

8TH INTERNATIONAL SYMPOSIUM ON NEUROVIROLOGY

SPEAKERS

Session 1: CNS response in NeuroAIDS

S.1

Anti-retroviral nanoformulations for enhancing CNS drug delivery

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The elimination of the human immunodeficiency virus inside its central nervous system (CNS) sanctuary is affected by variable antiretroviral therapy (ART) penetrance across the blood-brain barrier (BBB), complex dosing regimens, costs, toxicities, and limitations in biodistribution and pharmacokinetic drug patterns. Despite advances in ART NeuroAIDS morbidities continue. A principal issue obstacle in achieving maximal clinical responses is in maintaining ART drug levels in disease affected brain subregions. To address this issue, we developed nanoformulations of anti-retroviral drugs and delivered them inside circulating blood-borne monocyte-macrophages. The means to improve distribution of ART across the BBB included testing of nanoparticle (NP) drug formulation(s) for entry and secretion into and from bone marrow-derived macrophages (BMM) and monocyte-derived macrophages. Here, viral protease inhibitor(s) are being packaged into phospholipids coated NP. Cytotoxicity, anti-retroviral efficacy, mobility, and the functional consequences of macrophage carriage of the drug-laden particles are measured. Second, pharmacokinetics (uptake, release, plasma and tissue distribution) of the formulations is being investigated using BMM as a drug delivery system in mice. Third, optimal ways to enhance uptake of NP formulations are being developed. In this way, the abilities of drug to bypass the reticuloendothelial system and cross the BBB can be determined. High performance liquid chromatography analyses are used to measure drug levels in spleen, lymph nodes, liver and brain in NP-treated mice and provide confirmation of drug tissue penetrance. These tests are used in tandem with histology and imaging assays. Lastly, the NP are tested for anti-retroviral efficacy in humanized mouse models of human HIV-1 CNS disease. All together, our works explore the means to enhance therapeutic efficacy and BBB migration of ART so that they can be translated for human use to improve disease outcomes in NeuroAIDS.

S.2

Multifaceted response to SIV in the brain

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Although efficacious treatment greatly improves both the quality of life and overall lifespan in HIV infected individuals, CNS complications continue to affect those with HIV. HIV infects the brain early and persists. Using the SIV/rhesus model, we have assessed the functional, molecular, and immune consequences of chronic infection of the CNS. Experimental interventions, such as non-CNS penetrating antiviral therapy, and CNS immune cell manipulations, have allowed the assessment of peripheral and CNS components of the response to chronic infection in the primate brain, and their contribution to CNS pathophysiology.

S.3

Bioimaging and CNS responses to HIV-1 infection

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Despite treatment with potent antiretroviral medications and the significant decline in mortality and opportunistic brain diseases in patients with HIV, HIV-associated cognitive disorder remains a common and persistent morbidity for these individuals. With the longer survival and aging population of HIV patients, cognitive deficits in this patient population may be even more prevalent and may impact their quality of life further. Biomarkers that can reliably measure the brain changes associated with the infection is needed to assess the severity of brain injury, design rational therapy, evaluate treatment effects, and improve the quality of life of individuals living with HIV. A variety of in vivo structural and functional neuroimaging techniques, including magnetic resonance imaging (MRI), MR spectroscopy (MRS), diffusion tensor imaging (DTI), functional MRI (fMRI) and positron emission tomography (PET), can assess brain injury or inflammatory responses associated with HIV. Many of these measures can even detect brain injury during the neuroasymptomatic stages. In addition, quantitative

measurements with these techniques may serve as surrogate markers to monitor treatment effects.

DTI can measure brain water motion and thus provide quantitative assessment of the inflammatory changes in the brain. Correlation of DTI or MRS with other plasma or CSF markers, such as inflammatory cytokines or chemokines, or clinical assessments with neuropsychological tests, may further validate and provide confirmatory information regarding the effects of HIV infection in the brain. Evaluating inflammatory responses in the brain with DTI may be a sensitive measure to monitor the treatment effects. Findings from some of the neuroimaging studies, such as measures of dopaminergic function with PET, also provide insights into the pathophysiological mechanisms of HIV-associated brain injury and might lead to new therapeutic approaches.

S.4

STAT1 signaling modulates HIV-1-induced inflammatory responses and leukocyte transmigration across the blood-brain barrier

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The relationship between neuroinflammatory signaling pathways, the blood-brain barrier (BBB) and

progressive HIV-1 infection as they affect the development of cognitive and motor abnormalities is poorly understood. One possibility to link each of these is through the signal transducers and activators of transcription (STAT) pathways as they respond to pro-inflammatory and regulatory factors, and could affect neuroinflammatory responses from virus infected brain cells. Our previous works demonstrated that HIV-1 activate pro-inflammatory and interferon alpha-inducible genes in human brain microvascular endothelial cells (HBMEC) and that these genes were linked to the janus kinase (JAK)/STAT pathway. We now demonstrate that HIV-1 and HIV-1-infected monocyte-derived macrophages activate STAT1 and induce HBMEC IL-6 expression. The STAT1 inhibitor, fludarabine, blocked HIV-1-induced IL-6 production, blocked HIV-1 and IL-6-induced monocyte migration across in vitro BBB models. Enhanced expression and activation of STAT1 and decreased claudin-5 was observed in microvessels acquired from autopsy brains of patients with HIV-1-associated dementia. These data strongly support the notion that STAT1 plays an integral role in HIV-1-induced BBB damage and is relevant to viral neuropathogenesis. Inhibition of STAT1 activation could provide a unique therapeutic strategy to prevent HIV-1-induced BBB compromise and as such improve clinical outcomes.

Session 2: Clinical parameters & co-pathogens in NeuroAIDS

S.5

Neurologic disorders in patients co-infected with HIV and HTLVs

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Infection with HIV-1, HIV-2, HTLV-1 and HTLV-2 are heterogeneously distributed worldwide, with an estimated number of living infected carriers of 40, 2, 15 and 10 millions, respectively. While more than 90% of HIV-1/2-infected persons will develop symptoms of immunodeficiency lifetime in the absence of antiretroviral treatment, less than 5% of HTLV-1/2 carriers will suffer from neurologic or lymphoproliferative disorders in their life. Given their similar routes of transmission (sexual, parenteral and vertical), co-infection with several of these retroviruses is not uncommon. As result, their respective pathogenicities may be enhanced in co-infected individuals.

HTLV-1/HIV-1 co-infection is mainly seen in Central and South America. Tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) may occur more frequently, at younger ages and progress faster in co-infected patients than in HTLV-1-monoinfected carriers. A higher proviral load in the presence of HIV-associated immunodeficiency may explain these findings. Antiretroviral drugs used against HIV are not effective against HTLV.

HTLV-2/HIV-1 co-infection is mainly seen among injecting drug users in Western Europe and North America. Cases of TSP/HAM-like illness and peripheral neuropathies have been reported occasionally among HIV-1/HTLV-2 co-infected patients. These neurologic manifestations have been triggered following initiation of antiretroviral therapy in a few cases, suggesting that may be considered immune reconstitution inflammatory syndromes. On the other hand, a protective effect of HTLV-2 on CD4 depletion caused by HIV-1 has been noticed in co-infected patients.

S.6

Syphilis and HIV: are they co-pathogens?

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Syphilis and HIV can be considered to be co-pathogens for several reasons. Syphilis increases the chance of acquiring or transmitting HIV. During episodes of active syphilis in people infected with HIV, CD4 cell count

declines and plasma viral load increases. Thus, HIV-infected individuals with syphilis may be more infectious and even more likely to transmit HIV. Worldwide, the epidemiology of syphilis and HIV support their intimate connection. The WHO estimates that 12 million people acquire syphilis every year, and two-thirds of these individuals reside in sub-Saharan Africa. In Africa, the proportion of individuals with syphilis is 2–3 times higher in those who are HIV-infected compared to HIV-uninfected. Similarly, in the US in 2002, compared to all patients with primary and secondary syphilis, rates of primary and secondary syphilis were 80 times higher in patients who were also HIV-infected. One study estimated that in the US in 1996, syphilis accounted for 1000 new cases of HIV.

HIV-infected individuals may have suboptimal responses to treatment of early syphilis and to treatment of neurosyphilis. Neurorelapse, meaning development of early neurosyphilis after appropriate treatment of early syphilis, is more common in HIV-infected than HIV-uninfected individuals. After neurosyphilis treatment, CSF-VDRL is less likely to normalize in HIV-infected compared to HIV-uninfected individuals, particularly when the CD4 count is <200/ul. Preliminary findings show that HIV-infected persons who are taking antiretrovirals at the time of neurosyphilis treatment have faster and more complete normalization of CSF measures. Taken together, these findings suggest that an intact immune response is integral to success of treatment for any form of syphilis, even when antibiotics are given.

The appreciation that syphilis and HIV are co-pathogens offers opportunities to improve public and individual health. It highlights the importance of the host immune response in determining the neurological outcome of syphilis infection and suggests directions for future research.

S.7

Level of HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) increases with severity of neurocognitive dysfunction

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Background. HIV-1-infected monocytes are thought to play a role in the pathogenesis of HIV-1-associated neurocognitive disorders (HAND). Data by others suggest that a subset of circulating monocytes expressing

activated markers may harbor more HIV DNA. We hypothesized that the level of HIV DNA in PBMCs is incrementally higher as cognitive diagnosis worsens. We also posit that monocytes demonstrate a greater HIV-1 binding capacity for R5-tropic viral strains when compared to PBMCs.

Methods. We measured baseline HIV DNA in PBMCs by real-time PCR from subjects enrolled in the Hawaii Aging with HIV Cohort. Participants were diagnosed with normal cognition (NC), minor cognitive motor disorder (MCMD), or HIV-associated dementia (HAD) based on the American Academy of Neurology 1991 criteria. In-vitro viral binding assays were carried out with PBMCs and monocytes using R5 and R4 viral strains.

Results. PBMC HIV DNA levels increased in a step-wise fashion with worsening HAND diagnosis: NC [log HIV DNA copies/10(6) cells = -0.17 (standard deviation = 1.22); n = 99]; MCMD [0.42 (1.27); n = 78]; and HAD [1.38 (1.54); n = 39], $p < 0.001$. Among subjects with undetectable HIV RNA levels (n = 103), HIV DNA levels were significantly higher in those with HAD (n = 17) compared to subjects with NC (n = 52) and MCMD (n = 34), $p < 0.001$. In-vitro monocytes displayed higher binding capacity for R5 virus compared to R4 virus when compared to PBMCs.

Conclusions. These findings demonstrate for the first time that PBMC HIV DNA levels increase incrementally with worsening severity of cognitive dysfunction, based on diagnostic category. The in-vitro data suggest that peripheral monocytes preferentially bind R5 virus. We conclude that PBMC HIV DNA may reflect higher HIV DNA in the monocyte subset. If confirmed, a revised paradigm in our understanding of HIV-1 neuropathogenesis may be warranted.

S.8

Cerebrospinal fluid (CSF) soluble urokinase plasminogen activator (uPA) receptor (suPAR) in AIDS dementia complex, and its relationships with other uPA, virological, immuneactivation and neuronal CSF markers

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Background. The urokinase plasminogen activator (uPA) system may be involved in the pathogenesis of AIDS dementia complex (ADC) through interaction with HIV-1 and subsequent dysregulation of uPA system functions—extracellular proteolysis and chemotaxis. The aim was to investigate this hypothesis through measurement of CSF and plasma level of uPA molecules and comparison with levels of other HIV-1, immune activation and neuronal markers.

Study Design: Cross-sectional study of antiretroviral-untreated patients, including patients with neurologically asymptomatic HIV infection and no AIDS (n = 19); neurologically asymptomatic HIV infection and AIDS (n = 18); ADC (n = 17); and HIV-negative healthy controls (n = 16). CSF and plasma were assessed for uPA system molecules (suPAR, uPA, PAI-1, tPA); immune activation markers (CSF cells and protein, neopterin and the following cytokines and chemokines, measured by the Luminex technology—Bio-Plex, Bio-Rad Laboratories, Inc.: IL-1 σ , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, basic FGF, eotaxin, G-CSF, GM-CSF, IFN- τ ;, IP-10, MCP-1, MIP-1 Σ , MIP-1 σ , PDGF-BB, RANTES, TNF- Σ , VEGF); and neuron-associated markers (NFL, t-tau, p-tau, A σ 1-42, Σ -sAPP and σ -sAPP).

Results. ADC patients had significantly higher CSF and plasma levels of suPAR compared to each of the other groups ($p < 0.0001$ for all), slightly higher levels of uPA, and no different levels of PAI-1 or tPA. As expected, ADC patients had the highest CSF levels of HIV-1 RNA and MCP-1 and also showed CSF levels of IL-6, MIP-1 Σ ; and MIP-1 σ ; significantly higher than in each of the other groups. In the whole group of HIV-infected patients, CSF suPAR correlated strongly (Spearman r coefficient ≥ 0.50 or < -0.50) with CSF and plasma uPA, and with CSF HIV-1 RNA, MIP-1 Σ , MIP-1 σ , neopterin, NFL and tau (direct correlation); and with blood CD4 cells and CSF Σ -sAPP and σ -sAPP (inverse correlation).

Conclusions. These findings strongly support the role of the uPA system in the pathogenesis of ADC. The relatively low CSF levels of uPA and the unaltered CSF levels of PAI-1 and tPA do not argue in favor of proteolysis dysfunction as prevalent mechanism. The observation that increased CSF suPAR occurred in parallel with increased levels of HIV-1 and other cytokines and chemokines, seems to support a mechanism by which suPAR would promote migration of inflammatory cells into the CNS and further enhance viral replication. In addition, the strong association between CSF suPAR and neuronal markers suggests that suPAR might also exert direct effects on neuronal cells.

S.9

Oxidative stress markers may serve as a biomarker for the progression of HIV-associated cognitive impairment in women

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Objective: To determine if CSF oxidative stress markers serve as a biomarker for the development or progression of HIV-associated cognitive impairment.

Background: Prevalence of HIV-associated cognitive impairment is increasing as patients treated with antiretroviral treatment (ART) live longer. To date a biomarker(s) to identify HIV-infected patients at risk of developing cognitive impairment is lacking. Markers of CSF oxidative stress may identify HIV-infected patients at risk of developing cognitive impairment and may have a role in the progression of the disease.

Methods: CSF from 62 women followed at the Longitudinal HIV-infected women cohort were evaluated. The Memorial Sloan Kettering Scale (MSK) was used to categorize cognitive function into normal (MSK = 0, n = 8), mild impairment (MSK = 0.5, n = 29), and dementia (MSK \geq 1, n = 21). Protein carbonyls (PC) were determined by Slot-blot and Western-blot in 47 CSF samples and 4-hydroxynonenal (4-HNE) and sphingomyelin by ESI-MS/MS in 37 CSF samples. Predictive value was based on changes of cognitive status after 6 months and a Kaplan Meyer survival analysis.

Results: At baseline there was a difference in protein carbonyls (p = .048) with post-hoc testing showing that women with mild impairment (MSK = 0.5) presented elevated CSF PC versus women with normal cognition or dementia (MSK = 0 or MSK \geq 1) (p = .015). In the predictive analysis at 6 months follow up, there weren't any differences. From 20 women who presented at baseline with normal cognition (n = 10) or mild impairment (n = 10) a Kaplan Meyer survival analysis showed that those with the highest tertile of CSF PC were less likely to worsen their cognitive status in at least 18 months follow up (p = .042). A similar trend was observed with 4-HNE lysine although not significant. Findings were not affected by age or ART.

Conclusions/Relevance: In the ART era, oxidative stress as determined by CSF PC levels may represent an early marker for the progression of HIV-associated cognitive impairment. High baseline PC levels had a predictive value among women with stable mild cognitive impairment.

Paradigm builders lectureship

S.10

Influenza virus pandemics: past and future

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The influenza pandemic of 1918/1919 was a unique event in recorded history, costing on the order of 50 million lives within the time span of only several months. The virus is obviously extinct, but the advent of reverse genetics techniques for negative strand RNA viruses (a family of viruses which includes the influenza viruses) makes it possible to reconstruct infectious influenza viruses. Based on the nucleotide sequence that was obtained from RNA fragments present in lung samples of victims of the 1918 influenza virus, we succeeded in rebuilding the extinct pandemic virus entirely from commercially available oligonucleotides. This virus turned out to be highly virulent in the mouse model, more than any other human influenza virus strain tested. It also was shown to be highly pathogenic for chicken embryos and grew in human tissue culture cells to high titers. On the other hand, the 1918 virus was shown to be sensitive to FDA-approved antivirals (amantadine as well as the neuraminidase inhibitor oseltamivir). We were also able to show that vaccines worked perfectly well in protecting mice against a challenge with a virus containing

the 1918 hemagglutinin and neuraminidase genes. We also postulate that the human population, having experienced infections with currently circulating H1N1 viruses, is partially immune to a 1918 or 1918-like virus. The availability of the 1918 virus will help us to better understand the molecular basis of virulence and the mechanisms by which pandemic influenza viruses are transmitted from human to human and from one species to another. In addition, these studies will expand our knowledge of the biological and molecular properties of pandemic influenza viruses in general and this in turn will provide us with better prevention and treatment strategies for the future.

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Breakout session—NeuroAIDS—Emerging issues in neuropathogenesis and therapeutic targets: from preclinical models to clinical studies

S.11

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

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MicroRNAs (miRs) are short endogenous RNAs that regulate gene expression by incomplete pairing with messenger RNAs. An increasing number of studies show that mammalian miRs play fundamental roles in various aspects of cellular functions including differentiation, proliferation and cell death. HIV-1 encephalopathy (HIVE) is a manifestation of HIV-1 infection that often results in neuronal damage and dysfunction. While neurons are rarely, if ever, infected by HIV-1, they are exposed to cytotoxic viral and cellular factors including the HIV-1 transactivating factor Tat. Here, we show that treatment of rat embryonic primary neurons with recombinant Tat results in increased expression of miR-128a. Among all predicted gene targets for miR-128a, we found that expression of the synaptic proteins SNAP25, SV2a, and PLK2 can be regulated by the activity of miR-128a. We further show that expression of SNAP25 and SV2A proteins is highly reduced in Tat-treated neuronal cultures in a dose and time dependent manner. Finally, immunohistochemical analysis performed on brain samples revealed a dramatic decrease of SNAP25 and SV2A immunostaining in brain tissue from patients with HIV-encephalopathy compared to control tissues.

S.12

Inhibition of Mixed Lineage Kinase (MLK)-3 breaks HIV-1 neurotoxin-induced feedback loop between platelet activation and microglial stimulation

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Onset and progression of HIV-associated dementia (HAD) is linked with increased levels of proinflammatory mediators in the CNS and those of markers of platelet activation. Here we show that the circulating levels of sCD40L are elevated in cognitively

impaired HIV-1-positive subjects, and candidate HIV-1 neurotoxin, platelet-activating factor (PAF), triggers the synthesis of soluble CD40 ligand (sCD40L) in primary cultures of human platelets. Interestingly, this increase in sCD40L release from platelets, was profoundly blocked by addition of an inhibitor of MLK-3 (CEP1347). We also show that recombinant CD40L promotes the ability of HIV-1 Tat to hyper-activate monocytes and microglial cells, in MLK-3-dependent manner, leading to the secretion of neurotoxic inflammatory mediators (notably, TNF α). CEP1347 also enhanced survival of neurons after exposure to Tat, whereas, immunodepletion experiments revealed that TNF α is essential for the neurotoxic effects of conditioned medium recovered from Tat/CD40L-treated monocytes. Collectively, these observations corroborate with our previous findings, that PAF acts as an initiator step in HIV-1 neuropathogenesis, and suggest that HAD may be characterized by a positive feedback loop between platelet activation and monocyte/microglial stimulation. Our model also predicts that the drug regimens (such as the MLK-3 inhibitor, CEP1347, currently in development for Phase 1 trials of neuroprotection in patients with HIV-1 infection and cognitive deficits), which simultaneously target activation of both platelets and monocytes/microglia, may of therapeutic value in the treatment of HAD.

S.13

Novel expression of PINCH in the CNS and its potential as a biomarker for HIV-associated neurodegenerative processes

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Particularly interesting cysteine histidine-rich (PINCH) protein has recently been recognized as essential in integrin-linked kinase signaling, neuronal polarity, cell migration and survival. More recently, PINCH has been reported to function as a shuttling protein in Schwann cells during peripheral nerve damage, repair and remodeling. However, the presence

of PINCH in the human CNS has not been addressed. Given the fact that HIV-associated damage to the cells of the CNS involves dysregulation of neuronal signaling and white matter alterations, we hypothesized that PINCH may play a role in neuropathological processes during HIV infection. To address PINCH expression in the CNS, brain frontal cortex and CSF obtained at autopsy from HIV negative individuals with no CNS alterations, HIV patients without reported CNS alterations and HIV encephalitic patients were examined immunologically for PINCH. Our results show that PINCH is expressed in the brains and CSF of HIV patients; whereas, PINCH is nearly undetectable in the CNS of HIV negative individuals. Robust PINCH immunoreactivity was detected in the white matter and appeared to be predominantly extracellular. Compared to white matter, significantly less PINCH was detected in the gray matter and was largely neuronal. Moreover, in HIV patients with no reported CNS alterations, PINCH distribution tended to coincide with areas of inflammatory cell infiltration. In contrast, PINCH immunoreactivity in HIV encephalitis cases was evenly distributed through out the white matter, but levels in the CSF were decreased significantly. Our findings suggest a role for PINCH during HIV-associated neurodegenerative processes.

S.14

Multicompartment multiphoton NADH imaging: a neuroimaging method for the direct study of non-neural and neuronal pathologies in rodent models of neuroAIDS?

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HIV-1 frequently causes a common, progressive neurological disorder known as HIV-associated dementia (HAD). The neurocognitive symptoms are thought to arise from indirect damage from infected macrophages and microglia to neurons. Indeed, we have previously shown that the HIV-1 neurotoxin, Tat, is capable of decreasing NADH metabolism in cortical neurons in vitro (Norman et al., 2007). Thus, an experimental neuroimaging method capable of resolving functional processes in all cell types of the CNS would provide a powerful tool for the study of disease mechanism in in vivo models of neuroAIDS. Here, we suggest that multi-color two-photon imaging of metabolic (NADH) and physiological processes in existing mouse and rat models of neuroAIDS may provide a unique subcellular window for emerging pathogenesis of this viral CNS infection. For example, we have recently established the optical resolution of activity-dependent metabolic (NADH) processes in the neuronal, the astrocytic, and the vascular compartment of the mouse cortex, and can also be utilized as an indicator of relative tissue oxygenation. We hypothesize that changes in cortical oxygen tension, either through a decrease in oxygen delivery or through increased oxygen uti-

lization due to physiological activity, cause a distinct pattern of NADH transients. As two examples of this, we have applied two-photon NADH imaging to investigate metabolic transitions during whisker stimulation and global hypoxia in the mouse barrel cortex in vivo. We find remarkably heterogeneous NADH patterns in layer I of the barrel cortex under both conditions. These transients show an inverse relationship with oxygen tension within the brain, as shown by the use of an oxygen electrode. Furthermore, these NADH transients appear to be highly dependent on the relationship of the tissue to the distribution of arteries and veins within the microvasculature, leading us to speculate that transients in oxygen tension, and thus NADH transients, may play a role in cerebral blood flow regulation. These results provide novel insight into the coupling of cerebral blood flow to brain metabolism. With a better understanding of this complex relationship in physiological states, we can further investigate this relationship in neuropathological states. With the use of mouse transgenic lines with cell-specific expression of fluorescent proteins and novel fluorescent markers, our technique is easily adaptable to study the cellular interactions between macrophages, microglia and neurons in murine in vivo models of neuroAIDS. In addition, with the unique capabilities of functional imaging in both the vascular and cellular compartments of the rodent CNS, we may also provide a useful microscopic compliment to the emerging application of macroscopic neuroimaging techniques in clinical studies of neuroAIDS.

S.15

Cerebral blood flow (CBF) and cerebral metabolic rate of oxygen consumption (CMRO₂) are elevated in both early and chronically infected 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 cortical regions of the brain in health controls. 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 during a functional task. We used qfMRI to determine if physiological responses within the visual cortex to a simple flashing checkerboard task were different within acute and chronic HIV infection.

Methods: 20 seronegative healthy controls and 9 early infected HIV+ subjects (less than one year after known seroconversion) and 9 chronically infected HIV+ subjects (more than one year after seroconversion) 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 a flashing checkerboard stimulus at 8Hz. 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 visual cortex which included not only V1 but higher visual association areas. 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. An analysis of variance (ANOVA) was performed for both measured (CBF and BOLD) and calculated (CMRO₂) values with subsequent paired t- test performed with p values significant if $p < 0.05$.

Results: Both CBF and BOLD responses were reproducibly observed within the visual cortex for functional stimulation and mild hypercapnia in HIV+ subjects (early and chronic) and seronegative controls. Changes in CBF and CMRO₂ but not BOLD for functional activation were significantly greater within HIV+ subjects compared to seronegative controls. In particular these changes in CBF and CMRO₂ were observed in early infected HIV+ patients and remained elevated in chronically infected HIV+ subjects. There was no correlation between nadir CD4, current CD4, or HIV RNA levels and qfMRI measures within HIV+ subjects.

Conclusions: HIV has significant effects on CBF and CMRO₂ even within cortical regions. These findings are consistent with previous evidence of brain dysfunction early in HIV infection (e.g., Grant et al. 1987) demonstrated by neuropsychological testing and electrophysiological measures. These results extend previous work by suggesting that cerebral metabolic dysfunction is a final common pathway for both neuropsychological and electrophysiological disturbances in HIV-infected patients qfMRI may therefore act as a non-invasive surrogate biomarker for assessing effects of HIV in the brain.

S.16

Alteration of neuronal CXCR4 function by in vivo/ in vitro opiates treatments and its role in neuronal survival and HIV neuropathogenesis

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Studies suggest that opiates may exacerbate the neurological complications of HIV-1 through direct actions in the CNS. Our goal is to determine the effects of mu-opioid receptor (MOR) agonists on the signaling pathways activated by CXCR4—one of the major HIV co-receptors, which also plays critical roles in neuronal survival and differentiation. In particular, we use in vitro and in vivo approaches to establish the effects of MOR agonists on the coupling of CXCR4 to neuronal survival mechanisms. Our data show that long-term treatment of rat primary cortical cultures with DAMGO, morphine or endomorphin-1 inhibits activation of ERK1/2 and Akt by CXCL12—an effect that is not mediated by changes in CXCR4 levels on neuronal surface. Pretreatment with MOR agonists also prevents the neuroprotective action of CXCL12 in NMDA-treated cultures. MOR and CXCR4 are co-expressed in many cultured neurons suggesting a possible crosstalk between the two receptors. This is supported by GTPγS incorporation studies in rat brain slices and brain homogenates measuring levels of G-protein activation upon stimulation of CXCR4 or MOR. These studies also show that in vivo morphine treatments inhibit CXCR4-mediated G-protein activation. Experiments with glia-free cultures demonstrate that the inhibitory effect of opioids on CXCR4 signaling is independent of glia, and that opioid treatment blocks CXCL12-mediated phosphorylation on specific residues of the C-terminus of CXCR4, which is involved in receptor activation/desensitization. Down-regulation of phospho-CXCR4 was also found in brain tissue from HIV/HAD patients, suggesting that impairment of CXCR4 function by opioids may contribute to HIV neuropathology.

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Session 3: Drugs of abuse, aging, and other comorbidities in NeuroAIDS

S.17

Multifaceted influences on presentation of NeuroAIDS

Igor Grant

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Neurocognitive disturbances, i.e., difficulties with memory, attention, problem solving, and psychomotor performance, are commonly observed in HIV infected (HIV+) individuals and these can affect everyday functioning in areas such as employment and medication management. Despite effective ARV (eARV) treatment which has reduced medical complications and prolonged survival, these HIV-associated neurocognitive disorders (HAND) are still observed in 30–60% of HIV+. Cofactors such as increasing age, hepatitis C coinfection, and substance use may contribute to expression of HAND. Abuse of methamphetamine may increase likelihood of HAND via several mechanisms including facilitating neuroinflammation and reducing medication adherence. In international settings substance abuse, coinfections, and immune reconstitution syndrome may all play a role in persistence of HAND despite eARV.

S.18

Abnormal protein aggregation in the brains of long-term surviving HIV patients in the HAART era

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Background: Human immunodeficiency virus (HIV) positive patients under highly active anti-retroviral therapy (HAART) regimens show a markedly im-

proved general clinical status but the long-term impact on the brain of the antiviral therapies (e.g. hyperinsulinemia) has not been assessed. We hypothesize that increased longevity with chronic HIV infection and HAART may increase the risk of developing brain pathologies consistent with “early aging.” Markers of diseases in these patients may be abnormal axonal protein aggregates as a result of chronic neuronal stress. Our original autopsy studies showed both beta-amyloid [1] and Tau deposition in the brains of patients who died with HIV.

Methods: We used a positron emission tomography (PET) molecular imaging probe, FDDNP, to detect the distribution of amyloid and tau deposition in the brains of HIV patients with minor cognitive impairment (MCI), all males, age range: 40–65 y.o. The results were compared to aged matched controls and AD patients from ongoing studies at the UCLA using the same ligand. The study of FDDNP scans was performed for frames between 15 and 85 minutes using Logan graphical analysis with cerebellum as the reference region. The resulting distribution volume ratios (DVR) were used to generate DVR parametric images.

We performed immunohistochemistry (IHC) for amyloid, Tau and a-synuclein on autopsy brain tissues from 35 HIV infected patients who have died on HAART. The regions studied included neocortex, subcortical white matter and basal ganglia. Light microscopy analysis of single label staining regional distribution was complemented by double immunofluorescent stainings to determine the subcellular distribution of protein aggregates and association with markers of neuronal degeneration and glial activation.

Results: FDDNP-PET (targeting both b-Am and Tau aggregates) shows increased deposition in the brains of HIV patients with MCI (n = 3) compared to controls. The distribution of the FDDNP PET signal is quite different from that found in Alzheimer’s disease patients imaged with the same ligand [2]. All three HIV subjects had increased binding in the posterior cingulate gyrus, and in the (parieto-temporal) Brodmann areas 7, 40, 39 and 22.

From the expanded autopsy studies we now have evidence that intra-neuronal deposition of b-AM (34 positive cases out of 35 studied), Tau and a-synuclein in the HIV brain is abundant and the distribution follows the axonal tracts. Ultrastructural analysis shows that

intracellular b-AM is often found in structures consistent with autophagosomes.

Conclusions: FDDNP-PET detects early signs of brain degeneration in aging HIV infected patients on HAART. Furthermore, our recent autopsy studies suggest that the distribution of abnormal protein aggregates associates with disruption of the axonal homeostasis like accumulation of hyperphosphorylated Tau, and increased expression of brain immunophilins. Since the incidence of minor cognitive impairment in HIV patients is on the rise, studies of abnormal intra-neuronal protein aggregation may help in monitoring a key mechanism of brain disease and design neuroprotective strategies.

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2. Small, G.W., et al., PET of brain amyloid and tau in mild cognitive impairment. *N Engl J Med*, 2006. 355(25): p. 2652–63.

S.19

Opiates exacerbate HIV-1 neurotoxicity via DARPP-32 regulation of NMDA receptors

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Several pathogenic mechanisms underlie the exacerbation of HIV encephalopathy (HIVE) observed with opiate abuse. HIVE is initiated by neurotoxins released from activated cells within the milieu of the HIV-1 infected CNS and is mediated by activation of N-methyl-D-aspartate (NMDA), NR1 and NR2 receptors. Regions of the brain that are enriched in NMDA receptor subunits are likely to be more vulnerable to neurotoxins. Opiate induced dopamine-receptor stimulation leads to induction of transcription factors and phosphorylation of key signaling molecules such as DARPP-32 causing neurobiological changes. The DARPP-32 signaling pathway is important in the modulation of NMDA subunits by the dopamine D1 receptor. NMDA receptors also play a significant role in neurotoxicity induced by HIV-1 proteins. Thus, we hypothesize that opiate abuse by HIV-1 infected subjects may exacerbate the progression of HIVE as a consequence of the combined effects of HIV-1 induced neurotoxins plus opiate induced increases in the D1 receptor activation. Brain tissue samples taken at autopsy from the frontal cortex regions were obtained from the National NeuroAIDS Tissue Consortium (NNTC) from opiate abus-

ing HIV-1 patients with and without HIVE. Clinicopathological findings provided by NNTC show that HIVE+ subjects had marked gliosis and loss of cerebellar Purkinje cells, while the HIVE– subjects did not exhibit such pathology. Our specific aim was to compare the differential gene and protein expression of NMDA receptors (NR1, NR2A and NR2B), the D1 receptor, and DARPP-32 in human brain tissue samples from both opiate using HIVE+ and HIVE– subjects by real time, quantitative PCR and western blot analyses. Our results show the following. 1) A significant increase in the gene expression of DARPP-32 (45%, $p < 0.03$), D1 receptor (39%, $p < 0.05$), and NR1 (55%, $p < 0.01$) and NR2B (28%, $p < 0.05$) was observed in brain tissue of HIVE+ as compared to HIVE– subjects. 2) No significant difference was observed in NR2A gene expression in brain tissue of HIVE+ as compared to HIVE– subjects. 3) A significant increase in protein expression of DARPP-32 (52%, $p < 0.05$), D1 receptor (44%, $p < 0.05$) and NR1 (63%, $p < 0.05$) was observed in brain tissue of HIVE+ subjects as compared to the HIVE– subjects. Our results indicate that opiate-induced DARPP-32 activation may contribute to an increase in the expression of NMDA receptor subtypes thus facilitating HIV-induced neurotoxicity. Identification of the signaling mechanisms that modulate the activity of the NMDA receptors in the brain may be translated into the development of newer, more effective therapies for the prevention and treatment of HIVE, which continues to manifest significant morbidity and mortality among HIV-1 infected patients especially those who are addicted to opiates.

S.20

Nonhuman primate models of drug abuse and AIDS

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Determination of the mechanisms by which chronic exposure to drugs of abuse may alter the progression of HIV/AIDS requires animal models. The rhesus monkey in particular offers well established SIV models and has been employed in numerous studies of abused drugs. Nevertheless evaluation of the combined effects of immunodeficiency viruses and specific drugs of abuse requires close consideration of the criteria which should be used to select models of drug abuse. Consideration of routes of administration, dose and exposure schedules for individual recreational drugs may be critical to establishing relevant risks of co-exposure with immunodeficiency virus infection. Recent studies on the impact of chronic exposure to alcohol and methamphetamine, respectively, on the progression of SIV infection in rhesus monkeys will be discussed.

Investigators in Training I

S.21

Activation of the cell cycle protein CDC2 may cause calcium dysregulation in postmitotic neurons contributing to HIV-induced excitotoxicity and neuronal cell death

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The chemokine receptor CXCR4 is involved in HIV infection and contributes to HIV neuropathology in a direct and indirect manner. It has been suggested that abnormal activation of CXCR4 alters neuronal/glial signaling causing neuronal damage, whereas proper CXCR4 signaling promotes neuronal survival. Previous work from our group showed that dysregulation of cell cycle proteins, namely the CDK/Rb/E2F pathway, is involved in the neurotoxicity induced by X4-using HIVgp120s; we also reported that E2F1 and its transcriptional target *cdc2* (also known as *cdk1*), are up-regulated in the brain of HIV/HAD patients. This study aims to establish the role of *cdc2* in gp120-induced neuronal injury and determine the molecular mechanisms involved in neurotoxicity. We report that expression of a Cdc2 dominant negative mutant in rat cortical neurons rescues them from HIVgp120-induced apoptosis. Also, down-regulation of *cdc2* expression by shRNA in human cell lines reduced phosphorylation of apoptotic proteins downstream *cdc2*. Furthermore, as recent evidence suggests that Cdc2 is implicated in the regulation of intracellular Ca²⁺ homeostasis via phosphorylation of IP₃ receptors, we are currently testing the hypothesis that *cdc2* may also lead to aberrant Ca²⁺ signaling in neurons, by using calcium imaging and IP₃-uncaging. Alterations of Ca²⁺ homeostasis by gp120 and other HIV-1 proteins are well-documented, and known to affect neurotransmission and neuronal survival, which lead to HIV-associated neuronal deficits. These studies show the importance of *cdc2* in gp120-induced neurotoxicity and suggest that therapeutic approaches aimed at preventing stimulation of *cdc2* or its targets in neurons may turn useful in HIV neuropathology. (Supported by NIH grants DA 19808 and 15014 to OM).

S.22

T-cell infiltrates in HSV-1 latently infected human geniculate ganglia

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In humans, a primary infection with Herpes simplex virus type-1 (HSV-1) is followed by a lifelong viral persistence in the sensory neurons of the cranial nerve ganglia, especially in the trigeminal ganglia (TG), and less frequently in the geniculate (GG) and vestibular ganglia (VG). In some individuals HSV-1 reactivates repeatedly from the TG and causes 'herpes labialis'. In contrast, reactivation from the GG and VG is rather a once-in-a-lifetime event.

Immunohistochemical and quantitative RT-PCR studies in the TG have revealed that HSV-1 latency is accompanied by an active chronic immune response composed of CD8⁺ T-cells showing an effector memory phenotype. Although the infiltrating T-cells reflect an active immune response, it is not clear whether the T-cells control viral latency and reactivation or whether they represent an inflammatory residue after the frequent silent or overt reactivations in the TG.

In the present study, using immunohistochemistry, we found that T-cells are also present in the GG where the latency-associated transcript (LAT) was detectable by in situ hybridization. In the VG no T-cells could be found and LAT was detectable only after amplification by RT-PCR. Hence we suppose that the T-cell infiltration in the cranial nerve ganglia monitors HSV-1 latency and does not only represent an inflammatory sequel of a previous reactivation.

S.23

HIV-1 and cytokine (IFN- γ /TNF- α) synergy in CXCL10 release from astrocytes: implications in HIV-associated dementia

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There are 40 million people world wide infected with Human Immunodeficiency Virus-1 (HIV-1). Shortly after infection, HIV-1 penetrates the brain and can

eventually cause HIV-1 associated CNS disease, of which HIV-associated dementia (HAD) is the most severe manifestation. HIV encephalitis (HIVE), the pathologic correlate of HAD is characterized by astrogliosis, cytokine/chemokine dysregulation, and neuronal degeneration. The severity of HAD/HIVE correlates better with the presence of activated astroglial and microglial cells rather than with the presence and amount of HIV-1 infected cells in the brain. Also, correlated with the pathogenesis of HAD/HIVE are the increased levels of the proinflammatory cytokines, IFN- γ and TNF- α . These two cytokines are released by activated immune cells and have the ability to synergize with other cellular factors and HIV-1 viral proteins resulting in increased inflammation and a more severe disease state. Astrocytes, the most populous cell type within the brain, are activated not only by IFN- γ and TNF- α , but also by HIV-1. Once activated, astrocytes provide an important reservoir for the generation of inflammatory mediators including CXCL10, a neurotoxin and chemoattractant. CXCL10 is implicated in the pathophysiology of HAD since high levels of CXCL10 are also correlated with disease severity. The underlying central hypothesis of these studies is that the interplay of viral and cellular factors in astrocytes can result in the synergistic induction of CXCL10 expression resulting in neuronal degeneration. Our preliminary studies suggest a synergistic induction of CXCL10 protein in astrocytes exposed to HIV-1, IFN- γ and TNF- α . We observed that the HIV-1 proteins nef and tat were the key determinants involved in synergy with IFN- γ and TNF- α . Transcriptional and translational regulatory mechanisms involved in the synergistic enhancement of CXCL10 will be discussed.

This could have possible implications for enhanced CNS disease because of the neurotoxic functions of CXCL10 and its ability to enhance viral replication. Thus understanding the cellular and molecular mechanisms involved in the induction of proinflammatory responses by astrocytes is critical to the development of interventional therapies for the treatment of HAD.

S.24

The role of JC virus minor capsid proteins in the viral lifecycle

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JC virus (JCV) is a human polyomavirus for which 70% of the human population is seropositive. It is responsible for the fatal demyelinating disease Progressive Multifocal Leukoencephalopathy (PML). JCV contains an icosahedral capsid that consists of 72 pentamers of the major capsid protein Vp1 with a minor coat protein Vp2 or Vp3 in the center of the pentamer. It is known that Vp1 contributes to virus tropism through receptor interactions. However, little is known about the role the minor coat proteins in pathogenesis. Using site-directed mutagenesis we show that both Vp2, the

myristylation site on Vp2, and Vp3 are necessary for the correct packaging of viral DNA. We suspect that these proteins also play critical roles early in infection and have engineered tags in Vp2 and Vp3 to facilitate their detection in infected cells. The tagged viruses are viable and Vp2 and Vp3 localize to subcompartments within the nucleus. Co-localization studies with Vp1 show that Vp1 is uniformly distributed in the nucleus and Vp2 and Vp3 are excluded from the peripheral margins of the nucleus. We are currently using the tagged virions to study the trafficking of virus from the plasma membrane to the nucleus with the goal of understanding when and where the minor capsid proteins become exposed in the cell.

S.25

Transmigration of HIV infected monocytes/macrophages and lymphocytes into the central nervous system

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Human immunodeficiency virus 1 (HIV-1) invades the brain and results in neurologic dysfunction in a large percentage of individuals. Even with adequate suppression of systemic virus due to therapy, central nervous system (CNS) disease continues to evolve. Both the penetration of HIV into the brain and the subsequent damage are thought to be mediated by transmigration of infected monocytes across the blood brain barrier (BBB). The number of activated macrophages in the CNS appears to be a more reliable predictor of HIV associated cognitive impairment than viral load, suggesting that monocyte infiltration and CNS damage are tightly correlated. Monocyte/macrophage trafficking is of interest in studies of the pathogenesis of HIV infection because of their ability to cross the BBB, to elaborate factors that are harmful to the CNS, and to establish viral reservoirs. Monocytes may circulate in the peripheral blood for 1-3 days before entering and differentiating into macrophages in the brain. It is important to determine the phenotypic markers and the stage of maturation of monocytes as they enter the CNS, with the ultimate goal of therapeutic intervention to limit CNS infection and inflammation associated with NeuroAIDS. We first designed experiments to establish the optimal culture system for human peripheral blood mononuclear cells (PBMC) that promote monocyte/macrophage survival and examined the expression of specific phenotypic markers as cells mature in culture. Flow cytometry was used to determine monocyte/macrophage maturation using antibodies to CD14, CD71, CD68 and CD16. We found Macrophage Colony Stimulating Factor (MCSF) supports the survival of CD14+ isolated monocytes and their expression of maturation markers under non-adherent

conditions. However, when cultured as PBMC, the expression of CD14 and CD68 decreases over time when cultured with MCSF. In addition, HIV infection of PBMC does not affect the expression of monocyte markers. This knowledge was then used to characterize the transmigration of HIV infected monocytes and T cells across our in vitro model of the BBB using FACS analysis and confocal microscopy. Lastly, we examined the route, transcellular or paracellular, by which HIV infected leukocytes transmigrate, using various microscopic techniques. Preliminary data suggest that HIV infected cells transmigrate by both mechanisms. Data from these studies contribute to our understanding of how HIV infected monocytes/macrophages and lymphocytes transmigrate across and disrupt the BBB, rendering the CNS vulnerable to virus mediated inflammatory damage that leads to cognitive impairment.

S.26

Signal transduction mechanisms of HIV-1 gp120-induced proinflammatory cytokine interleukin-1 beta (IL-1b) production by primary human macrophages

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HIV-associated dementia (HAD) is a common neurological complication of AIDS that involves activation of macrophage/microglial cells in the brain, with subsequent release of toxins including proinflammatory cytokines that lead to neuronal death. In addition to direct infection, uninfected bystander macrophages can be activated by viral proteins such as the Env glycoprotein gp120 to induce cytokine production. Interleukin-1 beta (IL-1b) is one of the proinflammatory cytokines upregulated in brain, CSF and blood of HAD patients. Although gp120 has been reported to induce IL-1b production by macrophages in vitro, the signal transduction pathways responsible have not been defined. In this study, we set out to elucidate the mechanisms me-

diating gp120-induced IL-1b release by primary human monocyte-derived macrophage (MDM). Recombinant gp120 from CCR5-using (R5) HIV-1 stimulated IL-1b production by MDM as measured by ELISA in both time- and concentration-dependent manner. The gp120-induced IL-1b secretion was blocked by a CCR5 antagonist (M657) and inhibited by pertussis toxin, suggesting it is mediated by binding to CCR5 and coupling to Gi/o protein. Env-mediated IL-1b production was abrogated by upstream inhibitors of Pyk2 (AG17 & dantrolene) and direct inhibitors of PI3K (wortmannin & LY294002), as well as the src kinase inhibitor PP2 and a pseudosubstrate peptide inhibitor KRX123.302 specific for the src family kinase Lyn. We further demonstrated that exposure of MDM to gp120 activated Pyk2, PI3K & Lyn in a time-dependent fashion with direct (Pyk2 or Lyn phospho-specific immunoblot) or indirect (PI3K-dependent Akt activation) approaches. IL-1b release triggered by gp120 was also impeded by inhibitors of the MAP kinase ERK (U0126 & PD98059) and gp120 induced phosphorylation of ERK in a time-dependent manner, implicating MAP kinases in the HIV-1 envelope-mediated cytokine production. We then demonstrated via both coimmunoprecipitation and immunocytochemistry that gp120 triggered an activation-induced physical association between Pyk2, PI3K & Lyn, involving translocation of cytoplasmic Pyk2 and PI3K to membrane-bound Lyn. Finally, we demonstrated by immunofluorescent labeling and confocal microscopy that native gp120 on the surface of HIV-1 virions also induced colocalization of Pyk2, PI3K & Lyn in a CCR5-dependent fashion. Our results indicate that HIV-1 gp120 induces IL-1b release by macrophages through CCR5 coupled to Gi/o protein, subsequently activates multiple protein kinases including Pyk2, PI3K, Lyn and induces formation of a multimeric signaling complex and downstream MAP kinase activation. Defining the signaling pathways responsible for gp120-mediated proinflammatory cytokine production by macrophages could help understand the neuropathogenesis of HAD and contribute to the development of pharmacological agent that attenuate the progression of HAD by specifically blocking these signaling pathways.

Session 4: Antiviral therapy: emerging modalities and CNS implications

S.27

In search of antivirals for the treatment of neurovirus infections

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Numerous viruses have been associated with various neurological disorders; particularly, among the DNA viruses: polyomavirus [progressive multifocal leukoencephalopathy (PML)], herpes simplex virus (HSV) (herpetic encephalitis), human herpesvirus type 6 (HHV-6) (several CNS disorders); and, among the RNA viruses: enteroviruses (polio, Coxsackie and ECHO) poliomyelitis, meningitis, encephalomyelitis, alpha- and flaviviruses (encephalitis), rhabdoviruses (rabies), some arena- and bunyaviruses, and human immunodeficiency virus (HIV) (AIDS dementia). Which are the compounds that inhibit the replication of these viruses, and could eventually be useful in the treatment of the neurological disorders associated with these viruses? These are: (i) for polyomavirus, acyclic nucleoside phosphonates (such as cidofovir and derivatives thereof); (ii) for HSV, acyclic guanosine analogues (such as acyclovir, ganciclovir and penciclovir), and, putatively, helicase-primase complex inhibitors, (iii) for HHV-6, a variety of both acyclic nucleoside phosphonates and non-nucleoside types of compounds (i.e. CMV423); (iv) for polio- and other enteroviruses, a number of capsid binders as well as protease, polymerase, 3A and 2C inhibitors; (v) for flaviviruses, interferons and interferon inducers (such as amplitgen); (vi) for (-)RNA viruses, IMP dehydrogenase inhibitors (such as ribavirin) and SAH hydrolase inhibitors (such as 3-deazaneplanocin A); (vii) for arena- and bunyaviruses, the pyrazine derivative T-705 (which has also been known for its anti-influenza virus activity); and (viii) for HIV, the now more than twenty compounds which have been formally approved for clinical use and could be administered in various drug combination regimens.

S.28

Efficacy of antiretroviral therapy in the brain

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Epidemiologic evidence provides the best support for HAART's beneficial impact on primary HIV CNS disease. New cases of severe dementia have become rare

where HAART is widely available. At the same time, epidemiological evidence also argues for limitations in HAART's ability to fully restore brain function: There continue to be large numbers of individuals with milder degrees of neurocognitive impairment. Perspectives on the efficacy of HAART in treating primary HIV CNS disease are quite dependent upon an investigators model of the disease. For example, if one believes that much of the burden of neurocognitive impairment reflects irreversible injury, or if comorbidities are felt to explain the bulk of residual neurocognitive impairment, then the question is moot: Any residual neurocognitive impairment will not be impacted by antiretroviral therapy. However, setting these dogmas aside, the question remains whether HAART beneficially effects neurocognitive abilities to an extent that impacts on life quality, and whether different HAART regimens vary in their CNS effectiveness. Based on an analysis of an overarching model of HIV neuropathogenesis, we believe that research efforts should be focused on several areas. First, the field would benefit from the development of dynamic surrogate markers of neural injury and repair that would allow more rapid readout of therapeutic benefit than available measures such as neuropsychological test performance. Such surrogate measures would require validation against neurocognitive performance and real-world functioning. Second, research would be hastened by a reliable way to ascertain across individuals whether CSF is a better or poorer surrogate for brain tissue viral replication. Third, progress would be enhanced by the development of accepted metrics for comparing the relative CNS penetration of different HAART regimens.

S.29

Serotonin receptor 2A blocker (risperidone) has no effect on human polyomavirus JC infection of primary human fetal glial cells

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Background: No proven treatment is available for progressive multifocal leukoencephalopathy (PML), a

uniformly fatal, subacute demyelinating disease of the central nervous system (CNS) caused by human polyomavirus JC (JCV). A recent report demonstrated that JCV employs serotonin receptor 2A (5HT2AR) to infect the glial cells and suggested that 5HT2AR blockers may be useful in treating PML patients.

Objective: To assess the ability of a potent 5HT2AR blocker risperidone to inhibit JCV replication in primary human fetal glial (PHFG) cells.

Study design: PHFG cells grown in-vitro were treated with 0, 12.5, 50 or 200 ng/mL of risperidone and inoculated with JCV(Mad1) in the continuous presence of the drug. Toxicity of risperidone to the PHFG cells proliferation was determined by the CellTiter 96[®] Aqueous One Solution Cell Proliferation Assay, and JCV replication in treatment-naïve and risperidone-treated PHFG cells, was quantitated by real-time PCR, real-time RT-PCR and western blot.

Results: Risperidone up to 200 ng/mL for up to 15 days did not affect the PHFG cells viability and proliferation. There was no significant difference in JCV genome copies or mRNA transcripts and protein expression in treatment-naïve and risperidone-treated PHFG cells.

Conclusions: Our study suggests that risperidone, a potent 5HT2AR blocker does not inhibit JCV(Mad1) attachment, internalization and replication in PHFG cells and 5HT2AR blockers may not be effective in treating PML.

S.30

Detrimental contribution of the immuno-inhibitor B7-H1 to rabies virus encephalitis

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Rabies virus is the etiological agent of an acute encephalitis, which in absence of post exposure treatment is fatal in almost all cases. Virus lethality rests on its ability to evade the immune response. Here, we analyzed the role of the immuno-inhibitory molecule B7-H1 in this virus strategy. We showed that in the brain and spinal cord of mice, rabies virus infection resulted in significant up-regulation of B7-H1 expression, which is specifically expressed in infected neu-

rons. Correlatively, clinical rabies in B7-H1^{-/-} mice is markedly less severe than in wild type mice. B7-H1^{-/-} mice display resistance to rabies. Virus invasion is reduced and the level of migratory CD8 T cells increases into the nervous system, while CD4/CD8 ratio remains unchanged in the periphery. In vivo, neuronal B7-H1 expression is critically depending on TLR3 signalling and IFN-type 1 since TLR3^{-/-} mice—in which IFN-beta production is reduced—showed only a limited increase of B7-H1 transcripts after infection. These data provide evidence that neurons can express the co-inhibitory molecule B7-H1 after viral stress or exposure to a particular cytokine environment. They show that the B7-H1/PD-1 pathway can be exploited locally and in an organ specific manner—here the nervous system—by a neurotropic virus to promote successful host invasion.

S.31

Paroxetine treatment for HIV-mediated neurodegeneration

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The prevalence of both of HIV sensory distal neuropathy and HIV-Dementia has been increasing as effective antiretroviral therapy allows patients to live longer. These complications are a result of oxidative stress and neurotoxicity that results directly from HIV infection and from the exposure of CNS neurons to toxic viral proteins, such as Tat and gp120. These toxicities are complicated and exacerbated by aging and prevalent drug abuse in this patient population. We have mimicked the co-morbid effects of drugs of abuse on neurotoxic HIV-1 Tat and gp120 in in vitro assays by combining cocaine and/or morphine with Tat and gp120. Thus, identification and development of therapeutic strategies to protect against this neuronal damage and the ensuing clinical deficits is a major unmet medical need. We have screened more than two thousand compounds that included FDA approved compounds and natural products for protective efficacy against oxidative stress-mediated neurodegeneration and identified selective serotonin reuptake inhibitors (SSRIs) as potential neuroprotectants. Numerous SSRIs were then extensively evaluated as protectants against neurotoxicity as measured by changes in mitochondrial potential, neuronal cell death and induction of nitric oxide synthase elicited by HIV Tat and gp120 in primary rat mixed hippocampal cultures in the presence and absence of cocaine and/or morphine. While many of the SSRIs demonstrate neuroprotective actions, we find that paroxetine is potentially neuroprotective in this cell culture model, but may require concentrations greater than those required for inhibition of

serotonin transporters. Treatment of mixed hippocampal and human astroglial cells with Tat or Tat + Cocaine resulted in significant increases in numerous cytokines when evaluated by multiplexed protein array analysis of immune-related proteins (e.g. cytokines and chemokines). Some of the effects of Tat and drugs

of abuse on these these cytokine were reversed by paroxetine treatment. Therefore, neuroprotective compounds such as SSRIs, by acting on both neurons and astroglial cells, may provide an adjunctive therapy to treat HIV patients with HIV neuropathy and dementia.

Session 5: Emerging concepts on viral effects on the nervous system: virus as discovery tool

S.32

Pathogen surveillance and discovery in acute and chronic disease

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The roles of heredity and environment as disease determinants have been debated for millennia. The emphasis on one or the other has largely been driven by technology. Despite the periodic appearance of new pathogens, we have likely collected much of the “low hanging fruit” (microbes readily associated with diseases). The challenge now is to understand those disorders that reflect the interaction of environmental factors (microbes, toxins, other stressors) with susceptibility genes. The objectives of our research program are to investigate the role of infectious agents in the pathogenesis of acute and chronic diseases; dissect the mechanisms by which microbes and host responses result in damage and dysfunction; and find strategies to reduce the burden of morbidity and mortality due to infectious diseases. I will discuss a staged strategy for diagnosis of infection and pathogen discovery, efforts in zoonotic surveillance, and future perspectives based in prospective birth cohorts.

S.33

Activating the maternal immune system causes changes in the offspring resembling those in Schizophrenia and autism

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Maternal viral infection is associated with increased risk of schizophrenia and autism in the offspring. In a mouse model based on this risk factor, we found that respiratory infection with influenza virus leads to behavioral abnormalities in the adult offspring. These behaviors are consistent with abnormalities seen in schizophrenia and autism, including enhanced anxiety in the open field (OF), as well as deficits in social interaction (SI), latent inhibition (LI) and prepulse inhibition (PPI). The latter is corrected by anti-psychotic and exacerbated by psychomimetic drugs. The adult offspring display neuropathology in the cortex and hippocampus similar to that found in schizophrenia and a localized deficit in Purkinje cells that resembles that in autism.

The cause of these various abnormalities is the maternal response to viral infection, as treatment of uninfected, pregnant mice with the dsRNA, poly(I:C), which evokes an anti-viral-like immune response, also induces OF, SI, PPI and LI deficits and the Purkinje cell deficit in the offspring.

Exploring potential mediators of these effects revealed that injection of the cytokine IL-6 in normal pregnant mice causes PPI and LI deficits in the offspring, while co-injection of anti-IL-6 antibody with poly(I:C) in pregnant mice blocks the effects of maternal immune activation on the behavior of the offspring. Moreover, the offspring of poly(I:C)-treated IL-6 knockout mice do not display behavioral abnormalities. Furthermore, maternal anti-IL-6 blocks the changes in gene expression in the brains of adult offspring that are caused by maternal poly(I:C) treatment. Therefore, IL-6 is a key mediator of the effects of the activated maternal immune response on fetal brain development.

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S.34

Inhibition of the IFN- α/β response by mouse hepatitis virus (MHV) at multiple levels

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Murine coronavirus, mouse hepatitis virus (MHV) infection of the mouse causes acute encephalitis followed by chronic demyelinating disease and is used as a model for Multiple Sclerosis. We have been studying the interaction of MHV with the interferon(IFN)- α/β response. MHV strain A59 induces IFN- β gene transcription and low levels of nuclear translocation of the IFN- β transcription factor IRF-3, albeit late in infection of L2 mouse fibroblast cells. However, MHV does not induce IFN- β protein production during the course of infection in this cell type. In addition, MHV is able to significantly decrease the level of IFN- β protein induced by both Newcastle disease virus (NDV) and Sendai virus (SeV) infections, without targeting it for proteasomal degradation, altering the nuclear translocation of IRF-3 or decreasing IFN- β mRNA production or stability. Thus, MHV infection causes an

inhibition of IFN- β production at a post-transcriptional level, without altering RNA or protein stability. In contrast, MHV induces IFN- β mRNA and protein production in the brains of infected animals, suggesting that the inhibitory mechanisms observed in vitro are not enough to prevent IFN- β production in vivo. Furthermore, MHV replication is highly resistant to IFN- α/β action, as indicated by unimpaired MHV replication in L2 cells pretreated with IFN- β . However, when L2 cells are co-infected with MHV and NDV in the presence of IFN- β , NDV, but not MHV, replication is inhibited. Thus, rather than disarming the antiviral activity induced by IFN- β pretreatment completely, MHV may be inherently resistant to some aspects of the antiviral state induced by IFN- β . These findings show that MHV employs unique strategies to circumvent the IFN- α/β response at multiple steps; it both avoids induction of IFN- α/β and is resistant to the downstream effects of IFN induction. This is one mechanism by which MHV evades the host immune response.

S.35

Design of ICP0 deficient HSV oncolytic vectors for treatment of glioblastoma

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Oncolytic HSV vectors deleted for γ 34.5 and ICP6 are under evaluation in early phase trials for treatment of recurrent malignant glioblastoma. γ 34.5 encodes a virulence factor that interferes with the double stranded RNA activated protein kinase (PKR) pathway by inducing the inactivation of eIF2 α , an inhibitor of late viral protein synthesis. ICP6 encodes the large subunit of the viral ribonucleotide reductase, a product that enhances viral DNA synthesis in non-dividing cells. While this vector backbone has proven safe in both animal models and patients without evidence of encephalitis or serious adverse events, few complete responses have been observed. Impediments to effective therapy include inefficient vector replication in the tumor environment, a consequence of vector backbone design and innate immune responses to infection. Thus we have carried out experiments to (i) examine novel vector mutant backgrounds that show enhanced viral oncolytic activity relative to vectors commonly used in clinical trials and (ii) to study the role of innate immune responses in limiting vector growth in tumors. We are exploring the suitability of a new class of oncolytic vectors based on deletion of the immediate early gene ICP0 alone or in combination with other non-essential genes. Several of these vectors have shown a remarkable ability to replicate and destroy a variety of brain tumor cell lines and their

replication efficiency was 100 times more robust than the oncolytic vectors based on g34.5 and ICP6 deletions. These mutants also counteracted two innate immune response pathways, namely PKR and the IFN- α -inducible indoleamine-2,3-dioxygenase (IDO), that together inhibit the production of viral proteins. Experiments are underway to test the safety profiles of these vectors following intracranial injection of mice and for their ability to treat animal models of human glioblastoma.

S.36

Activation of microglia by a neurotropic RNA Borna Disease Virus is initiated by neuron-astrocyte interactions, leading to neurodegeneration

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Neonatal Borna disease virus (BDV) is a neurotropic negative-strand RNA virus that establishes persistent infection in the rat brain associated with microglial activation and damage to the certain neuronal populations. Since persistent BDV infection of neurons in vitro is non-cytolytic, activated microglia may be responsible for neuronal cell death in vivo. However, the mechanisms whereby microglia are activated and produce neuronal damage remain unknown. We found that neither purified virus nor BDV-infected neurons alone were able to activate microglia in vitro. In contrast, microglia were activated in organotypic BDV-infected mixed neuron-astrocyte-microglia cultures, suggesting an importance of interactions between infected neurons, astrocytes and microglia for neuroinflammation to occur. Co-culturing infected and uninfected primary neurons and glia cells in various combinations confirmed this suggestion by demonstrating that conditioned media from BDV-infected neurons activated uninfected astrocytes, which, in turn, were able to activate microglia. Remarkably, microglia activation was observed in the absence of overt neuronal toxicity in culture and two weeks before any signs of neurodegeneration in vivo. Taken together, our findings provide the first evidence that activation of microglia initiated by interactions between persistently BDV-infected neurons and uninfected astrocytes may be a leading event in chronic neuroinflammation that results in neurodegeneration in selected neuronal groups.

S.37

Association of HSV and amyloid precursor protein during intraneuronal transport: a time-lapse live imaging study

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Herpes simplex virus Type-1 (HSV1) transport during egress must involve recruitment of cellular proteins. Previously, we reported that HSV1 is physically associated with large amounts of amyloid precursor protein (APP) (Satpute et al. *Aging Cell*, 2003), a major component of Alzheimer's plaques and a candidate vesicle-motor receptor. We have also demonstrated that APP is sufficient to hitch cargo to motors for anterograde transport (Satpute et al. *PNAS* 2006). Thus, we hypothesize that APP is the link that hitches HSV1 to cellular motors for transport during egress. Here, we use live imaging and immuno-staining to examine the process by which HSV1 particles become associated with cellular APP during viral egress. For immuno-localization, synchronized infections of Vero cells with either hRr3-HSV or VP26GFP-HSV (Desai and Person, *J. Virol.* 1998) were fixed at 1.5, 4, 6, 9, 16 and 23 hr post-infection and stained for cellular APP and viral proteins, gE, gD, and VP5. For live imaging of APP, Vero cells were transfected with APP-mRFP. Synchronization of viral infection by a new method resulted in very few viral particles remaining in transit

towards the nucleus at 4 hr post infection, demonstrating that at later time points the great majority of cytoplasmic viral particles are nascent virus. We find that 90% of these intracellular virus stain for all three viral glycoproteins with 83% of capsids co-localizing with APP during the peak time of egress, 6.5–9.5 h post-infection, in quadruple-labeled 3-D images captured by confocal or deconvolution microscopy. Even prior to the formation of capsid, viral glycoprotein gE co-localized with APP in the cytoplasm. By live confocal imaging at 7–10 hr post infection, APP-mRFP vesicles actively associate with VP26GFP-viral particle and then move towards the plasma membrane. Preliminary quantification showed that the maximum and average velocity of APP-virus vesicles were $2.7 \pm 0.8 \mu\text{m/s}$ and $1.53 \pm 0.4 \mu\text{m/s}$. Using a gE-null virus with decreased transport in neurons (Wang et al. *J. Virol.* 2005), we tested the role of gE in APP recruitment. APP did not co-localize with viral particles in cells infected with a gE-null virus while normal gD-capsid co-localization occurred, suggesting that the virus may recruit APP through interaction of gE with APP. Our results suggest that HSV1 egress involves cellular transport machinery mediated by APP, and that APP recruitment depends on gE. Supported by NINDS RO1 NS046810 and NIGMS RO1 GM47368.

Neurological infections lectureship

S.38

Advancements in the diagnosis and management of CNS bacterial, fungal and tuberculous infections

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A number of advancements have been made in the diagnosis and therapy of bacterial meningitis, including:

Until recently, PCR could only detect viral nucleic acid in cerebrospinal fluid. A PCR assay is now available to detect bacterial nucleic acid in cerebrospinal fluid.

Serum procalcitonin is being investigated to distinguish bacterial from viral meningitis and may be useful in association with CSF cell count and glucose concentration in making the decision to discontinue empiric therapy for meningitis.

Dexamethasone is now part of the recommended empiric therapy of bacterial meningitis in children and adults as clinical trials have demonstrated its efficacy in reducing neurological sequelae and mortality. Concerns about the penetration of vancomycin through noninflamed meninges have been addressed by the recommendations for increasing the parenteral dose and the use and safety of intraventricular vancomycin. *Listeria monocytogenes* is increasingly recognized as a meningeal pathogen in immunocompetent individuals, and as the etiological organism of brainstem encephalitis:

There have been two significant advancements in the diagnosis and therapy of fungal infections:

Serum and CSF galactomannan are helpful in the diagnosis of fungal meningitis. Many experts now recommend high dose oral fluconazole as initial therapy for *Coccidioides immitis* meningitis.

Tuberculous meningitis may present as either a subacute meningitis with symptoms evolving over weeks, or a fulminant meningitis with the rapid development of coma. The development of a *Mycobacterium tuberculosis* ribosomal RNA test that can be performed rapidly on CSF is a critical advancement in the diagnosis of tuberculous meningitis. There is increasing understanding that a mildly decreased CSF glucose concentration in association with a CSF lymphocytic pleocytosis is evidence of tuberculous meningitis. Patients with acute fulminant tuberculous meningitis may initially have a CSF polymorphonuclear pleocytosis. Empiric therapy for tuberculous meningitis is warranted in patients with symptoms of meningitis, a CSF pleocytosis of either polymorphonuclear leukocytes or lymphocytes and a mildly decreased (30–40 mg/dl) CSF glucose concentration.

All of the above will be discussed with an emphasis on how they have changed the management of the patient with suspected bacterial, tuberculous or fungal meningitis.

Breakout session: MS and molecular observations workshop

S.39

The human endogenous retrovirus envelope glycoprotein, Syncytin-1, regulates neuroinflammation and its receptor expression in multiple sclerosis: a role for ER chaperones in astrocytes

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Retroviral envelopes are pathogenic glycoproteins, which cause neuroinflammation, neurodegeneration and endoplasmic reticulum (ER) stress responses. The human endogenous retrovirus (HERV-W) envelope protein, Syncytin-1, is highly expressed in central nervous system (CNS) glia of individuals with multiple sclerosis (MS). Herein, we investigated the mechanisms by which Syncytin-1 mediated neuroimmune activation and oligodendrocytes damage. In brain tissue from individuals with MS, ASCT1, a receptor for Syncytin-1 and a neutral amino acid transporter, was selectively suppressed in astrocytes ($p < 0.05$). Syncytin-1 induced the expression of the ER stress sensor, OASIS, in cultured astrocytes, similar to findings in MS brains. Overexpression of OASIS in astrocytes increased iNOS expression but concurrently downregulated ASCT1 ($p < 0.01$). Treatment of astrocytes with a nitric oxide donor enhanced expression of Egr1, with an ensuing reduction in ASCT1 expression ($p < 0.05$). siRNA molecules targeting Syncytin-1 selectively downregulated its expression, preventing the suppression of ASCT1 and the release of oligodendrocyte cytotoxins by astrocytes. A Syncytin-1 transgenic mouse expressing Syncytin-1 under the GFAP promoter demonstrated neuroinflammation, ASCT1 suppression and diminished levels of myelin proteins in the corpus callosum, consistent with observations in CNS tissues from MS patients together with neurobehavioral abnormalities compared to wild type littermates ($p < 0.05$). Thus, Syncytin-1 initiated an OASIS-mediated suppression of ASCT1 in astrocytes through the induction of iNOS with ensuing oligodendrocyte injury. These studies provide new insights into the role of HERV-mediated neuroinflammation and its contribution to an autoimmune disease.

S.40

Infection of the CNS need not occur to get inflammatory changes within the brain

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Multiple sclerosis (MS) is an immune mediated disease of the central nervous system (CNS). Environmental factors have been implicated in the disease. Epidemiologic studies suggest that some event early in life in genetically susceptible individuals can either prime or protect from MS later in life. Similarly, since the 1940s different viruses have been isolated from individuals with MS with the claim that some of these viruses were the etiologic agent of MS. Some acute and persistent viral infections in animals can cause demyelinating disease leading some investigators to hypothesize that MS is due to an infection within the CNS. Here, the immune response to the virus infection within the CNS could lead to inflammation and demyelination. We have been studying whether infections outside of the CNS, in the periphery, could lead to inflammatory changes within the CNS. A peripheral infection of a genetically susceptible mouse strain (SJL/J) with a virus having molecular mimicry with a CNS protein induces an antiviral immune response. This immune response leads to the clearance of the virus. Subsequent peripheral infection with a totally different virus induces an immune response activating previously primed immune cells which enter the CNS causing inflammation and demyelination. These data suggest that infection of the CNS may not be required for immune mediated CNS demyelination to occur.

S.41

A double-blind European Multicenter Study Evidences envelope protein from Human Endogenous Retrovirus W (MSRV-ENV or Syncytin) in serum from 73% of MS patients without selective clinical inclusion criteria.

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8% of the human genome consists in human endogenous retrovirus (HERV), but relatively little is known about the function and pathological potential of these retrotransposable elements (representing over 45% of the human genome!). Such a retroviral element, named Multiple Sclerosis associated RetroVirus (MSRV), which further unraveled the HERV-W multi-copy family, was discovered in the 90's.

Several studies have shown a significant association or a pathogenic rationale for MSRV and its HERV-W genetic family in MS pathogenesis. The envelope protein (ENV-MSRV or Syncytin) was found to display pro-inflammatory activities both *in vitro*, in human mononuclear cells and *in vivo*, in a humanized severe immuno-deficient mice either with an hyperacute T-lymphocyte mediated lethal syndrome, either with an ENV-induced "MBP EAE" model (Cf. abstract on pre-clinical model). MSRV RNA from extracellular virion was significantly detected in association with MS in patient's cerebrospinal fluid (CSF) and plasma. Prognosis of MS evolution was evidenced by the retroviral load in CSF at disease onset in 3 and 6 years retrospective studies. Numerous confirmatory epidemiological and experimental data from independent groups using various techniques now provide strong evidence of association with MS. The pathogenic ENV Protein itself has been evidenced in post-mortem brain plaques by different groups as well, but not yet in living patients.

We have thus developed an immunoassay with specific monoclonal antibodies for the detection of HERV-W antigens in human blood. Our results from a multicenter study with externalised and blind testing of HERV-W ENV antigenaemia in blind-coded serum, detected 73% of positive cases in about 80 MS patients, but not in 36 healthy controls. These results are thus providing the last missing link between virion RNA detection in MS serum or CSF and the immunotoxic ENV protein encoded by HERV-W elements.

This ELISA immunoassay is now dedicated to patients' follow-up in an extension of this first multicenter study. It also represents a positive inclusion criterion of MS patients for targeted therapeutic approaches under development (Cf. Pre-clinical therapeutic abstract).

S.42

Multiple sclerosis (MS)-associated retrovirus (MSRV) is inhibited during interferon-beta-therapy of MS patients and behaves as progression biomarker

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We have studied MSRV in MS patients in various stages of the disease (1), in follow up evaluations (2–3), as well as in the conversion of optical neuritis to full blown MS (4), in comparison to healthy and pathological controls. In all cases, the data showed a striking parallelism between MS behaviour and MSRV presence and/or load in patient's blood and spinal fluid. We found also that MSRV/HERV-W is highly expressed in MS brains, and that its env protein accumulates particularly in the core of active lesions, in cells resembling astrocytes (5). Cultured peripheral blood mononuclear cells from MS patients and from MSRV(+) healthy individuals release free virus in culture fluids (6), and MSRV production is stimulated by cell treatment with cytokines shown to be detrimental in MS patients, such as TNFalpha, IL-6 and interferon (IFN)gamma, while IFNbeta, that is used in MS therapy, causes a dramatic reduction of MSRV yields.

Based on these premises, also to investigate whether detection of circulating MSRV can constitute a biomarker of MS progression and therapy efficacy, we performed a longitudinal study on MS patients with relapsing-remitting disease, during one year of therapy with IFNbeta, by clinical examination and detection of cell-free MSRV and viral load in the blood by MSRVenv-specific fully quantitative real time RT-PCR. Routine clinical examination and blood withdrawal were blindly performed at study entry and every three months.

Results showed that at study entry MSRV load in the blood was directly related to MS duration; IFN therapy determined a complete clearance of the virus from blood; one patient had strong progression with therapy failure, that was accompanied by total MSRV rescue.

It is unknown so far whether MSRV is a MS cofactor or epiphenomenon. Our findings suggest that evaluation of plasmatic MSRV can be considered the first prognostic marker for the individual patient, to monitor disease progression and therapy outcome. Work supported partly by grants from Fondazione Italiana Sclerosi Multipla Onlus (grant No 2003/R/21, 2005/B/2), Ministero Università e Ricerca PRIN 2005.

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Session 6: Stem cell biology, nanomedicine, and therapy

S.43

Neurogenesis in the setting of chronic viral infection

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The past decade has seen the acceptance of conceptual descriptions of the central nervous system (CNS) as dynamic rather than rigidly deterministic, both in response to injury and through adult life. Localized stores of multipotent neuroepithelial progenitor cells (NEP) proliferate and then differentiate in response to growth factors or cell surface contact influences, giving rise to specialized, lineage-committed precursor cells, which in turn give rise to both glia (astrocytes, oligodendrocytes) and neurons. While NEP are most abundant during fetal development of the CNS, self-renewing populations continue to exist in the adult mammalian CNS, found predominantly in the brain subventricular zone and hippocampal dentate gyrus. Endogenous neurogenesis originating with these populations may be an important contributor to cognitive and behavioral function. However, inflammation, precipitated by CNS injury or viral infection, may influence neurogenesis in a complex manner. Chronic inflammation can lead to defective proliferation of neural precursors as well as failure of neuronal lineage differentiation. Thus inflammation may impair neurogenesis. Pro-inflammatory cytokines may inhibit proliferation of NEP but may also actually enhance neurite outgrowth and neurite development under differentiation conditions.

HIV-1 infection in the CNS represents the type of chronic viral infection that is capable of impacting the proliferation or differentiation of NEP. In hippocampal tissue specimens from HIV-associated dementia patients, numbers of proliferating NEP were reduced by 75% when compared to patients without dementia. In vitro studies have demonstrated that exposure to HIV-1 envelope protein reversibly inhibits human NEP proliferation. However, HIV-1 exposure does not appear to inhibit neuronal fate determination or the proliferation of neuronal-committed precursors during differentiation of NEP. But neurons developing in the microenvironment of HIV-1 exposure “fail to thrive,” even without direct infection of neural cells and without neuronal apoptosis. HIV-1 exposure may impair maturation of neuronal precursors in that late or post-

mitotic antigens (NF-L, Hu) are up to 40% lower when virus exposure commences with the start of differentiation. Neuronal microtubule antigen β -III-tubulin is up to 50% lower when virus exposure occurs during the first five days of differentiation. Neurofilament proteins, particularly NF-L, may be especially sensitive to viral exposure. NF-L shows depressed levels with exposure to envelope gp120 from HIV-1(SF2) as well as the intact HIV-1(SF2) virus. NF-L may have a role as CSF biomarker for neuronal dysfunction in HIV infection if it can be correlated with cognitive impairment.

Pathophysiologic mechanisms underlying neuronal “failure to thrive” may include non-viral factors such as oxidative and/or nitrosative stress, genetic susceptibility to oxidative stress or inflammation as mediated by apolipoprotein E, and alteration of cell responsiveness to neurotrophins due to factors secreted by immune-activated cells in the microenvironment of chronic viral infection. These indirect effects of HIV infection may impact endogenous neurogenesis and neuronal survival. As such, HIV-1 infection presents both clinical and biological parallels to other brain injury states such as trauma, radiation, or ischemia.

S.44

Lineage directed differentiation of human brain derived progenitor cells alters JC Virus and HIV-1 fate of infection: Implications of neuropathogenesis

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We have identified, isolated, and continually propagated neural progenitor cells from the developing human brain. These cells, NPCs, can be directed to multiple lineage pathways including neurons, astrocytes and oligodendrocytes by introduction of different factors into the cell culture medium. Treatment of NPCs with either the human polyomavirus, JCV, or HIV-1, resulted in only a small fraction of these cells showing infection. However, either at the time of delivery of virus or after 4 or more days after virus treatment, if the cells were directed to an astrocyte lineage, there was a substantial increase of virus synthesis indicating differentiation induced activation of either JCV or HIV-1. Differentiation toward a neuronal lineage reduced viral synthesis quickly until the infection was

eliminated. For JCV infection, molecular factors that are responsible for successful infection were altered during the course of lineage directed infection. In brain tissue of pediatric cases of AIDS encephalitis, HIV-1 infection was identified in nestin positive cells in the subventricular zone and in the hippocampus. These viruses may target neural progenitor cells in the brain contributing to the pathogenesis of neurodegeneration.

S.45

HIV persistence in neural progenitor populations

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HIV can reside in the brain for many years. While astrocytes are known to tolerate long-term HIV infection, the potential of HIV to persist in other neural cell types is unclear. Recent studies demonstrate HIV invasion of cultured fetal brain-derived neural progenitor cells and occurrence of HIV markers in nestin-positive cells in pediatric brain tissues. Here we investigated whether HIV can establish long-term infection in human neural progenitor populations.

As experimental system we used the human neural stem cell line HNSC.100, cultured either as self-renewing neural progenitor populations or under conditions known to induce differentiation to astrocytes. Several features were identified that allow reliable functional and phenotypical distinction of HNSC progenitor and astrocyte populations.

HNSC-progenitor populations were exposed to HIV-1 and various parameters of HIV infection were monitored for over 100 days. Analysis of HIV proviral copy numbers by quantitative real-time PCR confirmed persistence of HIV proviral genomes for the entire observation period. HIV-infected progenitor cultures released moderate amounts of HIV for over 60 days, after which HIV release declined. However, expression of early HIV gene products was still apparent at later time points. Differentiation of HIV-infected progenitor populations to astrocytes was associated with transient activation of HIV production. Long-term HIV infection of progenitor populations upregulated expression of GFAP and led to measurable, albeit moderate, changes in cell morphologies.

Our studies support persistence of HIV in neural progenitor populations and indicate that HIV persistence has the capacity to alter cellular properties in neural progenitor populations.

S.46

The homeostatic pressure exerted by stem cells in degenerative or injured CNS environments

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Dr. Snyder has most recently been studying an intriguing phenomena with possible therapeutic dividends that has begun to emerge from his observations of the behavior of neural stem cell (NSC) clones in various mouse and primate models of CNS injury & degeneration. During phases of active neurodegeneration, factors seem to be transiently elaborated to which NSCs may respond by migrating (even long distances) to degenerating regions & attempting to restore homeostasis. This may include differentiating towards the replacement of degenerating neural cells of multiple types, not only neurons but also requisite non-neuronal “chaperone” cells, all of which are essential for the proper development and reconstitution of function. NSC’s are drawn to inflammatory niches. These “repair mechanism” may reflect the re-expression of basic developmental programs (particularly during temporal “windows” following injury) that may be harnessed for therapeutic ends. There is an enormous amount of “programmed” cross-talk between stem cells and the milieu that add complexity but also enrich therapeutic promise to the system. In addition, NSCs in their native state (as well as following genetic-engineering) may serve as vehicles for protein delivery allowing for the possibility of simultaneous cell replacement & gene therapy (e.g., with factors that might enhance differentiation, neurite outgrowth, connectivity, neuroprotection, anti-inflammation, anti-scarring, and angiogenesis).

Cell-cell contact with communication through gap junctions appears to represent another mode of cross-talk. Multi-model approaches to most neurological conditions are likely required. The stem cell may serve as the “glue” for these. When combined with certain synthetic biomaterials, NSCs may be even more effective in “engineering” the damaged CNS towards reconstitution. Not only gene expression programs, but also an epigenetic chromatin modification programs seem critical for dictating plasticity and potency. These chromatin structures appear to influence the expression of various stemness genes, including some novel zinc finger proteins that influence the unfolding of various developmental programs along the continuum from pluripotency (as ES cells) to multipotency (as somatic stem cells, e.g., NSCs) to cell type commitment (e.g., as neural cell types).

S.47

HIV-1-infected and/or immune-activated macrophages affect human fetal cortical neural progenitor cell proliferation and differentiation through TNF- α

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Neurogenesis relies upon the proliferation, migration and differentiation of neural progenitor/stem cells

(NPC). Changes in neurogenesis have been associated with multiple neurodegenerative disorders, but how neurogenesis is affected during HIV-1 associated dementia (HAD) has not been fully addressed. Here we test the hypothesis that HIV-1-infected and/or immune-activated macrophages affect NPC proliferation and differentiation through the regulation of cytokines. Data shows that LPS-activated human monocyte-derived macrophages (MDM) conditioned medium (LPS-MCM) induced a substantial increase in NPC proliferation. HIV-infected MCM (HIV-MCM) did not show a significant effect but HIV-infected and LPS-activated MCM (HIV/LPS-MCM) induced most significant NPC proliferation. Moreover, LPS-MCM and HIV/LPS-MCM decreased σ -III-tubulin and increased GFAP expression, indicating inhibition of neurogenesis and increase of gliogenesis. The increase of NPC proliferation and gliogenesis corre-

lated with an increased production of TNF- α and IL-1 σ by infected/activated MDM. Although both IL-1 σ and TNF- α induced NPC proliferation and gliogenesis, these effects were only partially abrogated by soluble TNF- α receptors R1 and R2 (TNF-R1R2), but not by the IL-1 receptor antagonist (IL-1ra), indicating that the HIV-1-infected/LPS-activated MCM-mediated effect is partially through TNF- α . TNF- α induced an increase of the inhibitory bHLH transcription factor Hes1 and a decrease of the bHLH determination factor Mash1 expression in a dose dependent manner, suggesting that TNF- α and MCM could directly regulate NPC cell fate through bHLH regulation. These observations provide evidence that HIV-1-infected and immune-activated macrophages affect neurogenesis through induction of NPC proliferation, inhibition of neurogenesis and activation of gliogenesis.

Investigators in Training II

S.48

Clade specific variation in HIV-1 transactivating protein (Tat) induced neurotoxicity in human neurons

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Human immunodeficiency virus-1 (HIV-1) infection of central nervous system often results in mild to severe cognitive impairment and dementia due to significant neuronal death. The transactivator HIV-1 protein Tat, has been implicated in HIV associated neurological deficits. HIV-1 is classified into A-K clades that are unequally distributed in world of which HIV-1 clade B and C together account for almost all infections around the globe. Recently, clade B and C specific differences in Tat mediated cytokine and chemokine function have been demonstrated in human peripheral blood monocytes, however it is unknown if these differences affect neurons in human brain. The fact that HIV-1 clade C accounts for over 50% of HIV-1 cases in world today but its effect on neuropathogenesis is unknown, warrants an immediate need to investigate effect of Tat C on human neurons. We used human fetal brain derived neural precursor cell culture system to understand the pathological changes in terms of neuronal apoptosis, glial activation as well as elevation of certain inflammatory parameters. Our experimental data suggest that there are clade specific differences in Tat induced oxidative stress, degree of neuronal death which may be mediated due to disturbances in mitochondria membrane potential and release of cytochrome-c. We also observed that expression of Tat modulated cell growth and proliferation of neural precursor cells. We are currently studying the role of MAP kinase pathway as it plays an important role in neuronal cell function and differentiation. Our observations provide a better understanding of the neuropathogenesis and complications that may arise in human population infected with HIV-1C.

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S.49

JC virus latency in the normal human brain

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JC Virus, a member of the Polyomaviridi family is a neurotropic virus with wide distribution among the human population of the world, according to sero-epidemiological studies. After a sub-clinical primary infection in childhood, the virus remains in latent state, presumably in the kidney. Reactivation of JCV under immunosuppressive conditions results in Progressive Multifocal Leukoencephalopathy, a fatal demyelinating disease of the brain, result of the cytolytic destruction of oligodendrocytes. The histopathological features of PML are demyelinated plaques in the sub-cortical white matter, enlarged oligodendrocytes harboring intranuclear inclusion bodies, and bizarre astrocytes. These two cells represent the sites of active viral infection. Although JCV has been shown to infect lymphocytes, which can act as carriers into other organs, the time at which JCV enters the brain, and the mechanism of such entry are still unknown. Differences in the strain of JCV that is isolated from the kidney (CY) and the one that causes PML (Mad-1) suggest rearrangements in the control region during the time of latent and active infection. Another subject of controversy is the establishment of latency in the brain. To elucidate this question we have collected samples from 5 regions of 7 normal brains from individuals who died of non-neurological conditions, extracted DNA and PCR amplified viral sequenced from the early and late regions of JCV with specific primers. We found JCV DNA sequences in at least one region of all cases studied.

Furthermore, in order to demonstrate the cell type in which JCV is present, we have performed PCR amplification in laser-captured cells of different phenotypes labeled with specific markers for neurons, astrocytes and oligodendrocytes. Results from these experiments suggest that JCV establishes a latent infection in astrocytes and oligodendrocytes, but not in neurons. Finally, immunohistochemical studies showed no expression of viral proteins, T-antigen, capsid proteins (VP-1), and the accessory product Agnoprotein, ruling out productive infection.

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S.50

Non SIV-infected MAC387+ macrophages accumulate within encephalitic lesions of SIV-infected rhesus monkeys: MAC387 as a potential marker of disease

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HIV in humans and SIV in non-human primates cause immunodeficiency and can induce encephalitis. Monocytes/macrophages are targets of infection and play a key role in AIDS-associated neuropathogenesis. HIV/SIV enter the central nervous system (CNS) at primary infection (peak viremia), which corresponds to an increase of the number of CD14+CD16+ monocytes in peripheral blood and macrophages in the brain. We investigated the characteristics of macrophages present in brain lesions of SIV-infected CD8+ cell-depleted rhesus macaques treated or not with anti-retroviral drugs (PMPA and RCV). SIV-infected untreated animals were sacrificed with AIDS, whereas the treated animals had histopathological evidence of SIV infection, but not AIDS. We focused on MAC387 expression, a marker of newly infiltrating monocytes/macrophages. PMPA/RCV-treated SIV-infected animals showed no encephalitis and no MAC387+ cells (n = 4). By contrast, we detected encephalitis in all non-treated SIV-infected animals that showed SIV RNA and an accumulation of MAC387+ cells within brain lesions around blood vessels (n = 4). Interestingly SIV-infected cells were CD68-MAC387-cells. Moreover preliminary phenotypic analysis showed that peripheral blood CD14+CD16+ monocytes from uninfected macaques expressed MAC387 and CCR2. We also detected accumulation of MAC387+ cells in brain lesions of HIV-infected patients with dementia. These data suggest that MAC387+ cells detected in brain lesions of SIV-infected CD8+ cell-depleted macaques are newly infiltrating monocytes/macrophages that are not cellular reservoirs of SIV-productive replication possibly because of their differentiation stage. However they could serve as potential markers of CNS disease. Whether brain MAC387+ macrophages of SIV-infected CD8+ cell-depleted rhesus macaques are derived from blood CD14/CD16 monocyte subpopulations expanded after HIV/SIV infection remains to be determined. This should contribute to decipher the ontogeny of such populations in AIDS-associated neuropathogenesis.

S.51

Effects of minocycline and PMPA on dopamine and dopamine metabolite levels in the caudate nucleus of SIV-infected pigtailed macaques

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Acquired immunodeficiency syndrome (AIDS) and virus-related neurological disease are significant consequences of human immunodeficiency virus (HIV) infection. Even with the advent of highly active antiretroviral therapy (HAART), the prevalence of HIV-associated neurological disease has increased, as many HAART drugs do not cross the blood-brain barrier. Minocycline is an off-patent, tetracycline derivative that effectively crosses the blood-brain barrier and has been shown to be neuroprotective in animal models of several neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, and amyotrophic lateral sclerosis. Using our accelerated, consistent model of simian immunodeficiency virus (SIV) infection we showed that minocycline reduces the severity of neurodegenerative indicators such as macrophage activation and infiltration, astrogliosis, levels of beta-amyloid precursor protein, and macrophage chemoattractant protein-1 in addition to brain viral loads.

Previous studies on post-mortem AIDS patients showed decreased levels of dopamine (DA) in the caudate nucleus (Sardar AM et al 1996), which was also shown to occur in the putamen early in infection in a SIV model (Scheller C et al 2005). Our hypothesis was that minocycline would be able to abrogate the decreases in dopamine levels seen in the brains of SIV-infected macaques. We used high performance liquid chromatography (HPLC) with electrochemical (EC) detection to examine levels of dopamine and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate nucleus of terminally infected pigtailed macaques that were treated with varying regimens of minocycline with or without PMPA (tanofavir), a nucleotide reverse transcriptase inhibitor. Our data showed that uninfected animals had significantly higher levels of DA in the caudate than terminally infected animals and that this decrease was alleviated with early minocycline treatment or later treatment with minocycline in combination with PMPA. Surprisingly the level of DA in the caudate nucleus of terminally infected macaques did not correspond to severity of CNS lesions or several other markers of neurodegeneration.

S.52

Regulation of CXCR4 expression and impact on HIV-1 infection by the mu-opioid agonist DAMGO in a bone marrow progenitor cell line model

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Several studies now suggest that opioids act as a cofactor in enhancing susceptibility to HIV-1 infection in

immune cell populations as well as modulating innate, humoral, and cell-mediated immunity. Studies have also shown that under chronic exposure to mu opioid receptor ligands like morphine there is an increased detection in the amount of HIV-1 long terminal repeat (LTR) DNA in cells of the monocyte-macrophage lineage. CD34+/CD38 – progenitor cells within the bone marrow are refractile to HIV-1 infection, probably due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5. We have previously shown that the human CD34+/CD38+ TF-1 erythromyeloid progenitor cell line can be utilized as a model to study the differentiation process of hematopoietic progenitor cells and how this differentiation process effects the cell surface expression of the HIV-1 receptor and co-receptors and HIV-1 susceptibility. Given these observations, studies have been initiated to identify the presence of the mu opioid receptor on the TF-1 bone marrow progenitor cell line. Studies have also been initiated to determine the functional relevance of this receptor in altering HIV-1 co-receptor expression, susceptibility to HIV-1 infection, impact on HIV-1 LTR activity, and impact on HIV-1 replication during chronic opioid exposure.

S.53

Astrocytic activation causes impaired glutamate clearance and altered CD38/cADPR signaling: Mechanistic links to HIV-1-associated dementia

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Reactive astrogliosis is a key pathological feature of HIV-1-associated dementia (HAD) and is associated with neural injury. However, to date, molecular mechanisms through which astrocyte functions are altered

in HAD are incompletely understood. Glutamate is the primary excitatory neurotransmitter in the central nervous system. The regulation of synaptic glutamate concentration by high affinity transporter such as EAAT2 is crucial to avoid excitotoxicity. Astrocytes are known to express EAAT2 that is responsible for over 90% of synaptic glutamate clearance. CD38 is a 45 kD ectoenzyme involved in synthesis and translocation of the potent calcium (Ca²⁺) mobilizing agents, cyclic adenosine diphosphate-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP+). Recently, the expression of CD38 in astrocytes has been proposed to play a key role in glutamate-mediated bi-directional astrocyte-to-neuron communication. Our data suggest that the glutamate clearance capability of astrocytes is impaired in neuroinflammatory disorders such as HAD and the dysregulation of CD38 expression in astrocyte contributes to this impairment. Primary human astrocytes were cultured and treated with pro-inflammatory cytokine IL-1 σ or HIV-1gp120, or infected by HIV-1ADA. The glutamate uptake ability of the cells was determined using the Amplex Red Glutamic Acid/Glutamate Oxidase Assay. The astrocyte CD38 and EAAT2 levels were tested by real-time PCR, immunocytochemical and immunohistochemical analyses and confocal microscopy. Our results show that either IL-1 σ or HIV-1gp120, or HIV-1ADA induced astrocytic activation, and both IL-1 σ and HIV-1gp120 significantly upregulated astrocyte CD38 levels. Importantly, our data confirmed that IL-1 σ time-dependently downregulated astrocyte glutamate uptake, which was completely reversed by co-incubation with 8-Br-cADPR, a specific cADPR-antagonist. In contrast, 3-Deaza-cADPR, a non-hydrolysable analog of cADPR downregulated astrocyte glutamate uptake in a time-dependent manner. Downregulation of astrocytic EAAT2 expression induced by IL-1 σ was also reversed by co-incubation with 8-Br-cADPR. Our data provide new evidence that astrocyte activation is accompanied by impaired glutamate uptake in the context of CD38 dysregulation, thereby contributing to the pathogenesis of neuroinflammatory disorders such as HAD. Our data implicate that the dysregulation of astrocyte CD38/cADPR signaling pathway plays an important role in the maintenance of synaptic glutamate homeostasis.

Session 7: Innate and adaptive immunity in CNS disease

S.54

T cell control of herpes simplex virus type 1 latency in sensory neurons

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Growing evidence supports an important role for CD8+ T cells in maintaining herpes simplex virus type 1 (HSV-1) in a latent state in sensory neurons. Interferon gamma production by CD8+ T cells blocks HSV-1 reactivation from latency in some, but not in all latently infected neurons. We now demonstrate that the lytic granule components granzyme B (GrB) and perforin (Pfn) are required for a complete block of HSV-1 reactivation from latency in sensory neurons *ex vivo*. We also demonstrate that a portion of the HSV-specific CD8+ T cells in latently infected trigeminal ganglia (TG) express the lytic granule component GrB, can direct lytic granules to the junction with infected fibroblasts, and that lytic granule release leads to activation of the caspase system and morphologic signs of apoptosis in the infected fibroblasts. In contrast, interaction of these same HSV-specific CD8+ T cells with infected neurons, while resulting in directed lytic granule release into the CD8+ T cell/neuron junction, does not lead to activation of the caspase system of the neuron or to morphologic signs of apoptosis. We conclude that CD8+ T cells use lytic granules to block HSV-1 reactivation without destroying latently infected neurons.

S.55

Essential roles of innate and adaptive responses during coronavirus infection

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Infections by neurotropic coronaviruses trigger potent innate and adaptive immune responses which make distinct contributions in controlling CNS virus replication and pathogenesis. Although acute encephalomyelitis resolves, the inability to completely clear virus results in a chronic spinal cord infection characterized by persistent viral RNA and ongoing demyelination. Efforts to dissect virus host interactions in the CNS have revealed that CD8 T cells contribute to anti viral defense via both perforin and IFN γ -mediated mechanisms. While perforin mediated clearance is ef-

fective in microglia/macrophages and astrocytes, IFN γ signaling is necessary for viral control in oligodendrocytes. Increased infection of oligodendrocytes in mice expressing a nonfunctional IFN γ receptor decoy under control of the oligodendrocyte specific PLP promoter confirmed IFN γ as an anti viral mediator in this cell type. Cell type specific clearance mechanisms prompted a kinetic analysis of MHC class I expression on glial cells, the prominent targets of infection. Flow cytometric analysis of CNS derived cells demonstrated that class I was maximally induced on all glia cell types coincident with prominent T cell infiltration and IFN γ secretion. However, expression on microglia preceded detection on oligodendroglia and astrocytes and was initially IFN γ independent. Delayed expression of class I on oligodendrocytes coincided with strong, coordinated transcriptional induction of MHC genes encoding factors supporting the class I antigen processing pathway. By contrast, these mRNAs were detected at higher basal levels in microglia and were upregulated earlier, albeit only modestly during infection. Differences in class I expression on glia resided in distinct responsiveness to type I IFN. A crucial role of type I IFN in preventing viral early spread was revealed by uncontrolled viral replication and mortality in infected mice deficient in type I IFN signaling. Surprisingly, the absence of type I IFN signaling did not impair induction or CNS recruitment of virus-specific CD8 T cells, the primary adaptive mediators of virus clearance in wt mice. Rapid fatality was rather associated with expanded cell tropism to neurons, in addition to increased glia infection. The data overall highlight how distinct responsiveness of CNS cells to virus induced inflammation may determine their susceptibility to antiviral T cell function and/or viral persistence.

S.56

Low level expression of HSV-1 IE genes and clonal expansion of memory effector CD8T cells in human sensory ganglia

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The latent persistence of Herpes simplex virus type 1 (HSV-1) in human trigeminal ganglia (TG) is accompanied by a chronic CD8 T-cell infiltrate. The focus of the current work was to look for HSV-1 transcription activity as a potential trigger of the immune response and to characterize the immune cell infiltrates by this feature. We combined in situ hybridization, laser cutting microscopy, and single cell RT-PCR to demonstrate the expression of the HSV-1 immediate early (IE) genes ICP0 and ICP4 in human trigeminal neurons. Using CDR3 spectratyping, we showed that the infiltrating T-cells are clonally expanded, indicating an antigen-driven immune response. Moreover, the persisting CD8+ T-cells had features of the memory effector phenotype. The voltage-gated potassium channel Kv1.3, a marker of chronic activated memory effector cells, and the chemokines CCL5 and CXCL10 were expressed by a subpopulation of infiltrating cells. The corresponding chemokine receptors CCR5 and CXCR3 were coexpressed on virtually all CD8 T-cells. In addition, T-cells expressed granzymes and perforin. In contrast to animal models of HSV-1 latency, hardly any FoxP3-positive regulatory T-cells were detected in human TG. Thus, HSV-1 IE genes are expressed in human TG and the infiltrating T-cells bear several characteristics that suggest viral antigenic stimulation.

S.57

Innate immunity and virus persistence in Semliki forest virus encephalitis in the mouse

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Following high titer viremia, the A7(74) strain of SFV is efficiently neuroinvasive and initiates perivascular foci of infection in the CNS; infecting principally neurons and oligodendrocytes. The uninfected CNS has low levels of gene expression for several Toll-like receptors (TLRs) and high levels of expression for TLR3. CNS expression of several TLR genes, including TLRs 2,3,7,8, and 9 is increased upon infection as is interferon gene expression. Levels of interferon gene transcripts are proportional to virus RNA load. Upregulation of TLRs 3 and 9 but not TLR2 is dependent upon the interferon response. Interferon responses normally protect meningeal and ependymal cells from widespread SFV infection. The early innate response to infection, most probably interferon, changes the outcome of infection of oligodendrocytes from death by apoptosis to persistent infection but the restricted replication of the A7(74) strain of SFV is not mediated by interferon. Antibody alone is sufficient to clear infectious virus from the CNS but antibody alone cannot eradicate the infection.

S.58

Immunomodulatory effects of IVIG protect against fatal herpes simplex encephalitis

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C57BL/6 (B6) mice are genetically resistant to fatal HSV encephalitis (HSE) compared to susceptible 129S6 (129) and BALB/c mice. Fatal HSE in 129 mice results from hyper-inflammatory responses involving macrophages and neutrophils. Depletion of macrophages or neutrophils resulted in significantly enhanced survival for HSV-1 infected 129 mice though the effect was transient. In contrast, depletion of macrophages in B6 mice markedly decreased their resistance to fatal HSE whereas depletion of neutrophils had no effect on survival, emphasizing the conflicting roles of macrophages and neutrophils in HSV pathogenesis in these two strains.

Pooled human IgG (IVIG) has become increasingly important both as a replacement therapy for primary and acquired humoral immunodeficiency providing protection against viruses such as hepatitis B, cytomegalovirus, varicella zoster and west Nile virus. However, the mechanisms of IVIG protection against virus infection have not been delineated. We report that a single dose of IVIG given at 6–24 h PI protects 100% of 129 mice from fatal HSE. Protection was reduced when IVIG was given later and no protection was seen at 72 h PI. Counterintuitively, survival could not be ascribed to virus neutralization, rather IVIG potently inhibited infiltration of inflammatory monocytes into the brainstem and preserved the integrity of the blood brain barrier relative to untreated infected 129 mice. Results from ongoing studies of the mechanism(s) of IVIG anti-inflammatory effects in HSE will be presented.

S.59

Immunological synapses in the brain between virally infected astrocytes and CD8+ CTLs mediate profound morphological and functional changes in both T cells and target APCs during the clearance of a brain viral infection

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Immunological synapses (IS) are microanatomical structures mediate intercellular communication between T cells and APCs. T cell polarization at the IS consists of a peripheral supramolecular activation cluster (pSMAC), which is high in LFA-1, and low in TCR, and a central SMAC, high in TCR, and low in

LFA-1. Although studied for many years *in vitro*, until recently there was no information on their existence *in vivo* in the context of natural immune responses. We have recently described their existence *in vivo* during an antiviral immune response in the brain, and are currently exploring their *in vivo* function. Here we demonstrate that during an anti-viral immune response in the brain and the formation of an immunological synapse, both the T cell and the APC adopt a distinct polarized distribution of membrane protein, cytoskeleton, intracellular organelles and, in the case of the T cell, effector molecules such as IFN- γ . Astrocytes were infected with an adenovirus (Ad) expressing Thymidine Kinase (TK), a marker for the infected cells. 30 days later we induced a systemic immune response against Ads, and 14 days after the immunization animals were sacrificed and brains studied by ICC and confocal microscopy. Brain sections were immunostained for TCR, LFA-1, TK, EAAT2, α -Tubulin,

γ -Tubulin, GM130 and IFN- γ . Confocal analysis revealed that target infected astrocytes, normally a multipolar non-polarized cell, became unipolar cells, and thus had undergone profound phenotype changes. In response to the T cell attack astrocytes retracted most cellular processes and retained a single large protrusion towards the T cell. The MTOC and Golgi were also reoriented towards, and into, the protrusion. Confocal analysis also revealed that IFN- γ is polarized in T cells establishing close anatomical contacts with Ad infected cells. IFN- γ polarization was found in cells with or without polarized LFA-1 or TCR. This demonstrates that IFN- γ is selectively polarized towards the APC, and further suggests that maturation of the IS is not necessary for the polarization of IFN- γ . These results demonstrate that CTL signaling, potentially mediated through IS, induces profound morphological and functional changes in both T cells and target infected astrocytes, during a brain antiviral immune response.

Session 8: Immune cell trafficking, blood brain barrier and viral persistence

S.60

Mechanisms of transmigration of HIV infected leukocytes across the human BBB: a critical role in NeuroAIDS

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HIV enters the CNS early after infection, resulting in cognitive impairment and other neurological manifestations in a large number of individuals. The virus gains access to the CNS, in part, by the transmigration of HIV infected monocytes and perhaps T cells across the BBB. This results in infection of CNS macrophages/microglia and of some astrocytes, as well as in ongoing inflammation. Despite HAART, virus and inflammatory cells persist within the CNS, and the prevalence of the resultant cognitive impairment is increasing. Thus NeuroAIDS is among the most urgent public health concerns worldwide.

Our laboratory demonstrated that the chemokine CCL2 is critical to transmigration of HIV infected cells, and that HIV infection of leukocytes results in dysregulation of their surface protein expression, enhancing their passage across the BBB. In addition, this exuberant transmigration of HIV-infected cells results in BBB disruption and activation of CNS cells, as well as in the elaboration of several factors, including HIV tat protein, that cause neuron damage and cell death. Our findings of enhanced HIV-infected cell transmigration, BBB disruption and neuronal damage will be discussed in the context of potential therapeutic intervention.

S.61

Caveolae-associated signaling pathways in HIV-1 tat-induced activation of human brain microvascular endothelial cells

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Alterations of brain microvasculature and the disruption of the blood-brain barrier (BBB) integrity are commonly associated with HIV-1 infection. These changes are most frequently found in patients with HIV-related encephalitis and in HIV-associated dementia. Our research is based on the hypothesis that impaired function of the brain vasculature can contribute to HIV trafficking into the CNS and the development of neu-

rodegenerative changes associated with HIV infection. Indeed, activation or dysfunction of brain microvascular endothelial cells (BMEC) can lead to the breakdown of the BBB, induction of inflammatory responses and thus provide entry for HIV into the CNS. The leading hypothesis of our research is that the viral gene product, HIV protein Tat, can be responsible for BMEC injury and impaired normal function of the BBB. Tat is released by acutely HIV-1-infected cells and can affect the integrity of the brain endothelium and the BBB by induction of redox-activated signaling. Caveolae serve as the signaling platforms that co-localize various signaling components, including the Ras and Rho signaling cascades. These pathways are critical for the integrity of the BBB, because they regulate the rearrangement of cytoskeleton and permeability of cell junctions. Therefore, the present study was focused on the effects of Tat on the Ras and Rho signaling pathways in human brain microvascular endothelial cells (HBMEC) (Weksler et al., FASEB J 19:1872-4, 2005). Treatment with Tat markedly activated Ras and RhoA levels in cultured HBMEC. Interestingly, caveolin-1 protein levels were upregulated in parallel to Ras and Rho activation. Silencing caveolin-1 by specific siRNA resulted in diminished activation of Ras but not RhoA in response to Tat. Exposure to Tat also stimulates inflammatory response in BMEC. Therefore, we generated stably transfected cell lines of HBMEC which overexpress peroxisome proliferator-activated receptor (PPAR)-alpha or PPAR-gamma. Overexpression of PPAR-alpha or PPAR-gamma protected against Tat-induced mRNA levels of IL-1beta, TNF-alpha, MCP-1, and E-selectin. The present data indicate the importance of caveolar-mediated signaling in response to Tat exposure and suggest that targeting PPAR signaling may provide a novel therapeutic approach to attenuate Tat-induced dysfunction of brain endothelial cells. Supported by MH63022, MH072567, and NS39254.

S.62

Poor HIV-1 entry via endocytosis but highly productive transcriptional regulation leads to persistent viral replication in astrocytes in vitro

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The HIV infection of the central nervous system (CNS) is well documented but least characterized. HIV

infects microglia/macrophages productively, but, infection in astrocytes has been reported to be unproductive. Besides, there are conflicting reports such as no-infection to unproductive viral replication as well as sporadic reports on post-entry HIV-replication blocks in astrocytes. We therefore, characterized the HIV infection in astrocytes using sensitive and specific fluorescent reporter assays including GFP-expressing-HIV. Notably, transfection of infectious HIV proviral DNA in astrocytes revealed efficient production of Tat, Rev, Nef and p24. Unambiguously, regulatory proteins Tat and Rev were found to be functionally active in astrocytes. Further, human RNA helicase DDX3 was found to be critical in normal functioning of Rev and its inhibition by antisense-DDX3 impaired the Rev function which subsequently curtailed HIV replication in astrocytes. Despite absence of CD4, CCR5 and LFA-1 (CD11a), DC-SIGN and mannose receptor (MR), however, low-persistent (productive) viral infection occurred in human fetal astrocytes (HFA) and astrocytic cells (SVGA). Intriguingly, endosomal inhibitor Bafilomycin-A enhanced HIV-infection several fold suggesting viral entry via pH dependent endocytosis. To corroborate transfection and wild type HIV infection results, VSV-pseudotyped fluorescent HIV-NL4-3 virus infection in astrocytes revealed productive–non-cytolytic infection which developed into persistent state (productive) when serially followed-up for more than 3 months. Similarly, the HIV infection in SVGA and SVGA-reporter cells also developed into chronic state when followed up for more than 6-months. Further, persistently HIV-infected primary astrocytes did not show spread of infection to neighboring astrocytes, albeit, upon co-culture with lymphocytic cells revealed efficient transmission of infection. Our study led to a conjecture that HIV infection in astrocytes is restricted at the viral entry level but still low persistent infection occurs which could serve as a stable reservoir of the HIV in brain.

S.63

Subclinical reactivation and shed of infectious VZV in saliva of astronauts

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VZV enters the nasal/pharyngeal region as an aerosol and replicates in tonsillar T-cells, ultimately resulting in viremia and the development of chickenpox (varicella). After primary infection, VZV becomes latent in cranial nerve ganglia, dorsal root ganglia and autonomic nervous system ganglia along the entire neuraxis. Decades later, a decline in VZV host immunity

results in virus reactivation (zoster). An accompanying abstract by Mehta et al. describes the presence of VZV DNA in saliva of zoster patients. Herein, we extend the analysis of salivary VZV from the clinical setting to subclinical reactivation. We studied saliva obtained from three astronauts before, during and after space flight. Quantitative PCR detected VZV DNA in saliva from two of the three astronauts during and shortly after flight in space. No saliva sample was positive for VZV DNA before liftoff. From 2-6 days post flight, early morning saliva samples were also cultured on human fetal lung cells. After one subcultivation, a typical herpesvirus cytopathic effect was seen in samples taken from two of the three astronauts. Immunostaining and PCR revealed that both virus isolates were VZV and not HSV. ORF22-based PCR/sequencing along with FRET-based PCR assays that target specific subgenotype specific SNPs revealed both VZV isolates to be the European strain with an Msp restriction site in ORF62 (107,252 nt). Both of these rare genotypes were different from the only other VZV specimen in the lab at the time (a European strain lacking an Msp restriction site in ORF62 that had been isolated from vesicles of a patient with zoster). These findings extend our previous demonstration of VZV DNA in saliva of astronauts by showing that the virus is infectious. Overall, along with HSV-1 and HSV-2, VZV can reactivate and shed infectious virus in the absence of clinical disease.

S.64

Impaired maturation and function of HTLV-1 specific CTLs in HAM/TSP

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Despite significantly increased and chronically activated virus-specific CD8+ CTLs, they do not reduce the human T lymphotropic virus type 1 (HTLV-1) proviral load in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients. We hypothesized that failing immune control of HTLV-1 in HAM/TSP patients could be a result of impaired CTL maturation and function. To characterize the CD8+ CTLs in chronic HTLV-1 infection, we analyzed CD8+ T cells in PBMCs from age-matched HAM/TSP patients (n = 46), healthy HTLV-1 carriers (HCs) (n = 21) and normal controls (NCs)(n = 21) by flow cytometry based analyses. Expression of perforin, granzyme (Grz) A and GrzB, which is closely associated with CTL-mediated lysis, was significantly lower in total CD8+, CD8+CD28- and CD8+CD27- T cells in HAM/TSP patients than HCs and NCs, and was negatively correlated with HTLV-1 provirus load. Lower

perforin expression was evident in HTLV-1 specific CD8+ T cells but not in CMV specific CD8+ T cells in the same individuals. Based on the expression of CD28 and CD27 on virus-specific CD8 T cells (Nat Med. 2002; 8:379-85), HTLV-1 Tax-specific CD8+ T cells in HCs was mostly at the intermediate differentiated stage

(CD28-CD27+), which is significantly different from HAM/TSP patients (late differentiated stage; CD28-CD27-). These findings suggest that an impaired maturation and function of HTLV-1 specific CTLs might be associated with failing antiviral immune control and disease HAM/TSP.

Session 9: Host gene expression in neurologic infections

S.65

Host genetic determinants of HIV transmission and AIDS/neuroAIDS: Should we care?

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Borrowing from Shakespeare, (William Shakespeare, King Richard II. Act ii. Sc. 1)

“...this other Eden, demi-paradise, this fortress built by Nature for herself against infection ...”

Nature's fortress against infections such as HIV by necessity involves host factors that influence the early events of HIV-host interactions. In this seminar, I will show how we have used a genetic approach as a powerful tool to identify the host factors that underlie some of these early events. This approach has therefore permitted us to address the following clinical paradoxes: Why do some individuals when exposed to HIV-1 resist infection? Why do some individuals succumb to HIV-1 infection rapidly whereas others resist progression to AIDS and neuroAIDS? Why do some individuals have a better recovery of immune function after initiation of HAART?

Using this genetic approach we have (a) uncovered complex host gene-gene interactions that influence HIV-1 pathogenesis in vivo; (b) determined the relative contribution of some of these host genetic determinants to the HIV-1 epidemic at the population level. Specifically, in this seminar, I will focus on the role of CCR5 and its ligands in HIV/AIDS pathogenesis and neuroAIDS. In previous studies we uncovered some of the the CCR5 genetic determinants that contribute to inter-subject differences in HIV/AIDS susceptibility. Here, I will show that there are significant interindividual and interpopulation differences in the copy number of a segmental duplication encompassing the gene encoding CCL3L1 (MIP-1aP), a potent HIV-1-suppressive chemokine and ligand for the HIV coreceptor CCR5. Possession of a CCL3L1 copy number lower than the population average is associated with markedly enhanced HIV/AIDS susceptibility. This susceptibility is even greater in individuals who also possess disease-accelerating CCR5 genotypes. This relationship between CCL3L1 dose and altered HIV/AIDS susceptibility points to a central role for

CCL3L1 in HIV/AIDS pathogenesis and neuroAIDS. I will show that these genetic factors influence HIV pathogenesis by affecting both viral entry-dependent and -independent processes, one of which we identify as cell-mediated immunity. I will then place these findings in the broader context of issues such as CD4 recovery during HAART.

The work presented here is the culmination of a productive and synergistic collaboration/partnership between members of the VA HIV/AIDS Center/UTHSCSA and the following groups at (i) Wilford Hall Medical Center, San Antonio, TX, PI: Matt Dolan, MD; (ii) Barcelona, Argentina, PI: Luisa Sen, MD; (iii) UCSF (SCOPE; AIEDRP cohorts): San Diego, CA, PIs: PIs: Drs. Steve Deeks, Jeff Martin, Rick Hecht; (iv) UCSD (AIEDRP cohort), PIs: Susan Little, M.D. and Doug Richman, MD; (v) Harvard (Elite and Viremic controllers), PI: Bruce Walker, MD.

S.66

Host gene expression in the HIV infected brain

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In order to elucidate underlying pathological mechanisms associated with HIV brain infection and comorbid conditions we have been investigating the gene expression profiles. Specifically we have been assessing gene dysregulation associated with the presence of HIV encephalitis (HIVE), the effect of the prospectively assessed use of methamphetamine and the presence of the major depressive disorder. In a series of experiments to be described we found that HIVE was specifically associated with dysregulation of genes regulating the cytoskeleton and neuroimmune response. In contrast the use of methamphetamine during life was accompanied by significant up-regulation of interferon inducible genes, which in vitro we have demonstrated to result in glial cell toxicity. Finally examination of gene expression in HIV infected individuals with major depressive disorder showed substantial reduction in the neuromodulatory gene somatostatin. These observations reveal that there are discrete gene expression patterns which highlight pathological mechanisms involved as well as provide potential novel targets of therapeutic development.

S.67

HIV-1 infection: central dopamine and transporter gene expression

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Background: Human immunodeficiency virus type-1 (HIV-1) enters the central nervous system (CNS) early in the course of infection, and gets localized in various regions and causing a wide range of CNS complications. These complications include progressive neuroanatomical and neurophysiological abnormalities as well as neurocognitive impairments and psychiatric problems. It has been hypothesized that these HIV-1 mediated cognitive and behavioral abnormalities may be associated with the degenerative changes occurring in the monoaminergic neurotransmitters such as, dopaminergic and serotonergic systems that are known to regulate these functions. Since these neurocognitive and behavioral problems continue to persist even after intervention with the antiretroviral medication, it is pertinent to determine the status of these neurotransmitters in different brain regions associated with these functions.

Objectives: The main objective of this study was to investigate the status of dopamine (DA) and its major metabolite, homovanillic acid (HVA) in different brain regions of HIV-1 infected and non infected individuals. Furthermore, although, 5-hydroxytryptamine (5-HT, serotonin) has been considered as the main regulator of behaviors, according to the recent reports, interaction between DA and 5-HT involving DA-transporter (DAT) and 5-HT-transporter (5-HTT) activities are important events for regulating some of the cognitive functions and behaviors. We therefore, investigated DAT and 5-HTT gene expression in some of the brain regions of the same cases.

Methods: The postmortem brain tissues were procured from four centers of the NIH sponsored National NeuroAIDS Tissue Consortium (NNTC). We measured DA and HVA concentration in the tissue extracts of different brain regions of HIV-1+ and HIV- negative cases, using highly sensitive CoulArray HPLC-ECD system. We also investigated in this preliminary study the level of DAT as well as 5-HTT gene expression in a few samples of the same brain regions, using real-time RT-PCR.

Results: We found a significant decrease in dopamine and HVA concentrations in majority of the brain regions of HIV-1+ individuals. Although, a decrease in DAT as well as 5-HTT expression was observed in some of the regions investigated such as the frontal cortex-4 of HIV-1+ individuals, the change was not consistent in all cases, when compared to that in the brain regions of HIV-negative controls.

Conclusion: HIV-1 infection causes depletion in the central monoaminergic systems including that of dopamine and serotonin leading to reduced concentration and transporters expression.

This study was supported by the NIH grant #s RO1 NS 43982 and RO1 NS 055653.

S.68

The Pro-apoptotic transcription factor p53 influences the pattern of microglia activation in response to HIV-gp120

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We have previously reported that microglia from p53 deficient mice fail to transmit the toxicity of the HIV gp120 coat protein to neurons. Microglia, like macrophages, can adopt classical (M1) or alternative (M2) patterns of activation that lead to toxic or trophic actions respectively. HIV-gp120 causes M1 activation in microglia leading to TNF-alpha dependent neurotoxicity in mixed cerebrocortical cell culture. To determine how p53 influences microglia to develop a neurotoxic response to gp120, we compared whole mouse genome expression profiles in cultured microglia mRNA generated from strain matched p53 containing and p53 deficient neonatal mice. We observed that p53 has a dramatic effect on the regulation of gene expression in microglia and the gene expression changes induced by gp120 were distinct between the two genotypes. We also observed that p53 deficient microglia have increased expression of many genes associated with the M2 pattern of activation. To further assess the possibility that p53 influences whether microglia adopt an M1 or M2 pattern of activation, we measured the response of wild type and p53 deficient microglia to interferon-gamma, a specific inducer of M1 activation, and interleukin-4 (IL-4), a cytokine that promotes M2 activation. We observed that p53 deficient microglia display a blunted M1 cytokine response following exposure to interferon-gamma. In addition, we report that p53 deficient microglia express high levels of the M2 marker genes YM1 and FIZZ1 prior to treatment with IL-4. Taken together, these findings suggest that p53 is involved in promoting M1 activation and in the absence of p53, microglia default to an M2 pattern of gene expression, even without IL-4 stimulation for M2 activation.

S.69

HIV-1 Vpr causes oxidative stress through induction of HIF-1 α

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HIV-1 infection of brain microglia and perivascular macrophages results in chronic inflammation, secretion of toxins, and induction of oxidative stress.

Excitotoxicity and/or oxidative stress may ultimately lead to gene-directed neuronal death, apoptosis, or activation of latent HIV-1 gene expression and replication. Oxidative stress factors such as hydrogen peroxide, superoxide, and hypoxia inducible factor 1 alpha (HIF-1 α) have been shown to be involved in the activation of latent HIV-1. HIF-1 α is a transcriptional activator that function as a chief regulator of cellular and systemic as oxygen homeostasis. Histopathological evaluation of AIDS brain with encephalopathy revealed activation of HIF-1 α in several cells including, microglia, macrophages, and astrocytes all of which are infectable with HIV-1 and support its replication. Accordingly, results from infection of microglial cells with HIV-1 in a cell culture system showed elevated levels of HIF-1 α upon infection with HIV-1. Further examination of HIF-1 α expression in the presence of HIV-1 regulatory proteins, suggested a role for Vpr in

induction of HIF-1 α . The observed elevation in the HIF-1 α impacts on HIV-1 gene expression via cooperation with Vpr and NF- κ B. Interestingly, HIF-1 α failed to regulate the HIV-1 gene expression in the absence of NF- κ B. Further, in addition to inducing accumulation of HIF-1 α , Vpr was able to increase secretion of reactive oxygen species (ROS), promote phosphorylation of the mitogen-activated protein kinase p38 (p38MAPK), and positively regulate the tumor necrosis factor alpha (TNF- α) gene expression, all of which contribute to HIF-1 α activation and stabilization. In this project, we hypothesize that the interplay between HIF-1 α and the HIV-1 regulatory proteins plays a role in host homeostasis and viral gene expression and replication in CNS cells. The experimental design in this project will include a series of molecular, virological and histological approaches and unravel the role of HIF-1 α in HIV-1 gene expression and replication.

Session 10: Neurogenesis and neuroprotection

S.70

Minocycline: mechanisms of neuroprotection

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HIV-related CNS and PNS diseases are significant consequences of human immunodeficiency virus (HIV) infection. Even with the advent of highly active antiretroviral therapy (HAART), the prevalence of HIV-associated neurological disease has increased, at least partly because many HAART drugs do not cross the blood-brain barrier. Minocycline is an off-patent, tetracycline derivative that effectively crosses the blood-brain barrier and has been shown to be neuroprotective in animal models of several neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, cerebral trauma, stroke and amyotrophic lateral sclerosis. A preliminary study of minocycline treatment in people with multiple sclerosis suggested that minocycline reduced the reduced number of CNS lesions on MRI. In contrast, results of a large clinical trial testing the efficacy of minocycline in humans with ALS indicated that treated individuals progressed 25% faster than untreated controls.

Using our accelerated, consistent SIV/macaque model of HIV CNS and PNS disease, we showed that minocycline reduced the severity of neurodegenerative indicators such as macrophage activation and infiltration, astrocytosis, levels of beta-amyloid precursor protein in axons, levels of CSF and brain macrophage chemoattractant protein-1 (MCP-1 or CCL2), brain viral loads and levels of the neurodegenerative MAPKs p-p38 and pJNK. In addition, we demonstrated that minocycline-treated animals did not have the lowered dopamine levels in the caudate that are seen in SIV-infected macaques (and HIV-infected individuals). Further, minocycline, when given in combination with the antiretroviral drug Tenofovir, prevented the loss of neurons in the dorsal root ganglia that is associated with SIV infection, suggesting that the antibiotic is neuroprotective in the peripheral nervous system as well. These studies were the first to demonstrate efficacy of minocycline in a virus-induced neurodegenerative disease. Nonetheless, a recent report showed no efficacy of minocycline in reducing the neurodegenerative effects of minocycline in a mouse model of rabies encephalitis. These contrasting studies confirm the importance of under-

standing the mechanism by which minocycline acts in the CNS and PNS so that it might be used in rational therapeutic protocols and so that its structure might be modified to increase its neuroprotective capacity.

We therefore examined the mechanism by which minocycline modulates the cell signaling environment both in vitro and in vivo. In vitro, we used a nitric oxide donor to activate both p38 and JNK signaling in PMA-differentiated U937 cells and showed that pretreatment of these cells with minocycline resulted in dose-dependent suppression of p38 and JNK activation. Our findings further suggested that minocycline suppresses p38 and JNK activation by decreasing intracellular levels of, and hence activation of ASK1, a MAPKKK that leads to activation of both p38 and JNK. In vivo, these pathways were examined by quantitative immunohistochemistry of brain tissue from untreated and minocycline-treated SIV-infected macaques using an antibody that detects activated ASK1. We demonstrated increased activated ASK1 in the subcortical white matter of SIV-infected macaques with encephalitis and significant reduction of levels of activated ASK in minocycline-treated animals. Thus, minocycline appears to act in part by suppressing activation of ASK1, thus downregulating the activation of the proapoptotic MAPKs p38 and JNK.

S.71

HIV/gp120 decreases neural progenitor cell proliferation via checkpoint kinase-mediated cell cycle withdrawal and G1 arrest

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Impaired adult neurogenesis has been observed in several neurodegenerative diseases, including human immunodeficiency virus (HIV-1)-associated dementia (HAD). Here, we report that the HIV-envelope glycoprotein gp120, which is associated with HAD pathogenesis, inhibits proliferation of adult neural progenitor cells (aNPCs) in vitro and in vivo in the dentate gyrus of the hippocampus of HIV/gp120-transgenic mice. We demonstrate that HIV/gp120 arrests cell-cycle progression of aNPCs at the G1 phase via activation of a cascade consisting of p38 mitogen-activated protein kinase (MAPK)#8594;MAPK-activated protein kinase 2 (a cell cycle checkpoint kinase)#8594;Cdc25C. Our findings define a molecular mechanism that compromises adult neurogenesis in this neurodegenerative disorder.

S.72

Neurotrophin and neurotrophin receptor expression by macrophages: modulation by cell activation and HIV infection

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Neurotrophins (NGF, BDNF, NT3, NT4) and their high affinity (TrkA, TrkB, TrkC) and low affinity (p75NTR) receptors are key molecules implicated in the development of the central nervous system. They are recognized for their action in physiology and pathophysiology. On another hand, macrophages and microglial cells also express neurotrophins and their receptor with characteristic regulation features, but very limited data are available about this.

We investigated in vitro the expression of these molecules during monocyte-derived macrophage activation and infection with HIV. Expression was quantitated by means of qRT-PCR with GAPDH as a reporter gene. NGF was the most expressed ligand, followed by BDNF and NT4 (about ten times less) and NT3 (weak level). Among the receptors, TrkA and TrkB displayed mRNA levels comparable to the one of NGF whereas TrkC and p75NTR were ten times less abundant. Neurotrophin and neurotrophin receptor genes are thus differentially expressed by MDM. These levels of expression were modulated by pro and anti-inflammatory stimulants (TNFalpha, IL-1beta, LPS, IFNgamma, IL-4, PGE2, IL-10, dexamethasone). NGF mRNA increased with both pro- and anti-inflammatory factors, dexamethasone being the most potent (one log increase). Such a broad induction profile was also observed for TrkC but with weaker effect amplitudes. BDNF was induced by dexamethasone, and NT3 by TGFbeta, PGE2 and IL-4.

On the other hand, neurotrophins neither modulated macrophage ability to sustain lymphocyte proliferation, nor changed their basal or LPS-induced TNFalpha production. CD163 and CD206, two markers of macrophage alternative/suppressive activation were also studied. Results show that CD163 membrane expression was weakly stimulated by NGF, in contrast to CD206 one which was stimulated by BDNF, NT3 and NT4. HLA-DR expression was not modulated.

The expression of NT and their receptors by HIV-replicating macrophages was not modulated except NT3 which was stimulated by HIV infection compared with control starting from day 10 p.i., and TrkB which was inhibited starting from day 14. Treatment of HIV infected macrophages with neurotrophins or neutralizing antibodies did not exhibit any effect on virus replication or on cellular viability. The surface expression of CD4 and CCR5 was not modified, while CXCR4 was stimulated by NGF.

In conclusion, NT did not interfere with HIV replication in macrophages, nor did they modulate inflammatory properties of these cells. Macrophage expression of NT and NT-receptor genes is differentially modulated by cell stimulation, suggesting that NT and their receptors might participate in an adaptive response to macrophage activation and its related toxicity features. MDM ability to modulate activation-induced neurotoxic insults through this pathway is under investigation.

S.73

Leukocyte infiltration into murine brains during experimental herpes encephalitis

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Murine microglia produce innate proinflammatory immune mediators in response to infection of the brain with herpes simplex virus (HSV)-1, including chemokines and reactive oxygen species. Using an experimental murine model, we have previously shown that this proinflammatory mediator induction precedes the presence of brain-infiltrating systemic immune cells. In the present study, we used real-time bioluminescence imaging, along with adoptive transfer of splenocytes obtained from beta-actin-luciferase transgenic mice, to investigate the kinetics of leukocyte trafficking into HSV-infected brains. In these studies, trafficking of luc⁺-splenocytes into the brain was observed only in HSV-infected animals and mice receiving primed splenocytes demonstrated a vigorous brain-infiltrate which persisted up to 13 days post-infection (d.p.i.). Despite this vigorous immune cell infiltration, most animals succumbed to viral infection between 6 and 9 d.p.i. We then went on to isolate brain-infiltrating leukocytes from infected animals and characterize their phenotypes using flow cytometry at 5, 8, and 14 d.p.i. In these studies, both macrophage [CD45(hi)CD11b(hi)Ly6C(hi)] and T lymphocyte (CD3⁺) brain infiltrates were detected. In additional studies, RNA expression analysis of FACS sorted leukocyte populations isolated from the brains of animals with herpes encephalitis at 7 d.p.i. identified resident CD45(int)CD11b(hi) microglial cells as the predominant source of infection-induced iNOS and IL-1-beta, while brain-infiltrating CD45(hi)CD11b(hi) macrophages were identified as the primary source of TNF-alpha. Additional FACS studies identified CD45(hi)CD11b(dim) T-lymphocytes as the source of IFN-gamma in infected murine brains. Results obtained during these studies will help elucidate the beneficial as well as immunopathologic roles of immune cell infiltration into the brain during herpes encephalitis.

S.74

Molecular interaction between IRS-1 and Integrins in the Context of HIV-Encephalopathy

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HIV encephalopathy is a severe neurological disorder of the central nervous system, which develops in AIDS patients. Among numerous viral and cellular factors, tumor necrosis factor alpha (TNFalpha) released in the brain by activated and/or infected macrophages/macrogia is suspected to compromise stability of neuronal processes. Previously, we have demonstrated that insulin-like growth factor (IGF-I) signaling axis protects neurons from TNFalpha-induced damage. Since TNFalpha triggers serine phosphorylation of insulin receptor substrate 1 (IRS-1) and serine phosphorylated IRS-1 binds integrins, we asked how these molecules events affect neuronal stability. Here we confirmed that differentiated PC12/IGF-IR neurons, which express high levels of IGF-IR and IRS-1, and primary cortical neurons are well protected from TNFalpha-induced neuronal damage when cultured in the presence of IGF-I. We have also found that alpha1beta1-integrin, as well as serine phospho-

rylated IRS-1 are located in membrane rafts of differentiated neurons. In this subcellular compartment, IRS-1 co-precipitated with alpha1beta1-integrin, and this protein-protein interaction was facilitated by TNFalpha and inhibited by IGF-I stimulation. We have demonstrated also the presence of IRS-1 and alpha1beta1-integrin complexes by double immunocytofluorescence, and confirmed the binding by GST-pull down assay, which additionally demonstrated that the IRS-1 domain responsible for the integrin binding is localized within the central portion of IRS-1, between amino acids 462 and 740. Importantly, ectopic expression of the 426-740/IRS-1 mutant inhibited neuronal outgrowth in both differentiated PC12 cells and in primary cortical neurons. Our results demonstrate for the first time that the interaction between serine phosphorylated IRS-1 and beta1-integrin may affect formation and stability of neuronal processes in the paradigm of TNFalpha-induced neuronal damage. Since we have also demonstrated that IGF-I inhibits the formation of IRS-1-beta1-integrin complexes at membrane rafts of differentiated neurons, this could suggest a new mechanism of action for IGF-I, which in addition to its strong anti-apoptotic signal may support integrin-mediated cell attachment, counteracting detrimental effects of TNFalpha on neuronal processes.

Session 11: Animal models and viral pathogenesis

S.75

Studies of monocyte/macrophages in a monkey model of neuroAIDS define mechanisms of disease progression and potential therapies

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Macrophages are considered key targets of HIV and SIV infection in the CNS. Accumulation of macrophages correlates with CNS histopathology and neurological disease. Recent evidence underscores the expansion of monocyte/macrophage subsets in bone marrow and blood with pathogenesis. In fact, it has been suggested such expansion may be predictive of the rate of disease progression. This talk will review recent data using an SIV infected, rhesus macaque model of neuroAIDS to discuss changes in absolute numbers and the relative percentage of monocyte/macrophages in neuroAIDS. Data of the role of subsets as targets of infection, the contribution of plasma virus versus monocyte/macrophages driving disease will be discussed. Pharmacologic and antiretroviral approaches targeting virus and/or monocyte/macrophages will be discussed.

S.76

A protective role for the CXC chemokine receptor 2 (CXCR2) in host defense following viral infection of the central nervous system

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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 (MS). During both acute and chronic disease, transcripts specific for the CXC chemokine receptor 2 (CXCR2) and its binding ligands CXCL1 and 2 are elevated in the brains and spinal cords of mice suggesting a potential role in either host defense or disease. Expression of CXCR2 was restricted primarily to neurons, astrocytes, and oligodendrocytes and was not associated with infiltrating leukocytes. Antibody targeting of CXCR2 during acute disease resulted in 100% mortality that correlated with enhanced viral burden within the brain indicating that CXCR2 signaling is important in host defense. Although T cell infiltration into the CNS was

reduced in anti-CXCR2 treated MHV-infected mice, we do not believe that CXCR2 specifically enhances T cell trafficking as infiltration of other immune cell subsets was also limited compared to MHV-infected mice treated with control antisera. Rather, blocking CXCR2 signaling may diminish the permeability of the blood-brain-barrier that ultimately limits immune cell accumulation during acute viral-induced encephalitis. Antibody neutralization of CXCR2 during chronic disease in mice persistently infected with MHV and undergoing demyelination resulted in increased mortality compared to mice treated with control antisera. Antibody targeting of CXCR2 did not reduce infiltration of virus-specific T cells and macrophages into the 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 a large majority of the apoptotic cells in anti-CXCR2 treated mice were oligodendrocytes and oligodendrocyte precursor cells. Together, these findings highlight potentially differential protective roles for CXCR2 following viral infection of the CNS. During acute disease, CXCR2 is protective and influences inflammatory immune cell infiltration into the CNS while during chronic disease CXCR2 signaling influences oligodendrocyte survival.

S.77

Utility of SCID mouse model for HIV-1 encephalitis for studies of neuropathogenesis, blood brain barrier injury and development of new treatment approaches

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BBB compromise is one of the underlying causes of HIV-1 associated dementia (HAD). Brain tissues of HAD patients demonstrates diminished expression of tight junctions (TJ) of brain microvascular endothelial cells indicating BBB injury. We previously developed the hu-PBL-NOD/SCID model [non-obese diabetic (NOD)-SCID mice reconstituted with human peripheral blood lymphocytes (PBL)] that reproduces important features of HIVE including HIV-1 infection

of macrophages, glial activation, neuronal injury, BBB injury, cognitive impairment and acquired anti-viral immune responses. Now, using the 7T Magnetic Resonance Imaging (MRI) system, we developed in vivo imaging of BBB permeability using Gd-DTPA (Magnevist) as a contrast agent. Utilizing in vitro BBB model systems, we proved that interactions between HIV-1 infected monocytes and BMVEC led to activation of small GTPase (RhoA) and its downstream effector, Rho kinase (RhoK), resulted in phosphorylation of TJ proteins (occludin and claudin-5) and decreased tightness of the BBB. Using synthetic ligands activating the peroxisome proliferator-activated receptors (PPARs) we demonstrated that the enhanced adhesion and transendothelial migration of HIV-1 infected monocytes across inflamed BBB were markedly reduced by activation of PPAR τ ; via inhibition of Rho GTPases. Using SCID HIVE model we showed that PPAR τ ; agonist suppressed HIV-1 replication in MDM in brain tissue and reduced viremia by 50%. Effects of PPAR τ ; agonists on BBB permeability in vivo are currently being analyzed. These findings indicate that PPAR τ ; agonists could be attractive therapeutic targets for treatment HIV-1 CNS infection.

S.78

Monocyte heterogeneity underlying phenotypic changes in monocytes according to SIV disease stage

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Expansion of CD16+ monocytes during HIV infection may correlate with the development AIDS and incidence of HIV-associated dementia. To provide a potential mechanistic basis for the study of monocytes and monocyte subsets in HIV/SIV neuropathogenesis, we first identified 5 different mononuclear phagocyte subpopulations in blood of normal uninfected and SIV-infected rhesus macaques (CD14highCD16-monocytes, CD14highCD16low monocytes, CD14lowCD16high monocytes, CD14lowCD16-CD11c+ myeloid dendritic cells, and CD14-CD16-CD123+ plasmacytoid dendritic cells). Assays assessing SIV replication, TNF and granzyme B production, endocytosis and phagocytosis established the existence of phenotypically definable and functionally distinct subpopulations of monocytes. The phenotypic and functional heterogeneity of monocytes was further confirmed by gene array analysis of FACS-sorted subsets. Moreover, to evaluate the relative contribution of monocyte subsets to pathogenesis, we conducted a cross-sectional study of 45 uninfected control macaques and 54 rhesus macaques infected with either SIV or SHIV. Blood monocyte count and distribution of different monocyte subsets were analyzed in acute peak viremic macaques, chronically infected macaques with no detectable plasma virus

RNA, chronically infected macaques with detectable plasma virus RNA, and macaques that died with AIDS. Statistical analysis (Wilcoxon two-sample test, two-tailed $P < 0.05$) revealed that the absolute number of CD14highCD16low monocytes among different subsets was significantly high in acute peak viremic animals (median, 66 cells/microliter), infected animals with detectable plasma virus RNA (53 cells/microliter), and animals with terminal AIDS (96 cells/microliter), and not significantly different in animals with no detectable plasma virus RNA (33 cells/microliter) compared to uninfected controls (21 cells/microliter). Our study also demonstrates that there is a correlation of the number of total monocytes and CD14highCD16low monocytes with different disease stages (quantified by plasma viral load and CD4/CD8 ratio). Dynamic changes of blood monocytes, especially CD14highCD16low monocytes, are associated not only generally with lentiviral infection, but also specifically with the progression of the disease.

S.79

Hold me back: CXCL12 and CD8 T cell trafficking during West Nile virus encephalitis

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Recovery from viral encephalitis involves a delicate balance between successful viral clearance and the limitation of life-threatening immunopathology. However, the efficient migration of antiviral T cells into the central nervous system (CNS) during viral encephalitis is constrained, as most infiltrating leukocytes remain localized to perivascular spaces, unable to reach their parenchymal targets. Prior studies have suggested that this localization is regulated by the chemokine CXCL12, which is expressed along the basolateral surfaces of the CNS microvasculature. Using a murine model for encephalitis due to a neurovirulent strain of West Nile virus (WNV), we demonstrate that antagonism of the CXCL12 receptor CXCR4 improves antiviral T cell responses via enhanced intraparenchymal penetration of virus-specific CD8+ T cells within the CNS. Thus, CXCR4 antagonism led to significant decreases in CNS viral burden, glial cell activation and mortality during WNV encephalitis. In postexposure therapeutic trials in mice, administration of a CXCR4 antagonist was able to improve survival when given up to four days after WNV infection. These studies indicate that targeting CXCR4 improves outcome during WNV encephalitis and may also be a pan-antiviral therapeutic target.

S.80

Metabolic markers of neuronal injury correlate with SIV CNS disease severity and inoculum in the macaque model of NeuroAIDS

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The interrelation between the cellular changes relating to neuronal injury within the brain during neuroAIDS and the role of NAA/Cr as a metabolic marker of neuronal function remains unclear. In order to clarify the relationship between NAA/Cr and CNS disease severity, we utilized the SIV/macaque model of encephalitis. High-field 1H MRS was performed on extracted metabolites from frontal cortex samples of 29 rhesus macaques (6 healthy, 23 moribund with AIDS). Seventeen animals were classified as encephalitic, as determined by the presence of multinucleated giant cells (MNGCs), and further ranked as mild (n = 6), moderate (n = 4) or severe (n = 7) based on the quantity of MNGCs and size and frequency of perivascular lesions. Post-inoculation survival time ranged from

56 to 769 days with 15 animals designated as rapid progressors (RP, survival <200 days) and 8 normal progressors (NP, survival >200days). Histopathological determination of encephalitis severity was found to correlate with NAA/Cr levels. Macaques in any infected group exhibited significantly lower levels of NAA/Cr than uninfected age-matched controls (p < 0.0002 or smaller) ranging from -13% for animals with mild encephalitis (p = 0.01) to -26% for those with severe encephalitis (p = 0.000008) compared to healthy controls. To determine the effects of viral inoculum, MANCOVA of all metabolite ratios with inoculum and CNS disease severity was tested. NAA/Cr was significantly lower in animals infected with SIVmac251 than in those infected with SIVmac239 (p < 0.0003), even after controlling for the disease classification. Lastly, ANOVAs revealed significant differences in levels of NAA/Cr (p = 0.0004) with disease progression between controls and RP and controls and NP; however, although NAA was highest in controls and lowest in RP, there was no significant difference between RP and NP. These data support that some neuronal injury occurs in the absence of severe giant cell encephalitis, increasing CNS disease severity correlates inversely with decreasing NAA/Cr, and neuronal injury is more severe with SIVmac251 inoculum and possibly in rapid disease progressors.

Session 12: Late-breaking events in neurovirology and immunology: recent cutting-edge findings

S.81

Neuroimmunologic mediated disorders induced by non-cytolytic ribovirus persistent infection

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Chronic viral infections of the central nervous system (CNS) can affect neuronal function resulting in behavioral and cognitive impairment in the absence of the classical hallmarks of cytolysis and immune cell infiltrates within the brain parenchyma. This realization has led to the hypothesis that viruses may contribute to a variety of CNS disorders of unknown etiology, and thereby it is important to understand the mechanisms whereby non-cytolytic viruses can contribute to CNS dysfunction. To this end, the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) provides us with a superb model system. Mice with congenitally acquired LCMV persistent infection have impaired learning and memory, which are associated with altered host CNS gene expression in the absence of overt histopathological signs. Using gene expression profiling analysis we uncovered that LCMV persistence triggers chronic type I interferon responses within the host CNS. These host responses fail to clear the virus, but their sustained activation can however affect neuronal gene expression and contribute to abnormal CNS function. We have developed a reverse genetics system for LCMV that allow us to generate predetermined specific mutations within the virus genome and analyze their phenotypic expression in vivo. This provides us with a novel and powerful approach to investigate molecular and cellular mechanisms underlying non-cytolytic ribovirus persistence in the CNS and associated disorders.

S.82

MHC class I proteins in development and plasticity of the normal, uninfected brain part of symposium entitled proposed by M.B.A. Oldstone

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Members of the major histocompatibility complex class I (MHCI) are critical for adaptive immune function, where they permit recognition and destruction

of infected or cancerous cells by cytotoxic T lymphocytes. Although many adaptive immune responses are blunted in the brain, normal, healthy neurons can and do express MHCI mRNAs and proteins. mRNAs encoding specific classical and nonclassical MHCIs are expressed in distinct, overlapping patterns in the developing and adult brain, and MHCI expression is regulated by endogenous electrical signals in neurons. Furthermore, MHCI is unexpectedly required for the establishment and modification of precise, complex circuitry in the mammalian brain. In mice genetically deficient for most properly assembled cell surface MHCI (b2m^{-/-} or b2m^{-/-}TAP^{-/-}), the final, activity-dependent sculpting of developing visual system projections is disrupted. In addition, forms of hippocampal synaptic plasticity thought to underlie learning and memory are systematically altered in b2m^{-/-}TAP^{-/-} animals. This shift in synaptic plasticity is not secondary to immune compromise, since more severely immunocompromised (RAG1^{-/-}) mice are unaffected. Thus our findings demonstrate, for the first time, a role for MHC class I in non-pathological functions in the mammalian brain, specifically in the activity-dependent modification of synaptic structure and function.

S.83

Window on the diseased brain

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Meningitis is a potentially fatal disorder induced by a long list of human pathogens and is often associated with symptoms that include fever, headache, stiffness of the neck, and seizures. Presently, little can be done for patients with viral meningitis other than to relieve symptoms. We therefore propose that a detailed understanding of this pathogenic process in real time may foster the development of novel interventions to alleviate symptoms and prevent permanent neurological dysfunction/fatalities. Lymphocytic choriomeningitis virus (LCMV) is the prototypic member of the arenaviridae family and is particularly amenable to study because it is a murine as well as a human pathogen. Intracerebral inoculation of mice with LCMV Armstrong (Arm) induces fatal meningitis within 6 days that is mediated by cytotoxic lymphocytes (CTL). The precise mechanisms that mediate seizures and fatal injury during virus-induced

meningitis are not entirely understood. To gain advanced mechanistic insights into immune cell dynamics and function in the virally infected central nervous system (CNS), we examined disease pathogenesis in real time using intravital two photon microscopy. Our real time studies revealed highly dynamic immune cell activity in the meningeal space of LCMV-infected mice, which resulted in profound blood barrier breakdown and subsequent leakage of blood derived material into the subarachnoid space. Our studies also provide direct evidence that CTL divide within the CNS and that innate immune cells (e.g., microglia) respond rapidly to point sources of CNS damage caused by the inflammatory process. Thus, inflammatory reactions that unfold on the surface of the brain are amenable to study using real time imaging approaches. Moreover, examination and antagonism of immune cells through a “window on the brain” should rapidly advance our understanding of CNS viral pathogenesis.

S.84

Sickness syndrome: recent advances on interleukin 18

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Infections induce central and behavioral effects such as fever, loss of appetite, sleepiness, fatigue and withdrawal from social activity. This sickness syndrome is mediated by the action of cytokines on the central nervous system (CNS) and has been primarily investigated for interleukin 1 β (IL-1 β). The pro-inflammatory and pleiotropic cytokine interleukin-18 (IL-18) shares several similarities with IL-1 β and is expressed in both the periphery and the CNS. We recently found that IL-18 suppresses food intake in mice and modulated feed ef-

iciency and adiposity during adulthood. These effects were found in animals deficient in IL-18 (IL-18 $-/-$). Conversely, IL-18 administration suppresses food intake and weight regain in hungry mice when administered both centrally or systemically. Unlike IL-1 β , IL-18 did not influence core body temperature, suggesting that IL-18 may mediate loss of appetite in the absence of fever. IL-18 systems can influence of energy homeostasis and may be implicated in its physiologic or pathologic control during infection.

S.85

Early activation of an interferon signaling pathway in a mouse model of amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a late-onset motor neuron disease, currently without effective treatment and early diagnosis. Using expression profiling to study SOD1 mutant mice, a mouse model of ALS, we have identified a group of genes that were up-regulated at the region where motor neurons were degenerating. Among these genes, several interferon-stimulated genes were robustly induced in glial cells before the onset of disease symptoms. We have further shown that the activation of these genes was most likely stimulated by interferon Σ or σ through the type I interferon receptor. The response of glial cells to interferon was time- and dose-dependent, showing extreme sensitivity when tested in the in vitro system. Thus, we have revealed a novel signaling pathway between injured motor neurons and glial cells, which is activated in the pre-symptomatic stage of the disease. It warrants further investigation to determine whether this pathway represents a type of innate immune response in brain, which may be also activated in other types of neurodegenerative diseases.