-infected macrophages play an important role in the pathogenesis of HIV-associated CNS disease, we developed a BiFC assay using YFP as the fluorescent reporter to better study the interaction between Gag and Anx2. We created constructs with Gag, Anx2, CD63, and p11, the cellular binding partner of Anx2, fused to fragments of YFP. We confirmed our ability to detect protein-protein interactions using this assay by cotransfecting Anx2 and p11 fusion proteins into 293T cells and detecting YFP fluorescence with confocal microscopy and quantitatively using flow cytometry. We were then able to successfully detect fluorescence complementation between Anx2 and a Gag protein containing the matrix and capsid regions of the polyprotein (p41), again using both confocal microscopy and flow cytometry. The specificity of the assay was confirmed by the finding that cotransfection of CD63 and p41 fusion proteins produced no fluorescence, indicating that mere proximity of proteins without direct interaction does not result in nonspecific fragment complementation. This assay provides us with a sensitive tool to study the intracellular site of the Gag-Anx2 interaction as well as map the Anx2 binding site on Gag using mutagenesis.

## P.58

### Antibody blocking of IL-12/23 ameliorates autoimmune but not viral-induced encephalomyelitis

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The functional role of IL-12 and IL-23 in host defense and disease following viral infection of the CNS was determined. Instillation of mouse hepatitis virus (MHV, a positive-strand RNA virus) into the CNS of mice results in acute encephalitis followed by a chronic immune-mediated demyelinating disease. Antibody-mediated blocking of either IL-23 (anti-IL-23p19) or IL-12 and IL-23 (anti-IL-12/23p40) signaling did not alter virus-specific IFN-gamma-secreting T cell generation nor affect viral clearance. Treatment with anti-IL-23p19 did not modulate secretion of IFN-gamma or IL-4 in the brains of MHV-infected mice. Moreover, therapeutic administration of anti-IL-23p19 to mice with viral-induced demyelination did not attenuate disease. In contrast, treatment with anti-IL-12/23p40 or anti-IL-23p19 resulted in inhibition of experimental autoimmune encephalomyelitis (EAE). These data indicate that i) IL-12 and IL-23 signaling are dispensable in generating a protective Th1 response following CNS infection with MHV and ii) IL-23 amplifies disease in a model of autoimmune demyelination yet does not contribute to demyelination in a model independent of autoimmune T cell-mediated pathology. Therapeutic targeting of IL-12 and/or IL-23 for the treatment of autoimmune diseases may offer unique advantages by reducing disease severity without muting protective responses following viral infection.

## P.59

### Antibody blocking of IL-23 ameliorates autoimmune but not viral-induced encephalomyelitis

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## P.60

### Neuropsychological performance of HIV-infected individuals and its association with Antiretroviral Resistance, Methamphetamine Use, Hepatitis C Virus, and CSF HIV RNA levels.

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Background: Before the widespread use of combination antiretroviral therapy (ART), studies indicated that for individuals with AIDS, higher HIV RNA levels in cerebrospinal fluid (CSF) - but not in plasma—were associated with neurocognitive impairment. More recent studies have reported that HIV RNA levels in CSF are not as strongly associated with neurocognitive impairment. It has been suggested that ART lowers CSF HIV RNA levels and therefore lessens the association between CSF HIV RNA levels and neurocognitive function. ART can impact viral dynamics by inhibiting viral replication or by the selection of drug resistant variants that may be less neuropathogenic. We hypothesized that there would be significant differences in CSF HIV RNA levels and neuropsychological (NP) performance between individuals with drug resistance (DR+) and those without drug resistance (DR-).

Methods: A comprehensive NP battery was administered to 94 HIV-infected participants at the University of California, San Diego HIV Neurobehavioral Research Center. To assess Global Deficit the results from the NP battery were adjusted for age, education and ethnicity. Higher Global Deficit Scores indicate worse NP performance. Univariate and multivariate analyses included: age, ethnicity, duration of HIV infection, HIV RNA levels in CSF and blood, current

and nadir CD4 counts, drug resistance, hepatitis C virus (HCV) serostatus, past and current ART use, ART adherence, methamphetamine use and Global Rating. The Viroseq algorithm and Stanford HIV Resistance Database (April 2007) were used to interpret drug resistance from genotypic data.

Results: Logistic regression analysis revealed that the absence of drug resistance (DR-), history of methamphetamine use, lower CD4 count nadir, positive HCV serostatus (HCV+) and no prior ART use were independently associated with worse NP performance. An interaction between HCV status and drug resistance showed that individuals who were HCV+/DR- had worse NP performance than the rest of the study group who were HCV-/DR+, HCV-/DR- or HCV+/DR+. There was a similar interaction with current ART use (ART+) and HCV, which showed that individuals with ART+/HCV+ demonstrated worse NP performance than all other ART and HCV groups. Additionally, for individuals without drug resistance (DR-), higher CSF HIV RNA levels correlated with worse NP performance. However, in individuals with drug resistant HIV (DR+), CSF HIV RNA levels did not correlate with NP performance.

Discussion: In this study, we found complex multifactorial associations with NP performance, including CSF HIV RNA levels, HCV serostatus, methamphetamine use and drug resistance. In this era of increasing use of ART, the understanding of each of these factors will be important in fully evaluating HIV associated NP performance.

## P.61

### CXCL12-induced cleavage of CX3CL1: potential role in neuroprotection

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Chemokines are involved in various physiological and pathological processes in the CNS, including neuroinflammation/neuroAIDS. CX3CL1 (Fractalkine) and CXCL12 (SDF-1) are two chemokines that are constitutively expressed in the brain. CX3CL1 can exist as a transmembrane protein, which acts as adhesive molecule, or can be cleaved by ADAMs (A Disintegrin and Metalloproteinase) and act as a soluble protein. Both soluble and membrane-attached CX3CL1 interact with the specific CX3CL1 receptor, CX3CR1. In vitro and in vivo studies suggest that CX3CL1 and CXCL12 may have neuroprotective effects, though the mechanisms involved are not completely clear. The aim of this study was to establish whether neuronal production of CX3CL1 is regulated by CXCL12, and determine whether this regulation is involved in neuronal protection. Through the use of a pathway-specific DNA microarray, RT-PCR, and qRT-PCR, we show that the gene for CX3CL1 is up-regulated in neurons treated with CXCL12 (20nM, 18hr). Furthermore, treatment with CXCL12 stimulates cleavage of CX3CL1. Addition of different metalloproteinase inhibitors abolishes CXCL12-induced cleavage. We also found that treatment of neuronal cultures with NMDA can affect levels of soluble/cellular CX3CL1 in a time-dependent manner. This is important as we and others have previously shown that both CXCL12 and CX3CL1 can protect neurons from exitotoxicity. Survival assays will test the hypothesis that soluble CX3CL1 contributes to the action of CXCL12 on neuronal survival. Our findings so far uncover a novel interaction between the two chemokines, which may play an important role in the regulation of their individual functions in the brain (supported by NIH grants DA15014 and DA19808 to OM).

## P.62

### CXCR2 signaling protects against oligodendrocyte apoptosis during virus-induced demyelination

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Intracranial infection of susceptible mice with neuroadapted strains of mouse hepatitis virus (MHV) induces an acute encephalomyelitis followed by a chronic demyelinating disease similar to the human demyelinating disease multiple sclerosis. During the chronic stage of disease, transcripts specific for the CXC chemokine receptor 2 (CXCR2) and it's binding ligands CXC chemokine ligands (CXCL) 1 and 2 are elevated within the spinal cords of mice suggesting a potential role in either host defense or disease in mice persistently infected with MHV. Immunohistochemical staining revealed that expression of CXCR2 was restricted primarily to neurons, astrocytes, and oligodendrocytes and was not associated with infiltrating leukocytes. Antibody neutralization of CXCR2 during the chronic phases of MHV infection resulted in increased mortality compared to mice treated with control antisera. Blocking CXCR2 did not reduce infiltration of virus-specific T cells into the central nervous system (CNS) nor result in viral recrudescence. In addition, there was no difference in the severity of demyelination in mice treated with either anti-CXCR2 or control antisera. However, anti-CXCR2 treatment did correlate with a three-fold increase in TUNEL-positive cells that were restricted to the white matter of infected mice. Immunofluorescence combined with TUNEL staining revealed that a large majority of the apoptotic cells in anti-CXCR2 treated mice were oligodendrocytes (CNPase+) and oligodendrocyte precursor cells (Olig2+). Therefore, in mice persistently infected with MHV and undergoing demyelination, CXCR2 signaling influences oligodendrocyte survival and protects against apoptosis during chronic neuroinflammatory disease.

## P.63

### TNF-alpha mediates trail expression in primary human astrocytes: Relevance to HIV-1 associated dementia

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HIV-1 enters the brain within weeks of initial infection establishing a reservoir for viral replication in mononuclear phagocytes (MP, brain macrophage and microglia). Behind the blood brain barrier, these HIV-1-infected and/or activated MP drive the pathogenesis of HIV-1 associated dementia (HAD). Previously, we reported that TRAIL, a death ligand of the TNF superfamily, participates in MP-mediated neurotoxicity. In this study, we further examined the level of TRAIL in postmortem samples from HIV-1 encephalitic (HIVE) individuals. TRAIL levels in HIVE samples were found to be higher than HIV-1 serum-positive individuals without encephalitis or HIV-1 serum-negative samples. Astrocytes are the most abundant cell type in the central nervous system and human fetal astrocyte culture is a common model to study astrocyte in vitro. Using real time RT-PCR, we found astrocytes increased TRAIL transcription in response to inflammatory cytokine stimulation. Further, HIV-1-infected and LPS stimulated macrophage conditioned media (MCM) induced significantly higher levels of TRAIL as compared to LPS stimulated MCM alone. Upregulation of TRAIL expression was blocked by soluble TNF-alpha receptor, indicating a predominant role of TNF-alpha in inducing TRAIL expression in astrocytes. Upregulation of TRAIL expression by TNF-alpha was blocked by the c-Jun N-terminal kinase (JNK) inhibitor SP600125. In conclusion, HIV-1-infected and/or immune activated macrophages mediate TRAIL expression in astrocytes through the production of TNF-alpha. Defining macrophage-astrocyte interaction in the context of inflammation and HIV-1 infection may help to better understand the TRAIL-mediated neurotoxicity during HAD.

## P.64

### Methamphetamine induces increased levels of interferon-stimulated protein in human primary astrocytes

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Methamphetamine (MA) has been shown to be toxic to varying neuronal populations in the striatum, hippocampus and frontal cortex and this effect was greater with the presence of HIV encephalitis (HIVE). Exposure of mesencephalic, striatal and cortical astrocytes to MA has been shown to increase the presence of reactive oxygen species, suggesting a mechanism for astroglia-dependent MA neurotoxicity. We have reported that interferon stimulated gene, 15kD (ISG15) expression is increased in post-mortem tissue from the frontal cortex of patients with HIVE and HIVE + MA use, versus HIV alone, using microarray analysis, qRT-PCR, and immunohistochemistry. As astrocytic cells are immunocompetent and are capable of releasing proinflammatory chemokines and cytokines, the upregulation of ISG15 may play a role in the neurotoxicity of MA and HIV. Confirmation of this effect in an in vitro system would allow for further investigation of the cause and effect of ISG15 upregulation.

To further investigate the effect of MA on astrocytic cell ISG15 expression, we exposed human primary astrocytes (HPA) to 5uM and 20uM MA, an ISG15 transfection vector, and 1000U/ml interferon-beta, an inducer of ISG15 expression, for 24 hours. We assayed for ISG15 protein product levels using western blot (WB) and immunocytochemistry (ICC).

In response to an exposure of 20uM MA, HPA showed an increase in ISG15 expression, as measured qualitatively by ICC and WB. The same effect was also seen with interferon-beta and the ISG15 expression vector.

We have confirmed previous findings of ISG15 expression dysregulation in response to MA exposure at the protein level. The ability to investigate ISG15 dysregulation in an in vitro system will allow for more profound investigations of the molecular mechanisms involved with MA neurotoxicity and how these effects synergize with HIV infection.

## P.65

### Pre-treatment of mice with nocodazole delays viral entry into the brain following footpad inoculation of West Nile virus

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West Nile virus (WNV) infection in humans can cause neurological deficits including flaccid paralysis, encephalitis, meningitis, and mental status change. To better understand the neuropathogenesis of WNV in the peripheral and the central nervous system (PNS & CNS), we use a mouse footpad model to simulate a natural infection. Localization of WNV in the nervous system using this model suggested two routes of viral introduction into the CNS: retrograde transport from the PNS and hematogenous diffusion via a breakdown in the blood-choroid-plexus barrier. To test further the retrograde transport hypothesis, C57BL/6J mice were treated with nocodazole, a microtubule inhibitor that

blocks retrograde transport, and then infected with WNV. Nocodazole-treated WNV-infected mice developed a viremia at least one log greater than untreated WNV-infected controls at all time points tested (days 1-5 post-infection). Although viremia was greater in nocodazole treated mice, introduction of virus into the brain tissue (spinal cord, cortex, brainstem and cerebellum) as measured by real time RT-PCR occurred much later on day 7 instead of day 1. These results suggest that nocodazole decreases neuroinvasion during early stages of the infection (1-4 days post-infection). However at later time points (7 and 9 days post-infection), the nocodazole- treated animals had viral titers as high as the untreated WNV-infected controls. These data support a model of biphasic viral infection of the CNS and suggest that early CNS infection may also occur via retrograde transport of virus from the PNS.

## P.66

### Genome-wide screening of host genes involved in HIV-1-induced neurotoxic signaling via lentiviral siRNA library screening

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Viral infection-mediated neurotoxicity is a central pathologic mechanism for epidemic viral encephalitis, including HAD [human immunodeficiency virus (HIV)-1 associated dementia]. Survival of neurons during chronic viral infection is important for maintaining the cognitive, motor, and psychiatric functions of the brain. Identification of the host genes responsible for neurotoxic signaling will enable us to enhance cell survival and to develop a therapeutic intervention. To identify which genes are responsible for viral neurotoxic signaling, we will utilize high-throughput genome-wide screening of host genes in a human neuronal cell line. A lentiviral siRNA library that targets 47,400 human mRNA transcripts was utilized to identify which siRNA clones can prevent in vitro human neuronal cell death induced by pseudotyped HIV-1. The siRNA clones was identified by GeneChip microarray system. The cells protected by siRNA expression showed significant upregulation in the siRNA levels of 9 genes and downregulation of 5 genes. Each siRNA clones identified by the library screening was further tested for their neuroprotection efficacy after HIV-1 infection. This is the first report of such genome-wide functional screening of biologically significant neurotoxic signaling, with a therapeutic potential for NeuroAIDS.

## P.67

### Genetic variants within the HIV-1 LTR may be predictive of HAD

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Human immunodeficiency virus type 1 (HIV-1) proviral DNA with specific binding site sequence variants within the long terminal repeat (LTR), which arise during the course of infection may have functional implications relative to LTR activation, viral replication, as well predictive value relative to progressive neurological disease. The LTR regulates HIV-1 viral gene expression via its interaction with multiple viral and host factors, including members of the CCAAT/enhancer binding protein (C/EBP) and Sp transcription factor families. 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 representing increasing degrees of disease severity. The 3T configuration of C/EBP site I (C-to-T change at nucleotide position 3) and 5T configuration of Sp site III (C-to-T change at nucleotide position 5) were the only variants examined that were found in low frequencies in PB-derived LTRs derived from patients at early stages of HIV-1 disease, and at relatively high frequencies in patients in late stage disease. Sequence variation at these sites was also examined in LTRs derived from various brain compartments of patients both with and without HAD (the 3T C/EBP site I variant was identified in 25% of brain-derived LTRs from patients diagnosed with HAD, but was absent in patients not suffering from dementia). These results suggest that 3T C/EBP site I, and possibly 5T Sp site III may prove valuable in assessing the likelihood of HIV-1-infected individuals developing HAD.

## P.68

### Association of focal adhesion kinase (FAK) activation and lentivirus-induced disruption of blood-brain barrier

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Disruption of the blood-brain barrier (BBB) occurs with the development of HIV encephalitis (HIVE). While many studies have examined the role of viral strains and macrophages/microglia in the development of encephalitis, the molecular signaling pathways within the brain microvascular endothelial cells (BMECs) involved have not been examined. We have previously shown that there is activation and disruption of an in vitro BBB model using lentivirus-infected CEMx174 cells. Others have shown similar disruption

in vivo. Therefore, it was of interest to determine if the presence of these cells could disrupt intact cerebral microvessels immediately ex vivo, and if so, which signaling pathways were involved. We find a correlation between a transitory 10-fold upregulation of FAK expression and markedly decreased expression and altered localization of zo-1 in microvessels. Our data suggest that disruption of tight junctions between BMECs is mediated through activation of FAK by phosphorylation at TYR-397. Inhibition of FAK activation is sufficient to prevent tight junction disruption possibly via zo-1, a protein thought to recruit and organize structural elements (fibrils) within the tight junction. Thus, it may be possible to inhibit break down of the BBB associated with the development of HIVE by using inhibitors of FAK.

## P.69

### The clinical disease and outcome are explained by morphological changes in neurons in experimental rabies in yellow fluorescent protein transgenic mice

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Although human rabies can be effectively prevented, many questions remain unanswered about the pathogenesis of the disease. It is thought that the severe clinical illness and fatal outcome in rabies may result from neuronal dysfunction, rather than neuronal injury or death, because neuropathological changes are usually relatively mild and degenerative neuronal changes are not prominent. In this study the effects of rabies virus infection were evaluated on the structural integrity of neurons in experimentally-infected transgenic mice expressing the yellow fluorescent protein (YFP) in neurons.

Six-week-old transgenic mice (C57BL background) expressing YFP in neurons (H-line) were inoculated in the right hindlimb footpad with the CVS strain of fixed rabies virus or mock-infected with vehicle (PBS). Morphological and immunohistochemical studies using light and fluorescent microscopy were performed on regional brain areas with expression of YFP in neurons, including the cerebral cortex, hippocampus, cerebellum, and brainstem.

Routine histopathogical studies showed some inflammatory changes, but no significant neurodegenerative changes in the CVS-infected brains of moribund mice. However, with fluorescent microscopy dendrites and axons of pyramidal neurons in the cerebral cortex and mossy fibers in the cerebellum exhibited marked structural abnormalities, including beading, whereas the perikarya showed relatively few changes. In the cerebral cortex, 12% of dendrites and 41% of axons of pyramidal cortical neurons were affected in CVS-infected mice, whereas 0% of dendrites and 2% of axons were affected in mock-infected mice. Toluidine blue-stained plastic sections showed vacuolation in perikarya and neuronal processes, which correlated ultrastructurally with swollen mitochondria. There was prominent vacuolation in dendrites and in presynaptic nerve endings in the neuropil of the cerebral cortex. There was also swelling of Golgi apparati and of endoplasmic reticula. In contrast, perikarya and processes of CA1 pyramidal neurons showed only rare changes.

Thus we have found evidence of marked structural changes in neuronal processes in experimental rabies in mice. These findings indicate that injury prominently involving neuronal processes may be sufficient to explain the severe clinical disease with a fatal outcome in rabies rather than neuronal dysfunction without morphological changes.

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## P.70

### Modulation of dendritic cell maturation and function by the Tax protein of human T cell leukemia virus type 1: Implications for HTLV-1-associated neuroinflammatory disease

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HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by the generation of an intense cytotoxic T cell (CTL) response directed against the viral transactivator protein Tax. Additionally, patients diagnosed with HAM/TSP exhibit rapid activation and maturation of dendritic cells (DCs) likely contributing to the robust Tax-specific CTL response. In this study, extracellular Tax has been shown to induce maturation and functional alterations in human monocyte-derived DCs, critical observations being confirmed in freshly isolated myeloid DCs. Tax was shown to promote the production of proinflammatory cytokines and chemokines involved in DC activation process in a dose- and time-dependent manner. Furthermore, Tax induced the expression of DC activation (CD40, CD80, and CD86) and maturation (CD83) markers and enhanced the allogenic and antigen-specific T cell proliferation capability of DCs. Heat inactivation of Tax resulted in abrogation of these effects indicating a requirement for the native structure of Tax. Tax was found to efficiently bind to the DC membrane and was internalized within a few hours suggesting that extracellular Tax may possess an intracellular mechanism of action subsequent to entry. Finally, inhibitors of cellular signaling pathways NF-kappaB, protein kinase, tyrosine kinase and phospholipase C were shown to inhibit Tax-mediated DC activation. This is the first study reporting the immunomodulatory effects of extracellular Tax in the DC compartment. These results suggest that DCs, once exposed to

Tax by uptake from the extracellular environment, can undergo activation providing constant antigen presentation and costimulation to T cells leading to the intense T cell proliferation and inflammatory responses underlying HAM/TSP. Ongoing ex vivo investigations include frequency, activation state, and functions of various DC subsets in HAM/TSP patients.

## P.71

### The secretion of human T cell leukemia virus type 1 Tax protein is a regulated process involving components of cellular secretory pathway and critical amino acid signals within the viral protein: Implications for HTLV-1 neuropathogenesis

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HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a progressive neurological disorder. Many pathologic abnormalities associated with HAM/TSP have been attributed to the extracellular activity of the viral transactivator protein Tax. Cell-free Tax has been detected in the cerebrospinal fluid of HAM/TSP patients indicating its physiologic relevance in the neuropathogenesis. We have previously demonstrated the presence of a nuclear export signal within Tax and its active secretion in vitro. Studies reported herein elucidate the process by which Tax is secreted and identify domains of Tax that contribute to its subcellular localization and secretion. Tax was shown to co-localize with endoplasmic reticulum (ER) and Golgi, the cytoplasmic organelle relevant to protein secretion. An extensive Proteomics analyses followed by GST pull-down assay revealed the specific interaction of Tax with several proteins of the cellular secretory pathway including COPII, SCAMP1, SCAMP2 and SNAP23 in both the model cell line BHK (baby hamster kidney)-21 and a T cell line C8166 (relevant to HTLV-1 infection). The silencing of these proteins inhibited Tax secretion further confirming their involvement in this process. Comparison of the Tax protein sequence with amino acid signals known to target proteins to the secretory pathway yielded the identification of a number of putative secretory signals. The mutations in two signals DHE and YTNI resulted in aberrant subcellular localization of Tax with a large amount of Tax localized to areas immediately surrounding the nucleus. In addition, Tax constructs containing mutations in both signals significantly altered protein secretion. Together, these studies suggest that secretion of Tax is a regulated event facilitated by the interaction of Tax with cellular secretory pathway proteins and the presence of critical secretory signals within the carboxy-terminal domain of protein.

## P.72

### Presenilin and the Gamma-secretase Complex in HIV Neuroinflammation

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The Presenilin (PS) proteins, PS1 and PS2, form the catalytic core of the gamma-secretase complex that cleaves amyloid precursor protein (APP) to release the amyloid beta (Ab) peptide. Ab is deposited in amyloid plaques found in Alzheimer's disease (AD) as well as in HIV+ brain suggesting that pathways involved in AD neurodegeneration may also contribute to cognitive decline in HIV patients. In addition to APP, the gamma-secretase complex cleaves the transmembrane substrates Notch and ErbB4 through which PS's may regulate inflammation, development, and cell fate determination.

We tested the hypothesis that PS2 participates in HIV neuroinflammation. We found increased PS2 immunolabeling in microglia from both HAD and AD frontal cortex autopsy tissue compared to age matched controls. The gamma-secretase substrate ErbB4 is also increased in both the cytoplasm and nuclei of glial cells in HAD. Cultured primary murine microglia, and microglia or macrophage cell lines exposed to the HIV coat protein gp120 (400pM SF162, NIH AIDS Reagent Program) demonstrate increased PS2 protein. Furthermore, we observed an increase in the gamma-secretase cleavage product of Notch-1, Notch intracellular domain, in microglia stimulated with gp120 and Lipopolysaccharide (LPS). Pharmacological inhibition of gamma-secretase activity modulates nitric oxide production and release of tumor necrosis factor in stimulated primary microglia. Our data suggest that PS2 and gamma-secretase may participate in the CNS inflammatory response. Further studies into PS and gamma-secretase mediated cellular pathways may provide useful therapeutically relevant insights into immune mediated pathophysiology in human dementia.

## P.73

### Neurotropism and neuropathogenesis of a CAEV/SIV chimeric virus in goats

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The pathogenesis of AIDS and CNS disease caused by HIV revolves around the tropism of the virus for CD4+ T cells and macrophages, respectively. The macrophage phase of the disease, exemplified by HIV dementia, is contingent on elimination of CD4+ T cells by the virus and is thus a late aspect of the pathogenesis. SIV infection in macaques closely models HIV pathogenesis. Caprine arthritis encephalitis virus

(CAEV) is an ungulate lentivirus that replicates only in macrophages of infected goats and causes in some circumstances CNS disease in addition to other syndromes unique to goats, but there is no loss of T cells. We incorporated the vpr/vpx and nef genes of SIV into the genome of CAEV (1,2) and hypothesized that this chimera would create a caprine model of HIV encephalitis. This virus replicated productively in goat macrophages but not in T cells and caused increased cythopathic effects. Non infected lymphocytes in coculture with infected macrophages underwent apoptosis. We then undertook a study on pathogenesis of infection in juvenile and newborn goats using the recombinant virus and CAEV as control. The chimeric virus caused a persistent infection in the goats and was present in peripheral blood monocytes more often than CAEV. T cells underwent moderate apoptosis, but the cell count did not decrease dramatically as seen in HIV infections. Examination of tissues from the infected juvenile goats 24 weeks after virus inoculation and infected new born kids after 12 weeks postinoculation showed that CAEV caused minimal or no lesions in target tissues. However, the chimeric virus caused mild, but diffuse encephalitis that was associated with white and gray matter throughout the neuropil. All infected new born kids with the recombinant virus but not with CAEV have virus in most of the explant cultures derived from different regions of the CNS and encephalitic lesions were observed in some of the sections. This chimeric virus system therefore has a great promise for providing a simple and reproducible model of HIV CNS disease without causing AIDS.

1. Bouzar et al. Virology 2004.
2. Bouzar et al. Virology 2007.

## P.74

### Perturbation of the astrocyte integrated stress response (ISR) by HIV-1-infected macrophages

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Neuronal degeneration in HAD is mediated by soluble factors released by CNS-resident, HIV-infected and/or activated macrophages and microglia. Although many molecules have neurotoxic potential, primary rat mixed neuronal/glial cultures treated with HIV-infected, monocyte-derived macrophage supernatants (HIV-MDM) are protected from death by the NMDA antagonist MK801. However, our studies indicate that only ~30% of the toxicity of HIV-MDM supernatants is due to HIV-MDM glutamate content, suggesting the involvement of additional HIV-MDM excitotoxins or the modulation of excitotoxin production by astrocytes in these mixed cultures. The astrocytic cystine/glutamate antiporter (xC) is a key regulator of glutamate homeostasis, and can be modulated by the integrated stress response (ISR) by transcriptional upregulation of the xCT subunit via Nrf2. xC activity results in stoichiometric intake of cystine to synthesize the antioxidant glutathione in exchange for glutamate. Exported glutamate is scavenged by excitatory amino acid transporters (EAATs). We hypothesize that HIV MDM will activate the astrocytic ISR perturbing glutamate homeostasis. Consistent with this, HIV-MDM-treated rat astrocytes exhibited increased protein levels of ISR target genes ATF4, BIP, and CHOP, as well as the endogenous antioxidant response protein Nrf2 and its target, xCT, by immunoblotting. Increased xCT levels in astrocytes, by HIV-MDM or by classic ISR inducer thapsigargin, resulted in increased antiporter activity thereby increasing cystine uptake, and, presumably, glutamate export. HIV-MDM and thapsigargin also inhibited the scavenging of extracellular glutamate by the astrocytic EAATs, similar to effects of TNF-alpha. These findings suggest HIV-MDM-released factors may trigger a net efflux of glutamate from astrocytes due to ISR-induced increases in xCT-mediated glutamate export and concurrent decreases in EAAT-mediated glutamate import. Consistent with this mechanism, we have observed increases in ISR markers, BiP and ATF6B, and xCT in cortices from HIV-infected patients as compared to uninfected controls. Taken together, these data suggest that induction of the ISR in astrocytes by HIV-MDM may induce neuronal dysfunction and death by altering glutamate homeostasis in HAD.

## P.75

### Pur$\alpha$ as a cellular co-factor of Rev/RRE-mediated expression of HIV-1 intron-containing mRNA

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To ensure successful replication, HIV-1 has developed a Rev-mediated RNA transport system that promotes the export of unspliced genomic RNA from nuclei to cytoplasm. This process requires the Rev Responsive Element (RRE) that is positioned in the viral transcript encoding Env protein, as well as in unspliced and singly-spliced viral transcripts. We identified Pura, a single-stranded nucleic acid binding protein as a cellular partner for Rev that augments the appearance of unspliced viral RNAs in the cytoplasm. A decrease in the level of Pura expression by siRNA diminishes the level of Rev-dependent expression of viral RNA. Through its nucleic acid binding domain, Pura exhibits the ability

to interact with the multimerization and RBD domains of Rev. Similar to Rev, Pura associates with RRE and in the presence of Rev forms a complex with slower electrophoretic mobility than those from Rev:RRE and Pura:RRE. The interaction of Pura with RRE occurs in the cytoplasm where enhanced association of Rev with RRE is observed. Our data indicate that the partnership of Pura with Rev is beneficial for Rev-mediated expression of the HIV-1 genome.

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## P.76

### The kynurenine pathway metabolite quinolinic acid upregulates CCR5 expression

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Background: Activation of the kynurenine pathway (KP), especially the production of quinolinic acid (QUIN), has been pathogenetically linked to AIDS Dementia Complex (ADC). HIV isolated from the central nervous system (CNS) of ADC patients is both macrophage tropic and CCR5 dependent. Previously, we have shown that QUIN significantly upregulates CCR5 expression on astrocytes, and that the ability to induce the KP is dependent on the strain of HIV – greater in those derived from neurologically impaired patients. We therefore hypothesized that QUIN upregulates CCR5 expression on macrophages and is strain dependent. Methods: PBMCs were isolated from a blood pack by the Lymphoprep method. Macrophages were cultured in DMEM and 10% fetal bovine serum on coverslips. To observe if QUIN upregulates CCR5 expression, the cells were weaned to serum free conditions before the addition of QUIN at 150, 350, 500 and 1200nM. Negative controls without QUIN and positive controls with IFN$\gamma$ were also conducted. At days 1 and 3, cells were processed immunohistochemically for CCR5 (1:100) expression. 10 images per condition were taken and pixel intensities for each cell were measured. To observe if CCR5 expression is strain dependent the neurotropic strains JRFL, C124 (non-demented, primary isolate) and C158 (demented, primary isolate) and the non-neurotropic strain Bal were all added at the same viral IC50 concentration. Cells were immunohistochemically processed as before at day3 following. Results: There was no significant difference in CCR5 expression one day following the addition of QUIN. However, at day 3 there were significant differences between the control and QUIN (ANOVA, $F = 22.9$, $p = 0.0001$). There were also significant differences at day3 between the controls and the two neurotropic viruses C158 and C124 (ANOVA, $F = 121$, $p = 0.0001$). These strains also had a higher CCR5 expression compared to the non-neurotropic strain Bal (ANOVA, $F = 121$, $p = 0.0001$). Conclusions: These data add to the significant pathogenetic role of QUIN in ADC: not only does QUIN lead to neural damage, it also facilitates macrophage infection through an autocrine relationship. Further work will examine the effect of QUIN on microglia.

Key Words: Kynurenine Pathway, macrophages, neurotropic, QUIN

## P.77

### Astrocytes as potential source of cell-free Tax during human T cell leukemia virus type 1-associated myelopathy/tropical spastic paraparesis

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The mechanism of HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) pathogenesis is heavily reliant on the viral transcriptional transactivator protein Tax. The presence of cell-free Tax has been demonstrated in the cerebrospinal fluid of HAM/TSP and the effects of extracellular Tax has been reported in a number of cell types including microglia and neurons. Since astrocytes are the primary glial cells within the brain and are known to be infected with HTLV-1, we hypothesized that HTLV-1-infected astrocytes could play a critical role in HAM/TSP pathogenesis by releasing Tax. In the study reported herein, astrocytes were transduced with lentivirus vector expressing green fluorescent protein labeled Tax (Tax-GFP), and were observed under fluorescent microscopy to determine localization of the protein. Tax was found to be present in high concentrations within cytoplasm, with very little or no Tax being found in the nucleus, 48-hr post-transduction. Subsequently, both cell lysate and supernatant were collected from the transduced cells and analyzed for the presence of Tax via western immunoblot analysis. Results indicated that Tax is released by astrocytes within 48 hr of transduction, in accordance with visual confirmation of Tax presence in the cytoplasm. A cytokine array analyses was also performed to monitor the changes in selected cytokine levels within astrocytes in response to Tax. The Tax-mediated induction of proinflammatory cytokines and chemokines was observed indicating activation of astrocytes and alteration in their function. Future experimentation will determine the effect of Tax-transduced astrocytes on neuronal damage to correlate these observations with HAM/TSP pathogenesis.

## P.78

### Increased frequency of alpha-synuclein in the substantia nigra in HIV infection

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Objective: The frequency of neurodegenerative diseases markers among long surviving HIV infected individuals is unknown, therefore, the aim of the present study was to investigate the frequency of alpha-synuclein, beta-amyloid and HIV-associated brain pathology in the brains of older HIV infected individuals.

Design: Cross-sectional analysis of analysis of previously collected brain tissues from HIV infected persons enrolled in the National NeuroAIDS Tissue Consortium.

Methods: We examined the substantia nigra of 73 clinically well-characterized HIV infected individuals aged 50 to 76 years, who died after 1998. Additionally, we examined the frontal and temporal cortical regions on a subset of 36 individuals. The brain regions were examined for the presence of alpha-synuclein, beta-amyloid and HIV-associated brain pathology.

Results: Neuritic alpha-synuclein expression was found in 16% of the substantia nigra studied, which was far higher than previously reported in older controls. beta-amyloid was noted in 35 of the 36 cases examined. The beta-amyloid deposits were primarily intraneuronal with a few cases having additional staining of the vessel walls. Despite these increases of degenerative pathology, HIV-associated brain pathology was present in only 10% of cases.

Conclusions: Among older adults with HIV infection there was no increase in the frequency of HIV-associated brain pathology; however, there was a substantial increase in the frequency of both alpha-synuclein and beta-amyloid staining. The increased prevalence of alpha-synuclein and beta-amyloid in the brains of older HIV-infected individuals may predict an increased risk of developing neurodegenerative disease in this cohort.

## P.79

### Dynamics of NF-kB activation by HIV-1 tat and CD40L in microglial cells

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HIV-1-associated dementia (HAD) occurs in a significant number of HIV-1 infected individuals, in part, due to the inflammatory response to viral proteins in the central nervous system. We have shown that host factors, such as soluble CD40 ligand (sCD40L) that are found to be elevated in the cerebrospinal fluid and plasma of HAD patients, synergize with HIV-1 encoded Tat to activate microglial cells in an NF-kB-dependent manner. Here we demonstrate that, besides proteolysis of the inhibitory molecules IkB-alpha, the activation of NF-kB by Tat in microglial cells is associated with de novo synthesis of RelB, and processing of p100 (as defined by generation of the processing product p52), followed by translocation of RelB and p52 into the nucleus. However, under these conditions, neither RelB, nor p52, exhibited DNA binding activity at a cis-acting NF-kB responsive element. We further show that the simultaneous exposure to CD40L promotes DNA binding activity of p52, but not of RelB. Finally, ectopic expression of processing-deficient mutant p100, as well as RelB-specific short hairpin RNA (shRNA), revealed that the biogenesis of p52 and RelB in Tat plus CD40L-treated microglia is crucial for the synthesis of TNF-alpha. Taken together, these studies highlight the mechanism by which viral proteins and host factors (like Tat and CD40L) work in concert to activate NF-kB in microglial cells and explain how this process is dynamically controlled.

## P.80

### Impact of low affinity C/EBP and Sp binding sites on HIV-1 replication and relevance to commonly encountered LTR genetic variants observed early and late in HIV disease

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Recent studies have identified two genetic variants in the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) in the peripheral blood of infected patients that correlate with early and late HIV disease. One incorporates a C-to-T change in C/EBP binding site I (3T), and the other incorporates a C-to-T change at position 5 of Sp site III (5T). Interestingly, in peripheral blood-derived LTRs, 94% of patients who had the 3T variant also had the 5T variant within the same LTR. In LTRs isolated from the brains of HIV-infected patients, 3T C/EBP site I and 5T Sp site III were only found in patients who had HIV-associated dementia (HIVD), but were not found in patients without HIVD. The overall goal of these studies is to determine the effect of these particular genetic variants on viral replication. The 3T, 5T, and 3T5T double variant were incorporated into the HIV-1 LAI infectious molecular clone, and infectious viral particles were generated by transfection of the molecular clone into 293T cells. The results of infection of monocytic U-937 and lymphocytic Jurkat cell lines, as well as primary peripheral blood mononuclear cells and monocyte-derived macrophages with genetically altered viruses carrying the 3T, 5T, and 3T/5T C/EBP and/or Sp transcription factor binding site variants has suggested that the single and double base pair changes in the viral LTR have impact of viral replication capability. In addition, the effect of cytokine stimulation of these HIV-1 infected cell populations has also been examined.

## P.81

### Modulation of the NMDA receptor during HIV-tat induced neuronal apoptosis

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HIV infection of the CNS can result in neurologic dysfunction, including motor impairment and cognitive

deficits, in a significant number of individuals with AIDS. NeuroAIDS is characterized by neuronal injury and loss, yet there is no evidence of HIV infected neurons. Neuronal dropout must therefore be due to the indirect effects of HIV infection of other CNS cells, through elaboration of inflammatory factors and neurotoxic viral proteins, including the viral transactivating protein tat. Tat induces apoptosis in human neurons that is dependent on the lipoprotein receptor-related protein (LRP) and the NMDA receptor (NMDAR). We show that NMDAR positive neurons initiate tat induced apoptosis through the rapid formation of a cell surface complex that includes tat, LRP, PSD-95, and NMDAR channels. Neuronal nitric oxide synthase (nNOS) is subsequently recruited to this complex and activated. Blocking LRP mediated uptake of tat, NMDAR activation or nNOS activity with specific inhibitors results in a significant reduction in tat-induced neurotoxicity, suggesting that this complex plays an initial role in generating and amplifying tat toxicity. These data demonstrate the early mechanisms by which tat triggers apoptosis using the synaptic machinery expressed by NMDAR positive neurons.

In addition, tat treatment of neurons results in phosphorylation of the NMDAR. The NMDAR subunit 2A (NR2A) is rapidly phosphorylated after tat treatment in a Src kinase dependent manner. In vitro kinase assays suggest that Src can phosphorylate NR2A on at least 3 different tyrosine residues, and research is ongoing to determine whether these residues are phosphorylated in our human neuronal culture model. NMDAR phosphorylation is an important mechanism by which NMDAR activity can be modulated, by altering permeability of the channel to specific ions, the kinetics of channel opening and closing, and trafficking of NMDA receptors to and from the cell membrane. Preliminary data indicate that there may be a reduction in clathrin mediated internalization of surface receptors after tat treatment. These findings may provide further understanding of the neuropathogenesis of NeuroAIDS, and indicate avenues of treatment for those vulnerable to HIV cognitive impairment.

## P.82

### HIV-1 tat interaction with proteins at the cellular PCNA promoter and at JCV early and late promoters modulates JCV DNA synthesis and repair

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JC virus (JCV) us the etiologic agent of progressive multifocal leukoencephalopathy, a defining opportunistic infection in AIDS. JCV infects primarily oligodendroglial cells in the brain. The Tat protein encoded by HIV-1, which also infects the brain, interacts with cellular protein Pur-alpha to regulate several aspects of JCV gene transcription and DNA replication through binding of Pur-alpha to DNA regulatory sequences. Recent reports help elucidate the Pur-alpha replicative pathway. Pur-alpha plays a role in repairing double-stranded DNA breaks induced by stalled forks in replicating chromosomal DNA. Pur-alpha may thus act with cellular replicative proteins at both initiation and elongation stages of chromosomal DNA replication. Cellular protein PCNA is known to function as a DNA polymerase processivity factor during both DNA synthesis and repair, and PCNA is necessary for replication of all polyoma viral DNAs. We have thus asked whether Tat and Pur-alpha act to regulate expression of the PCNA gene in glial cells. Using chromatin immunoprecipitation (ChIP) we find that Tat, at concentrations as low as 10-12M, strongly enhances binding of Pur-alpha to the PCNA promoter. This result is highlighted by a potential unusual structure in the PCNA promoter. Using a new double ChIP method we find that Tat and Pur-alpha are bound together to a specific region of the PCNA promoter. Pur-alpha acts with JCV T-antigen and with Smad effectors of the TGF-Beta pathway at the JCV transcription/replication regulatory region. The TGF-Beta1 response is modulated by Tat. We find that Tat enhances binding of Smad 2/3, Smad 4 and Fast1 to the JCV regulatory region. We also find co-ChIP of Smad4 and Fast1 at the PCNA promoter. Functional aspects of Tat and its partner proteins at the PCNA promoter are detailed. Results indicate a mechanism by which Tat and Pur-alpha influence JCV DNA replication by facilitating the processivity of ongoing DNA replication forks, thus preventing the damage induced by stalled forks.

## P.83

### Gamma-herpesvirus infection of the CNS is independent of B-cells and leads to immune cell-triggered cellular damage

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We established a model system for neurologic gamma-herpesvirus diseases based on the murine herpesvirus 68 (MHV-68)/mouse system for investigation of Epstein-Barr virus (EBV)-associated neurological complications ranging from facial palsy to encephalitis. All types of inflammation observed in children in response to EBV can be observed in MHV-68 infected mice.

The kinetics of the viral appearance in the CNS and the related inflammatory response were determined by PCR, IFA, H.E. staining or immunhistochemistry in wildtype BALB/c-, wildtype C57BL/6-, and immunodeficient B6-(Rag1) mice and linked to each other.

In BALB/c mice viremia was maximal one week p.i. and decreased rapidly. The viral load in brain tissue exceeded viremia significantly six days post infection (p.i.) indicating intracerebral viral replication. Maximal viral load in brain was gained on day 12 p.i. and remained positive for months. Cellular infiltrates colocalizing with viral activity were initially detected 9 days p.i. (T-lymphocytes), their extent increased 16-48 days p.i. and decreased again 100 days p.i. A strong positive correlation was observed between CNS viral load and severity of inflammation, particularly in the temporal lobe. The inflammation reaction can be associated with severe cellular damages.

C57BL/6 mice showed also CNS inflammation, but with reduced cellular damage suggesting differences in the susceptibility to the virus in distinct mouse strains. In immunodeficient B6-(Rag1) mice developed from the identical genetic background higher CNS viral loads were observed compared to wildtype animals, but no cellular damages, which suggests that the viral entry to the CNS is B-cell independent and that the antiviral immune response contributed to the lesions.

Taken together, MHV-68 leads to delayed, persistent, B-cell independent CNS infection followed by inflammation patterns observed in patient with cerebral EBV infection. The antiviral immune response contributes to CNS lesions.

## P.84

### Targeted siRNA delivery into CNS using in vitro blood brain barrier model

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Background: RNA interference (RNAi) has emerged as a highly effective and conserved mechanism for the down regulation of gene expression. While gene silencing by RNA interference holds promises for new approaches for the treatment of a wide range of diseases, delivery of the RNAi to the right location has been a major obstacle, particularly in central nervous system (CNS) by the presence of blood brain barrier (BBB), regulated by a group of tight junction proteins (tjs) including ZO-1. Previous methods of delivery through BBB have been poorly effective. Production of chimeric peptides represents a physiological based strategy for targeted RNAi delivery into CNS by selective increase in blood brain barrier permeability without affecting the other tissues. We have developed a novel system for the targeted delivery of RNAi into CNS, using spleen necrosis viral (SNV) vectors.

Methods: Phage peptide library was purchased from New England Biolabs NEB and used to screen the peptides specific for the human brain micro vascular endothelial cells (hMVECS) using manufacturer protocol. MVECs were cultured in 60mm plates MVECs and biopanned with12-mer peptide libraries. After three round of successive biopanning and sequencing a12-mer peptide was selected for MVECs. The selected phage was amplified and the DNA sequences were cloned into the transmembrane region of the SNV envelope vector pTC53 using Nae1 site. Different short interfering RNA template sequences (siRNA) were designed using human ZO-I tight junction gene sequences. The 19 base pair hairpin template sequences were cloned into SNV transfer vector using humanU6 promotor(RNA polymerase III) with a Bbs1 and Hind111cloning sites. Antibodies against ZO-1 were purchased from to select the efficient siRNA by tranfecting the MVECs followed by western blot, RT-PCR and immunocytochmistry. Cell type specificity of the12-mer phage peptide cloned into SNV envelope was tested by generating SNV based viral particles transcribing siRNA against ZO-1 and displaying MVECs specific peptides on the surface of the virus, by using quadruple transfection method. Different CNS cells including astrocytes and MVECs were transduced and analyzed for targeting the MVECs by immunostaining and western blot. The ZO-1 inhibition was also tested by same methods.

Results: The results by of the transfected and transduced MVECs using immunocytochemistry,western blot and RT-PCR indicate that the 12-mer peptide highly selective for human MVECs and ZO-1 expression could be inhibited 60% by this targeted siRNA.

Conclusions: Targeted siRNA delivery into CNS by SNV based vectors could be ideal system to design the therapeutics against neurodegenerative disorders. To mimic the in vivo conditions, in vitro BBB system will be ideal for further studies.

Key wards: Blood brain barier, Spleen necrosis virus and ZO-1

## P.85

### Astrocyte-IL-8 regulation during HIV-1 infection of the CNS: The Yin and Yang of neuroprotection and glial activation

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Reactive astrogliosis is a key pathological feature of HIV-1-associated dementia (HAD) and is associated with neural injury. Activated astrocytes produce chemokines including Interleukin-8 (IL-8), a key mediator of neutrophils and monocytes recruitment and activation in the periphery. Recent research from our own and other laboratories suggests that IL-8 is involved in neuroinflammatory responses, and contributes to several neurodegenerative disorders, including multiple sclerosis, HIV-1-associated dementia (HAD) and Alzheimer's disease. Functional analysis with our gene microarray data revealed that IL-8 was one of the central mediators of the immune response in IL-1$\sigma$-activated astrocytes. The current study is designed to investigate the regulation of astrocyte IL-8 in the context of brain HIV-1 infection and its impact on neurodegeneration. Primary human astrocytes, microglia, and neurons were cultured and treated with different stimuli including, but not limited to, IL-1$\sigma$, TNF-$\Sigma$, HIV-1ADA, and HIV-1gp120. The cytokine and/or chemokine production from these cells was measured by real-time PCR and ELISA. The effects of IL-8 on neuronal survival were tested by double-stranded DNA fragmentation ELISA. The IL-8 expression in human brain tissues from HIV-1-infected patients was also examined. Our data show that 24 h treatment with HIV-1ADA, HIV-1gp120 or IL-1$\sigma$ cause 10- to 1000-fold increases in astrocyte IL-8 production as measured by ELISA. Both real-time PCR and ELISA data show that chronic activation of astrocytes with IL-1$\sigma$ for 7 days also significantly increased their IL-8 expression. Furthermore, while blocking the NF-$\chi$B pathway completely abrogated the IL-1$\sigma$-induced astrocyte IL-8 production, it shows no effect on HIV-1gp120–induced astrocyte IL-8 expression. Although, microglia and neurons may also produce IL-8, immunohistochemical analysis of brain sections from HIV-1-infected patients reveals that reactive astrocytes, but not microglia in the microglial nodules were positive for IL-8. While IL-8 by itself did not have any significant effects on astrocyte MCP-1 production, similar levels of IL-8 completely abolished the HIV-1ADA–induced neurotoxicity in primary human neurons. In summary, our data indicate that astrocytes are the major cellular sources of IL-8 in brain in the context of HIV-1 infection and this likely involves NF-$\chi$B regulation. The impact of IL-8 overexpression on neuronal survival is probably determined by direct effects on neurons and by microenvironment modulation through glial cell activation. How astrocyte-IL-8 regulation ultimately contributes to the pathogenesis of HAD is currently under investigation.

## P.86

### Macrophage-mediated dorsal root ganglion damage mediates altered nerve conduction in SIV-infected macaques

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Peripheral neuropathy is the most common neurological complication of HIV-1 infection, affecting over one-third of infected individuals including those treated with antiretroviral therapy. To study the pathogenesis of HIV-induced PNS disease, we established a model in which SIV-infected macaques developed changes closely resembling alterations reported in sensory ganglia of HIV-infected individuals including macrophage infiltration, SIV replication in macrophages, and neuronal loss. Significant declines in epidermal nerve fiber (ENF) density also developed in SIV-infected macaques ($P = 0.002$) similar to HIV-infected individuals with neuropathy. SIV-infected macaques also had significantly lower C-fiber conduction velocity in sural nerves than uninfected animals ($P < 0.001$) and the magnitude of CV decline correlated strongly with extent of DRG macrophage infiltration ($P = 0.006$). The finding that injury to neurons in the DRG mediated by activated macrophages was closely associated with altered function of unmyelinated nerve fibers in SIV-infected macaques suggests that macrophage-mediated DRG damage may be the initiating event in HIV-induced sensory neuropathy. Treatment of SIV-infected macaques with a combination of PMPA and minocycline initiated during early asymptomatic infection prevented both DRG macrophage activation and protected against loss of DRG neurons and epidermal nerve fibers. The SIV/macaque model will be valuable for defining mechanisms underlying HIV-induced PNS disease and for discovering novel therapies.

## P.87

### Place and Strategy Learning Deficits in the HIV-1 Transgenic Rat

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Infection with HIV-1 has been linked with several cognitive deficits. The nature of these cognitive deficits range from mild to profound and typically affect memory, attention, and motor function. In humans, these effects are difficult to study due to frequent co-morbidity with HIV. A new transgenic rat model has been developed that allows for more controlled investigations into the link between HIV and cognitive deficits. The HIV-1 transgenic (HIV-1 Tg) rat has almost the complete HIV-1 genome incorporated into its DNA with only the replication genes, gag and pol, deleted,

making this animal model of HIV non-infectious. The remaining HIV-1 Tg genes code functional viral proteins, including gp120 and Tat, both of which have been shown to be linked to cognitive deficits.

The HIV-1 Tg rats, however, have congenital cataracts that make visual information difficult for them to utilize during testing. For this reason, the rats were tested in the dark in a modified Morris water maze which provided rats with a combination of auditory, tactile, and olfactory cues arranged in such a way that each quadrant had a unique combination of cues. We previously found a learning deficit in HIV Tg rats that were tested in this modified water maze. In the present study we further characterize the nature of the learning deficit by making changes in the training procedures to test place, strategy, and reversal learning.

This experiment contained three distinct phases. Phase 1 was designed to test response learning. Rats were started from a fixed start point and were required to swim to a fixed platform location. Place learning and flexibility of behavior was tested following Phase 1 by starting the rats from randomized start locations and requiring them to swim to the same fixed platform location (Test 1) and to a moving platform in the same target quadrant (Test 2). Phase 2 tested the rats in the same procedure used in Test 2 but in the opposite target quadrant, and thus tested reversal learning. A single day (4 trials) of testing with the cues rotated 90 degrees from their original orientation tested the rats' reliance on the available cues. Place learning was made irrelevant in Phase 3 by requiring the animals to change their search strategy from a general quadrant search to a perimeter search.

The HIV-1 Tg rats showed subtle to strong impairments in all phases of the experiment. These results suggest that the HIV-1 Tg rat has deficits in place learning, strategy learning, and reversal learning as measured by this paradigm. These deficits are presumably due to viral interactions within the CNS. These results indicate that the HIV-1 Tg rat may be a good model for studying the cognitive deficits associated with NeuroAIDS. Future studies will investigate the relation between the behavioral deficits seen here and the levels of viral proteins within the brain.

## P.88

### The role of type 1 astrocytes in pro-inflammatory CNS signaling

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Astrocytes are the most abundant cells in the central nervous system (CNS), but astrocytes constitute a heterogenous population of cells with a variety of different functions. Astrocytes are divided into three major subgroups (type 1,2 and 3) but very little is known about the function of each subtype. Although it is known that astrocytes are capable of producing cytokines in response to a variety of stimulations including in response to pathogen infections, it is currently unknown which subtype is involved in immune functions in the CNS. To investigate the potential role of astrocytic subtypes in immune function we investigated the differential response of clones of the three subtypes of mouse astrocytes to neurotropic and non-neurotropic coronavirus infections. We studied the differential ability of astrocyte subtypes to mount pro-inflammatory immune reaction by analyzing by cDNA arrays cytokines, interferons and TNF profiles of each subtype of astrocytes and microglia in response to these infections. Although both type 1 and 2 astrocytes as well as microglia were infected by the viruses, only type 1 astrocytes and microglia cells produced different pro-inflammatory cytokine responses to neurotropic viral infection. The novel differential response of astrocyte subtypes suggests that only type 1 astrocytes serve along with microglia cells as the local immune system of the CNS.

## P.89

### Brain metabolism changes observed in subjects during primary/early HIV infection

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Introduction: HIV infection is believed to infiltrate and affect the brain early during the course of infection. Previously reported results in the SIV/macaque model of neuroAIDS have demonstrated neuronal injury during primary (acute) infection using both neuropathology and proton magnetic resonance spectroscopy (MRS). Temporal changes of metabolites in the frontal cortex of these animals led to the conjecture that SIV infiltration was occurring at this stage of infection. Until now, the translation of these results has not been verified in humans. Herein, we report both metabolic and immunologic differences between subjects deemed to be healthy controls and those with primary/early HIV infection.

Methods: Six subjects with primary/early HIV infection and eight healthy control subjects underwent magnetic resonance imaging and spectroscopy (1.5T GE Signa scanner). Subjects identified with primary HIV infection were imaged within 60 days of their western blot indicating indeterminate/positive results, and while they still had detectable viral RNA levels (on average, 105 copies of RNA/mL). Each subject had peripheral blood drawn within 48 hours of imaging and viral RNA levels were determined by RT PCR. Spectral analyses determining the quantities of N-acetylaspartate (NAA, the sum of NAA+NAAG), myo-inositol (MI), choline (Cho), glutamate + glutamine

(Glx), and creatine (Cr) in the frontal cortex were performed using LCModel software. Absolute concentrations and ratios were calculated. T-tests were used to confirm changes between controls and those with primary infection.

Results: Cho/Cr levels in the frontal cortex of subjects with primary HIV infection were 15% lower than those of healthy controls ($p < 0.03$). Similarly, absolute concentrations of Cho was found to be 11% lower in those with primary HIV infection ($p < 0.03$). Creatine levels were found to be similar between the two groups at this stage of infection in this brain region ($p = 0.14$). MI/Cr and absolute concentration of MI of those with primary HIV infection were not significantly altered from that of healthy controls. Levels of NAA and Glx were each discover to be 14% lower in subjects with primary HIV infection as well ($p = 0.01$, $p < 0.01$ respectively).

Conclusion: Within the frontal cortex of subjects with primary HIV infection, lower levels of both Cho and Cho/Cr were observed. These results confirm the previously reported decreases of Cho/Cr found within SIV infected macaques after peak virema. Choline decreases thus far have rarely been reported in neurologic diseases. Interestingly, preliminary results indicate that creatine, the central energy marker of neurons and astrocytes, does not appear to be different at this stage of infection in the frontal cortex. NAA, a marker of neuronal integrity, and Glx levels are decreasing, suggesting that the virus is capable of causing neuronal injury not only in the chronic stage of infection, but soon after HIV infection.

## P.90

### Olfactory nerve axotomy delays the onset of the acute phase response in mice inoculated intranasally with influenza virus

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The olfactory nerve is posited to be a possible route for viruses to reach the brain after intranasal (IN) infection. In previous studies with the mouse-adapted A/PR/8/34 (H1N1) (PR8) influenza virus we detected viral genomic RNA and viral replication intermediates as well as increased cytokine mRNA levels and cytokine immunoreactive cells in the olfactory bulb (OB) as early as 4 h (15 h for the cytokines) after IN infection. These results suggest that the olfactory nerve is likely being used by this strain of influenza virus to reach the OB. Furthermore, the OB has indirect connections with the hypothalamus, suggesting the possibility that the OB cytokines communicate with the hypothalamus to induce the acute phase response (APR). We hypothesized that surgically severing the olfactory nerves prior to IN infection with influenza virus will reduce or delay the APR. Adult C57BL/6 mice ($n = 16$) maintained at 24oC were surgically implanted with radio transmitters in the peritoneum to continuously monitor body temperature (Tb) and locomotor activity (LA). During the same surgical procedure the primary olfactory nerves were severed (OBA) using a flexible teflon transector blade inserted between the OB and the cribiform plate to sever all the olfactory axons projecting from the main olfactory epithelium ($n = 9$). Sham-operated mice ($n = 7$) received an identical surgical exposure of the OB as the experimental mice except the blade was not inserted. Following surgery mice were given 7 days to recover. After recovery, baseline temperature and activity were recorded for 3 days and after that all mice ($n = 16$) were infected IN at light onset (8:00 AM) with live PR8 virus. Mice were monitored for Tb and LA for 48 h after inoculation. Tb baseline values were not different between sham and OBA mice. Hypothermia was first apparent at 15 h post-infection (PI) in the sham group with the lowest value of 35oC recorded at 20 h PI. In contrast, in the OBA mice, hypothermia was first observed 26 h PI. After hypothermia was observed in the OBA mice, Tb behaved similarly in both groups with a decline that reached 33oC at 48 h PI. Normal diurnal fluctuations were observed only during the first 12-14 h PI. LA was diminished in the sham operated mice around the same time hypothermia was observed between 13 and 15 h PI. In OBA mice LA was higher until 26 h PI when it reached the same level of that in sham-operated mice; from that time on, both groups behaved similarly. These results suggest that the olfactory nerve route is important for early expression of the APR since severing the pathway delays the response to the infection.

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## P.91

### Altered recruitment of Sp1 and Sp3 isoforms during monocytic differentiation influences the activity of the HIV-1 LTR

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Infection of cells of the monocyte-macrophage lineage by human immunodeficiency virus type 1 (HIV-1) has been shown to be important in the pathogenesis of the acquired immunodeficiency syndrome. Viral replication in this cell lineage is, in part, mediated by interactions between the HIV-1 long terminal repeat (LTR) and a variety of host cell and viral proteins. Consistent with this logic, basal and activated LTR activity is dependent on interactions between the G/C box array within the HIV-1 LTR and the Sp family of transcription factors. The effect of monocytic differentiation on

Sp factor binding and transactivation has been examined with respect to the HIV-1 LTR. Primary monocyte-derived macrophages (MDM), as well as monoblastic (U-937 and THP-1) and myelomonocytic (HL-60) cell lines were utilized in the absence or presence of chemical differentiating agents, dimethylsulfoxide (DMSO) or phorbol myristate acetate (PMA), to model selected aspects of monocytic differentiation. The binding of Sp1, full-length Sp3, and truncated Sp3 to a high affinity HIV-1 Sp element was examined utilizing electrophoretic mobility shift (EMS) analyses. Sp1 binding increased relative to the sum of full-length and truncated Sp3 binding following PMA-induced monocytic differentiation in U-937, THP-1, and HL-60 cells. Relative Sp factor binding ratios obtained with nuclear extracts from PMA-induced cell lines were also shown to correlate with those derived from studies performed with extracts derived from primary MDMs. In addition, the altered Sp binding phenotype was shown to be associated with changes in the transcriptional activation generated by the HIV-1 G/C box array.

## P.92

### Seizures induced by Theiler's virus: a model for human seizure disorder

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We have established an infectious murine model for human seizure disorder. Infection of C57BL/6 mice with Theiler's murine encephalomyelitis virus induced transient seizures in about 50% of C57BL/6 mice. Seizures were not associated with fever and were very prominent between 3 to 10 days post infection. Coordination and motor function were impaired in the seized mice. Pyramidal neuronal loss and gliosis were prominent features of seized mice. Seizure activity inversely correlated with inflammation in the hippocampus. Due to the timing of the seizures, direct viral and innate immune responses contribute to the development of the seizures. The characterization of this model will allow us to study the involvement of virus and innate immune response in the central nervous system to the development of seizure disorders in humans.

## P.93

### Activation of the integrated stress response in patients with HIV-associated dementia

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Despite the introduction of highly active antiretroviral therapy (HAART), the prevalence of human-immunodeficiency virus associated dementia (HAD) has been steadily increasing. Neuronal injury and loss in HAD is initiated by HIV-infected, activated macrophages/microglia via soluble neurotoxic mediators, including reactive oxygen species (ROS). These mediators, which induce oxidative stress, are known to injure neurons directly and to alter astrocytic homeostatic functions such as glutamate homeostasis, which can lead to excitotoxic neuronal injury. A major downstream response to oxidative stress is induction of the integrated stress response (ISR), which includes both the ER stress response and the antioxidant response. We hypothesize that the ISR is activated in HAD patients. To begin to investigate ISR activation in HAD, we assessed the expression patterns of ISR proteins, Binding Protein (BiP) and NF-E2-related factor 2 (Nrf2), by immunofluorescence (IFA) and western blotting in autopsy tissue from HAD patients. BiP is a molecular chaperone protein whose expression is controlled by all three independently regulated branches of the ISR. Nrf2 is the primary regulator of the endogenous cellular antioxidant stress response, and is upregulated by the ISR. We saw a significant increase in total BiP levels by both IFA and western blot in HAD cortical tissue as compared with control tissue. Additionally, by phenotypic analysis of IFA, we saw cell-type specific increases in BiP levels in astrocytes. Nrf2 levels increased significantly in neurons, astrocytes, and overall in cortical tissue of HAD patients over that of control patients as determined by IFA. Further, analysis of IFA stained tissue revealed that, while Nrf2 levels increased in HAD tissue, 0 out of 5 HAD cases showed strong Nrf2 nuclear translocation. This trend suggests that, while Nrf2 protein levels may be upregulated by an activated ISR in HAD patients, the antioxidant response controlled by Nrf2 may not be successfully initiated, due to failure of this transcriptional regulator to translocate to the nucleus. Consequently, ROS released by activated macrophages, microglia, and astrocytes in this disease may overwhelm endogenous protection mechanisms resulting in neuronal death. Together these results suggest that the ISR is activated in HAD, although the endogenous antioxidant response, an important component of the ISR, may be aberrant in these patients.

## P.94

### Structure and function of a highly conserved HIV-1 LTR downstream regulatory element

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At least one CCAAT enhancer binding protein (C/EBP) site upstream of the TATA box is necessary for human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) activity in cells of the monocyte-macrophage lineage. However, no studies have been performed concerning C/EBP sites downstream (DS) of the start of transcription. TRANSFAC analyses of 100 subtype B LTRs have indicated three potential C/EBP

binding sites within the DS LTR. However, EMS analyses demonstrated that only one of the three sites (DS3, +158 to +175) was able to form DNA-protein complex(es) containing C/EBPbeta. Transient transfection studies utilizing the parental HIV-1 LAI-LTR or the LAI-LTR containing a knock-out 9C variant (LTR-DS3-9C, G-to-C change at position 9 of the site) demonstrated loss of binding to this site results in an approximate 50% decrease in basal LTR activity in the TF-1, U-937, and THP-1 monocyte-macrophage cell lines. In addition, C/EBP-induced activation of the LAI-LTR was reduced based on the loss of C/EBP binding to DS3. Interestingly, Tat-mediated transactivation of the LAI-LTR was elevated in the presence of the 9C knockout with co-expression of C/EBPbeta and Tat resulted in an even greater level of transactivation. Tat-mediated transactivation of the HIV-1 LAI-LTR was also enhanced in the presence IL-6 with the LAI-DS3-9C knockout. These results suggest that sequence alterations in the highly conserved DS3 element may influence the interaction of Tat with the HIV-1 LTR thereby affecting HIV-1 transcription. This interaction may be a critical element required for HIV-1 transcriptional regulation.

## P.95

### Perturbation of the viral co-receptor CCR5 by NB325 inhibits HIV-1 infection

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Despite the successes achieved using the latest therapies effective against HIV-1-associated immunopathogenesis, neurological disease associated with HIV-1 infection of the central nervous system remains a pressing problem. For this reason, the development of compounds effective against HIV-1, specifically the CCR5-utilizing strains predominant in the brain, remains a high priority. Our efforts in this area focus on compounds that inhibit HIV-1 infection by interfering with the role of CCR5 in viral binding and entry. NB325, which is a biguanide-based compound, is characterized by low toxicity and notable activity against HIV-1 BaL, a CCR5-utilizing viral strain. Results from flow cytometric analyses of primary human peripheral blood mononuclear cells (PBMCs) demonstrated increased CCR5 detection following exposure to NB325 despite effective inhibition of HIV-1 BaL infection. Differences in CCR5 detection were also noted between quiescent PBMC sub-populations and cells activated with PHA-P. We hypothesize that inhibition of HIV-1 BaL infection by NB325 may involve perturbation of CCR5 within the plasma membrane. These changes interfere with interactions with HIV-1, and make CCR5 more accessible to detection by CCR5-specific antibodies. Our current mechanism of action studies are building on the observation that NB325 also interacts specifically with CXCR4 extracellular loop 2 (ECL2). Ongoing investigations are exploring similar interactions between NB325 and CCR5 that may cause changes in co-receptor availability, localization, and/or conformation that result in inhibition of HIV-1 infection. These studies are being used to facilitate the development of novel compounds that can be used safely to inhibit HIV-1 CNS infection.

## P.96

### Chromatin immunoprecipitation of the HIV-1 LTR and sequencing of the HIV-ADA LTR

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Forty million people worldwide are infected with HIV, which causes AIDS and NeuroAIDS. NeuroAIDS includes behavioral and cognitive defects as well as motor abnormailities. Upon autopsy, over 90% of infected individuals show the characteristic pathology of NeuroAIDS, which includes neuronal damage and death, inflammation, elevated levels of the chemokine CCL2 in the CNS, and leukocyte infiltration of the CNS. Studies in humans and macaques indicate that cognitive impairment correlates with leukocyte presence and CNS inflammation. The cellular activities of HIV proteins are still being resolved. The viral protein Tat has pleiotropic activities and interacts directly with host cell chromatin. In astrocytes, Tat can directly interact with the CCL2/MCP-1 promoter, upregulating the expression of this chemokine. We hypothesize that Tat interacts with cellular proteins and chromatin directly in cells of the immune system, affecting transcription of host cell genes responsible for inflammation and chemotaxis of cells of the monocytic lineage into the CNS. To examine this, we will use ChIP on chip in order to identify host cell genes with which Tat directly interacts and Mass Spectroscopy in order to identify the proteins with which Tat interacts at the chromatin. To date we have successfully immunoprecipitated the HIVADA LTR using Tat antibody and sequenced this region of the virus, thereby demonstrating that direct immunoprecipitation of this protein while chemically crosslinked to DNA is possible. This project will identify components of the mechanism through which Tat mediates inflammation and chemotaxis. A more complete understanding of the effects of Tat on host cell function will contribute to the identification of therapeutic targets able to limit the devastating consequences of NeuroAIDS.

## P.97

### Functional synergy between CD40 ligand and HIV-1 tat contributes to inflammation: implications in HIV-1 dementia

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HIV-1 associated dementia (HAD) is believed to occur due to aberrant activation of monocyte-derived macrophages and brain resident microglial cells by viral proteins as well as by the pro-inflammatory mediators released by infected cells. To investigate the inflammatory aspects of the disease, we examined the levels of soluble CD40 ligand (sCD40L) in paired samples of plasma and cerebrospinal fluid (CSF) obtained from 25 HIV-infected individuals. A significantly higher level of sCD40L was detected in both CSF and plasma from HIV-infected patients with cognitive impairment, compared to their non-impaired counterparts. The contribution of sCD40L to the pathogenesis of HAD was then examined by in vitro experiments. Recombinant CD40L synergized with HIV-1 Tat to increase TNF-alpha release from primary human monocytes and microglia, in a nuclear factor kappa B (NF-?B)-dependent manner. The mechanistic basis for this synergism was attributed to a Tat-mediated upregulation of CD40 in monocytes and microglia. Finally, the CD40L-mediated increase in TNF-alpha production by monocytes was shown to be biologically important; immunodepletion experiments revealed that TNF-alpha was essential for the neurotoxic effects of conditioned medium recovered from Tat/CD40L treated monocytes. Taken together, our results show that CD40 signaling in microglia and monocytes can synergize with the effects of Tat, further amplifying inflammatory processes within the central nervous system and influencing neuronal survival.

## P.98

### Visualization of West Nile virus particles for examination on the cellular entry mechanism

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West Nile virus (WNV) is a flavivirus transmitted by Culex mosquitoes to vertebrate hosts and causes epidemics of febrile illness, meningitis, and, encephalitis. WNV is found throughout Africa, Europe, Central Asia, and, most recently in North America. Since the outbreak in New York during 1999, WNV has been subsequently spreading across the United States. In 2002, there were 4,156 reported cases of human illness, associated with 284 deaths in United States. Although virus entry into host cells is a crucial step of viral infection, the behavior of WNV at the entry step into host cells is still not clear.

As native WNV is limited to use in the biosafety level 3 (BSL-3), we initially attempted to synthesize subviral particles (SVPs) of WNV, which exclusively consist of WNV envelope proteins, M and E. We confirmed that the SVPs have similar characters as native WNV. To examine the cellular entry mechanism of WNV, we next tried to visualize the SVPs. We chose DiD a far-red fluorescent lipophilic dye to label the SVPs, because the long wavelength of DiD excitation shows a high S/N ratio, and succeeded in synthesis of the SVPs labelled with DiD (SVPs-DiD). We confirmed that fluorescent intensity level of the SVPs-DiD was enough high for observation by usual fluorescent microscopy. This system is useful for tracking the movement of the SVPs of WNV in living cells at the entry step using a time-lapse microscope.

## P.99

### NFAT4 is required for JCV infection of glial cells

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The human polyomavirus, JCV, infects 35-70% of the population worldwide. In immunosuppressed patients, JCV infection can lead to Progressive Multifocal Leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (CNS). JCV tropism is restricted to oligodendrocytes, astrocytes and B lymphocytes. The factors responsible for this limited tropism are not well understood. Previously our lab established a glial cell line (SVGR2) that is resistant to both wild-type JCV and a related polyomavirus, SV40, by selecting glial cells (SVG-A) that survived repeated rounds of JCV lytic infection. The block to viral replication in SVGR2 cells was identified to be at stage of viral transcription. Microarray comparison of SVG-A and SVGR2 cellular gene expression revealed a role for the transcription factor Nuclear Factor of Activated T-cell (NFAT) in both JCV and SV40 transcription and infection. In addition, viral activation of NFAT is important for the maintenance of viral transcription throughout the viral lifecycle.

## P.100

### Interplay of dendritic cell surface receptors and adhesion molecules with respect to HTLV-1 binding, entry, and transmission

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Despite the susceptibility of dendritic cells (DCs) to HTLV-1 infection and a demonstrated role of these cells in disease pathogenesis, the mechanisms of virus binding and entry have not been delineated. The identification of HTLV-1 receptor has been hindered due to its ubiquitous nature. Recently, a glucose transporter GLUT-1, heparan sulfate proteoglycans (HSPGs) and Neuropilin-1 (NRP-1) were demonstrated to facilitate HTLV-1 binding and entry, primarily in T cells. DCs express their own array of antigen receptors, most notably DC-specific ICAM-3-grabbing non-integrin (DC-SIGN), with respect to retrovirus binding. In this study, we analyzed the role of DC-SIGN and other known HTLV-1 receptors with respect to viral binding, entry,

and transmission using primary human monocyte-derived DCs and B cell lines stably expressing DC-SIGN. A comparative expression of DC-SIGN, GLUT-1, HSPG, and NRP-1 was examined and correlated with a number of biological endpoints. Inhibition of all these molecules dramatically reduced the binding of HTLV-1 virions as well as envelope glycoprotein gp46. With respect to HTLV-1 entry, GLUT-1 and HSPCs played an important role while inhibition of DC-SIGN and NP-1 had no apparent effect. Finally, the transmission of HTLV-1 from infected DCs to target T cells was analyzed and was found to be primarily mediated by DC-SIGN consistent with its known role for other viruses. These observations were also repeated ex vivo with myeloid and plasmacytoid DCs. This study represents the first comprehensive analyses of known HTLV-1 receptors on a major immune cell population that plays a critical role in viral binding and transmission

## P.101

### Bone marrow rearrangement of JC virus regulatory region in progressive multifocal leukoencephalopathy

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Background: JC virus (JCV), the agent of progressive multifocal leukoencephalopathy (PML), displays a stable archetypal regulatory region (RR) in the kidneys, where it remains quiescent. However, rearrangements of the RR are found in the CNS of PML patients and are associated with JCV neurotropism and neurovirulence.

Methods: JCV DNA viral load was measured in bone marrow (BM), PBMC, plasma and urine by QPCR using JCV T-specific primers. JCV RR was amplified by nested PCR, cloned and sequenced. Double immunohistochemical staining (IHC) was performed on BM biopsy tissue sections using an anti-VP1 and anti-CD138 ab. JCV-specific CD8+ cytotoxic T lymphocytes (CTL) was analyzed in blood samples by tetramer staining assay.

Case report and Results: A 70 year old man with rheumatoid arthritis (RA) treated with methotrexate developed PML.

Methotrexate was discontinued and the patient was treated with cytarabine and mirtazapine, but he passed away 13 weeks after disease onset. JCV specific CTL response was at limit of detection of the assay.

All JCV RR clones from plasma had a tandem repeat of 98bp elements consistent with the neurotropic Mad-1 RR, and urine samples contained an RR with partial truncation and duplications of the 98 bp element. Interestingly, the major form of BM RR (92% of the clones) contained a partial duplication of the 98bp element and truncation of the 23 and 66bp inserts, while a minor BM species of RR was similar to archetype (8% of the clones).

IHC on the BM biopsy sample showed that 94% of JCV-infected cells were CD138+ plasma cells, and these accounted for 9% of all plasma cells in the biopsy specimen.

Discussion: These results indicate that archetype JCV RR is present in BM and that rearrangements of the RR occur in this compartment. It is therefore possible that JCV gained its neurotropic phenotype in the BM of this patient, and this strain may have reached the CNS via hematogenous spread. Demonstration of productive JCV infection of BM plasma cells support this hypothesis. Finally this case illustrate that RR with partial tandem repeat can also arise from kidney.

Conclusion: This is the first report of a detailed analysis of JCV RR from BM of a PML pt. and represent evidence that rearrangements in the RR that may lead to PML can occur in this body site.

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## P.102

### Dendritic cells as a therapeutic vaccine against Progressive Multifocal Leukoencephalopathy

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Background: Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the brain caused by JC virus (JCV) which occurs in immunosuppressed individuals. There is no effective treatment for this disease, but PML patients who have JCV-specific cytotoxic T lymphocyte (CTL) in their blood have a better clinical outcome. One of the most promising strategies for boosting T cell immunity is through autologous dendritic cell (DC) immunization. In this study we sought to expand JCV- specific CTL ex-vivo using JCV antigen-loaded dendritic cells or peripheral blood mononuclear cells (PBMC) cultures from healthy individuals (HI), HIV+/PML – (HIV) and PML patients (PML).

Patients and methods: Peripheral blood samples were obtained from HLA-A*0201+ subjects, including 10 JCV-seropositive HI, 6 PML and 4 HIV+

patients. Monocyte-derived mature DC pulsed with HLA-A*0201-restricted JCV VP1p36 and/or VP1p100 epitopes were mixed with autologous T cells at a ratio 1:10. In parallel, JCV - VP1p36 and/or VP1p100 epitopes stimulated PBMC were cultured in the presence of IL-2. After 12 days, JCV specific CTL responses were compared in both cultures using tetramer staining assay.

Results: JCV-specific CTL responses against VP1p36 and/or VP1p100 epitopes, present in 5/10 HI, 3/4 HIV+ pts and 4/6 PML pts using peptide stimulation of PBMC, were enhanced when using Ag-loaded DC stimulation. After stimulation with Ag-pulsed DC a response was detected in 1 HIV+ pt who had no response with peptide stimulation of PBMC only. In 4 HI and 1 PML pts. no JCV-specific expansion was obtained either in PBMC or DC cultures. The mean percentage of JCV-specific CD8+ T cells in peptide-stimulated PBMC compared to DC cultures was 1.1% (0.2-2.6%) vs 29% (12.3-48.2%) in HI, 2.1%(0-4.2%) vs 5.2% (0.2-8%) in HIV+ pts and 0.5% (0.3-1.1%) vs 7.2% (0.4-26%) in PML pts.

Discussion: DC pulsed with HLA-A201-restricted JCV peptides were able to enhance the CTL expansion in the 3 study groups. The lack of any detectable JCV CTL expansion in PBMC or DC cultures of 4 HI and 1 PML pts. could be explained by an immune response directed toward other JCV epitopes.

Conclusion: Monocyte-derived mature DC loaded with JCV antigens can induce potent specific CTL expansion in vitro and offer a promising approach to PML immunotherapy.

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## P.103

### Interplay of host and viral factors in the clinical outcome of patients with Progressive Multifocal Leukoencephalopathy (PML).

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Objectives: To determine the role of immunological and virological factors in the clinical outcome of PML

Methods: We enrolled 60 PML pts (75% HIV+, 25% HIV-) in this study, as well as 20 HIV+ pts matched for CD4 count and plasma HIV VL and 15 healthy individuals (HI). JCV DNA viral load (VL) was measured in PBMC, plasma and urine by QPCR using JCV T-specific primers. JCV RR was amplified by nested PCR, cloned and sequenced. JCV-specific CD8+ cytotoxic T lymphocytes (CTL) were analyzed in blood samples of HLA A*0201+ subjects by tetramer staining assay for VP1 p36 and p100 and HLA A*0201- subjects by 51Cr release assay using pool of overlapping peptides covering the VP1 protein.

Results: The mean age of PML and HIV+ pts was 49 y and 35y for HI. HIV+/PML and HIV+ pts had a CD4 cell count of 263 vs 398 /$\mu$L, and a plasma HIV VL of 1.5 vs 2.2 log10 copies/ml, respectively. JCV DNA was detected in the urine of 48% PML, 61% HIV+ and 26% HI. JCV DNA was detected in plasma and PBMC of 39 and 54% PML, 10 and 30% HIV+ and 0 and 0% HI. JCV detection was significantly increased in plasma of PML vs HIV+ pts. ($p = 0.024$).The mean JC VL in blood was similar in PML and HIV+ pts and among the 3 study groups in urine.

In HLA A*0201+ PML pts there was a positive correlation between the magnitude of JCV VP1 p100 CTL response and JC VL in urine ($r = 0.92$ $p = 0.03$) or blood (plasma $r = 0.63$ $p = 0.05$; PBMC $r = 0.76$ $p = 0.04$). Among PML pts, survival at one year was 72 vs 42% for those with detectable vs undetectable JCV-specific CTL within one year of diagnosis, respectively ($n = 34$ Hazard Ratio of death (HR) 0.42, $p = 0.14$). Of 52 PML who had an MRI, 13 (25%) had contrast enhancement (CE) of PML lesions. Of these 13 pts, 12 (92%) survived more than one year, and 10 (77%) had detectable JCV CTL compared to 18/39 (46%) pts with no CE who had a CTL response (OR 3.9; $p = 0.064$). The analysis of JCV RR sequences is in progress and will be presented at the meeting.

Conclusion: JCV detection in plasma is significantly more frequent in PML vs. HIV+ pts. The presence of JCV CTL was associated with a higher rate of survival at one year. CE of PML lesions on MRI appears to be a useful prognostic marker of disease evolution.

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## P.104

### JC Virus Excretion Pattern in HIV infected Portuguese Patients

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Objectives: JC virus (JCV) is ubiquitous in human population. After initial infection that normally occurs during childhood, JCV seems to remain latent in renal tissue, and reactivation occurs under immunosupression conditions, with progeny shed in urine.

However, in severe immunosupresion situations as those resulting from advanced HIV infection, JCV

reactivation could result in PML, a fatal CNS disease. Actually PML is one of the most frequent CNS opportunistic infections among HIV-infected people, and AIDS represents it's most frequent underlying cause of immunossupression.

In this order, the present study intends to begin the evaluation of prevalence and pattern of JC virus excretion in HIV infected Portuguese patients. The results obtained will be compared with those described for non-HIV infected population, in order to evaluate if HIV infection affects the demographics of JCV urinary excretion.

Methods: Urine samples from 49 HIV infected Portuguese patients in different stages of the disease, were collected and screened for the presence of JCV DNA.

This patient group includes 10 women and 33 men, aged between 28 and 75 years old (43 mean age).

The median CD4 cell count was 350 cells/microL and the median viral load was 131062 copies/mL.

After DNA extraction, each sample was subjected to PCR amplification performed with specific primers from the VP1 coding region of JCV genome. Amplified products were visualized by agarose gel electrophoresis.

Results and Conclusions: Twenty-five (58%) out of 49 urine samples revealed the presence of the expected fragment from JCV genome. Thirty percent of women and 67% of men in this study excretes JC virus in urine.

The age groups presenting a greater prevalence of JCV viruria were 30 to 39 and over 60 years old, with 79% and 100%, respectively.

Rate of JCV urinary excretion don't seems to vary with CD4 cell count as well as with HIV RNA viral load.

Conclusions: The present study, although only at the beginning, points to a value of 58% of HIV-infected Portuguese population that excretes JC virus in urine, which is concordant with prevalence rates observed in other countries.

Our preliminary data also reveals and confirms that immunodeficiency as well as HIV plasmatic viral load don't seems to affect JC virus urinary excretion.

Although the excretion rate observed in HIV-infected Portuguese populations is similar to that described in immunocompetent population, age distribution doesn't seems equivalent, which could be due to the little number of samples evaluated.

## P.105

### CCR5-deficiency prevents injury to neuronal dendrites and synapses in a transgenic mouse model of HIV-1 neuropathology expressing viral envelope protein gp120 in the brain

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We investigated the potential role of coreceptors for human immunodeficiency virus-1 (HIV-1) in promotion and prevention of the neuropathology associated with HIV-1 infection. HIV-1 coreceptors CCR5 and CXCR4 are G protein-coupled chemokine receptors that interact, in conjunction with CD4, with the envelope protein gp120 of HIV-1. In earlier in vitro experiments using mixed neuronal-glial cultures from either wild-type or knock-out rodents (genetically deficient in one or both HIV-1 coreceptors), we found that depending on the viral strain, CCR5, CXCR4, or both mediated neurotoxicity of HIV-1/gp120. Conversely, physiological CCR5 ligands MIP-1beta or RANTES protected neurons from gp120-induced toxicity. For the present in vivo study we crossed mice expressing an HIV-1 gp120 transgene in brain (gp120tg; Toggas S. et al., Nature 367: 188, 1994) with CCR5KO mice only, since CXCR4-deficiency is perinatally lethal. One hallmark of HIV-1 neuropathology that brains of AIDS patients and gp120 transgenic mice share is the reduced number of neurites and synapses in comparison to wild type (wt) controls. This loss of neuronal processes and synapses correlates well with neurocognitive impairment in AIDS patients and is reflected by a diminished immunoreactivity of the neuropil for microtubule-associated protein (MAP)-2 and Synaptophysin, respectively. We estimated the percentage of MAP-2 or Synaptophysin positive neuropil by deconvolution microscopy in sagittal brain sections of 6 month old gp120tg mice both expressing and lacking CCR5 using CCR5KO and wild type animals as control. CCR5wt/gp120+ mice displayed a significant reduction in the percentage of MAP-2 positive neuropil in comparison to all of the other three genotypes (MAP-2 positive neuropil in % +/− SEM, n = 3 animals per group; CCR5wt/gp120+: 35 +/− 3, $p < 0.001$ (ANOVA and post hoc test) in comparison to CCR5KO/gp120+: 51 +/−2, or CCR5wt/gp120-: 47 +/− 2, or CCR5KO/gp120-: 50 +/− 2). Similarly, a reduction of Synaptophysin reactivity was observed in CCR5wt/gp120+ mice but not the other three genotypes. We thus propose that CCR5 is required for the HIV-1 gp120 transgene to cause damage to neuronal processes and synapses in this in vivo model, a finding consistent with the coreceptor usage of most HIV-1 strains previously isolated from brains of AIDS patients.

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## P.106

### Neuroprotective effects of small, non-peptide neurotrophin mimetics in neural cultures challenged with gp120 or feline immunodeficiency virus

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HIV-associated CNS disease is largely due to the development of an inflammatory response that subsequently damages neurons. Circulating cytokines, chemokines and viral proteins provoke dysfunctional regulation of calcium, mitochondrial reactive oxygen species generation and changes in receptor signaling that disrupt neuronal function and ultimately shift the homeostatic balance toward apoptosis. Signal transduction pathways that favor Akt activation and discourage GSK3$\beta$ signaling are thought to play an important role in reducing inflammation, maintaining normal neuronal function and discouraging apoptosis. Growth factors are strong modulators of this pathway but have had limited therapeutic application due to difficulties in delivery to the CNS. The development of several small, non-peptide mimetics of nerve growth factor and brain-derived neurotrophic factor have made it possible to more effectively exploit the potential benefits of neurotrophin receptor activation. To assess their therapeutic potential in the context of HIV neuropathogenesis, we tested three compounds on primary rat neural cultures challenged with gp120 or lipopolysaccharide/interferon-gamma. Addition of three prototype compounds, LM11A-31, BD2-4 and BD2-10, to the culture medium at concentrations of 10, 500 and 1000 nM resulted in a 50-100% reduction in cell death for each challenge. For a more sensitive assessment of neuronal dysfunction, we evaluated dendritic damage and neuronal loss in FIV-infected neural cultures over a period of 3-14 days post-inoculation. The appearance of dendritic beading, pruning and tortuosity, the loss of neurons, microglial proliferation and astrogliosis were all inhibited by these compounds. Neuron survival was completely restored by each compound although subtle differences in the reversal of dendritic damage and astrogliosis were seen. These compounds offer a novel therapeutic approach to prevent the destructive effects of lentiviral infection of the nervous system.

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## P.107

### Varicella zoster virus in saliva of patients with herpes zoster

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Varicella zoster virus (VZV) DNA has been found in saliva of healthy astronauts without rash during and after space flight as well as in saliva of patients with the Ramsay Hunt syndrome, which results from VZV reactivation in geniculate ganglia. It is not known whether VZV DNA is present in saliva of patients with herpes zoster in other dermatomes. We hypothesized that a prospective analysis of patients with zoster would reveal VZV in saliva. We treated 54 patients with herpes zoster with oral valacyclovir. On the first day of treatment, and 7- and 14-days later, pain levels were scored and saliva was examined for VZV DNA. Saliva from 6 control subjects with chronic non-dermatomal distribution pain and from 14 healthy control subjects was similarly studied.

For 50 of the 54 zoster patients, follow-up data were available. During the 14-day study period, pain decreased in 43 (86%), disappeared entirely in 37 patients (74%), recurred after it had disappeared in 3 patients (6%), and increased in 4 patients (8%). VZV DNA was found in saliva of every zoster patient on the day treatment was started. In 47 (94%), the salivary VZV DNA burden decreased. In 41 patients (82%), viral DNA disappeared. No patients exhibited any increase in viral DNA after it had begun to decline or disappear. There was a significantly positive correlation between the presence of VZV DNA in saliva and pain levels ($P < 0.0005$), as well as between the VZV DNA burden and degree of pain ($P < 0.0005$). Saliva of 2 zoster patients was cultured for virus isolation, and infectious VZV was isolated from 1 patient. VZV DNA was present in saliva in 1 patient before rash began and in 4 patients after pain resolved. No VZV DNA was detected in saliva from any control subjects. Analysis of human saliva is a non-invasive procedure with potential usefulness in diagnosing neurological disease produced by VZV in the absence of rash.

## P.108

### Spatiotemporal localization of TLR3 in human neurons infected with rabies virus

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Human neurons express the innate immune response receptor, Toll-like receptor-3 (TLR-3) as it has been shown in human post mitotic neurons and in Purkinje neurons of cerebellar cortex in postmortem human rabies cases (Prehaud et al, 2005) (Jackson et al, 2006). Here, we investigated further the expression and function of neuronal TLR3 in the time course of rabies virus infection in human neurons. In the absence of infection, TLR3 molecules are located in endosomes. Following rabies virus infection, TLR3 is not only present in endosomes but also in detergent resistant inclusions bodies located in the nucleus vicinity. Absence

of association with centrioles and absence of ubiquitinylated proteins suggested the inclusions bodies are not aggresomes, structures where proteins produced in excess or misfolded proteins are stored before elimination. However, by using nocodazole, we observed that the structures are tightly associated with the microtubule network. Besides TLR3, these inclusions bodies contain rabies virus proteins (N and P but no G). Detection of nucleocapsids in these structures suggests that inclusion bodies may correspond to sites involved in virus transcription/replication processes.

The size of the inclusion bodies (2–4 $\mu$m), their composition and the absence of surrounded membrane, suggest they correspond to the previously described Negri Bodies (NB). Confocal analysis and 3D modelisation indicate that NB structure is strictly organized with a nuclear core containing TLR3 surrounded by a corona composed with viral N and P proteins. The role of TLR3 in the course of rabies virus infection by using RNA silencing is under investigation.

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## P.109

### Agnoprotein of JCV inhibits oligodendrocyte differentiation and survival of oligodendrocyte-like cells

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We utilized a bipotential cell line that can be induced to differentiate into oligodendrocytes and type 2 astrocytes to investigate the biological impact of JCV agnoprotein. Replication of JCV in brain, an event that occurs upon immune suppression, leads to the destruction of oligodendrocytes and the development of the fatal demyelinating progressive multifocal leukoencephalopathy (PML). Our results show that in the absence of productive viral replication, agnoprotein is capable of affecting oligodendrocyte differentiation as evidenced by morphological changes due to delayed formation of processes and low complexity of outgrowth network. Differentiation of oligodendrocytes resulted in a high level of DNA damage in agnoprotein-positive cells as detected by DNA fragmentation and Comet assays. Analysis of the molecular profiles of differentiated cells revealed induction of proapoptotic and suppression of prosurvival signaling pathways in the presence of agnoprotein. These observations and our previous reports indicating involvement of agnoprotein in regulation of the cell cycle and DNA damage repair pathways suggest that, independent of productive viral infection cycle, agnoprotein may contribute to the destruction of myelin-forming cells that is observed upon JCV infection in PML.

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## P.110

### Treatment of acute flaccid paralysis caused by West Nile virus in hamsters with a humanized neutralizing monoclonal antibody

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Acute flaccid polio-like paralysis occurs during natural WNV infection in a small fraction of cases in animals and humans. To evaluate pathogenesis and the possibility for therapeutic intervention, we developed a uniform model of flaccid hind limb paralysis by injecting WNV directly into the sciatic nerve or spinal cord of hamsters. When WNV was injected into the thoracic (T8) or lumbar spinal cord (L1-4), 100% of hamsters developed paralysis within 6-18 days and then subsequently died from systemic infection. When the sciatic nerve was injected with WNV and either left intact or transected below the injection site, 50% and 21% of the animals became paralyzed, respectively, on the ipsilateral side of injection. In contrast, if the sciatic nerve was transected proximal to the WNV injection site, animals did not developed paralysis on the ipsilateral side. These data suggested that WNV undergoes axonal transport from the sciatic nerve into the lumbar spinal cord to cause paralysis. Confocal immunohistochemical staining of spinal cord sections from paralyzed hamsters indicated that WNV-infected neurons rapidly underwent apoptosis. WNV infection localized primarily to the ventral motor horn of the gray matter, consistent with the polio-like clinical presentation. Cell culture studies using a compartmentalized neuronal culture system established that WNV can rapidly spread via axonal transport in both retrograde and anterograde directions, and that this spread was disrupted by a neutralizing humanized monoclonal antibody (mAb), hE16. Accordingly, a single i.p. injection of hE16 (32 mg/kg) significantly reduced paralysis and mortality of hamsters injected at day 2 or 3 after WNV inoculation of the spinal cord at T8 vertebrae. Additionally, a single i.p. injection of hE16 administered as late as 5 days after WNV inoculation of the sciatic nerve prevented paralysis in all hamsters. Overall, our experiments establish that (a) WNV undergoes axonal transport, which leads to a specific clinical phenotype, acute flaccid paralysis; and (b) hE16 antibody therapy, prevents hind limb flaccid paralysis

in hamsters, even after WNV infects spinal cord neurons.

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## P.111

### Characterization of recombinant monoclonal antibodies directed against VZV IE63 protein expressed in eukaryotic and prokaryotic cells

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The varicella zoster virus (VZV) genome encodes two copies (ORFs 63 and 70) of the tegument-associated, immediate early 63 protein (IE63). During latency, IE63 is expressed in the cytoplasm of neurons, but is predominantly nuclear during productive infection. Phosphorylation of IE63 may account for protein localization and function, a hypothesis, which could not be tested due to lack of antibody that differentiates nuclear and cytoplasmic forms of IE63. Herein, we report the construction and characterization of recombinant monoclonal antibodies that bind different forms of IE63. To construct these antibodies, VZV ORF 63 was inserted into vectors which expressed N-terminally Flag-tagged IE63 either in bacteria (to identify unmodified forms of IE63) or in mammalian cells (to identify fully processed IE63). After expression of VZV IE63, IE63 was purified by Flag-affinity chromatography and used to immunize mice. When mice developed anti-IE63 antibodies (2 months), spleens were harvested, total RNA was extracted, mRNA was isolated and cDNA was synthesized. Both heavy and light chain IgG variable regions were PCR amplified and inserted into the phage-display vector pCOMB3H. Phage libraries which displayed recombinant IgG-Fab were panned against both prokaryotic or eukaryotic expressed Flag-tagged VZV IE63. The DNA sequence of enriched recombinant phage heavy and light chain variable regions were determined and shuttled into the mammalian expression vector pCEP4. Expression of the pCEP4 plasmids in human 293 cells permitted affinity purification of two monoclonal antibodies. Western blot analysis showed that the first monoclonal antibody detected VZV IE63 expressed in prokaryotic or eukaryotic cells as well as in VZV-infected MeWo cells. The second monoclonal antibody detected IE63 expressed predominately in eukaryotic cells (both transfected or infected); this antibody produced against eukaryotic expressed IE63 detected IE63 predominantly in the nucleus of VZV-infected MeWo cells, while the antibody produced against prokaryotic expressed IE63 detected VZV IE63 predominantly in the cytoplasm. The epitopes that are bound by these antibodies have been characterized and will be reported. These antibodies will allow the characterization of the post-translational modified forms of IE63 in VZV-infected cells.

## P.112

### Identification of the mechanisms used by p53 to inhibit HIV-1 gene expression and replication

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Increasing evidences point to the involvement of p53 in neurodegenerative diseases including HIV-1 associated dementia (HAD). p53 accumulation and DNA damage have been reported in neurons from HAD patients, as well as in non-neuronal CNS cells, including microglia and astrocytes. Taken together, these studies demonstrate that the CNS environment in HAD leads to a sufficient degree of injury to activate p53 in neuronal and non-neuronal cells. The mechanisms leading to p53 induction in HIV-1 infected patients are not well understood and remain to be identified. In this regard, we recently demonstrated that the protein kinase cdk9 phosphorylates p53 on serine 392 via their physical interaction (Claudio et al., J. Cell. Physiol. 2006). Therefore, we sought to investigate whether p53-cdk9 association affects HIV-1 gene expression and replication in CNS cells. By employing an adenovirus expression vector, we demonstrated that overexpression of p53 prevents cdk9-induced phosphorylation of polymerase II. Further, we demonstrated that wild type, but not mutant, p53 decreases the levels of Tat-induced activation of HIV-1 LTR. In addition, we showed that p53 significantly reduced HIV-1 replication in primary microglial. Using ELISA measurement, we demonstrated that p53 decreased HIV-1-induced cytokines production (e.g. TNF$\alpha$). To overcome the effect of p53, we demonstrated that cdk9 associate with Pirh2 to promote the ubiquitination and degradation of p53. These studies present a novel therapeutic approach for the inhibition of HIV-1 gene expression and replication and the treatment of AIDS in brain.

## P.113

### Role of p53 family in neuropathogenesis of AIDS

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Despite the use of highly active antiretroviral therapy (HAART), neuronal cell death remains a problem that is frequently found in the brains of HIV-1-infected patients. HAART has successfully prevented many of the former end-stage complications of AIDS, however, with increased survival times, the prevalence of minor HIV-associated cognitive impairment appears to be rising among AIDS patients. Further, HIV encephalitis (HIVE) is still prevalent in treated patients as well as attenuated forms of HIVE and CNS opportunistic disorders. HIV-associated cognitive impairment correlates with the increased presence in the CNS of activated, though not necessarily HIV-1-infected, microglia and CNS macrophages. This suggests that indirect mechanisms of neuronal injury and loss/death occur in HIV/AIDS as a basis for dementia since neurons are not themselves productively infected by HIV-1. Recent evidence indicates that the p53 tumor suppressor protein and its related family member, p73, play an essential role in regulating neuronal loss/apoptosis. The mechanisms involved in induction of p53 or p73 which lead to dendritic retraction and neuronal loss are not fully elucidated. Increased levels of p53 were observed in the neurons of AIDS patients, which may be connected to the occurrence of HIV-1-associated dementia (HAD) in those patients. Similar to p53 activation in neurons, p53 activation in microglia and astrocytes may also contribute to alterations in the physiology of these cells that eventually result in a neurotoxic environment and neuronal loss. Importantly, for p53 to be functional and stable, activated p73 is required. Based on this finding, we have studied the relationship between p73 and Tat, and have demonstrated the ability of HIV-1 in general, and of Tat in particular, to induce the endogenous levels of p73. P73 induction prevents acetylation of Tat on lysine 28 through their direct physical interaction, which also inhibits Tat's apoptotic activity. The association of Tat with p73 also reduces Tat-activation of the HIV-1 LTR, and prevents p73 from causing cell death in astrocytes. These data suggest a basis for the restricted replication of HIV-1 in astrocytes. We also investigated the interplay between Tat and p73 in neuronal cells. Surprisingly, Tat was unable to promote neuronal death in the absence of either p73 or p53, suggesting a strong link between Tat, p73, p53 and neuronal cell death. Hence, we now propose to study the mechanism(s) whereby Tat induces p73. This will include analysis of control of the p73 promoter and of p73 protein turnover. We will also examine whether induction of p73 by Tat leads to activation of p53 and induction of neuronal cell death. The outcome from the proposed studies should provide new information regarding mechanisms of neuronal loss in AIDS patients and suggest possible new therapeutic approaches.

## P.114

### Characterization of HIV-1 binding to peripheral blood mononuclear cells (PBMCs) and monocytes/macrophages (M/M): relationship to neuropathogenesis

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Background. Individuals with HIV-1-associated dementia (HAD) are characterized with increased percentages of circulating activated M/M with CD14/CD16 phenotype. Higher levels of HIV-1 DNA are detected in these activated cells, therefore we hypothesize that they are more permissive to infection and thus the magnitude of viral binding will be higher.

Methods. The study was performed following guidelines established by the local institutional review board. From a non-HIV-1-infected volunteer, peripheral blood mononuclear cells (PBMCs) and magnetic bead-separated monocytes were immediately placed in 96-well polypropylene plates [5x10(5) cells/well] and exposed to 2ng p24 units of LAI (X4 Strain) and p89.6 (dual tropic but preferentially X5 strain) for one hour at 37°C, 5% CO2 in triplicate. Unbound virus was washed off using PBS and the cells were lysed for RNA extraction. Viral binding capacity was assayed by RT-PCR using HIV GAG and beta-actin primers with appropriate positive and negative control RNA. Amplified fragments were resolved on 2.5% agarose gels and analyzed by densitometry. From the scanned gels, the ratio of HIV GAG light units/beta-actin light units was compared between the two cell populations and the virus strains. Differences in binding capacities between each of the two groups were considered significant by Student's t-test if $p < 0.05$.

Results. As expected, M/M displayed a higher HIV-1 binding to p89.6 than to LAI, 0.497 vs. 0.328 ($p < 0.05$), respectively. In the PBMCs, viral binding capacity was increased compared to M/M , for LAI: 0.492 vs. 0.328, respectively ($p < 0.05$); for p89.6: 0.878 vs. 0.497, respectively ($p < 0.05$). Of note was the significantly higher binding found with p89.6 (0.878) compared to LAI (0.492) ($p < 0.05$), since the PBMCs were from the same volunteer obtained at the same time.

Conclusion. These results demonstrate that peripheral M/M preferentially bind R5 virus suggesting that the high HIV DNA found in PBMCs represents bound virus on the M/M subset. The enhanced binding of R5 strains to M/M may lead to more permissive infection

of this PBMC subset. The theory that increased trafficking of HIV-1-infected M/M to the central nervous system is consistent with our findings. (Supported by NIH grants NS053345, U01A134853, and P20RR011091; and the Fulbright Staff Development Fellowship)

## P.115

### The varicella zoster virus vasculopathies

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Varicella zoster virus (VZV) vasculopathy produces transient ischemic attacks or stroke secondary to viral infection of cerebral arteries. Unfortunately, diagnosis is not always straightforward. Not all patients have rash before cerebral ischemia or stroke. Furthermore, other vasculitides produce similar clinical features, as well as comparable imaging, angiographic and CSF abnormalities. Herein, we review our 23 published cases and 7 unpublished cases of VZV vasculopathy. All CSFs were tested for VZV DNA by PCR and for anti-VZV IgG antibody by enzyme immunoassay and found to be positive for either or both. Among the 30 patients, rash occurred in 19 (63%), CSF pleocytosis in 20 (67%), and abnormalities on brain magnetic resonance imaging (MRI) or computerized axial tomography (CAT) scanning in 29 (97%). Conventional angiography or magnetic resonance angiography (MRA) in 23 patients revealed abnormalities in 16 (70%). Both large and small arteries were involved in 15 (50%), small arteries alone in 11 (37%) and large arteries alone in only 4 (13%) of the 30 patients. The average time from rash to onset of neurological symptoms and signs was 4.1 months. Disease was usually protracted so that the average time from neurological symptoms and signs to virological analysis of CSF was 4.2 months. The CSF of 9 (30%) patients contained VZV DNA while 28 patients (93%) had anti-VZV IgG antibody in their CSF; in each of these 28 patients, a reduced serum/CSF ratio of VZV IgG confirmed intrathecal synthesis.

Overall, the data reveal that rash or CSF pleocytosis is not required for diagnosis of VZV vasculopathy, whereas MRI/CAT abnormalities are seen in almost all patients. Furthermore, unlike earlier descriptions of exclusive involvement of large cerebral arteries, most patients had mixed large and small artery involvement. Detection of anti-VZV IgG antibody in CSF was a more sensitive indicator of VZV vasculopathy than detection of VZV DNA ($p < 0.001$). Most patients were treated with acyclovir, with or without steroids, and improved or stabilized. However, because our patients received different treatment regimens at different institutions in an uncontrolled setting, the determination of optimal dose, duration of antiviral treatment, and benefit of concurrent steroid therapy awaits studies with larger case numbers.

## P.116

### HIV induces complement C3 in primary human astrocytes indirectly through a NFkB-dependent pathway

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Innate immunity is believed to play an important role in protecting brain against HIV infection and development of HIV-associated dementia (HAD). Complement C3 is a key component of the innate immunity system but in models of other neurodegenerative diseases such as multiple sclerosis, uncontrolled C3 production can lead to tissue injury and inflammation. C3 is secreted by several cell types in the brain including astrocytes. We and others have previously shown that HIV interaction with astrocytes in vitro alters cellular gene expression, induces interleukin-6, and disrupts important physiological functions of the cells such as glutamate transport.

Here we investigated the mechanism of C3 induction in human fetal astrocytes (HFA) exposed to HIV in vitro. Using transient transfection assays with a C3 promoter-driven indicator gene, real-time PCR, and C3 ELISA we found that exposure of astrocytes to HIV increased C3 promoter activity, mRNA expression, and protein production, indicating that HIV acts

by transcriptional activation of the C3 gene. Unlike other effects of HIV in astrocytes, which are induced rapidly after the viral stimulus, elevated expression of C3 mRNA and protein peaked at 7-10 days, indicating an indirect pathway of induction. Studies with pharmacological inhibitors revealed that activation of C3 by HIV requires, among others, the NFkB and PTK signaling pathways; however, surprisingly, the C3 core promoter lacks NFkB and SP binding sites. In contrast, activation of the C3 promoter by HIV was absolutely dependent on the presence of the interleukin 1-B/interleukin 6 responsive element at -109 to -90 and its sub-domain -106 to -100. The requirement for functional NFkB for HIV effect on C3 was confirmed by overexpression of the NFkB inhibitor IkB-a in astrocytes; adenovirus-mediated transduction of IkB-a into HFA, but not of control virus, blocked nuclear translocation of NFkB and prevented induction of C3 promoter and mRNA. These results indicate that HIV activates C3 in astrocytes indirectly through NFkB-dependent induction of an unknown mediator that in turn activates C3 transcription via the -109 to -90 element in the promoter. Studies are in progress to identify the factor that mediates HIV activation of C3 in HFA. Activation and possibly dysregulation of astrocyte innate immune responses by HIV, as shown by induction of C3 in HFA here, may have deleterious consequences to the normal neuroprotective functions of astrocytes.

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## P.117

### Identification and characterization of C/EBP binding sites within the HIV-1 subtype C LTR

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Human immunodeficiency virus type 1 (HIV-1) has been transmitted worldwide and regional viral subtypes have been designated as subtype A through K. Subtype C, which is concentrated in Southeast Asia and sub-Saharan Africa, is the most prevalent subtype worldwide. To date, few studies have examined the role of CCAAT/enhancer binding proteins (C/EBP) with respect to long terminal repeat (LTR)-directed viral gene expression from the subtype C LTR. Within subtype B viruses, two functional C/EBP sites upstream of the TATA box have been shown to be required for efficient viral replication in cells of monocyte-macrophage lineage. In order to assess the roles of C/EBP sites within the subtype C viral LTR, 211 HIV-1 subtype C LTR sequences were collected and aligned via the Clustal V method. From these analyses, three putative C/EBP binding sites were identified: two upstream binding sites 1 and 2 (US1 and US2) and one downstream binding site (DS1). Interestingly, the putative downstream site (DS1) is highly conserved between subtypes B and C, suggesting the presence of a functionally important cis-acting element that has yet to be characterized. Electrophoretic mobility shift analysis demonstrated that two of three sites within the HIV-1 subtype C were able to bind C/EBP factors (US1 and DS1). Additional studies focused on examining relative binding affinities of naturally occurring variants of these two sites are currently underway. Future studies will examine the molecular architecture of these sites relative to the functional properties of the HIV-1 LTR.

## P.118

### Apelin, an endogenous neuronal peptide, protects hippocampal neurons against HIV-associated excitotoxic injury

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Several G-protein couple receptors (GPCRs) have been shown to mediate neuronal cell migration and survival upon activation by their native peptide ligands, and to activate death-signaling pathways when activated by certain non-native ligands. We recently described, in cultured neurons, expression of the unique seven-transmembrane (7TM)-GPCR, APJ, which is also strongly expressed in neurons in the brain as well as different cell types in other tissues. We have now demonstrated the ability of the endogenous APJ peptide ligand, apelin, to activate signaling in rat hippocampal neurons and to modulate neuronal survival. We found that 1) Both APJ and apelin are expressed in hippocampal neurons; 2) apelin peptides induce phosphorylation of the cell survival kinases AKT/PKB and Raf/ERK-1/2 in hippocampal neurons; and 3) apelin peptides protect hippocampal neurons against N-methyl-D-aspartate receptor (NMDAR)-mediated excitotoxicity, including including that induced by human immunodeficiency virus type 1 (HIV-1). Thus, apelin/APJ signaling likely represents an endogenous hippocampal neuronal survival response, and our results suggest that apelin should be further investigated as a potential neuroprotectant against hippocampal injury.

## P.119

### Pharmacological cdk inhibitor suppresses JC virus proliferation

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JC virus (JCV) utilizes cellular proteins for viral replication and transcription in the host cell nucleus. These cellular proteins represent potential targets for antiviral drugs against the JCV. In this study, we examined the antiviral effects of the pharmacological cyclin-dependent kinase (cdk) inhibitor, which has been shown to have antiviral activity against other viruses. We found that cdk inhibitor significantly suppressed the viral production and cytopathic effects of the JCV in a JCV-infected cell line. It attenuated the transcriptional activity of JCV late genes, but not early genes, and also prevented viral replication via inhibiting phosphorylation of the viral early protein, large T antigen. These data suggest that the JCV requires cdks to transcribe late genes and to replicate its own DNA. Pharmacological cdk inhibitor exhibited antiviral activity in JCV-infected cells, suggests that it might have therapeutic utility in the treatment of progressive multifocal leukoencephalopathy (PML).

## P.120

### Expanded plasma cell clones in multiple sclerosis cerebrospinal fluid do not produce antibody against abundant myelin proteins

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Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of humans. A hallmark of disease is increased intrathecal IgG synthesis and persistence of oligoclonal IgG in CSF and brain. To better understand the nature of humoral immunity in MS, we used FACS and single-cell RT-PCR to analyze IgG variable (V) region sequences of CD138+ plasma cells recovered from the CSF of MS patients. Clonal expansion, somatic hypermutation and the preferential use of VH4 family heavy chain gene segments were characteristic features of the 11 repertoires examined, supporting the notion of a targeted B cell response within the CNS. To identify their antigenic targets, we produced over 40 different monoclonal human IgG1 recombinant antibodies (rAbs) by co-expression of heavy- and light-chain V region sequences from plasma cell clones in mammalian cells. Because MS is considered a putative autoimmune disease, rAbs were first assayed for immunoreactivity to myelin basic protein, proteolipid protein and myelin oligodendrocyte protein. Whereas mouse mAbs and rAbs produced from anti-myelin hybridomas readily reacted with myelin antigens in multiple immunoassays, none of the MS CSF rAbs displayed clear immunoreactivity. Our findings indicate that common myelin antigens are not the major target of the humoral immune response in MS. The identification of novel MS-specific antigens is being pursued.

## P.121

### Measles virus-specific plasma cell clones are prominent in subacute sclerosing panencephalitis CSF

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Chronic infectious and inflammatory diseases of the CNS are often characterized by a robust B cell response that manifests as increased intrathecal IgG synthesis and the presence of oligoclonal bands. Further, the oligoclonal IgG is directed against the agent that causes the disease. To demonstrate the specificity of expanded CD138+ plasma cell clones recovered from the cerebrospinal fluid (CSF) of a patient with subacute sclerosing panencephalitis (SSPE) for measles virus (MV), variable (V) region sequences of CD138+ cells sorted from SSPE CSF were amplified by single-cell PCR and analyzed. Human IgG1 recombinant antibodies (rAbs) were produced from four expanded CD138+ clones and assayed for immunoreactivity against MV proteins. Clonal expansion was a prominent feature of the SSPE plasma cell repertoire, and each of the four rAbs assayed were specific for either the MV fusion or MV nucleocapsid protein. Thus, expanded plasma cell clones in the CSF of patients with SSPE produce disease-relevant antibodies and provide a link to our previous observation of MV-specific plasma cell clones in the parenchyma of SSPE brain. Because CSF is readily accessible, recombinant Abs derived from B cell clones could provide a tool to identify target antigens in idiopathic inflammatory disorders.

## P.122

### Stereology of dorsal root ganglia neurons and perineuronal macrophages in HIV infected tissues

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The most abundant types of HIV-Neuropathies are the distal sensory polyneuropathy (DSP) and antiretroviral toxic neuropathies (ATN). The pathological features of DSP and ATN include macrophage infiltration in peripheral nerves and dorsal root ganglia (DRG). Increased activation of macrophages and proinflammatory cytokines appear to be the principal immunopathogenic factors in DSP.

Perineuronal inflammation in the DRG leads to damage of DRG neurons. Our preliminary data have demonstrated a reduction of DRG neurons in HIV tissues with significant infiltration by CD68+ and CD14+ macrophages. We suggest that this aberrant inflammatory response plays a critical role in damaging or sensitizing DRG neurons and axons, and subsequently affects the inputs to central pain pathways.

Therefore, we used stereological techniques to quantify DRG neurons and perineruonal macrophage cells in HIV samples in order to determine the virus's effect on the DRG.

## P.123

### Importance of the second exon of HIV-1 Tat on the survival of neuronal progenitors

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HIV-Encephalopathy (HIVE) is a neurological disorder associated with HIV-1 infection and AIDS. Among the molecular mechanisms thought to participate in neuronal dysfunction, the viral trans-activating protein Tat has been shown to be neurotoxic in various cellular models. Tat gene consists of two exons that encode for two variants of the protein, which are 72 and 101 amino acids in length, respectively. Although the 101 aa Tat is considered the natural full-length isoform of Tat, shorter peptides of 72 and 86 amino acids are often used for in vitro experiments. While it is clear that the first exon (72 aa in length) is required for viral replication, the function of the carboxy-terminal region of Tat is less clear. Here, we utilized a rat neuronal progenitor cell line as a model of retinoic acid induced neuronal differentiation and various plasmids for the expression of C-terminus truncated mutants of Tat protein: Tat-72, Tat-86, and Tat-101. Our results strongly suggest that: (i) truncation of the C-terminus correlates with higher toxicity in both proliferating and differentiating cells; and (ii) survival of Tat-transfected cells is enhanced upon retinoic acid treatment and induction of cellular differentiation. Altogether, these results indicate that neuronal toxicity of Tat is attenuated by the presence of a sequence in the second exon and by the activation of neuronal differentiation.

## P.124

### Structure-activity relationships of biguanide-based compounds with activity against HIV-1

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Combination antiretroviral therapy, which is used as a highly effective treatment of HIV-1-associated immunopathogenesis, has also had a positive impact on neurologic disease caused by HIV-1 infection of the central nervous system (CNS). However, limits on treating or preventing HIV-1-associated neuropathogenesis due to reduced CNS drug penetration and drug-associated neurologic side effects underscore the importance of developing new drugs with greater efficacy within the CNS. In this regard, we have focused on the development of biguanide-based HIV-1 inhibitors. Our investigations, which demonstrated that biguanide-based compounds have activity against HIV 1, also suggested a relationship between biological activity and the lengths of hydrocarbon linkers surrounding the positively charged biguanide unit. To better define biguanide structure-activity relationships (SAR), biguanide oligomers with select linker lengths were evaluated for cytotoxicity, anti-HIV-1 activity, and in vivo toxicity. Results of the in vitro experiments demonstrated that (i) increases in linker length (and, therefore, increases in compound lipophilicity) were generally correlated with increases in cytotoxicity and antiviral activity against HIV-1, and (ii) polyethylene hexamethylene biguanide (PEHMB; biguanide units spaced between alternating ethylene and hexamethylene linkers) provided the greatest in vitro therapeutic indices (CC50/IC50) among the compounds tested. Additionally, the negligible toxicity of PEHMB relative to polyhexamethylene biguanide (PHMB; biguanide units alternating with hexamethylene linkers) in a murine model of cervicovaginal microbicide toxicity was consistent with considerable differences in cytotoxicity between PEHMB and PHMB observed during in vitro experiments. These SAR investigations represent an essential step in the development of biguanide-based inhibitors effective against HIV-1 CNS disease.

## P.125

### Platelet-derived growth factor protects neurons against Gp120-mediated toxicity

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The human immunodeficiency virus (HIV)-1 envelope glycoprotein gp120 has been implicated in mediating neuronal apoptosis, a hallmark feature of HIV-associated dementia (HAD). Mitigation of the toxic effects of gp120 could thus be a potential mechanism for reducing HIV toxicity in the brain. In this study we hypothesized that neurotrophic factor, such as platelet-derived growth factor (PDGF), could protect the neurons against gp120-mediated apoptosis. The in vitro model system we have used are the neuronal SH-SY5Y cell culture exposed to gp120 with and without pretreatment with PDGF. Cells treated with gp120 exhibited increased cell death when measured by TUNEL assay with concomitant loss of neurites and increased cell rounding. Pre-treatment with PDGF, however, reduced gp120-associated neurotoxicity and rescued the neurite outgrowth. Additionally, gp120-mediated activation of caspase-3, was also significantly reduced in cells pretreated with PDGF. Taken together these studies suggest that PDGF may be considered as a therapeutic agent to reduce gp120-mediated neurotoxicity in HAD.

## P.126

### Development of a novel animal/human hybrid model for multiple sclerosis in SCID-mice with functional human lymphoid system: experimental allergic encephalomyelitis induced by envelope protein from human endogenous retrovirus "W"(MSRV-ENV/Syncytin).

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Multiple Sclerosis associated RetroVirus element (MSRV), which further unraveled the HERV-W multicopy family, was discovered to encode a powerful imunopathogenic envelope protein (MSRV-ENV or HERV-W Syncytin copy), causing sequential activation of a pro-inflammatory and autoimmune cascade through initial interaction with Toll-Like receptor 4 on antigen-presenting cells (APC), accompanied by gliotoxicity causing blood brain barrier (BBB) breakdown and further triggering superantigen-like dysregulation of T-lymphocytes.

Initial animal studies in severe combined immunodeficiency mice (SCID) grafted with a functional human lymphoid system (thus creating a mouse/human hybrid) resulted in an hyperacute BBB breakdown with lethal brain haemorrhage was associated with peaks of TNF-alpha, but was inhibited by T-cell depletion. This dramatic effect was obtained with live IP-injected MSRV concentrated viral particles.

Using recombinant ENV protein at much lower doses in this hybrid model with human immune system, we have now been able to cause an "experimental allergic encephalomyelitis" (EAE) using human MBP as autoantigen (highly homologous to mouse MBP). Clinical symptoms and neurological deficits started about 10 days post-inoculation and partial remission was observed in the following week. Repeated injections with ENV caused new aggravations reaching complete paralysis with, e.g., spastic retraction of paralyzed forelegs(s). This pattern was relatively similar to that obtained in positive controls series with complete Freund's adjuvant (CFA) whereas controls with Incomplete Freund's adjuvant (IFA) remained normal. Nonetheless weight curves indicated a continuous loss in ENV series, when ACF mice rapidly compensated weight loss during the phase of recovery.

Histopathological examination of CNS sections from sequentially sacrificed mice, after perfusion with PBS + 4% Paraformaldehyde before dissection and tissue embedment in paraffin, revealed massive demyelination prominent in the spinal cord of "ENV series". Numerous lymphoid cells were associated with tissue destruction in the vicinity of blood vessels, whereas very large macrophage cells were seen at distance surrounded by large plaques of demyelination. Brain lymphoid infiltrates were also seen, with more restricted demyelination. Such features appeared less extended with much fewer activated macrophage cells in demyelinated areas in CFA series. Moreover, Magnetic Resonance Imaging (MRI) was performed at regular intervals, evidencing apparition and dissemination of characteristic T2-weighted hypersignals in white matter of both spinal cord and brain of ENV+ animals. IFA controls remained normal in all techniques.

Lympocytes recovered from spleen dissection before animal perfusion, confirmed a time- and dose-dependent increase in MBP autoreactive T-cells in ENV or CFA, but not IFA, series.

We have thus developed a novel animal model of MS characterized i) by the induction of EAE with the autologous immunotoxin as detected post-mortem in MS brain plaques by independent immunohistology studies as well as in MS serum by our recent multi-center study with immunoassay dosage (Cf. Epidemiology Abstract) in spite of the heterologous and "MS-irrelevant" M. tuberculosis extract currently used in CFA for classical EAE models and ii) by the development of an autoimmunity against mouse CNS mediated by human immune cells activated against homologous human MBP, from which human anti-MBP autoreactive T-cell clones can be isolated.

This novel model caused by an MS "immunotoxin" and mediated by human immune cells, is now used for pre-clinical studies with therapeutic monoclonal antibodies (Cf. therapeutic antibody abstract).

## P.127

### Monoclonal antibody against envelope protein from Human Endogenous Retrovirus "W" (MSRV-ENV or Syncytin) inhibits TLR4-initated immunotoxicity cascade in human peripheral blood mononuclear cells and displays therapeutic effects in novel pre-clinical models for Multiple Sclerosis

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HERV-W endogenous retroviral family encodes a powerful imunopathogenic envelope protein (MSRV-ENV or Syncytin), causing sequential activation of a pro-inflammatory and autoimmune cascade through initial interaction with Toll-Like receptor 4 (TLR4) on antigen-presenting cells, accompanied by gliotoxicity causing blood brain barrier (BBB) breakdown and further triggering superantigen (Sag)-like dysregulation of T-lymphocytes. The specific association of this protein with MS brain lesions post-mortem, as evidenced by independent immunohistological studies, its presence in about 75% of MS sera ex-vivo versus none

in healthy controls (Cf. Epidemiology abstract) and its ability to reproduce the "EAE" MS animal model associated with demyelination and autoimmunity, has conducted us to select anti-ENV specific monoclonal antibodies (mAb) capable to block pathogenic effects of this protein in vitro. Further confirmation of therapeutic efficiency on clinical effects in vivo (animal models) was also pursued.

We can now present an anti-ENV mAb blocking ENV interaction with TLR4 (preventing IL6 and IL12-P40 release by human blood mononuclear cells), and inhibiting later Sag-like activation of T-lymphocytes (preventing IFN-Gamma release in the same culture). Similar effects are obtained with anti-TLR4 blocking antibody, which also prevent the immune system from intercepting bacteria (by LPS) and thus may cause serious opportunistic infections if used as therapeutic agent. Anti-ENV blocking mAb do not block TLR4 and thus, do not interfere with LPS activation of such innate immunity receptors.

Anti-ENV mAb was further tested in two MS animal models (EAE induced by ENV in either C57/Bl6 mice with MOG, or in SCID mice grafted with a human lymphoid system with MBP-Cf. Abstract on animal model), with significant inhibition or prevention of clinical symptoms compared to untreated controls and to treated animals in EAE models induced by complete Freund's adjuvant. Therapeutic efficiency in the human/mouse hybrid SCID model was even obtained during a third "challenge" after two separate ENV injections had already triggered previous "relapses".

We have cloned the variable chains of this mAb and tested resulting activity of recombinant scFv, which showed complete preservation of affinity.

Such results in vitro and in vivo, now pave the way to the molecular humanization of this antibody for preclinical and clinical trials.

## P.128

### Activation of anti-apoptotic protein survivin upon JCV infection in glial cells. Implications for the development of progressive multifocal leukoencephalopathy

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Progressive Multifocal Leukoencephalopathy (PML) is a sub-acute and fatal disease of the Central Nervous System, result of the productive infection of glial cells, particularly oligodendrocytes, by the opportunistic JC Virus. The lytic destruction of oligodendrocytes and the activation of astrocytes in response to JCV infection results in the characteristic histopathological landmarks of PML; extensive and confluent areas of myelin loss, in which numerous bizarre reactive astrocytes with atypical and pleomorphic nuclei, and enlarged oligodendrocytes harboring intra-nuclear eosinophilic inclusion bodies, which represent the site of active viral replication.

Apoptosis is a host defence mechanism to dispose of senescent, damaged or potentially harmful cells, including virally infected cells, however, certain viruses have the ability to de-regulate apoptotic pathways in order to complete their vital cycles. Although apoptosis has been extensively demonstrated in a variety of viral infections of the brain, including Herpes, Polio and HIV-encephalopathy, there is little evidence to support the occurrence of apoptosis in PML, suggesting that the cellular machinery that controls programmed cell death may be disrupted by the presence of JCV. One of these pathways involves a novel protein, Survivin, a member of the inhibitors of apoptosis family, which is abundantly expressed during embryonic development in proliferating tissues, but is normally completely absent in terminally differentiated cells. Immunohistochemical experiments performed on a collection of 20 cases of PML, revealed the increased expression of Survivin in the intra-nuclear inclusion bodies of JCV infected oligodendrocytes, and in the cytoplasm of bizarre astrocytes. Corroborating these findings, in vitro experiments showed the activation of Survivin expression at the transcriptional and translational levels in JCV infected primary cultures of astrocytes and oligodendrocytes, compared with uninfected cultures, which, as expected, lack expression of Survivin. Furthermore, in order to identify the JCV protein responsible for the activation of the Survivin promoter, we transfected primary astrocytes with the Survivin promoter driving a Luciferase reporter gene, which was activated in the presence of JCV T-antigen. Cell cycle analysis and TUNEL assay demonstrated a significantly lower number of cells undergoing apoptosis upon JCV infection compared with uninfected cultures. Finally, the specificity of these events was tested by siRNA inhibition of Survivin, which resulted in a dramatic increase of apoptosis in JCV infected cultures.

This is the first time that expression of the anti-apoptotic protein Survivin has been demonstrated in clinical samples of PML and in JCV infected glial cell cultures. Based on these observations we hypothesize that upon its reactivation, JCV attempts to prevent cells from entering the apoptosis pathway by activating the Survivin gene, which results in expression of the normally silent anti-apoptotic protein, which in turn disrupts the apoptotic machinery, allowing JCV to successfully complete its lytic cycle and resulting in the development of PML. Understanding of this pathway may lead to the development of more effective therapeutic strategies against a thus far incurable and fatal disease.

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## P.129

### Induction of brain tumors by human neurotropic JC virus in a transgenic animal model

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JC Virus, a member of the Polyomaviridi family of viruses, is a neurotropic virus with wide distribution among the human population of the world, according to sero-epidemiological studies. JCV is the established opportunistic infectious agent of Progressive Multifocal Leukoencephalopathy, a fatal demyelinating disease of the brain, result of the cytolytic infection of oligodendrocytes. Mutations in the regulatory region of JCV are responsible for the viral strain. The prototype strain or Mad-1, which has been associated with PML contains a promoter with two 98 base pair repeats, whereas the archetype strain or CY, isolated from the urine of healthy individuals contains only one 98 tandem with two insertions of 62 and 23 base pairs respectively. JC Virus has been shown to possess oncogenic potential, as demonstrated by the development of a wide variety of brain tumors upon direct inoculation of viral particles into rodents and non-human primates. Furthermore, JCV DNA sequences and expression of viral proteins have been detected in a variety of brain tumors, including medulloblastomas, oligodendrogliomas, astrocytomas, anaplastic glial tumors, glioblastomas, ependymomas and CNS lymphomas.

To further understand the oncogenicity of JC virus and its role in the development of human neoplasia we have generated a transgenic mouse colony, which contains the early region of the archetype strain of JC virus (CY), under the regulation of its own promoter. These transgenic animals developed a wide variety of neural tube and neural crest origin tumors, including: Primitive Neuroectodermal Tumors (PNETs) (173 cases), Medulloblastomas (4 tumors), Pituitary Tumors (21 tumors), Malignant Peripheral Nerve Sheath Tumors (18 cases), Adrenal Neuroblastomas (6 tumors), and Glioblastoma Multiforme (3 cases). The most frequently observed phenotype thus far is the PNET. The neoplasms originate from different areas of the brain, including in order of frequency, the lower part of the Brainstem and upper portion of the Cervical Spinal Cord, the Basal Ganglia, the Cerebellum, the Frontal Cortex, and the Temporal Cortex (including the Hipocampus). Rarely the tumors arise from the olfactory bulb. The tumors range from small foci of few neoplastic cells to huge masses, which substitute up to 80% of the brain parenchyma. Interestingly, in 5 cases a combination of PNETs and Pituitary tumor was observed, and in 7 cases, PNETs and MPNSTs were developed concurrently.

Immunohistochemical studies for the detection of the JCV early product, T-antigen demonstrated the nuclear robust expression in the nuclei of neoplastic cells in all the different phenotypes of tumors. Furthermore, the cell cycle regulatory protein p53 was also detected in the nuclei of neoplastic cells, and double labeling demonstrated co-localization of both proteins in the nuclear compartment of neoplastic cells. Expression of the accessory product Agnoprotein has been also constantly detected in the cytoplasm of neoplastic cells, demonstrating the active transcription of the viral sequences. Interestingly, while some cells within the tumors express abundantly T-Antigen, some other cells demonstrate weak expression and other are completely negative. Alterations in several pathways are currently investigated including the Wnt signaling pathway, the IGF-1 / IRS-1 axis, and the antiapoptotic Survivin pathway. Results from these experiments will help us further understanding the mechanisms of JCV oncogenicity.

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## P.130

### Cooperative inhibition of HIV-1 by drug combinations containing the biguanide-based inhibitor NB325

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Combination antiretroviral therapy (ART), which continues to be used as highly effective treatment of HIV-1-associated immunopathogenesis, has also had a positive impact on neurologic disease caused by HIV-1 infection of the central nervous system (CNS). However, limits on treating or preventing HIV-1-associated neuropathogenesis due to reduced CNS drug penetration and drug-associated neurologic side effects underscore the importance of developing new drugs and combinations with greater efficacy. Our efforts in this direction have focused on NB325, which is a biguanide-based compound that is an effective inhibitor of both R5 and X4 strains of HIV-1. Assays of drug combinations have demonstrated that NB325 can be cooperatively paired with several types of HIV-1 inhibitors, including nonnucleoside and nucleoside reverse transcriptase inhibitors (efavirenz and tenofovir, respectively), and a fusion inhibitor (cyanovirin). Assays of cytotoxicity and anti-HIV?1 activity demonstrated that these compound combinations were minimally cytotoxic and highly effective against HIV-1 entry and replication. Further analyses demonstrated additive or synergistic activity against HIV-1, and that the nature of the activity was dependent on the partner for NB325 and the combination ratio, as confirmed through the use of two distinctly different software programs-CalcuSyn and MacSynergy II – that are used specifically to analyze activities of combined agents. These results suggest that cooperative antiviral activity between two agents may be influenced by similarities or differences between each agent's mechanism of

action. Furthermore, these results support the further testing of combinations that may be used effectively to treat HIV-1 CNS infection.

## P.131

### Sequence variation within the HIV-1 LTR correlates with brain regionalization and HIV-1-associated dementia

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The long terminal repeat (LTR) regulates human immunodeficiency type 1 (HIV-1) viral gene expression during the course of infection. The activity of the LTR is regulated by the relative availability of viral and cellular transcriptional control proteins and the genetic variation that accumulates during replication. The C/EBP site II consensus B (conB) variant has been shown to be highly conserved in brain-derived HIV-1 LTR populations, and that LTRs containing C/EBP site II 4C and 6G variants were only found in brain tissue of patients with HIV-1-associated dementia (HIVD). A statistically significant difference was also found in the regional distribution of LTRs containing the C/EBP site II conB, 4C, or 6G variant in brain regions derived from patients with and without HIVD. A low affinity C/EBP site II 4C variant accumulates in the cerebellum, a region exhibiting little viral gene expression, while the high affinity C/EBP site II 6G variant accumulated in the mid-frontal gyrus, a site of highly productive replication. A 3T C/EBP site I variant was identified in 25% of brain-derived LTRs from patients diagnosed with HIVD, but was absent in patients without dementia. LTRs containing a C/EBP site I 6G variant were found to accumulate in a highly specific manner in the head of caudate and the C/EBP site I 3T variant accumulated in the mid-frontal gyrus. In conclusion, distinct LTR populations with specific C/EBP site I and II configurations were found in different neuroanatomical regions of the brain and correlated with neurological disease.

## P.132

### Increases CD14+/CD16+ monocytes in Hispanic women with asymptomatic HIV-associated cognitive impairment with HAART

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HIV-associated dementia (HAD) has remained prevalent despite highly activated antiretroviral treatment (HAART). The advent of HAART has affected the phenotype of HAD which now present in a milder form termed cognitive impairment (CI) and with variable progression patterns. Prior to HAART, this disease has been associated with an increased subset of monocytes (CD14+/CD16+/CD69+). In the post HAART era this phenotype has been reported to decrease significantly with HAD. In a recent study, similar of expression of CD14+/CD16+ was reported between HIV-seropositive and HIV-seronegative controls. To our knowledge no similar study has been conducted in a cohort of HIV-seropositive women. Therefore, we designed this study to compare the expression of this monocyte subset in HIV-seropositive Hispanic women on HAART. Sixty-three HIV-seropositive women and 15 HIV-seronegative controls stratified for cognitive function using the American Academy of Neurology HAD criteria modified to include an asymptomatic group (mAAN) into 17 (32%) women with normal cognition , 12 (23%) with asymptomatic cognitive impairment, 11 (21%) with MCMD, and 13 (24%) with HAD. Co-infection with HCV was present in 24% cases and drug abuse was present in 6%. For the purpose of this study MCMD and HAD were grouped together as symptomatic CI. Monocyte subsets were determined in whole-blood samples of HIV-seropositive women by using antibodies against CD14, CD16, and CD69 antigens and flow cytometry analysis. We found no significant differences in age, CD4 cell count, plasma or CSF viral load between the three groups. Increase percentage of CD14+/CD16+ positive monocytes was found in HIV-seropositive women compared to seronegative controls ($p < .05$). In HIV-seropositives after adjusting for age, education, CD4 cell count, and viral load using a generalized lineal equation model we found that the percentage of CD14+/CD16+ positive monocytes significantly increased with the degree of cognitive impairment; being higher in the asymptomatic group when compared with those with normal cognition. Likewise, CD14+/CD16+ expression in monocytes from women with symptomatic CI was higher than those with asymptomatic and those with normal cognition. In conclusion, our result show that monocyte activation occurs in early stages of CI an remain elevated in HIV-seropositive women while using HAART.

## P.133

### A novel humanized mouse model for HIV-1 encephalitis

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A novel rodent model was developed for studies of HIV-1 neuropathogenesis. Human hematopoietic stem cells derived from umbilical cord blood were used to reconstitute newborn irradiated NOD/scid-IL-2Rgc-/- mice (hu-NSG). Thirty hu-NSG animals developed human lymphoid tissue and showed migration

of blood-derived macrophages into the brain. HIV-1 infection with CCR5 utilizing viruses induced lymphadenopathy and viral replication with sustained plasma viral loads. At 3 months after infection, human HIV-1p24+ macrophages were present in the meninges and perivascular spaces. Depletion of CD8a+ cells one week before and 3 weeks after infection by cM-T807 antibodies, increased viral replication and induced a profound meningoencephalitis. Brain infiltration by lymphocytes, formation of perivascular cuffs and the presence of HIV-1p24+ cells with microglial-like morphology were documented. These neurovirological and neuropathological disease features corresponded to reduced CD4/CD8 T cell ratios in circulation and lymphoid tissues with concomitant humoral immune responses against HIV-1 gp120. We conclude that hu-NSG mice show sustained viral replication, immunosuppression and macrophage ingress across the blood brain barrier. These are reflective of what can occur early after viral exposure in an infected human host.

## P.134

### Perturbation of glutamate metabolism in HIV-infected macrophages: Implications for the CNS

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Central nervous system disorders in HIV infection are mostly the consequences of an inflammatory macrophage activation and relate to glutamate-mediated excitotoxicity. However, recent studies also suggest neuroprotective aspects of macrophage activation through the expression of glutamate transporters and glutamine synthetase. We thus aimed to study whether HIV infection or activation of macrophages could modulate glutamate metabolism in these cells. We assessed the effect of HIV infection on glutamate transporter expression as well as on glutamate uptake by macrophages and showed that glutamate transport was partially decreased (30%) in the course of virus replication whereas EAAT-2 gene expression was dramatically increased (x20). The consequences of HIV infection on glutamine synthetase was also measured and for the first time we show the functional expression of this key enzyme in macrophages. This expression was repressed during virus production. We then quantified EAAT-1 and EAAT-2 gene expression as well as glutamate uptake in differentially activated macrophages (TNFalpha, IL-1beta, LPS, IFNgamma, GM-CSF, IL-4, PGE2, M-CSF, IL-10, dexamethasone) and show that the effects of HIV are not directly related to pro- or anti-inflammatory mediators. Indeed, EAAT-2 gene expression was upregulated by LPS and PGE2 and EAAT-1 one by dexamethasone. Of note, the function of EAATs was not directly correlated to gene expression levels, as both TNFalpha and LPS increased it, dexamethasone exhibiting a weaker effect.

We finally showed that the glutamate transport by macrophages is less affected than what has been described in astrocytes. Macrophages may thus play a role in neuroprotection against glutamate in the infected brain, through their expression of both EAATs and glutamine synthetase. As glutamate metabolism by activated macrophages is sensitive to both HIV infection and inflammation, it may thus be of potential interest as a therapeutic target in HIV encephalitis.

## P.135

### Simian varicella virus induces apoptosis in monkey kidney cells by an intrinsic pathway in which BCL-2 expression is downregulated

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Like varicella zoster virus (VZV) infection in humans, SVV causes chickenpox in primates, becomes latent in ganglionic neurons and reactivates to produce zoster. Thus, SVV has been a useful model of varicella infection. We showed that the cytopathic effect in SVV-infected monkey kidney (Vero) cells in tissue culture is apoptotic as evidenced by nuclear condensation. Apoptosis is triggered by sequential activation of a group of cysteine-rich proteases (caspases). To study this caspase cascade, we searched for markers of apoptosis by Western blot analysis in virus-infected and uninfected cells at 24 h, 40 h, 48 h and 64 h post infection (p.i). At 40 to 64 h p.i., there was a significant increase in the level of active caspase-3. This increase was accompanied by a parallel increase in caspase-3 activity measured with DEVD-pNA, thus confirming apoptosis. Apoptosis proceeds through extrinsic or intrinsic pathways. The extrinsic pathway is mediated by death receptors, and the intrinsic pathway is caused by an imbalance between pro-apoptotic proteins (e.g., Bad, Bax) and anti-apoptotic proteins (Bcl-2 and Bcl-XL) in mitochondria. The active form of caspase -9 (an intrinsic pathway marker) was elevated in infected cells 40-64 h p.i., while active caspase 8 (an extrinsic pathway marker) was not detected at any of the time points. Bcl-2 mRNA levels in SVV-infected cells decreased in a time-dependent manner with a maximum decrease of 90% at 64 p.i. compared to uninfected cells. Bcl-2 protein in infected cells was decreased 40-60% at all time points while Bcl-XL, Bad and Bax were unchanged. These findings suggest that SVV infection of Vero cells

leads to induction of apoptosis thru an intrinsic (mitochondrial) pathway, as evidenced by decreased expression of the anti-apoptotic gene Bcl-2. Because VZV has been shown to induce apoptosis in cultured human fibroblasts, but not in human sensory neurons, varicella-induced apoptosis requires further analysis in neurons and non-neuronal cells both in vitro as well as in vivo.

## P.136

### JC virus T-antigen transforms circulating bone marrow mesenchymal stem cells

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Bone marrow-derived stromal cells (MSCs), also referred to as mesenchymal stem cells, have the ability of trafficking and multilineage differentiation into different tissues and organs. JC virus is a human polyomavirus and the etiological agent of progressive multifocal leukoencephalopathy. The JC virus early protein, T-antigen, has been described to have oncogenic potential in several animal models and has been detected in the variety of human tumors. In this study, we demonstrate that MSCs can be infected with JC virus, and can undergo transformation after transfection with JC virus T-antigen. Cultured T-antigen transfected MSCs lost their original morphology and contact inhibition. These cell when transplanted subcutaneously in nude mice formed tumors, but T-antigen negative MSCs did not form tumors. This study shows for the first time the infection and transformation of MSCs, a globally accessible adult stem cell, with a naturally occurring human viral oncoprotein. In light of earlier reports on the association of JCV with the variety of human tumors our data contributes to identifying a possible common origin for a variety of cancers. Moreover JCV T-antigen transformed MSCs provide a powerful tool for study of molecular pathways and animal models of oncogenesis.

## P.137

### Regulation of HTLV-1 gene expression in primary versus secondary target cell population in the context of chromosomally integrated viral promoter

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HTLV-1 exhibits a broad cellular tropism due to the ubiquitous nature of its receptor. In addition to the CD4+/CD25+ T cells other cell populations, including the cells of the monocyte-macrophage lineage, are also susceptible to HTLV-1 infection. Viral-induced alterations in these cells and their trafficking to the brain may play important roles in the progression of HAM/TSP. However, very little information exists concerning the regulation of HTLV-1 promoter or long terminal repeat (LTR) in secondary target cell populations. Moreover, most of the studies of HTLV-1 gene expression have been performed using transiently transfected LTRs that differ physically from a chromosomally integrated provirus, which like eukaryotic DNA, is wrapped with histone and nonhistone proteins to form the nucleosome, the basic unit of chromatin. Hence it is important to understand how the viral promoter is regulated when formatted in the context of chromatin. To this end, we have generated a number of clones of CD4+ T cell line Jurkat and monocytic line U-937 (representative primary and secondary target cell type, respectively) stably integrated with an HTLV-1 LTR. The single cell clones from both the cell types were propagated and examined for LTR integration by a reporter gene (luciferase) assay. Selected clones with varying luciferase expression were analyzed for integrated LTR copy number by real time PCR with higher levels of LTR expression shown to correlate with higher LTR copy number. An equal number of cells from the clones with comparable copy numbers were pooled and transfected with a Tax-expressing plasmid and analyzed for the luciferase expression as well as for global microRNA profiling. Tax has demonstrated a clear differential pattern in the activation and suppression of the cellular miRNA pathway indicating that the differential interplay of viral and cellular factors in primary versus secondary target cell populations regulates viral activation post-integration. Our results also demonstrated, for the first time, that HTLV-1 Tax protein can interfere with miRNA pathway as shown previously with other viral transactivator proteins such as HIV-1 Tat. These observations are currently being confirmed in the primary T cells and monocytes and are being extended to the B cell (Raji), and bone marrow progenitor cell (TF-1) lines. Future investigations will analyze the role of individual miRNAs and potential transcription factors in regulating Tax-mediated integrated LTR activation in selected cell types.

## P.138

### Monocyte protein sialoadhesin facilitates HIV-1 trans infection and induces inflammatory chemokine expression

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Monocyte/macrophages are mediators of HIV-1-associated neuronal dysfunction by actively transporting HIV-1 to the CNS and through the release of neurotoxic factors. Using high-density microarrays to examine how HIV-1 infection dysregulates the immune system and alters gene expression in circulating monocytes, we found increased expression of sialoadhesin (Sn, CD169) in CD14+ monocytes from subjects

infected with HIV-1 compared to non-infected controls. When analyzed by flow cytometry, Sn expression on freshly isolated monocytes correlated with the subject's HIV-1 viral load and could be induced in cultured human monocytes by interferon. Sialoadhesin is a 190 kDa transmembrane protein that binds sialic acid and when expressed on monocytes is capable of binding HIV-1 through interaction with the sialic acid residues on gp120. When evaluated in an infectivity assay, HIV-1 bound to Sn-expressing monocytes, infected target cells in trans within 48 hr. Infection of the target cells was dependent on CD4+ receptor and CXCR4+ coreceptor expression and occurred in the absence of productive infection of the Sn-expressing monocytes. In addition to binding HIV-1, Sn expression triggered the elaboration of cytokines from transfected THP-1 cells. THP-1 cells either stably or transiently transfected with a CMV-SN construct secreted proinflammatory chemokines IL-8 (CXCL8), MIP-1alpha (CCL3), MIP-1beta (CCL4) and IP-10 (CXCL10) into the culture medium. These findings suggest that monocyte expression of Sn in the periphery may facilitate transport of HIV-1 to the CNS and that transmigration of Sn-expressing monocyte/macrophages could impair neuronal function through secretion of inflammatory chemokines.

## P.139

### Modulation of galectin-1 by drugs of abuse

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It is well established that drug abuse is a significant risk factor for contracting human immunodeficiency virus (HIV-1) infection and its progression to HIV-1 associated encephalopathy (HIVE). Astrocytes, integral components of the central nervous system (CNS), maintain a homeostatic environment and participate in a bidirectional communication with neurons. Upon activation of astrocytes, the expression of various immunoregulatory proteins involved in the development of HIVE is modulated. Proteomic analyses (difference gel electrophoresis) of heroin treated normal human astrocytes (NHA) demonstrated the differential expression of several biologically significant proteins, including galectin-1, as identified by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Galectins are a family of $\beta$-galactoside binding lectins that regulate cell to cell and cell to matrix interactions, cell adhesion, and cell signaling. We observed that treatment of NHA with heroin, morphine or methamphetamine increased the gene and protein expression of galectin-1 in a time and dose dependent manner. Gene and protein expression of galectin-1 was assessed by quantitative real-time PCR and western blot analyses respectively. Addition of HIV-1 envelope protein, gp120, to treatment of NHA with heroin, morphine or methamphetamine results in a further increase in galectin-1 expression. Recent evidence indicates that galectin-1, acting as a soluble adhesion molecule, enhances HIV-1 infectivity and replication in immune cells. Our studies demonstrate that drugs of abuse enhance HIV-1 replication in NHA. The mechanism underlying the association of HIVE with drug abuse may be mediated by the increased expression of galectin-1 which, in turn, enhances HIV binding to target cells. Our findings may lead to novel strategies for preventing HIVE in high risk, drug abusing populations.

## P.140

### MCP-1 levels and neurocognitive performance in HIV seropositive marijuana users

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Chronic marijuana use among Human immunodeficiency virus (HIV) patients is common, however, its effects on the brain are not fully known. The aim of this study is to evaluate whether marijuana use in HIV infection alters neuroinflammation, which in turn might alter cognitive function. Neuroinflammation is vital for normal function and protection of the central nervous System (CNS); however, excess inflammatory response may be neurotoxic and contribute to the pathophysiology of many neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease and HIV encephalitis. The inhibitory effect of cannabinoids on reactive oxygen species, glutamate and tumor necrosis factor suggests that they may be potent neuroprotective agents. Monocyte chemotactic protein-1 (MCP-1) is a chemokine that attracts monocytes. MCP-1 has been implicated as an important mediator of monocyte and T lymphocyte infiltration of tissues in several inflammatory diseases. We measured CSF MCP-1 levels in HIV patients with and without marijuana use and determined whether their CSF MCP-1 level correlates with neuropsychological test performance. Methods: We evaluated 94 subjects (46 HIV without marijuana use, 48 HIV with any recent marijuana use). CSF MCP-1 levels were measured with a commercial ELISA kit (human MCP-1 by R&D Systems) and a battery of neuropsychological tests was performed in each subject.

Results: The two subject groups were well-matched in terms of age ($p = 0.63$ ), education ($p = 0.50$) and estimated verbal IQ ($p = 0.90$), and the two HIV subject groups (marijuana vs non-marijuana) had similar CD4 count ( $241 \pm 23$ vs.$258 \pm 25$ /mm3, $p = 0.61$), Log plasma viral load ($3.90 \pm 0.24$ vs. $3.39 \pm 0.21$ $p = 0.11$), HIV dementia scale ($13.6 \pm 0.4$ vs. $13.0 \pm 0.47$, $p = 0.35$) and Karnofsky score ($88 \pm 1.3$ vs.$87 \pm 1.5$, $p = 0.55$). MCP-1 levels (pg/ml) showed a trend for higher levels in HIV patients without marijuana use

(487 ± 29 pg/mL) compared to HIV patients with marijuana use (474 ± 30) . MCP-1 levels correlated significantly with KS ($p = 0.015$ Spearman Correlation; and grouped as scores of 100, 90, 80, and 70 & 60 for ANOVA $p = 0.002$). MCP-1 also correlated with three tests of psychomotor speed Trails A ($r = 0.33$, $p = 0.0016$), Trails B ($r = 0.27$, $p = 0.009$), Symbol Digit ($r = -0.25$, $p = 0.019$), Stroop tasks ($r = 0.21$ to $0.25$, $p = 0.01$ to $0.03$) and response reversal on a computerized (CalCAP) reaction time test during working memory ($r = 0.2$, $p = 0.02$). Higher MCP-1 consistently associated with slower performance, and with trends for poorer executive function.

Conclusion: Trends for immunosuppressive effects of was observed in HIV-infected individuals who had any marijuana usage compared to those who did not use the drug. Since higher MCP-1 levels were associated with poorer cognitive performance and general functioning, cannabinoid drugs may have neuroprotective effects and may have therapeutic values in HIV patients. Further evaluation of other chemokines and cytokines are underway to better delineate the neuroinflammatory patterns associated with these individuals. Relationship between drug usage (dose, duration, and recency) and inflammatory changes in these subjects also will be evaluated.

## P.141

### Mechanisms of HIV infected monocyte transmigration across the blood brain barrier

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HIV infected individuals are living longer due to the success of highly active antiretroviral therapy (HAART). Despite control of peripheral viral replication, HAART is relatively ineffective at crossing the blood brain barrier (BBB). As such, HIV persists in the central nervous system (CNS) and viral-mediated damage to the parenchyma accumulates. Consequently, clinical manifestations of CNS damage, including motor and neurocognitive impairment, are increasingly presenting as devastating sequelae of HIV infection. Early during primary infection, HIV gains entry into the CNS, at least in part, by the transendothelial migration (TEM) of infected monocytes across the BBB, and establishes a viral reservoir in resident macrophages and microglia. CCL2, the predominant monocyte chemoattractant, is significantly elevated in the cerebral spinal fluid (CSF) of patients with HIV Associated Dementia (HAD) and HIV Encephalitis (HIVE). Thus, HIV may establish a CNS microenvironment that promotes recruitment of monocytes. As a mediator of both inflammation and viral replication, monocytes are thought to be a major contributor to the neuropathogensis of HIV infection. In fact, autopsy specimens of seropositive individuals demonstrate BBB disruption and suggest that HAD and HIVE more directly correlate with monocyte recruitment, macrophage and microglia activation, and neuroinflammation than with CNS viral load.

In non-pathologic states, monocytes traffic across the BBB at low levels without barrier disruption. This process is regulated by interactions between homotypically interacting adhesion proteins present on both the monocyte and the endothelial cell (EC) of the BBB including PECAM-1, CD99, and PrPC. However, studies from our lab using a human BBB cell culture model demonstrate that monocyte infection with HIV and exposure to CCL2 work together to disrupt the homeostasis of this system, causing both an increase in monocyte TEM across the BBB and a loss of barrier integrity. HIV and CCL2 appear to act in concert, as neither variable on its own alters base line monocyte recruitment or disrupts barrier integrity to the same extent. Therefore, we hypothesize that HIV infection and CCL2 stimulation result in aberrant interactions between intercellular adhesion proteins on the monocyte and EC during diapedesis, promoting neuroinflammation and viral entry into the brain. The goal of this research includes characterizing the changes to whole cell protein expression, cellular localization, membrane dissociation, and phosphorylation of the intercellular adhesion molecules PECAM-1, CD99, and PrPC in both the monocyte and EC during HIV infection and CCL2-mediated TEM. Changes in expression, localization, or structural integrity of these proteins could alter the balance of maintaining barrier integrity while accommodating monocyte TEM, while phosphorylation of cell adhesion proteins is known to affect their localization, interactions with adaptor proteins and the cytoskeleton, and activity as mediators of cell signaling. Preliminary data suggest that monocytes increase their whole cell expression of PECAM-1 and CD99 in response to HIV infection, potentially increasing the migratory capacity of the monocyte. In response to CCL2, EC demonstrate an early increase in PECAM-1 whole cell expression, followed by a decline, while CD99 is redistributed away from intercellular junctions. These changes to EC adhesion molecules could disrupt the resealing of the BBB behind the transmigrating monocyte.

## P.142

### Proteomic fingerprints for HIV-1 cognitive impairments

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The need for suitable biomarkers in cerebrospinal fluid (CSF) and sera to predict the onset and tempo of cognitive impairments (CI) linked directly to advanced HIV infection cannot be overstated. To these ends

a "state of the art" proteomics platform integrating 2-dimensional electrophoresis (2DE) with Difference Gel Electrophoresis (DIGE) and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was developed within our laboratories. A bioinformatic system was used for identifications of differential expression of proteins retrieval and identification using (DeCyder® 6.5 software, GE Healthcare, Inc.) and protein identification (BioWorks® 3.2 utilizing Sequest algorithm). To facilitate analysis of low abundance proteins, we removed the 6 most abundant proteins: Albumin, IgG, IgA, $\alpha$1-Antitrypsin, Haptoglobin, and Transferrin from CSF samples and 12 most abundant proteins from sera samples: 6 proteins listed above and additional 6: Fibrinogen, $\alpha$2-macroglobulin, IgM, Orosomucoid, Apolipoprotein A-I, Apolipoprotein A-II. Subsequently, we used a 2DE DIGE for proteomic profiling. Analysis was performed using DeCyder® 6.5 software (GE Healthcare, Inc.). Protein spots showing statistically significant differences ($p < 0.05$) were subjected to protein identification using LC-MS/MS. Altogether 20 CSF samples, 10 from demented and 10 from patients with normal cognition were used. Within the CSF, proteins with regulatory functions (complement C3 and its fragments, neuronal cell adhesion molecule (NrCAM), cystatin C, vitamin D binding protein, clusterin, gelsolin, procollagen C-endopeptidase enhancer) were found to be differentially expressed and have been validated by Western-blot analysis. 14 sera samples, 7 from demented and 7 from patients with normal cognition were used. Several proteins with regulatory functions have been initially identified as differentially expressed and are being validated by western blot analysis. In conclusion, these biomarkers while preliminary in number of samples tested provide new means to follow disease progression and to pinpoint therapeutic needs in an HIV-1-associated cognitive impairment.

## P.143

### Novel host defense mechanisms in the neuropathogenesis of HIV infection

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We have discovered two novel host defense mechanisms, which prevent excitotoxicity in the setting of HIV infection. We treated human fetal neuronal cultures with recombinant Tat, matrix metalloproteinases (MMPs), antisera to Tat, and NMDA, individually or in various combinations.

Surprisingly, we found the combination of Tat and MMPs produced significant attenuation of neurotoxicity, as measured by cell death and mitochondrial potential. Using protein electrophoresis techniques, we found that MMP-1 can degrade Tat, an effect blocked by MMP inhibitors. MMP-2 and -9 also prevented Tat-toxicity but did not degrade Tat. The degradation by MMP-1 was specific for Tat and not other viral proteins.

Interestingly, monoclonal antibodies against the C- or N-terminal of Tat not only prevented Tat-toxicity, but the Tat-antisera complexes also attenuated NMDA-mediated excitotoxicity. This effect was specific for NMDA and was not seen for kainate.

Further characterization of these MMP and antibody mediated defense mechanisms will be important in developing new therapeutic strategies for HIV dementia, and may also be operative in other viral encephalitides.

## P.144

### Toll-like receptors play a crucial role in protection against West Nile virus infection

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West Nile virus (WNV) is a neurotropic flavivirus that appeared as an emerging infectious disease in the northeastern United States in 1999. WNV, now endemic throughout North America, has become a significant global cause of viral encephalitis, primarily in the elderly and immunocompromised individuals. Prior studies have established an essential protective role of both innate and adaptive immune response components. Toll-like receptors (TLR) play an essential role in initiating innate responses by sensing non-self components, and are shown to decreased in aged animals. It has been shown that TLR3 deficient mice are more resistant to lethal infection. However, TLR signaling pathways in host defense against WNV infection have not been fully explored. In the present work, we evaluated the role of MyD88 and TRIF, two key adaptor molecules of TLR signaling, in WNV susceptibility and spreading into the brain. The absence of TLR signaling through MyD88 and TRIF simultaneously resulted in increased vulnerability to WNV infection with a rise in mortality from 68% (wild-type mice) to 100% (MyD88/TRIF dKO mice), and a decrease in the average survival time. MyD88/TRIF dKO mice showed increased virus replication in the brain and in associated lymphoid tissues. Brain-infiltrating macrophages were more abundant in infected MyD88/TRIF dKO mice, and expressed higher CD86 than in WT mice. However these cells failed to up-regulate MHC Class II, suggesting a defective activation of selective pathways. Interestingly, mice lacking only MyD88 (with intact TLR3 signaling pathway) also exhibited higher mortality and increased levels of virus replication in the brain than WT mice. Our observations suggest the dominant protective role of Toll-like receptors against WNV infection.

## P.145

### Higher relative frequencies of HIV-specific CD8+ T cells in cerebrospinal fluid (CSF) versus peripheral blood in antiretroviral-naive immune controllers

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Background: HIV-specific CD8+ T cell responses play a central role in controlling HIV replication. While many studies have evaluated CD8+ T cell responses in the peripheral blood and tissues, relatively little is known regarding qualitative and quantitative characteristics of HIV-specific T cell responses in the central nervous system (CNS). It has been speculated that activated virus-specific T cells may participate in the pathogenesis of CNS dysfunction during HIV-associated dementia (HAD). Conversely, these responses may be critical for controlling HIV replication in the CNS.

Methods: Seven asymptomatic antiretroviral-naive HIV+ immune controllers were assessed for the frequency of HIV-specific (tetramer+) CD8+ T cells and their maturation phenotypes (CD45RO, CD57 and CCR7) in cerebrospinal fluid (CSF) and peripheral blood using a 9-colour flow cytometry panel. In addition to direct study of unexpanded cells, CSF cells were expanded with phytohaemagglutinin (PHA) and assessed for frequency of HIV-specific T cells by flow cytometry and for IFN-gamma production to HLA-class I-restricted optimal peptides by ELISpot. Approximately 40 mL of CSF was analyzed per participant.

Results: Plasma HIV-1 RNA ranged from 60 to 16,800 copies/mL and CD4 T cells were >500/cubic mm. CSF HIV-1 RNA was <50 copies/mL in 6 of 7 subjects, and less than the plasma HIV-1 RNA levels in all participants. HIV tetramer positive T cells could be directly enumerated and phenotyped with as few as 10,000 total CSF mononuclear cells. Frequencies of tetramer+ CD8+ T cells in CSF, assessed by direct ex vivo staining, were higher than in blood ($p = 0.0004$). The expansion potential of tetramer+ CSF T cells was similar to those from blood, and the relative higher frequencies of HIV-specific T cells in CSF was maintained in expanded cell populations ($p = 0.0016$). There were also higher frequencies of IFN- producing T cells in expanded cells from CSF compared to the blood in response to HLA-restricted optimal peptides ($p = 0.012$). By direct ex vivo evaluation of T cell maturation markers, both CD8+ and CD4+ T cells in CSF had a higher frequency of memory CD45RO+ T cells than in blood. Among HIV-specific T cells, frequencies of CD45RO+, CD57+ (a marker of replicative senescence) and CCR7+ T cells were similar between CSF and blood.

Conclusions: Asymptomatic HIV+ controllers have higher relative frequencies of HIV specific T cells in CSF compared to blood and lower concentrations of virus in CSF. These cells are capable of expansion and IFN-gamma production. This pattern is consistent with homing of HIV-specific T cells to the CNS and/or preferential expansion within this compartment with resultant control of virus.

## P.146

### Dephosphorylation of JC virus agnoprotein by protein phosphatase 2A: Inhibition by small t antigen

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Previous studies have demonstrated that the JC virus (JCV) late regulatory protein agnoprotein is phosphorylated by the serine/threonine-specific protein kinase-C (PKC) and mutants of this protein at the PKC phosphorylation sites exhibit defects in the viral replication cycle. We have now investigated whether agnoprotein phosphorylation is regulated by PP2A, a serine/threonine-specific protein phosphatase and whether JCV small t antigen (Sm t-Ag) is involved in this regulation. Protein-protein interaction studies demonstrated that PP2A associates with agnoprotein and dephosphorylates it at PKC-specific sites. Sm t-Ag was also found to interact with PP2A and this interaction inhibited the dephosphorylation of agnoprotein by PP2A. The interaction domains of Sm t-Ag and agnoprotein with PP2A were mapped, as were the interaction domains of Sm t-Ag with agnoprotein. The middle portion of Sm t-Ag (aa 82-124) was found to be critical for the interaction with both agnoprotein and PP2A and the N-terminal region of agnoprotein for interaction with Sm t-Ag. To further understand the role of Sm t-Ag in JCV regulation, a stop codon was introduced at Phe86 immediately after splice donor site of the JCV early gene and the functional consequences of this mutation were investigated. The ability of the replication of this mutant virus reduced substantially compared to WT. Next, the functional significance of PP2A in JCV replication was examined by siRNA targeting and down-regulation of PP2A caused a significant reduction in the level of JCV replication. Collectively, these results suggest that there is an interplay between agnoprotein, Sm t-Ag and PP2A with respect to the regulation of JCV life cycle and this could be important for the progression of the JCV-induced disease, PML.

## P.147

### JC virus small t antigen targets the host translation system

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Sm t-Ag is produced by the translation of the alternatively spliced transcripts of the JCV early pre-mRNA. Unlike LT-Ag, the function of Sm t-Ag in JCV life cycle is unclear. To understand the role of Sm t-Ag in JCV regulation, a stop codon was introduced at Phe86 immediately after splice donor site of the JCV early gene so that the unique region of Sm t-Ag is not produced and the functional consequences of this mutation were investigated. Results showed that the ability of the replication of this mutant virus significantly reduced compared to WT, confirming the critical importance of this protein in JCV life cycle. To investigate the mechanism by which Sm t-Ag exerts its function during the infection cycle, we analyzed the protein complexes that interact with the unique region of Sm t-Ag by Mass Spectroscopy. This analysis revealed the presence of protein complexes that are involved in host translation, including, translation elongation factor 1 and 2 suggesting that Sm t-Ag might function as a regulator of host translation machinery during the infection cycle. The specificity of the interaction between Sm t-Ag and host elongation factor was further confirmed by coimmunoprecipitation assay. To gain more insight into the functional significance of this targeting, we will further characterize the role of small t antigen in host translation during the viral replication cycle.

## P.148

### Kinetics of cytokine expression in brain and spleen following intracerebral and intraperitoneal challenge of JEV in BALB/c mice

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Introduction: Japanese encephalitis is a serious CNS disease due to its complexity, lack of specific treatment and long term neuro-psychiatric disabilities in the survivors. Immune mechanisms during JEV infection are complex and need further elucidations for the development of safe and efficacious vaccines.

Methods: We investigated iNOS and cytokine expression in spleen and brain of BALB/c mice challenged intracerebrally (IC) with 3x106 pfu and intraperitoneally (IP) with 1x108 pfu of JEV at various time points during disease development at mRNA and protein level. Histopathology and viral growth assays were performed in brain.

Results: In IC group, there is a significant upregulation of proinflammatory cytokines like IFN-g ($P = 0.03$) and TNF-$\alpha$ ($P = 0.009$) while downregulation of anti-inflammatory IL-10 and IL-4 ($P = 0.017$ and $<0.001$) both in transcript as well as protein expression with the disease progression, histopathological changes and increased viral load in the brain. iNOS mRNA was downregulated following IC challenge. In spleen however, TNF-$\alpha$, IL-4 and iNOS mRNA was upregulated till 3 d.p.i. while IL-10 mRNA was upregulated from 1 to 5 d.p.i. but IFN-g was not detected. In IP group, spleen had a gradual, significant upregulation of all cytokines till 5 d.p.i. followed by decrease at 7 and 11 d.p.i. IL-10 expression outcompeted both IFN-g and TNF-$\alpha$. In the brain, there is no significant difference in iNOS and cytokines' transcript as well as protein between control and infected mice. But at 11 d.p.i., all the cytokines showed significant increase. No virus was isolated from brain but histopathology shows slight meningeal inflammation at 11 d.p.i. and real-time PCR analysis for JEV E protein was positive.

Conclusion: An insufficient anti- inflammatory cytokine response in the brain is associated with increased tissue pathology and viral load and regulates inflammatory responses driven by IFN-g in concert with TNF-$\alpha$, to cause brain tissue damage. Deleterious effect of IFN-g in brain is independent of NO induction. TNF-$\alpha$ is involved in pathogenesis of JEV and role of IL-10 needs further investigations.

## P.149

### Impact of selegiline transdermal system on measures of brain metabolism and markers of oxidative stress in hiv infected individuals with cognitive impairment

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Objectives: This substudy of A5090, " Phase II, Placebo-Controlled, Double-Blind Study of the Selegiline Transdermal System (STS) in the Treatment of HIV-Associated Cognitive Impairment" examined the effectiveness of STS in reversing metabolic brain injury, and the relationship between the oxidative stress marker protein carbonyl and brain metabolite changes, as measured by 1H-MRS.

Background: HIV-associated cognitive impairment has been associated with oxidative stress induced neuronal injury. Oxygen radicals can attack proteins, deoxynucleic acids, and lipid membranes resulting in cellular dysfunction and cell death. Among the products of oxidation, elevated CSF levels of protein carbonyl have been reported in HIV infected subjects with cognitive impairment. Furthermore, brain metabolites including N-acetyl aspartate (NAA), choline (CHO), and myoinositol (MI), measured non invasively by magnetic resonance spectroscopy (MRS), have been reported to be affected in early stages of HIV infection and cognitive impairment.

Methods: One hundred twenty-eight HIV infected subjects with cognitive impairment were enrolled in A5090 and randomized to receive STS 3mg/24h, STS 6mg/24h, or matching placebo for 24 weeks. Randomization was stratified by plasma HIV viral load (undetectable, <200 copies/ml; versus detectable, ≥200 copies/ml) and clinical stage of the AIDS dementia complex (ADC; 0.5 versus 1.0 or higher). Subjects with ADC stage 1 or higher were eligible to be co-enrolled in this 1H-MRS substudy. Cognitive impairment was defined as performance at least one standard deviation below the mean on two or more independent neuropsychological tests, or at least two standard deviations below the mean on one neuropsychological test using a standard neuropsychological battery. Cognitive performance was evaluated using the NPZ-8 (average z-score of 8 neuropsychological tests) and NPZ-6 (average z-score of 6 neuropsychological tests), as well as cognitive domain scores (average z-score of neuropsychological tests corresponding to cognitive domain). Sixty-two subjects (25 placebo, 19 STS 3mg/24h, and 18 STS 6mg/24h) were co-enrolled in the substudy, and underwent proton MRS at study entry, and weeks 12 and 24. MRS-measured metabolites were obtained from the midline of the frontal lobes, right (or left) mid-frontal centrum semiovale, and right (or left) basal ganglia. Metabolites measured included NAA, CHO, MI, creatine (Cr) and glutamate/glutamine (Glx), and were expressed as a ratio relative to Cr. CSF was obtained at baseline and week 24. CSF protein carbonyls were detected and quantified using a slot blot technique and an Oxyblot protein detection kit from Chemicon.

Results: The sixty-two subjects enrolled were predominantly male (87%), African American (55%) and Caucasian (39%). Median age at baseline was 46, with a median education level of 12.5 years. The three treatment groups did not differ significantly at baseline with respect to these demographic characteristics, ADC stage, HIV viral load, CD4+ count, NPZ scores, or cognitive domain scores. No significant differences in 1H MRS metabolite ratios were detected between treatment arms at baseline, with the exception of Glx/Cr in the centrum semiovale (STS 3mg/24 h mean = 1.95, SD = 0.29; STS 6mg/24h mean = 1.72, SD = 0.21; placebo mean = 1.81, SD = 0.21; $p = 0.037$). Change in MRS metabolite ratios from baseline to weeks 12 and 24 was evaluated using an analysis of covariance adjusted for baseline metabolite levels and multiple comparisons. There was a slight increase in NAA/Cr (a marker of neuronal function) in the basal ganglia and centrum semiovaleof the placebo group compared to the STS groups ($p = 0.023$ and $p = 0.072$ respectively). The placebo group also demonstrated a greater increase in Cho/Cr (a glia marker) in the mid-frontal cortex ($p = 0.002$), when compared to the combined STS groups. Evaluation of the change in NPZ-8 and NPZ-6 scores, as well as cognitive domain scores from baseline to weeks 12 and 24 revealed no significant differences between treatment arms. Protein carbonyl analysis will be available and included in the presentation of this communication at the meeting.

Conclusion: The results of this 24-week study show that STS had no effect on brain metabolites as measured by MRS. The lack of effect on brain metabolites was also reflected in the lack of cognitive improvement in the STS groups compared to placebo.

## P.150

### MMP-7 cleaves the NR1 NMDA receptor subunit and modifies NMDA receptor function

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Matrix metalloproteinases (MMPs) are zinc dependent enzymes that play a role in the inflammatory response. These enzymes may be substantially increased in association with viral infection of the brain. MMPs are known to cleave proteins of the blood brain barrier basement membrane and accumulating evidence suggests they can also target proteins critical to synaptic structure and neuronal survival, including integrins and cadherins. Here we show that one member of the MMP family, MMP-7, which may be released from cells including microglia, can cleave a protein critical to synaptic function. Through analysis of extracts from murine cortical slice preparations, we show that MMP-7 cleaves the NR1 subunit of the NMDA receptor to generate an N terminal fragment of approximately 65 kDa. Moreover, studies with recombinant protein show that MMP-7 mediated cleavage of NR1 occurs at amino acid 517, which is extracellular and just distal to the first transmembrane domain. Data suggest that NR2A, which shares sequence homology with NR1, is also cleaved following treatment of slices with MMP-7 while select AMPA receptor subunits are not. Consistent with a potential effect of MMP-7 on ligand binding, additional experiments demonstrate that NMDA mediated calcium flux is significantly diminished by MMP-7 pretreatment of cultures. These data suggest that synaptic function may be altered in neurological conditions associated with increased levels of MMP-7.

## P.151

### Impact of single nucleotide sequence configurations in stably integrated HIV-1 LTRs on gene expression, under basal and activating cellular phenotypes

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Human immunodeficiency virus type 1 (HIV-1) gene expression is driven by the long terminal repeat (LTR), which has a variety of binding sites for the interaction with multiple viral and host factors, including members of the CCAAT/enhancer binding protein (C/EBP) and Sp transcription factor families. Previous studies have identified specific nucleotide

sequence configurations within C/EBP site I and Sp site III (3T, C-to-T change at position 3 of the binding site, and 5T, C-to-T change at position 5 of the binding site, respectively) which correlate with increased severity of HIV-1 disease and HIV-1-associated dementia. To begin exploring the LTR phenotype, associated with these genotypic changes, from an integrated chromatin-based microenvironment, a series of stably transfected cell lines have been developed utilizing bone marrow progenitor, T, and monocytic cell lines (TF-1, Jurkat, and U-937, respectively). Macrophage-, T cell-, and dual-tropic LTRs were coupled to the gene encoding green fluorescent protein (GFP) and polyclonal HIV-1 LTR-GFP stable cell lines were developed and examined under basal, and chemical or cytokine activation, as well as in the presence of Tat. The results demonstrate that the cell type and LTR backbone have a significant effect on the expression profiles. In addition, TF-1 cells the 3T/5T-containing LTR is associated with a lower expressing phenotype, however it can be induced to similar levels as the parental LTR, following stimulation and/or in the presence of Tat. Current studies are underway to examine the integration pattern and proviral DNA level utilizing uncloned and cloned HIV-1 LTR-GFP stably expressing cell lines.

## P.152

### West Nile virus induced flaccid paralysis mediated by apoptosis in the ventral horn of the spinal cord in hamster model

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Symptoms of West Nile Neurological Disease (WNND) in human patients include tremors, myclonus, parkinsonism, cerebral ataxia, limb atrophy and acute flaccid paralysis. Since a subset of patients with WNND develop acute flaccid paralysis caused by poliomyelitis-like lesions, we hypothesize that West Nile virus (WNV) induced paralysis was due to infection and death of motor neurons in the ventral horn of spinal cord. Here we developed a hamster model for West Nile Virus (WNV) infection showing the hind limb flaccid paralysis. To develop the paralysis we injected NY WNV 1) sub-cutaneously, 2) into the spinal cord at the 8th thoracic vertebrae by laminectomy or 3) into the sciatic nerve. By 8 or 9 days post-viral infection (dpi) a small proportion of hamsters developed the hind limb paralysis after sub-cutaneous infection. Hamsters injected into the thoracic spinal cord developed ipsilateral hind limb paralysis at day 8 – 9 followed by bilateral paralysis or death by injection into the thoracic spinal cord. With the sciatic nerve injection, the hamsters developed paralysis on day 8. Confocal microscopy was performed using Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) and WNV and neuron specific enolase (NSE) immunohistochemistry. The virus predominantly infected neurons in the gray matter of the ventral horn, analogous to human infection, most particularly in the thoracic and lumbar spinal cord. Co-localized staining of specific anti-WNV antibody, NSE and TUNEL assay and the anatomy of the cell indicated that the dying cells were infected motor neurons. Some infected animals that were not paralyzed had WNV infected cells, but TUNEL staining was not extensive. This suggested that motor neurons stained with WNV and TUNEL are markers for the progression of paralysis in WNV infected hamster. (Supported by PHS NO1-A1-15435 and PHS 1-U54-A1-06357 (JDM)).

## P.153

### West Nile virus infection in central nervous system of hamster

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West Nile virus (WNV) infects the Central Nervous System (CNS) and induces a variety of neurological disease symptoms in human. To investigate the neurological disease, hamsters were sub-cutaneously (s.c.) injected using prototypic NY99 WNV strain. CNS tissues were obtained by cardiac perfusion with paraformaldehyde. Histopatholgic analysis of 3-$\mu$m thick sections revealed mild gliosis, neuropil vacuolation, myelin sheath dilatation, axonal swelling, infiltration of lymphocytes around the vessels, and glial cuffing around neurons in brain stem area. In spinal cord, there was a moderate bilateral ventral horn gliosis, neuronophagia, satellitosis and hypereosinophilic tissues in the cervical and thoracic cord. Particularly neurons in the ventral horns were diffusely hypercellular, and were infiltrated by increased numbers of glial cells and neutrophils. Inflammation was most severe in cervical and thoracic segments, and less severe in lumbar segments and in the brain stem. Immunohistochemical analysis showed that WNV focally infected the different nuclei of brain stem, such as lateral alpha-gigantocellular nuclei, para-gigantocellular nuclei and lateral spinal nuclei. In the medulla and pons of the brain, the facial nuclei, parvicellular reticular nuclei, intermediate reticular nuclei, pontine reticular nucleus, paraolivary nuclei and superior olive were infected. In thoracic and lumbar spinal cord, motor neurons in the ventral horn were profoundly infected. In few cases, neurons in the dorsal horn were also infected. Apart from these areas, the next intensely infected areas were the

hippocampus, cornu ammonis 1, 2, 3 and dentate gyrus. Neurons in the cerebral cortex and Purkinje in cerebellum were also positive to a lesser extent. In this WNV animal model, we observed a clear cytoplasmic immuno-staining on the neuronal cell body, whether it was a neuron in the brain stem or motor neuron in the spinal cord. Even though, the histoplathologic evidence showed the infiltration and accumulation of lymphocytes and glial cells in brain stem or mid brain, these cells were not stained for WNV. This information may be useful to understand the WNV induced neurological disease in human and animal models. (Supported by PHS Contract NO1-A1-15435 and PHS 1-U54- A1-06357 (JDM)).

## P.154

### Molecular mimicry in inducing DNA damage between HIV-1 Vpr and the anti-cancer agent, cisplatin

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The human immunodeficiency virus type 1 (HIV-1) vpr gene is an evolutionarily conserved gene among the primate lentiviruses. Several functions are attributed to Vpr including the ability to cause cell death, cell cycle arrest, apoptosis, and DNA damage. The Vpr domain responsible for DNA damage as well as the mechanism(s) through which Vpr induces this damage is unknown. Using site directed mutagenesis, we identified the helical domain II within Vpr (aa 37–50) as the region responsible for causing DNA damage. Interestingly, Vpr ?(37-50) failed to cause cell cycle arrest or apoptosis, to induce Ku70 or Ku80, and to suppress tumor growth, but maintained its capability to activate the HIV-1 LTR, to localize to the nucleus, and to promote NHEJ. In addition, our cytogenetic data indicated that helical domain II induced chromosomal aberrations, which mimicked those induced by cisplatin, an anti-cancer agent. This novel molecular mimicry function of Vpr might lead to its potential therapeutic use as a tumor suppressor.

## P.155

### Unique role of ARMS in neurotrophin-mediated activation of NF-kB and neuronal protection against HIV-1 encoded gp120

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Activation of the transcription factor NF-kB is a key feature of the signaling triggered by neurotrophins, and this activity has been shown to be critical for neuronal survival examined in in vitro models of neuroAIDS. However, the precise mechanism by which neurotrophins activate NF-kB is not well understood. In an effort to dissect this process, we analyzed the role of brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, on NF-kB activity in cortical neuronal cultures. Here we show that TrkB expression is developmentally regulated in primary cortical cultures, and expression levels correlate with the ability of BDNF to activate NF-kB. This action of BDNF requires functional Trk since addition of K252a, a potent inhibitor of Trk, completely abrogated NF-kB activation. Additional experiments were performed to understand the exact mechanism of NF-kB activation by BDNF. It has been shown that Trk interaction with an accessory protein, ankyrin-rich membrane-spanning protein (ARMS), is necessary for persistent Rap1-dependent MAPK activation (Embo Journal, 2004, 00, 1-11). We report that the activation of NF-kB by BDNF requires normal expression of ARMS and its association with Trk. Notably, an overexpression of ARMS augments BDNF-induced NF-kB signaling in cortical neurons, while dissociation of TrkB:ARMS interaction by overexpression of the membrane -spanning region of ARMS blocks NF-kB signaling. These analyses were further extended through the generation of an ARMS siRNA expressing lentivirus and essentially supported our conclusions. Finally, we examined the potential role of ARMS in BDNF-mediated neuroprotection. We found that ARMS is required for BDNF to protect neurons from toxicity induced by HIV-1 encoded gp120. Thus, we propose that the interaction of TrkB with ARMS is essential for NF-kB activation by neurotrophins and subsequent neuroprotection against candidate HIV-1 neurotoxins.

## P.156

### Cannabinoid rescue of striatal neurogenesis in chronic encephalitis in rats

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Although neurotropic viruses and inflammatory diseases target neural progenitor cells, neurogenesis can be induced in the adult brain by various

pharmacological treatments. Cannabinoids confer neuroprotection, promote cell survival in various experimental paradigms, and increase neurogenesis in hippocampus in rat models of neural injury. Borna Disease virus (BDV) infection reduces neurogenesis in hippocampus of adult rats (Solbrig et al. Brain 2006), reduces numbers of BrdU labeled cells in subventricular zone (SVZ) of striatum and causes disorderly migrations of newly generated SVZ cells to prefrontal cortex (Solbrig unpublished). The hypothesis that cannabinoids rescue SVZ neurogenesis during encephalitis was tested by examining the ability of cannabinoid treatments to increase number and survival of newly generated cells in the striatal complex of adult rats with BDV, using pharmacologic probes, immunohistochemistry and cell counting techniques. After BrdU labeling of mitotic cells in S phase followed by cannabinoid treatment of rats (Win-55,212-2 1mg/kg ip twice daily x 1 week), there are significant increases in survival of newly generated cells in SVZ dorsal striatum in cannabinoid-treated BDV rats compared to BDV-infected vehicle-treated rats. Both BD groups have fewer BrdU positive cells than age-matched uninfected control rats. There were no apparent cannabinoid effects on cell birth in infected rats, as BDV rats pretreated x 1 week with cannabinoid or vehicle prior to BrdU administration showed no differences in SVZ cell labeling the day after BrdU injection. Therefore, cannabinoids have the potential to improve striatal motor and procedural learning functions compromised by viral injury, not only as modulators of neurotransmission in motor and behavior circuits, but also by neuroprotective effects on new cell survival in this region.

## P.157

### Paroxetine treatment for HIV-mediated neurodegeneration

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## P.158

### HIV-1 tat enhances localization of smad proteins to the JCV regulatory region and modulates transcriptional activity in oligodendroglial cells

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HIV-1 infection alters pathways of production and signal transduction of immunomodulators, including TGF-Beta1, in the CNS. Therefore, we have examined effects of HIV- Tat on binding of TGF-Beta1 effectors, Smads, to the JCV transcription and replication control region. Immunoblotting reveals endogenous expression of Smad2/3, Smad4 and Fast1in KG-1 oligodendroglioma cells. Fast1 is reportedly a mediator of DNA binding of Smad4. Of these proteins, Smad4 is detected at significantly lower levels than the others. Even upon transfection to overexpress Smad4, that protein is only detectable at low levels. Yet, Smad4 is readily detected on the JCV regulatory region using chromatin immunoprecipitation (ChIP). Results so far indicate a situation in which Smad4 not bound to chromatin is rendered less detectable, either through instability of the protein, its mRNA, or through masking of epitopes. Real-time PCR allows detection of 10-16 moles of a 0.3 kb segment representing the JCV control region in $<30$ cycles. ChIP demonstrates that Smad2/3, Smad4 and Fast1 each bind in living KG-1 cells to a specific segment of the JCV control region. Smad4 and Fast1 were detected on the control region at 10 to100-fold higher levels than were Smad2/3. Tat stimulated binding of Smad2/3 and Smad4 to the JCV control segment each $>10$-fold and also statistically significantly stimulated binding of Fast1. In the presence of Tat levels of binding of Smad2/3, Smad4 and Fast1 to the control region were virtually equal. At picomolar concentrations Tat stimulates JCV early and late gene transcription. At high levels of overexpression, however, Tat inhibits early gene transcription. This inhibition is reversed by expression of Smad2, 3 and 4, which stimulate transcription more than 1000-fold. The present results reveal a mechanism by which HIV-1, through interaction with immunomodulators, can significantly influence the infection of oligodendroglial cells by JCV.

## P.159

### Histone deacetylase inhibitors suppress the astrocyte innate immune response

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Astrocytes are important modulators of immune and inflammatory reactions in the CNS participating in both innate and acquired immune responses. Astrocytes express certain members of the toll-like-receptor (TLR) family, in particular, the receptor for double stranded RNA (dsRNA), TLR3. The synthetic TLR3 ligand, polyinosinic-polycytidylic acid (PIC), is a potent activator of human astrocytes resulting in the activation of specific sets of transcription factors (IRF3 and NF-$\kappa$B) that leads to the induction of cytokines, chemokines and antiviral proteins. Acetylation of various histones is a posttranslational modification involved in the regulation of gene expression or silencing. Histone acetylation is regulated by histone acetyl transferases (HAT) and histone deacetylase (HDAC). In general, HAT induces gene transcription, whereas HDAC suppresses

the transcription. Surprisingly, recent studies have demonstrated that type I IFN (IFNalpha)-stimulated anti-viral gene expression is downregulated by the general HDAC inhibitors such as valproic acid (VA) or trichostatin A (TSA). Using a well-established model of primary human glial cell cultures, we investigated the effect of TSA and VA on PIC-induced gene expression. We hypothesized that HDAC inhibitors will inhibit transcription of genes involved in innate immune response by inhibiting the activation of the critical transcription factor IRF3. Our results show that TSA and VA suppress the PIC-induced expression of cytokines, chemokines and antiviral proteins in astrocytes. TSA and VA also suppressed the expression of IFNbeta as well as IRF7 and TLR3, implying IFNbeta as the converging point. These data suggest that the site of inhibition is in the TLR3 signal cascade upstream of IFNbeta production. Remarkably, microarray analysis demonstrated that the proteins belonging to the IFN-inducible gene family are globally restricted and are among the most severely downregulated genes. Our results have clinical implications as they predict that the clinical use of HDAC inhibitors will lead to a compromised innate immune system. (Supported by MH55477, NS07098 and AI051519)

## P.160

### HIV/HCV co-infection has a monocyte phenotype that induces MIP3alpha and reactivates MCP-1

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Nearly 24% of HIV-infected veterans on highly active antiretroviral therapy (HAART) are co-infected with HCV. While neuropsychological (NP) consequences in HIV infection are well established, HCV-infected subjects also have NP abnormalities and co-infection may cause a greater impairment of NP functions. Since monocytes normally traffic into the brain and activated monocyte/macrophages significantly impact cognition in HIV infection, we explored monocyte gene expression profiles from subjects with HIV/HCV co-infection and HCV mono-infection.

Four HIV/HCV co-infected (HIV, low viral load, [LVL], <100 copies/mL; HCV >200,000 IU/mL), 4 HCV mono-infected (>200,000 IU/mL), 17 HIV mono-infected (LVL) and 7 healthy controls were enrolled. Total RNA from CD14+ monocytes was hybridized to microarrays. Data were analyzed using R/BioConductor and GeneSpring.

HCV mono-infection induced 550 differentially expressed (DE) genes. Co-infection induced 736 DE genes, which included MIP-3alpha (CCL20), MCP-1 (CCL2), CD83, IL6, and IP10 (CXCL10). CCL20 was up regulated >100 fold in HCV mono-infected and in HIV/HCV co-infected subjects but not in HIV mono-infected subjects. CCL20 has been implicated in enhanced inflammatory cell recruitment to the liver in HCV subjects. CCL20 can be produced by astrocytes during inflammatory conditions in the CNS, where it may play a role in monocyte and dendritic cell recruitment. CCL2 was up-regulated 3 fold in co-infection but not in LVL HIV or HCV mono-infected subjects. CCL2 appears to be reactivated by HCV infection in HIV-infected subjects with LVL.

HIV/HCV co-infection altered the monocyte phenotype compared to HIV or HCV mono-infection. This synergistic effect may play a role in NP impairment seen in HIV/HCV co-infected subjects. CCL20 plus CCL2 induction may cause an increase in monocyte chemotaxis to the CNS.

## P.161

### Biological effects of Natalizumab on JC virus infectivity in its permissive human neural cell lines in vitro

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The human Polyomavirus JC virus (JCV) infects the 70-80% of humans and establishes latent infection in the kidney. In immunosuppressed patients JCV can reactivate and result in a fatal and progressing neurological disease known as progressive multifocal leukoencephalopathy (PML). Over the past three decades, PML has become one of the important neurological complications in acquired immunodeficiency syndrome (AIDS) patients. Recently, it has been reported that patients being treated with therapeutics targeted at the integrin, very late antigen (VLA)-4, are unexpectedly found to have an increased risk for development of PML. However, there have not been interpreted determinately the relationship between sideration of PML and natalizumab. Therefore, we have investigated the biological effects of Natalizumab on the growth of JCV in its permissive human neural cells, IMR-32 cells with inoculation of the virus. Initially we have determined the effective concentration of Natalizumab using the cell adhesion assay in the IMR-32 cells. Natalizumab did not affect on the expression levels of the viral proteins by immunblotting using the specific antibodies, nor hemmaglutination activities of the cellular lysates from the infected cells. These results suggested that Natalizumab did not have an influence on JCV infectivity in IMR-32 cells in vitro.

## P.162

### JC virus agnoprotein modifies plasma membrane permeability and facilitates

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Viruses inflict a number of injuries as they infect susceptible cells. Some of these injuries affect the plasma and intracellular membranes, resulting in modification of their functions. A typical feature observed during the animal virus infection is enhanced permeability of infected cells. Viroporins are a group of proteins that participate in the promotion of release of viral particles from cells, and interact with cellular membranes modifying permeability. These proteins are not essential for the replication of viruses, but their presence enhances virus growth.

Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease resulting from lytic infection of oligodendrocytes by the polyomavirus JC virus (JCV). The genome of JCV encodes six major proteins including agnoprotein. Previous studies from our and other laboratories indicated that the JCV small auxiliary protein, agnoprotein, plays an important, though not fully understood, role in the propagation of JCV.

Agnoprotein encoded in the late coding region of JCV is a highly basic protein and contains a hydrophobic domain in the central portion of the protein.

Here, we demonstrate that agnoprotein possesses properties commonly associated with viroporins. Our findings indicate that: (i) Agnoprotein localizes to the ER, Golgi apparatus, and plasma membrane in transfected cells; (ii) Agnoprotein can homo-oligomerize in transfected cells; (iii) Agnoprotein enhances permeability of cells to the translation inhibitor hygromycin B; (iv) Agnoprotein induces the influx of extracellular Ca2+; (v) The N-terminal basic amino acid residues of agnoprotein are critical for intracellular membrane targeting and modification of the membrane permeability. These viroporin-like properties which enhances membrane permeability, thereby disrupting the intracellular Ca2+ homeostasis and ultimately causing the dysfunction of the membrane may contribute to the role of agnoprotein in virus release and may have important implications for pathogenicity of JCV infections.

## P.163

### Anti viral and anti inflammatory effect of rosmarinic acid in an experimental murine model of Japanese encephalitis

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Flaviviruses are important human pathogens causing a variety of diseases ranging from mild febrile illnesses to severe encephalitis and hemorrhagic fever. Among them, Japanese encephalitis virus (JEV) targets the central nervous system (CNS) and is a major cause of acute encephalopathy in children. Rosmarinic acid (RA) a phenolic compound found in various Labiatae herbs, such as Ocimum basilicum In the present study, we investigated the efficacy of RA as a therapy against murine JE. Four to five weeks old BALB/c mice of either sex were infected with intravenous (through tail vein) inoculation of lethal dose of 3x105 p.f.u of JE virus strain GP78). After one day following virus inoculation animals started receiving RA intra-peritoneally, twice daily (25 mg/kg of body weight) until the first animal died from the group of infected animals, which did not receive any RA treatement. Rosmarinic acid (RA) reduced the mortality of adult BALB/c mice infected with Japanese Encephalitis virus (JEV). ). RA treatment completely reduced the presence of viral protein and significantly reduced the viral transcripts as compared to infected animal without any treatment. RA also decreased the level of proinflammatory mediators like TNF-a, IL-12, IFN-g, IL-6 and MCP-1 ($p < 0.001$) in infected animals. Thus RA presents a potential for therapy for JE.

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## P.164

### Molecular mechanism of neuronal apoptosis in Japanese encephalitis

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Japanese Encephalitis (JE) is an acute viral infection of the central nervous system caused by a mosquito-borne flavivirus called JE virus. JEV is a neurotropic virus, which causes apoptosis in immature neurons. The molecular cascades of neuronal death following JEV infection are not known in literature. In this work, we investigated the molecular events leading to JEV induced neuronal apoptosis. JEV induced apoptosis is initiated by activating membrane-bound tumor necrosis factor receptor-1 (TNFR1/CD120b). The adapter protein TNFR1-associated death domain (TRADD), which is an essential component of TNFR1 complex, is involved in recruiting other members of the complex to the receptor. TNFR-associated factor (TRAF2) is one on these proteins and is involved in initiating the apoptosis cascade. Overexpression of TRADD can initiate neuronal apoptosis even in the absence of TNF-a. Since TRADD-knockout animals or deficient cell lines are unavailable, it has been difficult to definitively address the physiological role of TRADD in diseases

pathology following JEV infection. We circumvented this problem by silencing TRADD expression with small-interfering RNA (siRNA). We have found that following in vitro infection with JE virus, TRADD is required for TNFR-1 initiated neuronal apoptosis. Interestingly, siRNA against TRADD also decrease the viral load in Neuro2a cells. Furthermore, siRNA against TRADD increased the survival of JEV infected mice by altering the expression of pro apoptotic versus anti apoptotic molecules. These studies show that TNFR-1 TRADD engagement following JEV infection plays a crucial role in neuronal apoptosis. Overexpression of the TRAF2 stimulates Apoptosis Signaling Kinase-1 (ASK1, which initiates the signaling leading to the activation of caspase-3, DNA fragmentation and ultimately cell death. Increased neuronal death then triggers microglial activation and increased microvascular permeability. While Blood Brain Barrier disruption leads to the entry of leukocytes into brain, our studies indicate that this phenomenon can be prevented in TRADD siRNA mice. TRADD siRNA mice also had reduced levels of inflammation and reduced astrogliosis.

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## P.165

### A Prospective cohort study in JCV and non JCV-related leukoencephalopathies in hiv patients: clinical and MRI findings

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Objective: to describe the clinical and radiological features at the diagnosis and during the follow-up of a cohort of HIV related Leukoencephalopathies (LE).Background: The spectrum of LE in HIV+ patients has changed in the recent years due to multiple reasons directly or indirectly linked to the introduction of highly active antiretroviral therapy (HAART). In both JCV and non-JCV related LE the clinical features, the survival time and the possible role of immune-reconstitution are discussed. Methods: in 2002 we started the enrolment of HIV+ patients with at least 7 years of disease; 185 patients underwent brain MRI. Twentyseven patients (16%) showing non opportunistic CNS involvement, underwent the full clinical, neuropsychological, immunological and virological CSF and serum analyses, MRI assessment at the diagnosis stage and during the follow-up. Depending on the detection of JCV in the CSF, 14 were diagnosed as Not Determined LE (NDLE), 13 as Progressive Multifocal Leukoencephalopathy (PML). Results: Median follow-up was 27 months. All patients were HAART treated. We classified both NDLE and PML in different swubgroups according to the disease course. Different MRI patterns, according to the presence and the anatomical distribution of lesions, were identified: multifocal, inflammatory, diffuse and vascular-like. Moreover, non conventional MRI techniques (NCMT) were applied (spectroscopy MRI and diffusion weighted imaging), in order to detect microscopic damage of the brain tissue, and to possibly identify distinctive features of PML and NDLE. DWI showed a diffuse white and grey matter damage, more severe in HIV patients than in healthy subjects (HS), and in HIV patients with leukoencephalopathy (HIV LE+) than in HIV patients without leuokoencephalopathy (HIV LE-). Concerning spectroscopy, all HIV LE+ showed a lowered N-Acetyl Aspartate/Creatine ratio in frontal white matter and thalamus with respect to HIV LE- and HS; a decreasing gradient of severity from PML to HIV LE- was demonstrated. No difference between HS, HIV LE-, HIV LE+ was found regarding Choline/Creatine ratio.

Conclusions: In our series, the amount of atypical PML cases with good outcome is surprisingly high and can occur in every stage of HIV infection. These preliminary data suggest that the slow PML variant should be distinguished from the PML with benign outcome, the former being related to an intrinsic feature of the disease, the latter being treatment-related. Finally, PML show a worse phenotype than NDLE. NCMT could be a useful tool in detecting early involvement of the brain tissue, and in understanding the pathogenetic mechanisms underlying different forms of LE.

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## P.166

### Inhibition of HIV-1 infection by NB325 may be independent of the neuroglial functions of the chemokine receptor CXCR4

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Within the central nervous system (CNS), CXCR4 and its ligand SDF-1alpha (stromal cell derived factor-1alpha) participate in neurogenesis, neuronal survival, and axonal pathfinding; inhibition of HIV-1 infection by a CXCR4 inhibitor may adversely affect normal neuronal functions. For this reason, chemokine receptor (CCR) inhibitors developed for use in the CNS should specifically inhibit HIV-1 infection while permitting normal CCR functions. Our efforts in this area have focused on compounds that inhibit HIV-1 infection

by interfering with interactions between the virus and cell surface proteins. NB325 (a biguanide-based compound) is characterized by low toxicity and considerable activity against HIV-1 IIIB, which uses CXCR4 as a viral co-receptor. The particular potency of NB325 in the presence of both virus and target cells led us to hypothesize that NB325 may interfere with viral binding and entry mechanisms. Flow cytometric assays demonstrated that NB325 altered the detection of CXCR4 on activated primary human CD4+ T lymphocytes in an epitope-specific manner. Further mechanistic studies demonstrated that NB325 (i) did not inhibit CXCR4 binding by its sole ligand, SDF-1alpha, (ii) did inhibit SDF-1alpha-induced chemotaxis, (iii) was competitively inhibited from binding CXCR4 by a peptide derived from CXCR4 extracellular loop 2 (ECL2), and (iv) did not induce CXCR4 internalization. Ongoing investigations will further explore the mechanism of NB325 activity and its impact on normal CXCR4 functions in both immune and neuroglial cells. These studies will facilitate the development of novel compounds that can be used safely in the CNS to inhibit HIV-1 infection.

## P.167

### The HIV-1 infected monocyte-derived macrophage proteome: Analysis following infection with primary viral isolates from infected women with cognitive impairment

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The signature for HIV-1 neurovirulence remains a subject of intense debate. One hypothesis revolves around the idea that specific viral strains can induce robust neurotoxic secretions from monocyte-derived macrophages (MDM) and as such incite progressive neurodegeneration. We hypothesize that neurovirulent HIV-1 variants will influence the macrophage proteome and as such affect the onset and progression of NeuroAIDS. To test this hypothesis, primary HIV-1 isolated from blood of women with cognitive impairment (CI) were compared to isolates with identical replication properties but from noncognitive impaired (NC) individuals. The neurotropic isolate, SF-162 served as a control. HIV-1 isolates were inoculated onto MDM and viral replication tested by levels of HIV-1 p24 antigens in culture fluids. Uninfected and HIV-1 infected MDM were lysed at 14 days post-infection, fractionated, analyzed by surface enhanced laser desorption ionization time of flight (SELDI-TOF) for protein profiling. Spectral peaks were assessed by Generalized Estimated Equations statistics. Gel cubes were cut from 1D-SDS PAGE and proteins identified by tandem mass spectrometry (LC-MS/MS). Levels of HIV-1 replication were similar amongst isolates, although the highest levels of viral replication were seen from isolates obtained from patients with CI. Significant differences were found in protein profiles between MDM and MDM infected with NC, CI and SF-162 ($q < 0.10$). We identified 6 and 7 proteins unique to NC and SF-162. Three proteins were found common to SF-162 and CI strains, and 20 to CI HIV-1. The MDM proteins linked to viral replication with CI strains were related to redox, apopototic processes, signaling, and intracellular trafficking. We conclude that different viral isolates may influence the macrophages proteome.

## P.168

### Neutralizing antibody to interferon-alpha treatment in SCID mice with HIV encephalitis

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The pathogenesis of HIV associated dementia (HAD) is incompletely understood. Since highly active antiretroviral therapy is only partially effective, new treatments are needed based on delineation of HAD pathogenesis. It is hypothesized that HIV infected cells produce putative neurotoxins that damage neurons. Interferon Alpha (IFNa) is a pleomorphic cytokine produced by nucleated cells in response to viral infection. Treatment with IFNa in patients has side effects including cognitive impairment resembling subcortical dementia, which is the hallmark of HAD. It has been shown that IFNa is increased in the cerebrospinal fluid of HIV dementia patients compared to HIV patients without dementia symptoms. We hypothesized that the inhibition of IFNa in the central nervous system (CNS) would ameliorate the cognitive dysfunction function and pathological abnormalities seen in SCID mice with HIV encephalitis. In addition, we have shown a correlation between IFNa in the CNS and cognitive performance in this model with higher levels of IFNa correlating with increased cognitive deficits during testing. In this study, HIV mice treated with intraperitoneal (i.p) injections of IFNa neutralizing antibodies demonstrated significantly improved cognitive function ($p < 0.05$) as determined in the water radial arm maze compared to HIV mice that received either isotype matched control antibody treatment or a saline injection. Pathological analysis showed presence of IFNa neutralizing antibodies in the brain. Anti- IFNa antibody treated HIV mice exhibited decreased microgliosis ($p < 0.05$) compared to HIV mice with control antibody or saline injections. Also, anti- IFNa antibody treated mice showed improvements in loss of dendritic arborization

surrounding HIV infected cells compared to other HIV infected mice.

## P.169

### The signaling cooperation between IGF-IR and EGF-R in human glioblastoma cell lines with different PTEN background

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Glioblastoma is the most aggressive and frequent brain tumor, which accounts for approximately 25% of all primary intracranial neoplasms. Despite recent advances in surgical and clinical sciences, glioblastomas are always associated with poor prognosis. Patients with glioblastoma have a median survival of one year despite of aggressive therapies, and fever than 5% will survive 5 years. The important characteristic of glioblastoma is its unusual ability to invade surrounding brain tissues, and its autonomy in respect to growth factors requirements, the feature which is often associated with PTEN mutations. In this study we evaluate two tyrosine kinase receptors, IGF-IR and EGF-R, and their possible cooperation in supporting growth, survival and invasiveness of glioblastoma cell lines, characterized by different PTEN background. We have utilized five human glioblastoma cell lines: U-87 MG and U-118 MG lacking PTEN expression; LN-18 and LN229, which express wt PTEN; and T98G, which express mutated PTEN. We have observed that in the absence of functional PTEN, levels of expression of IGF-IR, EGFR, as well as the major IGF-IR signaling molecule, IRS-1, are about 10 fold lower in comparison with cells expressing PTEN. Despite that, IGF-I or EGF stimulation mediated similar responses at the levels of Akt, Erks and GSK3beta phosphorylation in all cell lines examined. Another observation is that independently of the PTEN background EGF is more effective than IGF-I in phosphorylating ERKs, and IGF-I seems to be more efficient in Akt phosphorylation. Interestingly, all five cell lines have similar growth requirements. They grow exponentially in the presence of 10%FBS, slow down substantially in serum-free medium, and surprisingly, do not respond well with increased cell proliferation when stimulated singly by IGF-I or EGF. In contrast, the addition of both IGF-I and EGF strongly supported cell proliferation in the absence of serum. In a similar manner, single inhibition of IGF-IR by NVP-AEW541 (Novertis), or inhibition of EGFR by PD153035 (Calbiochem) had only marginal effects on cell survival, or on the inhibition of cell proliferation. In contrast, combination of NVP-AEW541 and PD153035 applied at relatively low doses inhibited serum -dependent cell proliferation, and efficiently challenged survival of glioblastoma cells in anchorage-independence. In summary, our results indicate that in five human glioblastoma cell lines examined there is a signaling synergy between IGF-IR and EGFR, which supports malignant growth of glioblastoma, and that the absence of functional PTEN, although decreases growth factors requirements, does provide growth factors independence.

## P.170

### Dysregulation of Cu/Zn superoxide dismutase-1 in the CSF and monocytes of Hispanic women with cognitive impairment

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Cognitive impairment (CI) occurs in advanced stages of HIV-infection and despite the wide spread use of antiretroviral medicines. Disease is linked to activation of brain mononuclear phagocytes (MP; perivascular blood-borne macrophages and microglia) that results in neuroinflammation and secretion of toxic substances. These include pro-inflammatory cytokines, chemokines and immune products that affect oxidative stress and neuronal death. Our prior works have demonstrated that alterations in the macrophage and CSF proteome are associated with CI. These studies revealed that among other candidates, CSF Cu/Zn Superoxide Dismutase (SOD-1) in HIV-1 seropositive Hispanic women with CI. Since MP activation is a hallmark of neuronal injury in HIV associated CNS disease, we tested the hypothesis that SOD-1 is dysregulated in the CNS, and HIV-infected macrophages contribute to this effect during CI. For this purpose SOD-1 expression was measured in CSF, monocytes, monocyte-derived macrophages (MDM), and plasma from a group of HIV seropositive Hispanic women characterized for cognitive function by ELISA and Western blots. SOD activity was measured in the CSF from 32 patients by a colorimetric assay based on inhibition of xanthine oxidase by SOD (Calbiochem). We found that SOD-1 expression was not significantly different in the CSF and macrophages of women with CI compared with NC. When stratified by the degree of CI, we found that SOD expression in the CSF decreased in asymptomatics (A) compared to normal cognition (ND) and HIV associated dementia (HAD), while SOD activity in the CSF increased in A compared to ND and HAD ($p < 0.5$). These results indicate that there is dysregulation of SOD-1 in the CNS with increased activity in early stages of CI.

## P.171

### Lipopolysaccharide protects against neurological disease in cats infected with feline immunodeficiency virus

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Infection by the human immunodeficiency virus (HIV) results in neurodegeneration caused by aberrant activation of glial cells with ensuing neuronal death. Since patients with HIV/AIDS often develop opportunistic infections, we studied the role of systemic immune activation on neurological function and viral replication in the brain. For this purpose, we repeatedly administered lipopolysaccharide (LPS), a component of the membrane of gram-negative bacteria, to cats infected with feline immunodeficiency virus (FIV), as an animal model of HIV encephalopathy. After behavioral assessment, animals were sacrificed and proteins and mRNA were extracted from cerebral cortex and basal ganglia, for immunoblotting of neuronal markers and RT-PCR analyses of genes for inflammatory cytokines and neurotrophic factors.

FIV-infected cats treated with LPS developed less severe neurobehavioral deficits (mean deficit score $1.4 \pm 0.2$ in FIV-LPS group versus $3.6 \pm 0.2$ in FIV group, $p < 0.05$) and showed lower brain viral load than the FIV-infected control group. Moreover, we observed reduced loss of the presynaptic protein synaptophysin and the vesicular acetylcholine transporter, and up-regulation of the mRNA for brain-derived neurotrophic factor (BDNF) in the basal ganglia, despite high levels of the pro-inflammatory cytokine IL-1beta. Other neurotrophins (IGF-1, GDNF), as well as the mannose receptor, were not affected by LPS treatment.

Thus, systemic LPS improved neurological function and synaptic architecture in FIV-infected animals through a mechanism involving increased expression of BDNF and reduced viral replication. Our findings emphasize the beneficial aspects of selective activation of innate immunity in neurological disease.

## P.172

### Expression of CXCL10 from the mouse hepatitis virus genome results in protection from viral-induced neurological and liver disease

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Using a recombinant murine coronavirus (mouse hepatitis virus, MHV) expressing the T cell-chemoattractant CXCL10 (MHV-CXCL10), we demonstrate a potent antiviral role for CXCL10 in host defense. Instillation of MHV-CXCL10 into the central nervous system (CNS) of CXCL10 deficient mice (CXCL10−/− mice) resulted in viral infection and replication in both brain and liver. Expression of virally-encoded CXCL10 within the brain protected mice from death and correlated with increased infiltration of T lymphocytes, enhanced IFN-? secretion, and accelerated viral clearance when compared to mice infected with an isogenic control virus, MHV. Similarly, viral clearance from the livers of MHV-CXCL10-infected mice was accelerated in comparison to MHV-infected mice, yet was independent of enhanced infiltration of T lymphocytes and natural killer (NK) cells. Moreover, CXCL10-/- mice infected with MHV-CXCL10 were protected from severe hepatitis as evidenced by reduced pathology and serum alanine aminotransferase (sALT) levels compared to MHV-infected mice. CXCL10-mediated protection within the liver was not dependent on CXC-chemokine receptor 2 (CXCR2) signaling as anti-CXCR2 treatment of MHV-CXCL10-infected mice did not modulate viral clearance or liver pathology. In contrast, treatment of MHV-CXCL10-infected CXCL10−/− mice with anti-CXCL10 antibody resulted in increased clinical disease correlating with enhanced viral recovery from the brain and liver as well as increased sALT levels. These studies highlight that CXCL10 expression promotes protection from coronavirus-induced neurological and liver disease.

## P.173

### E2F1 expression is changed by activation of the NMDA receptor pathway in an in vitro model of HIV Associated Dementia

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In the pre-HARRT era, about 15∼25% of HIV infected individuals develop HIV associated dementia (HAD), which is characterized by neuronal and dendritic damage, neuronal loss, microgliosis and astrogliosis in the central nervous system. Although neurons are damaged in HIV infected patients, HIV does not infect neurons directly. Instead neuronal damage is mediated by neurotoxic factors from infiltrating, activated and /or infected macrophage/microglia. Neuroinflammation and the associated oxidative stress seen in patients with HAD is also seen in other neurodegenerative diseases including Alzheimer disease and Parkinson disease. Interestingly, the pro-apoptotic cell cycle protein, E2F1, exhibits increased immunoreactivity and altered subcellular distribution in several neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis and HIV encephalitis. However, in these

diseases E2F1 localized predominantly to the cytoplasm, a localization distinct from its well-defined nuclear role as a transcription factor during apoptosis and cell cycle progression. Based on these observations we hypothesized that cytoplasmic E2F1 might also contribute to neuronal death in HAD. We used an in vitro model of HAD in which we treated primary rat neuroglial cultures with supernatants from human monocyte-derived-macrophages infected with a neurovirulent strain of HIV-1 (HIV M/M). Unexpectedly, using a concentration of HIV M/M that produced 50% neuronal loss by 20 hours, we observed a lower molecular weight E2F1 expression, accompanied by increased calpain activation by western blotting. Besides, this generation of lower band E2F1 was reversed by NMDA receptor antagonist and Calpain inhibitor. Furthermore, both of the mRNA and protein expression of E2F1 targets didn't change in the in vitro model. And there is no change of E2F1 targets protein expression in the mid-frontal cortices of patients with HIV and those with HIV associated cognitive impairment. Based on our in vitro observations, we propose that the generation of lower band of E2F1 is mediated via the activation of NMDA pathway, probably through Calpain, but whether this change of E2F1 has its functional significance is still needed to be further explored.

## P.174

### CART results in decreased brain virus burden and reversal of neuronal injury in treated SIV-infected CD8-depleted rhesus macaques

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The clinical entity of HIV-associated dementia is due to a combination of neuronal injury, dysfunction, and loss, which may be partially reversible. Although antiretroviral drugs have been shown to be effective in decreasing the HIV/SIV burden in plasma, the effect of these drugs on HIV/SIV replication in the brain is not clearly known. Because many antiretroviral agents do not cross the blood-brain barrier (BBB), it has been suggested that the brain may serve as a proviral reservoir in HIV patients treated with antiretroviral agents. To quantitatively determine the effect of short-term antiretroviral therapy on virus replication and neuronal injury in different regions of brain we used QC real time RT-PCR to accurately analyze brain virus burden in eight rhesus macaques that were inoculated intravenously with SIVmac251 and CD8-depleted. Four animals were treated with CART, comprised of daily subcutaneous injections of PMPA (30 mg/kg) and RCV (10 mg/kg), beginning 28 days post-inoculation (dpi) and continuing until the animals were sacrificed at 57 dpi. Neither PMPA nor RCV cross the BBB. Untreated animals were sacrificed when moribund with AIDS (57 to 85 dpi). Parenchymal brain virus burden was quantified in the frontal cortex, putamen, hippocampus and brain stem. Significantly less virus replication was observed in frontal cortex of animals that were treated with CART compared to those that were untreated (median values of 3.7 vs. 5.1 log10 SIV RNA copies/mg, respectively; $p < 0.03$). The median virus burdens were lower in the CART animals than untreated macaques in putamen, hippocampus, and brain stem, as well (5.0 vs. 5.8, 5.1 vs. 5.4, and 4.3 vs. 5.5, respectively); however, the differences were not statistically significant in these regions. Longitudinal and terminal magnetic resonance spectroscopy (MRS) analysis of NAA/Cr from frontal cortex demonstrated an inverse relation with increasing NAA/Cr and decreasing brain virus burden in treated animals (mean NAA/Cr untreated $0.666 \pm 0.137$, treated $0.707 \pm 0.103$). Taken together, these data suggest that short-term systemic antiretroviral agents are effective in reducing replicating virus in the brain, and can result in partial reversal of neuronal injury.

## P.175

### Role of C/EBP-beta isoforms in JC virus latency and reactivation

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The human neurotropic JC virus (JCV) causes the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) when it reactivates from a latent state to destroy oligodendrocytes in the brain. It is thought that this reactivation may involve positive regulation through cellular transcription factors that become activated by the action of cytokines. The JCV non-coding control region (NCCR) contains elements that regulate transcription including an NF-kappa-B binding site (kappa-B element) that may be involved in this transcriptional activation. We now report that isoforms of the C/EBP-beta transcription factor, especially the LIP isoform, also bind to the kB element but that this binding inhibits both basal and NF-kappa-B-stimulated JCV transcription. Gel shift analysis showed that C/EBP-beta LIP bound this region in vitro but not to other regions of the NCCR. ChIP assays confirmed LIP binding in vivo. Co-immunoprecipitation experiments with oligonucleotides to this site indicated the formation of ternary complexes of NF-kappa-B p65, C/EBP-beta LIP and JCV DNA. Mutagenesis analysis of the JCV kappa-B element indicated that p65 and LIP bound to adjacent but distinct sites both of which are required for basal and

p65-stimulated transcription. Finally, immunohistochemistry for C/EBP-beta on tissue from a PML lesion and from a normal brain showed that C/EBP-beta is expressed in the cytoplasm of oligodendrocytes harboring viral inclusion bodies, which are negative for C/EBP-beta. In contrast, normal uninfected oligodendrocytes show robust nuclear C/EBP-beta expression. These observations indicate a novel inhibitory role for C/EBP-beta isoforms in the regulation of JCV, which may be important in controlling latency and the transcriptional reactivation that occurs in PML.

## P.176

### The early growth response-1 protein is induced by JCV infection and binds and regulates the JCV promoter

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JC virus (JCV) is a human neurotropic polyomavirus that infects many people subclinically but then enters a latent state. Under conditions of immunosuppression, usually AIDS, JCV can emerge from latency to cause the cytolytic destruction of glial cells of the brain in the fatal demyelinating disease, Progressive Multifocal Leukoencephalopathy (PML). Previous studies described an important cis-acting transcriptional regulatory element in the JCV non-coding control region (NCCR) that is involved in the response of JCV to cytokines and may have a role in viral reactivation. This consists of a 23 base pair GGA/C rich sequence (GRS) near the replication origin (5112 to +4) that contains predicted potential binding sites for the cellular transcription factors Sp1 and Egr-1. Gel shift analysis showed that Egr-1, but not Sp1, bound to GRS. Evidence is presented that the GRS gel shift seen on cellular stimulation is due to Egr-1. Thus, TPA-induced GRS gel shift is blocked by antibody to Egr-1. Furthermore, the TPA-induced GRS DNA/protein complex band was isolated from non-denaturing gels and found to contain Egr-1 by Western blot. No other Egr-1 sites were found in the JCV NCCR. Functionally, Egr-1 was found to stimulate transcription of the JCV late promoter but not the early promoter. JCV infection of primary astrocytes induced nuclear Egr-1 expression that was maximal at 5-10 days post-infection. Finally, upregulation of Egr-1 was detected in the nuclei of JCV-infected cells in clinical PML samples by immunohistochemistry. These data suggest that Egr-1 induction may be important in the life cycle of JCV and in the pathogenesis of PML.

## P.177

### Development of lentiviral encephalitis

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A quarter of terminally ill AIDS patients develop a severe neurological disease term HIV Associated Dementia (HAD). The neuropathological substrate of this disease is termed HIV encephalitis and consists of abundant activation and infection of brain macrophages. Diagnosis of this disease is complicated by a myriad of minor cognitive impairments that are seen in earlier stages of HIV infection. We examined the utility of the peripheral benzodiazepine receptor ligand PK11195 in positron-emission tomography (PET) imaging to assess microglial/macrophage activation in the brains of HIV infected subjects with minor neurocognitive impairment. None of the 12 HIV-infected subjects nor 5 controls demonstrated increased retention of [11C](R)-PK11195 in the brain parenchyma. These results suggest that macrophage activation is not the pathological substrate of this minor cognitive impairment. To develop better biomarkers for the development of lentiviral encephalitis, we studied the SIV non-human primate model. We longitudinally followed Rhesus and Pigtailed macaques infected with the B670 examining blood, CSF and lymph nodes in addition to serial MRI and PET scans. These studies permitted us to identify the onset of encephalitis and examine the pathogenesis of neurodegeneration associate with lentiviral infection.

## P.178

### The role of IGF-IR–p66Shc-FOXO3a signaling axis in high glucose-mediated accumulation of reactive oxygen species (ROS) and neuronal damage

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It has been demonstrated that diabetes mellitus and associated hyperglycemia alter the redox status of cells through the overproduction of ROS by the mitochondrial electron transport chain and NADPH oxidase. Our results indicate that high glucose (HG) concentration (25mM for 16 hours) elevated the accumulation of ROS in differentiated PC12 neurons in comparison to the cells kept in normal glucose (NG; 5mM). The accumulated ROS was found in neuronal processes and in perinuclear cytoplasm. This abnormal ROS accumulation in HG was associated with a gradual (over a period of 10 days) loss of neuronal extensions. Interestingly, the addition of IGF-I to PC12 neurons cultured in HG prevented ROS accumulation and significantly improved the stability of neuronal processes. Since, p66Shc – FOXO3a signaling axis is

involved in ROS metabolism, and Shc proteins are direct substrates for the IGF-IR kinase activity, it was reasonable to speculate that the IGF-IR may affect ROS metabolism by changing the status of p66Shc phosphorylation and FOXO3a subcellular localization. We have selected several clones of PC12 cells, which stably express a dominant negative mutant of p66Shc (DNp66Shc) in which Ser36 was replaced with alanine. We have analyzed protein levels for Shc proteins including p42, p52 and p66; DNp66shc, as well as IRS-1, IGF-IR, and FOXO3a. Importantly, in the presence of HG, PC12 neurons stably expressing DNp66Shc showed significantly lower levels of ROS in comparison to parental PC12 neurons, further confirming the importance of functional p66Shc in ROS accumulation in differentiated neurons. We have evaluated also FOXO3a nuclear localization in PC12 cells cultured in NG, HG and HG+IGF-I. The purpose of this experiment was to determine whether FOXO3a, which is the key transcription factor for superoxide dysmutase (SOD), translocates to the nucleus when ROS accumulation is attenuated. Digital images collected from mid sections of the nuclei of PC12 neurons, show an apparent immunolabeling for FOXO3a detected in the cytoplasm of examined cells. In normal glucose concentration (NG) an apparent FOXO3a nuclear foci were also detected. In high glucose (HG), the number of nuclear FOXO3a foci decreased nearly 10-fold compared to NG. Importantly, an average number of nuclear foci of FOXO3a in HG was restored when the cells from HG cultures were treated with IGF-I. This corresponded well with ROS levels in these three experimental conditions and with the detection of nuclear FOXO3a in cortical neurons from HIV encephalopathy clinical samples.

## P.179

### Identification of Epstein Barr virus epitopes in multiple sclerosis cerebrospinal fluid using phage-displayed random peptide libraries

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of unknown cause. The most common laboratory abnormality associated with MS is increased intrathecal IgG synthesis and the presence of bands of oligoclonal IgG in brain and cerebrospinal fluid (CSF). However, the major antigenic targets of the antibody response are unknown. Recent interest has focused on a possible role for Epstein Barr virus (EBV) in the development of disease. The risk of MS is increased after infectious mononucleosis, and MS patients have higher serum titers of EBV antibodies than control populations. To identify disease-relevant antibodies, we screened phage-displayed random peptide libraries with IgG purified from an acute MS brain. Two different phage-displayed peptides were selected that share linear sequence homologies with EBV nuclear antigens 1 and 2 (EBNA-1 and EBNA-2), respectively. The specificity of the EBV epitopes to panning MS brain IgG were confirmed by ELISA and competitive inhibition assays. Using a highly sensitive phage mediated immuno-PCR assay, we measured specific binding of each phage-displayed EBV epitope to IgG from 50 MS CSFs and 5 inflammatory control (IC) CSFs. IgG binding to the EBNA-1 epitope, but not the EBNA-2 epitope, was found in 25 of 50 (50%) MS CSFs, and in 1 of 5 IC CSFs. We also studied antibody titers directed against the purified EBNA-1 protein and lysates of EBV-infected cells. On average, EBV titers in MS CSF ($n = 50$) were significantly greater than in inflammatory controls ($n = 9$). Although we detected increased antibody to an EBNA-1 epitope as well as to purified EBNA-1 protein in MS CSF, it remains to be determined whether EBV antibodies are synthesized intrathecally and correspond to bands of oligoclonal IgG in MS CSF and brain.