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P1

Infection of primary mouse neural cells in vitro and ex vivo by wild type and vaccine strains of measles virus

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Murine models have been used to study the fatal CNS complications of measles virus (MV), subacute sclerosing panencephalitis and measles inclusion body encephalitis. Normal mice are only productively infected by rodent adapted virus, while IFNAR+/+ transgenic SLAM or CD46 mice, show restricted infection by wild type (WT) and vaccine (V) strains, respectively. Infection in SLAM or CD46 negative, IFNAR−/− mice allows a limited degree of infection with these viruses which suggests that restrictions other than cell entry are also involved. To examine virus entry and replication in the CNS of normal mice we compared infection, in vitro and ex vivo, of primary mixed neural cell cultures from C57/BL6 mice by WT, V and rodent adapted strains of MV. Infection of murine cerebral endothelial cells (Ec) was also examined. Cultures derived from 16 day foetuses were not susceptible to infection by any of the virus strains. However, all viruses infected specific neural cell subsets from 2 to 3 day old neonatal mice in vitro, although the infection with WT and V strains was non productive. Although negligible numbers of infected cells were observed in brain sections of WT and V infected mice, primary cultures derived from these brains and allowing whole brain sampling, showed infection. All 3 virus strains infected Ec but only WT virus gave a productive infection. The results suggest that murine receptors present on neonatal neural cells allow infection by WT and V strains in vivo but virus replication and spread is subsequently restricted by other factors.

P2

The cholesterol depleting drugs statin protect post-mitotically differentiated human neurons against ethanol and HIV-1-induced oxidative stress in vitro

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Background: Human immunodeficiency virus type I (HIV-1) infected monocytes and T-cells cross the blood-brain barrier (BBB) into the central nervous system (the 'Trojan Horse' pathway) to spread the infection or triggers infection related responses that hamper proper synaptic activity of neurons. Among several others, alcohol has been reported as a major factor in pathogenesis of HIV-1 due to its easy metabolic and systemic availability and the potential to cross the BBB. Because a majority of HIV-1-infected individuals are either alcoholics or prone to alcoholism, we sought to determine stress responses induced by the interaction of HIV-1-infected primary cells (CD14+ monocytes, CD3+ T-cells) with postmitotically differentiated neurons in vitro. We also determined whether or not the cholesterol depleting drugs Statin protect these cells against ethanol and HIV-1-induced oxidative stress.

Methods: We utilized either uninfected controls or co-culture of neurons with supernatant generated from HIV-1-infected versus uninfected neuronal cells. Using ELISA and Western Blotting analyses, we determined the release of Hsp70, 8-iso-Prostane-F2-alpha, and nitric oxide, well-characterized oxidative stress markers from the supernatants of primary post-mitotic differentiated neurons. The statins, atorvastatin and simvastatin were added at concentrations of 10 mM when needed.

Results: It was observed that co-culture of HIV-1-infected primary monocytes over a time period of 72 hours elevated the production of heat shock protein 70 (HSP70), 8-iso-Prostane F2-alpha, and nitric oxide 4–7 fold compared with uninfected controls. The presence of 0.1% ethanol (physiological concentrations in plasma) showed similar responses, however, 0.3% EtOH alone elevated the levels of hsp70 in uninfected controls but had no significant effects on isoprostane production in this group. Neurons exposed to supernatants generated from HIV-1-infected monocytes showed slight elevation of these oxidative stress markers compared with control. Co-culture of infected T-cells with neurons or neurons exposed to HIV-1-infected primary CD4+ T-cells supernatant showed similar elevation of HSP70 responses as well as 8-isoprostaglandin-F2-alpha and nitric oxide 4-5 fold. We also tested the ability of the clinically available inhibitors of HMG-CoA reductase, statins, to inhibit HIV-1 induced release of these compounds from HIV-1-infected cells. We observed that both atorvastatin and simvastatin were able to inhibit the release of HSP70, nitric oxide and 8-isoprostaglandin-F2-alpha.

Conclusion: The results of this study provide new insights into HIV-1 neuropathogenesis aimed at the development of future HIV-1 therapeutics to eradicate viral reservoirs from the brain.

P3

Use of human antigen-presenting cell gene array profiling to examine the effect of human T cell leukemia virus type 1 Tax on primary human dendritic cells

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Human T cell leukemia virus type 1 (HTLV-1) is etiologically linked to adult T cell leukemia and a progressive demyelinating disorder referred to as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). One of the most striking features of the immune response in HAM/TSP centers on the expansion of HTLV-1-specific CD8+ cytotoxic T lymphocyte (CTL) compartment in the peripheral blood and cerebrospinal fluid. More than 90% of the HTLV-1-specific CTLs are directed against the viral Tax (11-19) peptide implying that Tax is available for immune recognition by antigen presenting cells, such as dendritic cells (DCs). DCs obtained from HAM/TSP patients have been shown to be infected with HTLV-1 and exhibit rapid maturation. Therefore, we hypothesized that presentation of Tax peptides by activated DCs to naïve CD8+ T cells may play an important role in the induction of a Tax-specific CTL response and neurologic dysfunction. In this study, a pathway-specific antigen presenting cell gene array was used to study transcriptional changes induced by exposure of monocyte-derived DCs to extracellular HTLV-1 Tax protein. Approximately 100 genes were differentially expressed including genes encoding toll-like receptors, cell surface receptors, proteins involved in antigen uptake and presentation and adhesion molecules. The differential regulation of chemokines and cytokines characteristic of functional DC activation was also observed by the gene array analyses. Furthermore, the expression pattern of signal transduction genes was also significantly altered. These results have suggested that Tax-mediated DC gene regulation might play a critical role in cellular activation and the mechanisms resulting in HTLV-1-induced disease.

P4

HSV-1 ICP4 deleted, Us3 defective mutant d120 infection failed to induce apoptosis in NGF-differentiated PC12 cells

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It has been suggested that neuronal cells respond differently in many aspects to the HSV-1 infection as compared to mitotic cells. For instance, the HSV-1 infection establishes a latent infection in neuronal cells but a lytic infection in mitotic cells. HSV-1, ICP4-deleted, Us3-defected, mutant d120 has been known to induce classical apoptosis in a variety of mitotic cell lines that have been tested. Here we report that the d120 mutant virus failed to induce apoptosis in neuronal-like, nerve growth factor (NGF) differentiated PC12 cells. More strikingly, the genome of the d120 mutant remained linear inside of the nuclei for the course of the infection, with a surprisingly long half-life of 30 days. This contrasted with the half-life of linear DNA inside of the nuclei of mitotic cells which is less than 2–3 days. The failure of the mutant d120 virus to induce apoptosis in neuronal-like cells might be due to its inability to translocate the NF-kB from the cytosol to the nuclei after viral infection. Furthermore, the mutant d120 virus was able to prolong the life of neuronal-like cells in the culture flask as compared to the mock-infected NGF-PC12 cells. This was suggested since approximately 50% of d120-infected NGF-PC12 cells remained in the culture after 45 days of infection while less than 10% of mock-infected cells were left in the culture after 45 days of infection as compared to the amount of cells at the time of infection. The possible mechanisms regarding this distinct difference in the outcome of host cells (neuronal-like NGF-PC12 cells vs the mitotic Vero cells) after mutant d120 infection are discussed.

P5

Phosphatidylinositol-3 kinase (PI3K)/Akt/mTOR pathway is activated in an in vitro model of HIV Encephalitis

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HIV Encephalitis (HIVE), the pathological substrate of HIV Associated Dementia (HAD), is characterized by formation of multinucleated giant cells, astrogliosis, neuronal loss and dendritic damage. While the mechanisms of neuronal death in HIVE remain only partially defined, neuronal damage has been linked to soluble factors released by HIV infected, and non-infected, activated macrophages/microglia in the brain. Among these factors, glutamate is of particular interest. Glutamate plays an important role in long term memory formation and neuronal plasticity as well as neurodegeneration. Glutamate excitotoxicity, triggered by excess glutamate is thought to be a component of neuronal death in a range of neurological disorders including Multiple Sclerosis (MS), Alzheimer's Disease (AD), Parkinson's Disease (PD), Amylotropic Lateral Sclerosis (ALS) and HIVE. In HIVE, other excitotoxins in addition to glutamate are thought to contribute

to neuronal damage. To begin to define the signaling pathways involved in HIVE-associated excitotoxicity, we used an in vitro model of HIVE where we treated primary rat neuroglial cultures with supernatants from human monocyte-derived-macrophages infected with a neurovirulent strain of HIV-1 (HIV M/M). We assessed phosphorylation of a number of signaling cascade molecules. Using a supernatant dilution of HIV M/M that produced 25% neuronal loss by 4 hours and 50% neuronal loss by 20 hours, we observed 70% increase in mTOR, 50% increase in Erk-1 and 105% increase in Erk-2 levels by 2 hours, as detected by western blotting. An early, rapid phosphorylation of Akt-1 (152% by 4 hours) as well as a late increase in S6K activity (52% by 20 hours) was also observed. NMDA exposure also induced these changes in Akt-1 and S6K2 by 4 hours. In contrast, BDNF treatment induced Akt-1 phosphorylation by 20 hours, but no S6K activity. Recently, increased mTOR immunoreactivity in autopsied human brain tissue of patients with HAD has been substantiated. Thus, we hypothesize that our in vitro model of HIVE reflects the findings of HIVE in vivo, and we propose that activation of the phosphatidylinositol-3 kinase (PI3K)/Akt/mTOR pathway may be involved in the excitotoxic injury in HIVE.

P6

PML presenting as dementia and diffuse leukoencephalopathy in non-immunosuppressed hosts

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Progressive multifocal leukoencephalopathy (PML) is an opportunistic infection of oligodendrocytes by JC virus usually occurring in immunosuppressed hosts. Rarely, PML has been associated with no identifiable underlying immunosuppressive illness.

We report two middle-aged men presenting with chronic cognitive disturbance classifiable as dementia and diffuse leukoencephalopathy on MR imaging without identified underlying immunosuppression. In a 63-year-old man, the four month history of dementia was associated with diffuse leukoencephalopathy and negative PCR for JC virus in the spinal fluid. Although brain biopsy failed to demonstrate PML on morphological grounds, autopsy findings seven months after onset were diagnostic of PML. A variety of leukoencephalopathies were considered in life, but not PML. In the second case, a 57-year-old man presented with ten months of progressive cognitive changes, language difficulty, and slow thinking without focal motor or sensory symptoms. MRI showed multifocal subcortical white matter abnormalities. Spinal fluid was positive for JC virus by PCR. Further testing revealed serologic markers of mixed connective tissue disease and depression of CD4 and CD8 counts.

The first patient had autopsy proof of PML but no identified underlying immunosuppression or systemic illness, even at autopsy. Both patients had evidence of nonmalignant colonic ulceration of uncertain cause.

PML can present as a dementing illness with a diffuse leukoencephalopathy without overt immunosuppression. PCR is incompletely sensitive for JC virus detection in the spinal fluid. PML can be considered in the differential diagnosis of late onset diffuse leukoencephalopathy.

P7

Transcriptional competency of HIV-1 LTR genetic variants in an integrated chromatin-based environment

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Human immunodeficiency virus type 1 (HIV-1) gene expression is driven by the long terminal repeat (LTR), which has a variety of binding sites for the interaction with multiple host and viral factors, including members of the CCAAT/enhancer binding protein (C/EBP) and Sp transcription factor families. We have previously identified specific nucleotide sequence configurations within C/EBP site I (C to T change at position 3; 3T) and Sp site III (C to T change at position 5; 5T) which correlate with increased severity of HIV-1 disease and HIV-1-associated dementia. As one approach to begin exploring the LTR phenotype associated with these genotypic changes with respect to LTR-directed transcription from an integrated chromatin-based microenvironment, a series of stably transfected cell lines have been developed utilizing bone marrow progenitor (TF-1), T (Jurkat), and monocytic cell lines (U-937). To this end, macrophage-, T cell-, and dual-tropic LTRs were coupled to the gene encoding green fluorescent protein (GFP). The resulting HIV-1 LTR GFP stably expressing cell lines were examined under basal, and chemical or cytokine activation, as well as in the presence of HIV-1 Tat. The results demonstrate that the cell type within which these integrated LTRs were expressed produced different expression profiles. In TF-1 cells the 3T/5T-containing LTR drives transcription in all cells but with a lower expressing phenotype, however it can be induced to similar levels as the parental LTR, following stimulation and/or in the presence of Tat. In addition, the backbone, within which this sequence configuration is incorporated, also plays a role in the resulting phenotype. Future studies will examine the impact of specific LTR sequence variation on viral gene expression

both in primary cells as well as in viral replication studies.

P8

Effects of valproic acid on markers of HIV disease progression

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Background: We evaluated the possible beneficial or adverse effects of concurrent treatment with antiretroviral (ARV) and valproic acid (VPA) treatment on markers of HIV disease progression—HIV viral load (VL) in plasma and cerebral spinal fluid (CSF), and CD4 lymphocyte counts. HIV-infected patients often receive concurrent treatment with VPA due to comorbidities such as seizures, headaches, and mood disorders. In vitro, VPA activates expression of latent HIV provirus and may eliminate latently infected, HIV reservoir in resting CD4 cells. However, activation of latent provirus might accelerate HIV replication or deplete CD4 cells, both of which could be clinically deleterious.

Methods: We performed a retrospective case-control study to identify 30 HIV+ individuals, half of whom who initiated VPA. For these two groups approximately half of each group took stable antiretroviral therapy. Cases and controls were matched for ARVs, CDC stage, sex, education, and time period between measurements. Using commercially available assays CSF VL, plasma VL, and CD4 lymphocyte counts were assessed prior to and after starting a stable VPA regimen for both groups. To determine if differences were present simple paired t-tests were performed for each variable ($p < 0.05$) across both groups. Subset analysis was performed to determine the interaction between VPA and ARVs.

Results: The median duration between visits was 14 $\pm$ 3 months for both groups. A trend towards a decrease in plasma CD4+ lymphocyte counts (from 420 cell/uL to 331 cells/uL) was observed in the group initiating VPA compared to matched controls. Similar results were seen for patients on ARVs compared to those not taking medications. No differences were seen in plasma or CSF VL within the two groups or within the subsets on or off ARVs.

Conclusions: With addition of VPA, we found no significant adverse effects on HIV VL in plasma or CSF. A trend towards a decrease in CD4 lymphocyte counts was observed after starting VPA. This decline may reflect the loss of latently infected lymphocytes or the effects on this medication on the bone marrow. Whether these CD4 reductions may reduce immunocompetence and translate to significant clinical disease progression is not yet determined.

P9

HSV-infected microglial cells undergo apoptosis following production of proinflammatory immune mediators

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Herpes simplex virus (HSV)-1 efficiently infects glial cells (i.e., microglia and astrocytes) as well as neurons. While microglial cells are non-permissive for viral replication, productive infection is observed in both astrocytes and neurons. Microglial cells respond to HSV by rapidly initiating a burst of proinflammatory cytokine and chemokine production during the early stages of infection. In contrast, astrocytes and neurons were not found to produce these same immune mediators, although viral replication is productive in these cell types. In the present study, we investigated the effect of HSV infection on cell survival immediately following the rapid induction of immune mediators. Preliminary studies using real-time PCR showed that mRNAs for caspases 3 and 9 were induced in HSV-infected microglia, but no expression of pro-apoptotic genes was seen in astrocytes. Further studies using microarray analysis indicated that the TNF-α signaling pathway was active in microglial cells at a time when most cellular mRNAs, including those of housekeeping genes and caspases, were not present in astrocytes. Taken together, these findings indicate that HSV infection leads to degradation of cellular mRNA in productively-infected astrocytes, while cellular mRNAs, including those encoded by genes involved in apoptotic cell death, were not shut off during non-productive infection of microglia.

P10

When does the HI-Virus entry into the human brain achieve clinical relevance?

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Objectives: During the last years knowledge on HIV effects on the central nervous system (CNS) in vitro and in animal models has grown considerably. However, less information is available on the course of virus related CNS disease in vivo. Recent findings provide evidence that HAART should also be aimed at

suppression of viral load in cerebrospinal fluid (CSF) but CSF analysis has not yet been integrated in therapy decision and patient care guidelines. Consequently, whereas it is known that HIV enters the brain very early in the course of the disease, it remains to be established, when viral replication in the CNS achieves clinical relevance for the individual patient. Thus, the present study has been designed to further clarify this issue.

Methods: 111 HIV-1-positive individuals without apparent neurological abnormalities (27 from early CDC stages = A1, A2, B1, B2 without highly active antiretroviral therapy (HAART), 29 from early stages with HAART, 5 from late stages = A3, B3, C1-3 without HAART and 50 AIDS-defined individuals on HAART) have been recruited consequently at three study sites. They underwent clinical examination, a neuropsychological test battery, magnetic resonance imaging (MRI) as well as viral load (VL) analysis in plasma and CSF.

Results: There was a low viral replication in CSF accompanied by neuropsychological deficits in some of the early and late stage patients on HAART underlining that this therapy is not in every case effective in protecting the CNS against HIV. In contrast, both early and late stage patients without therapy revealed very high CSF VLs in the majority of the cases. CSF-VLs correlated with the duration of known HIV-1-positivity and with psychomotor slowing. Surprisingly, the majority of patients without HAART had no hints for blood-brain-barrier (BBB) disruption, but relevant CSF pleocytosis.

16.1%/111 patients had a higher CSF than plasma VL, with higher prevalence among untreated patients. In 19% of the patients with complete suppression of viral replication in the periphery by HAART limited viral replication could be detected in the CSF.

Conclusions: HIV does not only invade the human brain very early, but replicates there actively and provokes neuropsychological deficits. CSF analysis has to be integrated into routine care for HIV carriers and therapy planning. It has to be investigated, whether patients with higher CSF- than plasma-VL will have to start effective HAART earlier. Early and not late stages of the disease are relevant for impending brain damage.

P11

Expression of APOBEC family members of cytidine deaminases in the central nervous system

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Background: In the human genome the apolipoprotein B mRNA-editing enzyme catalytic polypeptide (APOBEC)3 gene has expanded into a tandem array of genes termed APOBEC3A-G. Two members of this family, APOBEC3G and APOBEC3F, have been found to have potent activity against virion infectivity factor deficient (DeltaVif) human immunodeficiency virus 1 (HIV-1). These enzymes become encapsidated in DeltaVif HIV-1 virions and in the next round of infection deaminate the newly synthesized reverse transcripts. The lentiviral Vif protein prevents the deamination by inducing the degradation of APOBEC3G and APOBEC3F. The expression and distribution of APOBEC family members of cytidine deaminases in the central nervous system (CNS) is currently unknown.

Methods: In the current study we isolated RNA from primary human brain microvascular endothelial cells (MVECs), and primary human astrocytes, which are key components of the blood-brain barrier (BBB) and the CNS. Reverse transcriptase (RT)-PCR was employed to detect the presence of APOBEC-3A/3B/3C/3D/3F and 3G mRNA, and cell lysates were analyzed via Western blot using monoclonal and polyclonal antibodies against APOBEC3G and APOBEC3F. In our studies we also included human NT2 neuronal precursor and differentiated post-mitotic mature neuronal cells, as well as H9 (positive control) and 293T (negative control) cells.

Results: Our RT-PCR assays demonstrate that APOBEC-3B/3C/3F and -3G mRNA is present in all CNS cell systems, namely MVECS, astrocytes, NT2 and differentiated post-mitotic mature neurons. In addition, Western blot analysis of lysates prepared from the above cells clearly demonstrates APOBEC-3G and APOBEC-3F protein expression in all CNS-cell components.

Conclusions: Our findings demonstrate that both APOBEC-3G and -3F host restriction factors known to block HIV-1 replication, are widely expressed in CNS and may function as innate immunity against retroviral infection.

P12

Effect of cannabinoids on astrocytes: A proteomic analysis

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Delta(9)-THC, the main active component of marijuana and other synthetic cannabinoid receptor agonists, is known to dysregulate various immune responses. CB1 and CB2 receptors have been expressed mainly on neuronal and immune cells respectively. Since brain being the target organ for cannabinoids, and neuronal cells play a significant role in various immune responses,

it is reasonable to suggest that cannabinoids play a significant role in the molecular profile of neuronal cells. The present study has been undertaken to investigate the proteomic profile of astrocytes treated with delta(9)-THC by 2-Dimensional Difference Gel Electrophoresis (DIGE) and HPLC—MS/MS analysis. Our results show that more than 20 proteins were differentially dysregulated by astrocytes treated with delta(9)-THC compared to untreated control. Expressions of most of the proteins were confirmed by Q RT-PCR at mRNA levels and western analysis at protein levels. Among the identified proteins, Glutathione Peroxidase was highly upregulated and the significance of other dysregulated proteins with respect to HIV- 1 infection of astrocytes and their role in neuropathogenesis will be discussed.

P13

Axonal transport of human immunodeficiency virus-1 envelope protein GP120 causes neuronal apoptosis

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A better understanding of the pathogenic mechanisms mediating HIV-1 neurotoxicity is crucial for developing effective neuroprotective therapies against Acquired Immune Deficiency Syndrome-associated Dementia Complex (ADC), a disorder characterized by a broad spectrum of motor impairments and cognitive deficits. The HIV-1 envelope glycoprotein 120 (gp120), which is shed from the virus, is one of the agents causing neuronal cell death. In this study, we examined the molecular and cellular mechanisms underlying its neurotoxic effect. In cerebellar granule cells gp120 is internalized and axonally transported. Blocking intracellular trafficking by nocodazole prevented neuronal cell death. To further prove that internalization and axonal transport are crucial for gp120-mediated toxicity, gp120 was injected into the rat striatum. Gp120 was sequestered by neurons and subsequently retrogradely transported to dopaminergic neurons in the portion of the substantia nigra that projects to the striatum. Cleaved caspase-3 and TUNEL, hallmarks of apoptosis, were seen in neurons internalizing and transporting gp120. Furthermore, colchicine abolished gp120-mediated apoptosis. The retrograde transport of gp120 and apoptosis were mediated by the chemokine receptor CXCR4 because AMD3100, a selective CXCR4 inhibitor, blocked both events. These results indicate that axonal transport of gp120 is a crucial mechanism whereby HIV-1 promotes widespread neuronal cell death.

P14

Associative and predictive biomarkers of changing cognitive status in HIV-1 infected patients

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Infection with HIV can result in a debilitating CNS disorder known as HIV-associated cognitive-motor impairment or HIV-dementia (HIV-D). Since the advent of highly active antiretroviral therapy (HAART), the incidence of HIV-D has declined but the prevalence continues to increase. In general, the cognitive manifestations of HIV-D are less severe and the course of HIV-D appears to be more variable. Traditional biomarkers such as CSF viral load and MCP-1 levels are less likely to be associated with dementia in patients on HAART, and other biomarkers that can predict HIV-D have not yet been identified. In order to identify associative and predictive biomarkers of HIV-D we differentially grouped HIV-infected patients from the North Eastern AIDS Dementia (NEAD) cohort based on changes in cognitive status over a 1 year time period and analyzed sphingolipid, sterol, triglyceride, antioxidant and lipid peroxidation levels in CSF. We found that increases of the antioxidant vitamin E and triglyceride C52 predicted the onset or worsening of dementia while the catabolism of sphingomyelin to ceramide and the accumulation of 4-hydroxynonenals were associated with an actively progressing dementia. We interpret these findings to indicate that early in the pathogenesis of HIV-D there is an up-regulation of endogenous antioxidant defenses in brain. The failure of this attempted neuroprotective mechanism leads to the accumulation of sphingomyelin and moderate neuronal dysfunction that manifests as an inactive dementia. The breakdown of this enlarged pool of sphingomyelin to ceramide and the accumulation of highly reactive aldehydes is associated with neuronal degeneration and an active dementia. Thus, increased levels of endogenous antioxidants in CSF may identify HIV-infected patients who are at higher risk for the development or progression of dementia.

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P15

Modeling HIV-1 infection of differentiating populations of CD34+ progenitor cells

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Previous studies have suggested that the bone marrow compartment may play an integral role in the pathologic events associated with HIV-1 dementia (HIVD). Interestingly, CD34+/CD38- progenitor cells within the bone marrow are refractile to HIV-1 infection, possibly due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5, which upon differentiation are upregulated, potentially increasing susceptibility to infection. The CD34+/CD38+ TF-1 bone marrow progenitor cell line was selected as a model to study HIV-1 infection during the differentiation of hematopoietic progenitor cells. TF-1 cells were treated with a number of metabolic activators including PMA, conditioned media from PMA-treated cells, as well as cytokines such as GM-CSF, M-CSF, IL-1beta, TNF-alpha, IL-4 and their maturation was monitored through their expression of surface markers by flow cytometry. Interestingly, IL-1beta, alone or in combination with TNF-alpha leads to CXCR4 and CCR5 upregulation and preservation of CD4 expression providing a window of opportunity for HIV-1 infection to occur. Moreover, transient and stable transfection analysis demonstrated that the HIV-1 LTR activity was significantly increased following treatment of TF-1 cells with IL-1beta and conditioned media. These results indicate that progenitor cell differentiation potentially increases HIV-1 susceptibility and leads to altered LTR activity, viral transcription, and possibly productive replication.

P16

Post-HAART HIV infection of the brain—A new disease

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Post-HAART HIV Infection of the Brain—A New Disease Bell JE, Anthony IC, McCrossan M, Willey, S, Simmonds P

The dynamics of HIV infection of the brain in the pre-HAART era were studied in the context of a systemic immune system in terminal free fall. Now that compliance with HAART arrests that immune collapse, a different balance is created in the brain between viral replication on the one hand, and partial systemic/CNS immune control on the other. The challenge posed to the neuroAIDS community is to determine how this precarious balance is maintained and what the predictions might be for the future. We have shown previously that neuroinflammation persists in HAART treated individuals and that premature hyperphosphorylated Tau deposition also occurs.

The closest comparison group for HAART-treated subjects from the pre-HAART era might be presymptomatic HIV infected individual dying from non-HIV related causes. Our recent studies comparing these two groups show that brain proviral load is generally low in both (<60 proviral copies per 106 cells). The HIV brain population is frequently genetically distinct from viral variants isolated from lymphoid tissues and shows varying CD4 affinities which may reflect neuroadaptation. A direct relationship is present between the brain proviral load and the extent of CD8 infiltration but no similar relationship is detected in lymphoid tissue. CD8 lymphocyte infiltration in the brain is also significantly associated with macrophage activation. We consider it likely that HAART protects against the development of HIV encephalitis by inducing a moderate rise in lymphocyte influx to the brain. However the role of vigorous microglia/macrophage responses is still unclear in this scenario and taken together with other accessory factors such as illicit drug intake, hepatitis induced encephalopathy and possible toxic effects of HAART itself, suggests that neurodegenerative features (Tau deposition) are part of a pattern of novel CNS pathology emerging in chronic HIV infection.

P17

JCV T-antigen stabilization of beta-Catenin at the cell surface requires small GTPase Rac

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Interaction between various signaling pathways regulates wide variety of cellular responses. Perturbation of one or more of inter connected pathways may promote uncontrolled cellular processes leading to abnormal cell growth and diseases. Role of viral oncoprotein from human neurotropic polyoma viruses JCV have gained considerable attention in recent years due to its association with several human CNS tumors, including medulloblastomas and broad range of glial-origin tumors. Most importantly viral T-antigen (T-Ag) has been shown to possess the ability to transform cells of neuronal origins. T-Ag has been shown to interact with a wide variety of cellular proteins with varied functions. Two of the most recently observed interacting proteins of T-Ag are oncogenic beta-Catenin and tumor suppressor Merlin (NF-2). beta-Catenin and NF2 show diverse subcellular localization. NF2 is an ERM-related protein and has significant cytoskeletal-binding property. Similarly beta-Catenin

was first identified as a binding partner of membrane bound E-cadherin. beta-Catenin has gained enormous interest due to its ability to promote gene transcription leading to oncogenesis. It is a downstream effector of Wnt-signaling pathway. Level of beta-Catenin is kept low through APC and GSK3beta mediated phosphorylation and subsequent degradation. Mutation in APC or GSK3beta promotes beta-Catenin stabilization and translocation to the nucleus to promote gene transcription. T-Ag of JCV has been found to bind and stabilize beta-Catenin. Stabilization of beta-Catenin is an important step for its transcription activity. We observed for the first time that T-Ag positive mouse medulloblastoma BsB7 cells show significant localization of beta-Catenin at the cell surface compared to T-Ag negative Bs1b cells. We further observed that membrane localization of beta-Catenin in BsB7 cells is abrogated to a large extent when cells were expressed with dominant negative(DN) RacGTPase, RacN17. DN mutant of closely related GTPase, RhoN19, has no effect on the membrane staining of beta-Catenin in BsB7 cells. Oncogenic G-proteins, Galpha12/13 and Galphaq have been shown to promote small GTPases Rho, Rac and Cdc42. Overexpression of constitutively active Galpha12/13QL and GalphaqQL shows strong staining of endogenous beta-Catenin at the cell surface in BsB7 cells. As expected, DN Rac but not Rho promoted T-Ag induced beta-Catenin activity at the nucleus, as measured by TOP-Flash reporter assay in U-87MG cells. Galpha12/13QL and GalphaqQL completely abrogated T-Ag mediated TOP-Flash activity in agreement with subcellular localization of beta-Catenin.

Finally, the above observations provide us the first evidence that stabilization of beta-Catenin by JCV T-Ag occurs, in part, at the cell surface via GTPase Rac. Further study to understand how membrane associated stable beta-Catenin transduces nuclear signaling during T-Ag mediated cellular transformation, will provide valuable insight for effective therapeutic strategy.

Supported by grant from NIH to KK.

P18

Gestational age determines the outcome of congenital LCM virus infection in humans: confirmation in an animal model

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Lymphocytic choriomeningitis virus (LCMV) can severely damage the human fetal brain. We studied 20 cases of human congenital LCMV and found a wide range of pathology and outcomes among infected infants. We hypothesized that gestational age affects the pathogenesis of LCMV. We tested this hypothesis by infecting neonatal rats on postnatal day (PD) 1,4,6,10,21,30 or 60. Viral titers and pattern of infection were determined on post-inoculation day (PI)14, and neuropathology on PI 25. The pattern of infection depended strongly on the age of the animal. Neurons and glial cells that were readily infectable at one age were no longer infectable at another. For example, cerebral cortical neurons and hippocampal pyramidal cells were readily infectable on PD1, but were no longer targets of infection by PD4. Peak viral titers also changed dramatically based on the age of the host at the time of infection. Over the course of several days, brain infectability fell multiple orders of magnitude. The severity and nature of neuropathology also depended strongly on age. Infection at certain developmental ages interfered with neurogenesis and produced focal brain hypoplasia, while infection at other ages led to an acute inflammatory response and caused tissue destruction. Brain pathology was a function of viral titer and CD8+ T-cell infiltration, both of which varied among brain regions and depended on host age. All of the various pathologic changes in humans with congenital LCMV infection were reproduced in the rat model by altering the age of the animal at the time of infection. Thus, the variation in outcome in human congenital LCMV infection reflects differences in gestational age at the time of infection.

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P19

HIV-1 infection of astrocytic progenitor cells

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Potential targets for HIV in the central nervous system include multipotent neural stem cells that can give rise to astrocytes, oligodendrocytes and neurons. The infection of these cells is poorly understood.

The aim of our study was to determine whether human neural stem cell populations are capable of long-term HIV infection and thus may serve as HIV reservoirs.

We used a human neural progenitor cell line (HNPC) as a cell culture model for multipotent self-renewing neural stem cells populations. Growth-factor withdrawal and CNTF-treatment induced targeted differentiation of these cells to astrocytes.

In infection studies with HIV-IIIB we demonstrate that within the first two weeks of HIV exposure proliferating progenitor cells show continued moderate virus production, whereas HNPC-derived astrocytes stopped producing HIV. Proliferating progenitor cells sustained HIV infection for a long time period (>4 months) while retaining the capacity to differentiate into astrocytes. Differentiation of infected progenitor cells to astrocytes led to a transient burst of HIV production, followed by renewed restriction of HIV production.

The HIV regulatory factor Rev showed increased cytoplasmic accumulation and diminished activity in HNPC-derived astrocytes, compared to progenitor cells. This suggests that differentiation of progenitor cells to astrocytes influences HIV functions. Our results suggest that HIV may chronically infect human neural stem cell populations, which may serve as reservoirs and disseminators of HIV in the brain.

P20

The national NeuroAIDS tissue consortium

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The National NeuroAIDS Tissue Consortium (NNTC) established in 1998 is a unique, NIH-supported multisite repository that collects and distributes well-characterized antemortem and postmortem tissue specimens with clinical and serological data from HIV-infected individuals, thereby facilitating neuroAIDS research. The NNTC collects, stores, and distributes samples from nervous system (brain, spinal cord, peripheral nerve, CSF), muscle, liver, blood, and other tissues from HIV-infected individuals. Neuropsychological (e.g., cognitive testing), neuromedical (e.g., neurological deficits, history of ART use), neuropathological and laboratory (e.g., viral loads, CD4 counts) data are also provided. To date 538 post-mortem cases have been placed in repository and antemortem data exists on 336 cases. Participating sites include: The Manhattan HIV Brain Bank, NYC (S. Morgello, PI); Texas Center for AIDS Neuropathogenesis Research, Galveston (B. Gelman, PI); California NeuroAIDS Tissue Network, San Diego (I. Grant, PI); and The National Neurological AIDS Bank, Los Angeles, (E. Singer, PI). Brain tissues from specified anatomic sites are provided as either embedded, fixed sections or as freshly frozen blocks. Plasma and PBMCs are also available. All specimens and associated data are available to qualified investigators at no charge (except shipping) upon approval of his/her application (www.nntc.org). NNTC specimens may also be accessed to investigate non-neurological HIV processes as appropriate. Consultative services for tissue utilization are available upon request. Recent studies by NNTC site-affiliated investigators utilizing these resources address HIV-associated channelopathies, Hep C co-infection, PML and neurocognitive impairment. This unique and comprehensive resource is thus designed to enable individual researchers to more fully develop timely investigations addressing the natural history, the neuropathogenesis, and the clinical consequence of CNS HIV infection.

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P21

Minocycline protects macaques from SIV-Encephalitis and is immunomodulatory in SIV-infected macaques

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World Health Organization figures indicate that in 2003 over 40 million adults and children were infected with HIV globally. Current anti-AIDS therapies are expensive, require complex dosing regimens with significant side effects and toxicity and do not readily cross the blood brain barrier. We recently demonstrated that minocycline, a safe, readily available, inexpensive, semisynthetic tetracycline derivative prevented the development of SIV encephalitis in macaques. Minocycline-treated macaques had lower levels of viral RNA in the brain and lower numbers of lymphocytes and macrophages infiltrating the brain. These findings prompted us to examine the potential effects of minocycline on the immune system of SIV-infected macaques, and in particular, the potential effects of minocycline on T lymphocyte proliferation, function, and homing capabilities in macaque primary blood lymphocytes in vitro. Our studies demonstrated that minocycline inhibited T cell proliferation and activation, down-regulated CCR5 expression, reduced cell surface LFA-1 expression and reduced loss of CD45RA after SEB stimulation. Other in vitro studies have suggested that minocycline decreased production of certain cytokines, including IL-2, IFN-(gamma), and TNF-(alpha). These findings suggest that the therapeutic effect of minocycline is likely associated with down-regulation of CD4+ T cell turnover and decreasing susceptibility of CD4+ T cells to infection by SIV.

P22

Cocaine: A co-factor for HIV-Dementia

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While HIV infection is the leading cause of death among Americans 25–44 years old, injection drug use now accounts for about one-third of all new US AIDS cases reported each year. Cocaine, often abused by HIV-infected patients, has been suggested to worsen the HIV-associated dementia (HAD) via unknown mechanisms. The brain is a target organ for both, cocaine and HIV-1.

The goal of the present study was to explore the effect of cocaine on HIV-1 replication in

monocyte-derived macrophages (MDM) and in the chronically infected promonocytic cell line (U1). Cocaine enhanced virus production in both U1 cells and in HIV-1-infected MDMs and this effect was at the level of transcriptional activation of HIV-1. Analyses of chemokines/cytokines in cocaine-treated macrophages by Real-Time RT-PCR and Luminex assays suggested increased expression of CXCL10 & CCR2 and the cytokine, IL-10, all of which are known to promote HIV-1 replication in MDMs. In addition, cocaine also caused an upregulation of macrophage activation markers, HLA-DR and CD40 and the viral coreceptor CXCR4. Thus the synergistic effects of cocaine on virus replication and cytokine/chemokine regulation suggest that cocaine operates at multiple pathways to aid in acceleration of HAD.

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P23

The immunosuppressive activity of MLD-disintegrins as inhibitors of alfa4beta1 integrin-dependent invasion of T-lymphocytes in chronic inflammation

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The chronic inflammatory disease such as multiple sclerosis (MS) is characterized by the infiltration of central nervous system (CNS) through activated T-cells and macrophages. The alfa4beta1 integrin that is expressed on leukocyte is essential for transmigration of these immune cells by blood brain barrier (BBB) and became a pharmaceutical target for modern therapy of MS. Recently, humanized monoclonal antibody (natalizumab, Tysabri, Elan and Biogen Inc.) against alfa4 subunit of integrin was introduced to the therapy of MS and Crohn's disease, showing significant reduction of risk of the sustained progression of disability in MS patients. However, treatment with natalizumab is associated with the possibility of activation JC virus (JCV) leading to development of progressive multifocal leukoencephalopathy (PML). In presented work we propose alternative approach to blocking of alfa4beta1 integrin by monoclonal antibody, using biologically active peptides isolated from snake venom. These low molecular weight compounds belong to disintegrin family and in the integrin binding site they contain MLD sequence. We performed series of in vitro experiments showing effectiveness of MLD-disintegrins in blocking of alfa4beta1 integrin. These disintegrins potently inhibited binding of purified receptor to its endogenous ligand VCAM-1 in ELISA assay, and adhesion of cells expressing this integrin to immobilized VCAM-1. Moreover, they were also effective in blocking of interaction of human cultured T-lymphocytes (Jurkat cell line) with primary endothelial cells. That interaction is fundamental for trafficking of leukocytes through the vessel wall during an immune response, which occurs following simulation of endothelial cells by pro-inflammatory cytokines. This stimulation leads to expression of VCAM-1 on endothelia cells. MLD-disintegrins inhibited also transmigration of calcein-labeled Jurkat cells through the cytokines-stimulated endothelial cell layer in the Boyden chamber with fluoroblock membrane. Further studies with MS animal models are required to proof activity of MLD-disintegrins in vivo. Our work may lead to development of an alternative to monoclonal antibody, peptide-derived MS therapy that may be less risky in activation of JCV.

P24

Both non-clonal and clonal populations of plasma cells dissected by laser capture microdissection from SSPE brain produce IgG directed against disease-relevant antigens

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A prominent feature of subacute sclerosing panencephalitis (SSPE), a chronic fatal encephalitis cause by measles virus (MV), is the presence of increased IgG and oligoclonal bands (OGBs) in brain. The oligoclonal IgG is antibody synthesized in the CNS and most is directed against MV. To determine the specificity of individual immune cells in SSPE brain that produce oligoclonal IgG or any other intrathecally synthesized antibody, and to develop techniques to analyze the specificity of brain IgG in other chronic inflammatory CNS diseases of unknown etiology, we microdissected individual plasma cells from SSPE brain, identified specific IgG sequences expressed by each cell and their cognate disease-relevant antigens.

Plasma cells were identified in frozen 7-micron sections of postmortem SSPE brain by immunostaining for CD38. Individual cells were isolated by laser capture microdissection with a nondestructive infrared laser. cDNA was synthesized from the unfractionated cell lysates, and heavy (H) and light (L) chain pairs of Ig sequences expressed by each plasma cell were determined by nested RT-PCR. Analysis of 65 plasma cells from a single SSPE brain revealed features of antigen-driven selection and affinity maturation in both over-represented clonal populations and also in non-clonal populations encountered only once in the repertoire. H and L chain variable region sequences were cloned into expression vectors to produce functional recombinant IgG (rIgG). Most rIgG reacted specifically with

MV: five of eight overrepresented clonal populations of cells recognized the nucleocapsid protein of MV; rIgG from four of eight non-clonal cells also stained MV-infected cells, but did not recognize specific MV proteins in several assays, indicating that background IgG may be lower affinity or directed against less abundant antigens. While both clonal and non-clonal populations of plasma cells in SSPE brain produce IgG directed against disease-relevant antigens, analysis of clonal populations is more valuable than non-clonal populations for determining specificity. These techniques can readily be applied to other chronic inflammatory CNS diseases of unknown cause, such as multiple sclerosis, neurosarcoid and Bechets, to determine disease-relevant targets of the immune response.

P25

The HIV-1 proteins gp120 and Tat promote the phosphorylation and clustering of NMDA receptors into lipid raft domains

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HIV-dementia (HIVD) is clinically characterized by deficits in cognitive and motor functions. Pathological findings include white matter pallor, gliosis, dendritic pruning and neuronal loss. Dysfunctional sphingolipid and sterol metabolism have been associated with the severity and progression of HIVD in clinical populations suggesting that interventions designed to stabilize sphingolipid metabolism may be neuroprotective. Indeed, in vitro models have shown that the neurotoxic HIV-1 coat protein gp120 and the transacting protein Tat promote neuronal dysfunction and cell death by ceramide dependent mechanisms and by IP3-sensitive enhancement of NMDA-receptor mediated calcium flux. Because ceramide-dependent mechanisms have previously been implicated in the clustering of proteins into lipid raft domains, we sought to determine if gp120 and Tat promote the clustering of NMDA receptors into lipid rafts. Hippocampal neurons were exposed to Tat, gp120 or vehicle for 0, 6 and 12 h and the phosphorylated NMDA receptor subunit NR1S896 and the gangloside GM1 were quantified in primary neurites by immunofluorescence techniques. At 6 h. Tat increased the total number of NR1S896+ receptor subunits but did not alter the number of GM1+ lipid rafts or the number of NR1S896+ subunits that co-localized with lipid rafts. After a 12 h. exposure to Tat, there was no difference in the number of GM1+ lipid rafts and the number of NR1S896+ subunits returned to basal levels. However, the number of NR1S896+ that co-localized with GM1+ domains remained elevated. Gp120 did not alter the number of GM1+ lipid raft domains or the total number of NR1S896+ subunits at 6 or 12 h. Gp120 did increase the number of NR1S896+ subunits that co-localized with GM1+ domains after 6 h of treatment; the number of NR1S896+ subunits that co-localized with GM1+ domains remained elevated at 12 h. The Tat-induced increase in total NR1S896+ subunits was not altered by the inhibition of IP3 mediated ER calcium release but was prevented by inhibition of de novo ceramide synthesis with the serine palmitoyl transferase inhibitor ISP-1. Tat-induced increases in the number of NR1S896+ subunits that co-localized with GM1+ domains was reduced by ISP-1 and by inhibition of neutral sphingomylenase with GW4869 and by inhibition of IP3-sensitive ER calcium release. Gp120-induced increases in the number of NR1S896+ subunits that co-localized with GM1+ domains was reduced by ISP-1 and by inhibition of neutral sphingomylenase with GW4869 and by inhibition of IP3-sensitive ER calcium release. In neurite microdomains, gp120 and Tat increased the amplitude of NMDA-evoked increases in cytosolic calcium 5-fold. These findings suggest that gp120 and Tat promote the clustering of phosphorylated NMDA receptors into lipid rafts and can result in focal calcium bursts of sufficient magnitude to activate death cascades.

P26

Harnessing Wnt signaling to restrict HIV replication: A lesson learned from astrocytes

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The major targets of HIV infection in the brain are resident microglia and infiltrating monocytes/macrophages. In contrast, astrocytes which are the predominant cell type in the brain, demonstrate an intrinsic restriction to HIV replication. This HIV restriction is defined by low-level of HIV replication in vitro, at best, or even abortive HIV replication. We recently established that this restriction can be overcome by priming astrocytes with IFNg, which is elevated in the cerebral spinal fluid of HIV-associated dementia patients. Despite IFNg-mediated up-regulation of D6, restriction to HIV replication in astrocytes is neither at the level of HIV entry nor at the level of condensed chromatin. Rather, we demonstrate that active Wnt signaling is associated with restricted HIV in astrocytes. Specifically, we show that the down-stream effector of Wnt signaling, TCF-4, is part of a transcriptional complex that is immunoprecipitated with HIV TAR in untreated astrocytes but not in IFNg-treated cells. Blocking TCF-4 activity with a dominant negative mutant overcomes this restriction to HIV replication, so that astrocytes now support high levels of HIV replication similar to those documented by priming astrocytes with IFNg. We also directly demonstrate that Wnt signaling is active in astrocytes and is markedly reduced by IFNg

treatment. Understanding this intrinsic molecular mechanism of restricted HIV replication in astrocytes and how IFNg can overcome this restriction is critical in harnessing this natural limitation to HIV replication within and outside of the brain.

P27

The rabies virus p protein synthesis and distribution are altered by nerve growth factor in infected sensory

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Infection caused by rabies virus continues to be a public health problem in underdeveloped countries. Rabies virus P protein participates as a regulating factor in viral transcription and replication; its interaction with a cellular protein implicated in retrograde transport suggests that this also participates in some physiopathological mechanisms. Recent studies carried out in our laboratory found an anti-transcriptional and anti-replicative effect of Nerve Growth Factor (NGF) and Neurotrophin-3 (NT-3) in dorsal root ganglion neuron cultures infected with rabies virus. This study was aimed at investigating P protein participation in viral replication, using recombinant P protein expression and the production of an anti-P polyclonal antibody in rabbits as an experimental strategy. P synthesis was evaluated in adult mouse dorsal root ganglia neuron primary cultures infected with rabies virus and treated with NGF and NT-3 by a fluorometric cell-ELISA assay. Its sub-cellular distribution was also evaluated by fluorescence microscopy 16, 26 and 36 hours post-infection. The results revealed that NGF but not NT-3 caused a significant increase in the quantity of P protein and also an accumulation of protein in neuronal bodies, revealing changes in transport to the neuritic processes. It can thus be concluded that P protein expression and sub-cellular distribution is modulated by the effect of NGF.

P28

Cerebral endothelial cell response to HIV challenge is affected by exposure to protease inhibitors

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To maintain viral suppression, HIV patients adherent to anti-retroviral (ART) medications experience fairly consistent plasma levels of the drug(s). In this scenario, the cerebral endothelial cells (CEC) of the blood-brain interface (BBI) are bathed regularly in ART drugs. Our hypothesis, based on the fact that many components of the highly active ART regimen are not benign, is that chronic exposure of CEC to ART drugs will alter signaling among components of the BBI, and ultimately contribute to changes in cellular responses to viral challenge at rebound. While reported deleterious effects of ART on host cells are diverse and drug and cell type specific, commonalities such as mitochondrial toxicity and dysfunction are observed. Numerous studies describe mechanistically the effects of ART on host cell fitness and signaling, however, relatively few studies consider the impact of chronic exposure to components of ART on the host's response to viral challenge. Studies show that while fibroblast growth factor 2 (FGF2) can protect CEC from the toxic effects of HIV gp120 (1), chronic exposure of CEC to some HIV protease inhibitors abolishes the ability of FGF2 to protect from gp120. Likewise, autopsy studies in HIV encephalitic patients indicate that individuals with low levels of astrocyte-derived FGF2 expression have markedly increased CEC apoptosis (2). To address the potential effects that exposure to ART may have on CEC signaling and fitness, we investigated changes in gene expression patterns in CEC treated with PIs and exposed to HIV. Our results indicate that exposure to HIV PIs induces changes in the expression of gene families important in maintaining CEC fitness and that the cell's signaling response to HIV exposure is affected by exposure to PIs.

P29

QPCR measurement of HIV-1 DNA and RNA during primary infection of mice

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We have constructed two chimeric HIV-1 clones that carry ecotropic MLV gp80 in place of gp120 in the virus genome. The viruses, EcoHIV (based upon NL4-3) and EcoNDK (based upon Clade D NDK) have been shown to productively infect mice, induce immune responses, enter the brain, and modulate brain cell gene expression (PNAS 102:3760, 2005). Here we describe our new methodology for quantitation of viral DNA and RNA in infected mice as convenient, reproducible, and sensitive measures of infection. We employ quantitative real time PCR amplifying DNA or RNA isolated from spleen or peritoneal macrophages. Our first generation primer and probes were customized for NL4-3 gag and were found highly suitable for amplification and detection of EcoHIV. Detection of EcoNDK with this set was poor due to a total of 5 mismatches between NL4-3 and NDK in these regions. Conversely, the customized primer/probe set for NDK gag detects EcoNDK efficiently but not EcoHIV. Variables of DNA isolation methods, reaction volumes, and DNA

normalization were investigated. The method is applied to measurement of EcoHIV and EcoNDK burden in spleen and peritoneal macrophages from infected mice. The QPCR method of detection of cDNA complementary to viral RNA in infected tissues has also been optimized including use of spliced versus unspliced mRNA as template and construction of appropriate standards, among other variables. Our study provides well-defined methods for highly sensitive and specific detection of HIV DNA and RNA in infected mice suitable for investigation of disease course and evaluation of therapeutics or vaccines.

P30

Targeting of the HIV Reservoirs in vitro by TAT-Sirna using PTD-Fusion peptide as a delivery vehicle

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Viral reservoirs are established early during an HIV infection; remain unaffected by highly active antiretroviral therapy (HAART) for their whole life and virus reemerge after treatment failure or interruption in treatment. It is often proposed that the brain serves as an important HIV reservoir. While macrophages/microglia (MM) in the brain undergoes a productive infection following virus entry, HIV infection in astrocytes becomes dormant after a brief period of viral replication. Given their life pan, astrocytes may harbor the viral genome for their whole life. In the presence of HAART, viral transcription and translation proceeds normally in the infected cells. To reduce the impact of viral proteins and viral replication and emergence of drug resistance, inhibition of HIV transcription is of the utmost importance. We used Tat-siRNA to target HIV in latently infected monocytic cells, HIV infected macrophages and astrocytic (SVGA) LTR-GFP reporter cells. We developed a PTD peptide based method to deliver siRNA. Interestingly, PTD of Tat has been recently shown to confer inhibitory effect on HIV replication. However, we did not observe any inhibitory effect of PTD on Tat transactivated LTR in astrocytic cells. Moreover, PTD peptide was incapable of transducing siRNA in macrophages and astrocytes. We then modified the PTD by attaching a stretch of 13 amino acids and monitored for siRNA transduction behavior. PTD fusion peptide based transduction of siRNA was very efficient in macrophages and astrocytes without any toxicity. Tat-siRNA strongly inhibited Tat mediated LTR transactivation up to 120 hrs in SVGA-LTRGFP reporter cells in a dose dependent manner (0.1 μM-0.4 μM). Further, Tat-siRNA strongly suppressed TNF-α reactivated HIV in latently HIV infected monocytic cells (THP89) for 72 hrs. To verify further whether Tat siRNA can inhibit HIV infection in primary macrophages, we established HIV infection in macrophages and transduced Tat-siRNA on 2nd day post infection. Indeed, strong inhibition of HIV replication was observed upto day 21 post infection, suggesting that peptide might have stabilized and enhanced the half life of siRNA in intracellular environment.

Conclusion: PTD fusion peptide mediated Tat-siRNA transduction in macrophages and astrocytes strongly inhibited HIV replication.

P31

A critical role for interleukin-10 during cytomegalovirus encephalitis

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We have previously shown that murine cytomegalovirus (MCMV) brain infection induces a transient increase in chemokine and cytokine production, but this immune mediator induction by endogenous brain cells alone is insufficient to protect immunodeficient mice. The cooperation of CD8+ lymphocytes, via a perforin-depended mechanism, was found to be essential in preventing viral replication, intracerebral spread, and disease. In the present study, we hypothesized that an absence of anti-inflammatory cytokines, in particular interleukin (IL)-10 and IL-4, would result in sustained proinflammatory responses during MCMV brain infection. Data obtained during these studies showed that direct intracerebroventricular (icv) injection of MCMV into IL-10 knockout (KO) mice resulted in an infection that was 100% lethal by 5 d post infection. On the other hand, similar icv infections into IL-4 KO or wild-type animals did not induce lethal brain disease (100% survival). Subsequent adoptive transfer experiments in which MCMV-primed splenocytes from either IL-10 and IL-4 KO animals were injected into lethally infected MHC-matched SCID/beige mice showed that these splenocytes retained their ability to protect against viral brain infection, data which suggested that the presence of IL-10 was critical during viral clearance rather than during development of the T-cell response. To further characterize the role of IL-10 in neuroimmune responses to MCMV, infected brain tissue from IL-10 KO and wild type animals was assessed for viral gene expression as well as cytokine production using quantitative real-time PCR. Results obtained from these studies showed consistent 2- to 5-fold increases in mRNA expression for IL-6, CXCL10, and CXCL9 in brain tissue obtained from IL-10 KO animals. Expression of MCMV glycoprotein B remained similar in both groups, indicating comparable levels of viral brain infection. Based on these results, studies are underway to further characterize MCMV-induced neuroinflammatory responses in the absence of IL-10,

and test the corollary hypothesis that development of CTL activity remains unaltered in IL-10 KO mice.

P32

Characterization of chemokine receptor CCR5-mediated signal transduction pathways in primary human macrophages

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The beta-chemokine receptor CCR5 is critical for normal inflammatory responses and is utilized as an entry co-receptor for macrophage-tropic strain of human immunodeficiency virus-1 (HIV-1), the predominant virus type found within the CNS of people with HIV-associated dementia (HAD). Activation of extracellular signal-regulated kinases (ERK) has been shown to mediate proliferation, differentiation, survival and apoptosis of macrophages. Despite the emerging role of CCR5 in immune functions and HIV infection, the signaling pathways by which CCR5 regulates ERK activation is not well-defined. In this study, we set out to delineate the signal transduction pathways activated by CCR5 upon binding to its endogenous ligand, macrophage inflammatory protein-1 beta (MIP-1 beta) in primary human monocyte-derived macrophage (MDM). MIP-1b induces phosphorylation of ERK-1 and 2 in both time- and concentration-dependent manner. The activation of ERK by MIP-1 beta can be blocked by phosphatidylinositol 3-kinase (PI3K) inhibitors LY294002 and wortmannin, Lyn-specific peptide inhibitor KRX123.302, as well as proline-rich tyrosine kinase 2 (Pyk2) inhibitors AG17 and dantrolene. CCR5 mediates ERK phosphorylation in response to MIP-1 beta at least in part via coupling to Gialpha since such response is significantly attenuated by pertussis toxin treatment. In addition, we demonstrated that HIV-1 virions that utilize CCR5 are able to elicit ERK activation in a CCR5 dependent-fashion. Taken together, these results indicate that CCR5 induced ERK phosphorylation is mediated via signaling molecules PI3K, Lyn and Pyk2. Current studies are aimed at defining the relationship between PI3K, Lyn and Pyk2 in this cascade. Characterizing CCR5 intracellular signaling pathways in macrophages could help us understand its role in normal immune function and mechanisms of neuroAIDS pathogenesis in syndromes such as HAD.

P33

Peroxisome proliferators-activated receptors (PPARs) attenuate IGF-1–mediated growth responses in very aggressive mouse medulloblastoma cell line, which express JCV T-antigen, and in several human medulloblastoma cell lines

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PPARs are nuclear receptors belonging to the superfamily of steroid hormone receptors. In the cerebellum three cellular layers: molecular, Purkinje cell and granular, are all positive for PPAR-alpha and PPAR-gamma transcripts; however in granule neurons which undergo malignant transformation to form medulloblastoma, PPAR-alpha predominates. Recent reports indicate a potential role of lipid lowering drugs, fibrates and statins in anticancer treatment. One candidate for the tumor chemoprevention is fenofibrate. It has been widely used to lower plasma levels of triglycerides and cholesterol, and prevent development of arteriosclerosis mainly through the regulation of apolipoprotein genes expression. Fenofibrate is also a potent ligand for PPAR-alpha. Recent studies revealed its broad function as differentiation inducer, inflammatory response modulator, and potential anticancer agent. Our results indicate that very aggressive mouse medulloblastoma cell line, BsB8, which expresses JCV T-antigen, as well as human medulloblastoma cell lines, D283, D384 and D425 express high quantities of PPAR-alpha. In addition, medulloblastoma cells responded to the fenofibrate treatment by a significant upregulation of the PPARs responsive elements. The same treatment attenuated several IGF-I –induced signaling pathways including attenuation of IRS-1, Akt, and GSK3beta phosphorylation. Importantly, we have observed that the fenofibrate treatment resulted in a severe attenuation of cell growth responses to IGF-I, evaluated by the monolayer and soft agar assays. Interestingly, growth retardation effects of fenofibrate were accompanied by a severe G1 cell cycle arrest and low level of apoptosis. In conclusion, our results show that PPAR–dependent growth inhibition by fenofibrate involves down–regulation of several IGF-I - activated signaling pathways, and suggest that supplementation with this non-toxic drug may improve effectiveness of radio and chemotherapy regiments against medulloblastoma.

P34

Cdk9 phosphorylates p53 on serine 392 independently of CKII

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The tumor suppressor p53 is an important cellular protein, which controls cell cycle progression. Phosphorylation is one of the mechanisms by which p53 is regulated. Here we report the interaction of p53 with another key regulator, cdk9, which together with cyclin T1 forms the positive transcription elongation complex, p-TEFb. This complex cooperates with the HIV-1 Tat protein to cause the phosphorylation of the carboxyl terminal domain (CTD) of RNA polymerase II and this facilitates the elongation of HIV-1 transcription. We demonstrate that cdk9 phosphorylates p53 on serine 392 through their direct physical interaction. Results from protein-protein interaction assays revealed that cdk9 interacts with the C-terminal domain (aa 361-393) of p53, while p53 interacts with the N-terminal domain of cdk9. Transfection and protein binding assays (EMSA and ChIP) demonstrated the ability of p53 to bind and activate the cdk9 promoter. Interestingly, cdk9 phosphorylates serine 392 of p53, which could be also phosphorylated by casein kinase II. Kinase assays demonstrated that cdk9 phosphorylates p53 independently of CKII. These studies demonstrate the existence of a feedback-loop between p53 and cdk9, pinpointing a novel mechanism by which p53 regulates the basal transcriptional machinery.

P35

Varicella zoster virus (VZV) Immediate early 63 Protein (IE63)

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VZV, a ubiquitous neurotropic human herpesvirus, has three distinct stages of pathogenesis: primary infection, latency and reactivation. VZV IE63 is expressed in all three stages. In biopsy or autopsy samples, IE63 is located in the nucleus during productive infection, as compared to the cytoplasm of latently infected neurons. IE63 is a 278 amino acid tegument-associated phosphoprotein whose complete function is unknown. IE63 is both essential and dispensable for VZV growth in vitro, modulates gene transcription by forming a complex with IE62 (the major VZV immediate-early transcriptional transactivating protein) and cellular RNA polII, and protects human sensory neurons from apoptotic cell death in vitro. Cells infected in culture with a recombinant VZV in which both copies of ORF 63 were deleted, over-express VZV IE62 and cell genes associated with chromatin structure and transcription regulation.

An unbiased (bacterial 2-hybrid) search of a mouse brain cDNA library for proteins that interact with IE63 found more than 100 cDNA candidate clones. Of the mouse specific cDNA inserts identified in the 2-hybrid screening, 12.5% have homology to members of the COX family of proteins (mitochondrially located, cytochrome C oxidase). Fractionation of VZV-infected MeWo cells and Western blot analysis shows that IE63 colocalizes with the vacuole fraction which is enriched for mitochondria but also contains lysosomes and peroxisomes. Confocal microscopic imaging of VZV-infected cells shows that IE63 accumulates in the nucleus early in virus infection, but later, when multinuclated syncitia form, IE63 is generally excluded form the nucleus and accumulates in the cytoplasm. Finally, late in VZV infection, when IE63 is predominately cytoplasmic, IE63 is also associated with mitochondria.

In summary, IE63 is a multifunctional protein with specific nuclear and cytoplasmic activities.

P36

A novel role for the Src family kinase Lyn in macrophage activation and TNF-a secretion triggered by HIV-1 gp120: Implications for macrophage activation in HIV-associated dementia

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Neuronal injury in HIV-associated dementia (HAD) is mediated by multiple mechanisms including direct toxic effects of viral proteins and indirect effects of neurotoxins released by activated monocyte/macrophage (M/M) cells. Both uninfected and infected M/M are activated, and the extent of activation correlates with neuronal injury and disease. While many studies have addressed the mediators and neurotoxic mechanisms of macrophage activation products, how HIV actually causes M/M activation is unknown. CCR5 is a receptor for several b-chemokines and the entry co-receptor utilized by macrophage-tropic (R5) isolates of HIV-1, including most isolates from the CNS. In previous studies we showed that in addition to supporting viral entry, CCR5 ligation by the HIV-1 envelope glycoprotein gp120 can elicit intracellular signals in macrophages and trigger inflammatory mediator release independent of infection. In this study we addressed the signal transduction pathways activated by gp120 through CCR5 and, in particular, the pathways responsible for release of TNF-a, which is upregulated in HAD and implicated in its pathogenesis. Using a combination of in vitro kinase assay, Western blotting for phospho-specific proteins, pharmacologic inhibition, CCR5 knockout (CCR5delta32) cells, and kinase-specific blocking peptide, we found that signaling through CCR5 in primary human macrophages is linked to the Src family kinase (SFK) Lyn. Stimulation of monocyte-derived macrophages with R5 HIV-1 gp120 as well as with MIP-1b results in

CCR5-mediated activation of Lyn and the concomitant Lyn-dependent activation of the mitogen-activated protein (MAP) kinase ERK-1/2. Furthermore, activation of the CCR5/Lyn/ERK-1/2 pathway is responsible for gp120-triggered production of TNF-a by macrophages. This is the first evidence for SFK involvement in CCR5 signaling, links Lyn to upstream molecules we previously identified as activated by gp120 including Pyk2 and PI3K, and begins to develop a map for the signal transduction pathways involved in gp120-CCR5-M/M activation. Lyn kinase may play an important role both in normal CCR5 function in macrophages, and in HAD where HIV-1 gp120 contributes to inappropriate macrophage activation, mediator production and secondary injury.

P37

The genetic control of early traits in rat EAE

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The genetic control of EAE and MS is multigenic, and more than 20 quantitative trait loci (QTL) are likely to regulate the severity and susceptibility of EAE in inbred mice. Each of the identified QTL likely represents a natural allele of a gene with a small overall effect on the disease. For gene identification, it would help to know what that individual gene effect is, and when in the course of the disease induction and manifestation it is likely to act. This level of understanding is missing in MS and EAE research, and, given that EAE must be induced in most rodent models, no current study explains the very first aberrant immune responses that characterize resistance vs. susceptibility. Especially valuable in this regard would be the description of the genetic basis for the loss of immune tolerance to self, as this may have homology to a similar situation in human disease. It should be possible to construct a pathway of early gene activity leading eventually to EAE, and by extension, to MS. The studies in this report are designed to link results from ongoing mapping experiments to the observed component phenotypes of the EAE process that show important differences between the EAE-S and EAE-R inbred strains at very early time points after immunization and EAE induction.

LEW EAE-susceptible rats display very polarized responses as early as two days post-immunization, unlike the MHC-identical LER EAE-resistant inbred strain. This early phenotype is likely to be controlled by fewer genetic loci responsible for its manifestation, whereas the genetic architecture underlying the cascade of deleterious events that follows this early distinction is likely to be much more complex. We have investigated the phenotype of early immune responses in the genetically susceptible LEW rat by studying the relevant biomarkers of immune activation in LEW, LER, and LER.chr4 congenic rats induced for EAE with MBP in a minimal adjuvant, CpG. We have also determined the genetic control of the early polarization by mapping the early immune response in LER × LEW F2 intercross rats, and elucidate the mechanism by which this difference occurs. An effort will be made to link the loci for the early immune response polarization and the clinical EAE loci.

P38

Identification of the mechanisms used by Vpr to regulate HIV-1 gene expression

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Expression of the viral protein R, Vpr, of HIV-1 can affect many biological events in host cells including cell cycle progression, and can modulate HIV-1 gene transcription. Earlier studies implicated p21WAF1 (p21), a cellular protein that controls progression of cells through the cell cycle, in regulating HIV-1 transcription. These observations led us to investigate the functional and physical interaction of Vpr and p21 and further its influence on HIV-1 gene expression and cell cycle progression. Our results show that Vpr modestly activated HIV-LTR in cells lacking p21 gene. We described the mechanisms of p21 and Vpr interaction for stimulating transcription of HIV-1. Data from the protein-protein interaction experiments revealed the ability of Vpr, p21 and p300 to form a ternary complex. Furthermore, we showed that, Vpr interacted with the N- and the C-terminal (aa 1-90, and 140-164, respectively) domains of p21. Unlike control cells, where p21 was predominantly localized in the nucleus, in cells expressing Vpr, whereas p21 was found in both cytoplasm and nucleus. Further, expression of Vpr alleviates p21-mediated inhibition of cell departure from G1 phase. Interestingly, expression in cells of a mutant Vpr, with arginine 73 altered to serine, did not affect p21 ability to cause cell arrest or its sub-cellular localization. These observations revealed a new cellular partner for HIV-1 Vpr. Additionally, the use of R73S mutant of Vpr provided a new therapeutic avenue for controlling HIV-1 expression.

P39

p53 inhibits HIV-1 gene expression and replication

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In HIV-1-infected individuals the virus enters the central nervous system (CNS) at an early stage of the disease. At a late stage of the disease, the presence of HIV-1 in CNS of 15–20% of the patients causes clinical abnormalities defined as HIV-1-associated dementia (HAD). The exact mechanism by which HIV-1 causes HAD is not completely understood. Recent studies suggest that p53, which is an important factor in the neuropathogenesis of AIDS, has the ability to suppress activation of HIV-1 LTR by the viral transactivator, Tat. Our recent results showed the ability of p53 to associate with the protein kinase cdk9, which together with cyclin T1 forms the positive transcription elongation complex, pTEFb. This complex, in cooperation with Tat, is responsible for the phosphorylation of the carboxyl terminal domain (CTD) of RNA polymerase II, facilitating the elongation of HIV-1 transcription. Therefore, we attempted to investigate whether p53-cdk9 association affects HIV-1 gene expression and replication in CNS cells. By employing an adenovirus expression vector, we demonstrated that p53 could decrease the levels of Tat-induced activation of HIV-1 LTR in brain cells. In addition, we showed that p53 significantly reduced HIV-1 replication in primary microglial. We also showed that p53 decreased the activity of cytokines produced (e.g. TNF-alpha) by HIV-1-infected cultures as measured by ELISA. To overcome the effect of p53, we demonstrated that cdk9 promotes the ubiquitination and degradation of p53 through a novel pathway. These studies present a novel therapeutic approach for the inhibition of HIV-1 gene expression and replication and the treatment of AIDS in brain.

P40

Interactions between JCV large T-antigen, beta-catenin and Rad51 deregulates DNA repair by Rad51

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The JCV early gene product large T-antigen is a multifunctional oncoprotein essential for viral replication. T-antigen has the ability to activate viral transcription, to bind and break DNA, and has helicase and ATPase activities. T-antigen is also able to transform cells, most likely by affecting function of several tumor suppressors such as p53 and Rb proteins. This makes T-antigen unique in its ability both to disrupt chromosomal integrity and to inactivate the cell cycle checkpoints that would limit clonal expansion of such damaged cells. By creating a T-antigen inducible glial cell line, we explored interplay between T-antigen, Rad51 (a major component of homologous recombination-directed (HR) DNA repair) and beta-catenin, the central protein of the Wnt signaling pathway, that transactivates several genes including c-Myc and TCF-4. Expression of T-antigen increased cell survival following cisplatin treatment as measured by colony formation assay, but over-expression of T-antigen and beta-catenin inhibited cell growth. In reporter assays, T-antigen and beta-catenin activated the c-Myc promoter. However cells over-expressing both proteins revealed reduced c-Myc transcriptional activity. T-antigen also was able to increase activation of c-Myc promoter by Rad51 protein. Immunoprecipitation/Western blot analyses indicated that both Rad51 and beta-catenin bound to T-antigen and that Rad51 and beta-catenin bound to each other in the absence of T-antigen. Rad51, as well as T-antigen, was able to stabilize wild-type beta-catenin and an N-terminal deletion mutant. In cells co-expressing both T-antigen and beta-catenin, a majority of phospho-Histone 2A.X was co-localized with Rad51 foci, suggesting that there was an increased DNA damage and homologous recombination in such cells. Taken together, these studies demonstrate that T-antigen, Rad51 and beta-catenin can combine to regulate cellular processes and this interplay may be an important factor in T-antigen disruption of HR DNA repair.

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P41

Creation of a novel bidirectional protein delivery system (BPDS) that is capable of secretion and nuclear import of Rad51 protein to perform DNA DSB repair by homologous recombination

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Efficient intracellular delivery and desired localization of pharmaceutical macromolecules remains problematic because of the barrier of cell membranes impeding the passage of molecules. An 11-amino acid region of the HIV-1 encoded Tat protein, YGRKKRRQRRR, is sufficient for intracellular transduction and nuclear localization. Sec is a region of murine Igk, a domain that exhibits secretion of fusion proteins. Here we report the creation of a novel system that is capable of directing the secretion and internalization

of recombinant proteins in eukaryotic cells. We have developed a GFP-11Tat-Sec fusion protein to assess its ability to deliver fusion protein into cells. Our results show efficient entry of fusion proteins into the nucleus when fused with the 11-Tat peptide. To examine the efficiency of this Tat-Sec transport system in delivering functional proteins, we created a vector that contains Rad51 protein, a major component of homologous recombination-directed (HR) DNA repair, fused with 11-amino acid of Tat and Sec domain. Results from protein transduction experiments using secreted Rad51 protein in BPDS revealed cellular internalization and nuclear appearance of the Rad51 after 16 hours and its detection in nuclei up to 24 hours after treatment. Cells, incubated with antisense Rad51 (in order to inhibit endogenous Rad51), and treated with conditioned medium containing secreted Rad51, exhibited the ability of Rad51 in BPDS to enter into the cells and perform DSB repair by HR. Reconstitution of functional GFP in an HR reporter assay indicated the presence of functional Rad51. Thus we demonstrated that double strand break repair by homologous recombination was regulated by functionally active Rad51 secreted via BPDS. The BPDS expression system thus facilitates export and import of proteins from one cell to another with no cell-cell contact, which may be useful for the delivery of therapeutic agents. Bidirectional Protein Delivery System may be useful for targeted protein delivery in the therapy of neorological disorders.

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P42

Inhibition of neuronal cytoskeleton network and dysregulation of NGF-signaling by tat through activation of mitochondrial porin in neuronal cell culture

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Examination of signal transduction pathways that modulate neuronal cell differentiation and protection against apoptosis has revealed a central role for the activation of MAPK/Erk cascade through the TrkA NGF receptor. The activation of neuronal cytoskeleton network that modulate neuronal cell differentiation, is critical for growth and survival of neuronal cells. Interactions between cytoskeleton-associated proteins and mitochondria, and a role of tubulins in HIV-1 Tat-mediated apoptosis through a mitochondria-dependent pathway have provided a rationale to examine the mitochondrial outer membrane protein Porin (also known as VDAC, voltage dependent anion selective channel) expression in neuronal cells. Here, we utilized SK-N-MC neuroblastoma cells, expressing HIV-1 Tat, to investigate the impact of Tat on the activation of Porin in NGF-signaling pathway. We demonstrated that expression of Tat in these cells inhibits cell growth and induces apoptosis by deregulating various steps involved in the NGF pathway including suppression of MAPK activity. Our results demonstrate the ability of MAPK to phosphorylate Puralpha, a cellular protein that plays an important role in neuronal cell function and differentiation, and the inhibitory effect of Tat in MAPK-mediated phosphorylation of Puralpha. Our data also indicate that expression of Tat decreases NGF-induced Egr-1 levels in SK-N-MC cells, and effects on binding of Puralpha to Egr1 promoter. Expression of Tat in these cells inhibites activity of the p35/CDK5 complex and phosphorylation of neurofilaments and MAP2. Tat also dysregulates the subcellular localization of the Shc isoforms which mediate many of the signaling pathways. Levels of expression of beta-catenin in Tat-expressing cells were dramatically reduced, but recovered upon treatment with the MG-132 proteasomes inhibitor. This suggests that Tat can act to promote beta-catenin degradation. Results from Comet assays indicated enhanced single-strand DNA breaks in Tat-expressing cells. Finally we showed that Tat was able to induce apoptosis and caspase-3 cleavage by activating of mitochondrial Porin. Inhibition of Porin by anion transport inhibitor, DIDS, in Tat-expressing cells reduced caspase-3 cleavage measured by the GLO assay. All of these observations support a model where Tat has multiple effects on neuronal cell growth and apoptosis through its interactions with cellular proteins including Puralpha, Porin, beta-catenin and the NGF/Shc/MAPK signaling pathway. The interplay between Tat and its cellular partners can result in dysregulation of the NGF pathway affecting the MAPK/Erk network and reducing the expression and activity of cytoskeleton network in neuronal cells.

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P43

p27SJ, a novel protein in St. John's Wort, is capable of inhibiting of HIV-1 gene transcription and viral replication via bidirectional protein delivery system

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Transcription of the HIV-1 genome is controlled by the cooperation of viral regulatory proteins and several host factors, which bind to specific DNA sequences within the viral promoter spanning the long terminal repeat, LTR. Here, we describe the identification of a novel protein, p27SJ, present in a callus culture of Hypericum perforatum (St. John's Wort) that suppresses transcription of the HIV-1 genome and its replication in several human cell types including primary cultures

of microglia and astrocytes. We demonstrate that p27SJ associates with C/EBPb, a transcription factor that regulates expression of the HIV-1 genome in macrophages and monocytic cells, and the viral transactivator, Tat. Association of p27SJ with C/EBPb and/or Tat prevents their subcellular localization, causing their accumulation in the perinuclear cytoplasmic compartment of the cells. Results from infection experiments demonstrated the ability of p27SJ to suppress HIV-1 replication. To achieve an efficient intracellular delivery and desired localization of p27SJ, we have developed Bidirectional Protein Delivery System (BPDS) that is capable of intracellular transduction, nuclear localization, and secretion of proteins from cells. BPDS consists of two functional domains, an 11-amino acid NLS region of the HIV-1 Tat protein, YGRKKRRQRRR, and the Sec domain of murine Igk, that exhibits secretion of fusion proteins from cells. To examine the efficiency of this transport system in delivering functional proteins, we cloned a novel protein p27SJ in BPDS and studied its effect on HIV-1 gene transcription and viral replication. Here, we show that secreted p27SJ protein suppresses transcription of the HIV-1 genome in several human cell types in reporter assays. Secreted p27SJ was able also to suppress HIV-1 replication in macrophages. These observations indicate the potential for the development of a therapeutic advance based on p27SJ protein to control HIV-1 replication in cells associated with HIV-1 infection in the brain. We demonstrated that in this expression system, export and import of proteins from one cell to another occurs with no cell-cell contact. This system can be useful for the delivery of therapeutic agents.

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P44

Regulation of puralpha promoter by E2F-1 plays an important role in the control of the cell cycle

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Puralpha is a ubiquitously expressed multifunctional nucleic acid-binding protein that is involved in many cellular processes including transcriptional regulation, the cell cycle, oncogenic transformation and postnatal brain development. Puralpha also plays an important role in neuronal cell function and differentiation. Moreover, Puralpha has the capacity to interact with and alter the activity of JCV-T-antigen and HIV-1 Tat in glial cells. Previously, Puralpha protein was found to bind to E2F-1, an important cell cycle regulator, and inhibit E2F-1 transcriptional activity. In addition Puralpha binds to a GC/GA-rich sequence within its own promoter and inhibits gene expression, i.e., Puralpha is autoregulated. We now report that the Puralpha promoter is induced by E2F-1 and that this activity maps to a consensus E2F-1 binding motif that is juxtaposed to the Puralpha binding site. Deletion mutants of the E2F-1 protein showed that the region between amino acid residues 88-241 is important for this activity. E2F-2 also was able to induce the Puralpha promoter. E2F-1-associated activation of the Puralpha promoter was inhibited by co-expression of Puralpha, pRb and an RNA species with specific binding to E2F-1. ChIP assay using primers that flanked the juxtaposed Puralpha and E2F-1 binding sites verified the presence of Puralpha and E2F-1 on the Puralpha promoter in vivo. In a Tet-inducible cell line, Puralpha delayed cell cycle progression. Thus E2F-1 and Puralpha interplay appears to be involved in the regulation of Puralpha expression and control of cell cycle.

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P45

Alterations of DNA damage repair pathways resulting from JCV infection

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Progressive multifocal leukoencephalopathy (PML), caused by JCV infection, is a disorder characterized by multifocal areas of demyelination, distributed throughout the CNS and cytological alterations in both astrocytes and oligodendrocytes. Patients often present with neurologic deficits, with or without changes in mental status. Almost all patients have underlying disorder of immune system, including AIDS, lymphoproliferative and myeloproliferative disorders, immune deficiency states. Previous studies have shown the ability of JCV agnoprotein and T-antigen to interfere with DNA damage repair pathways. In this report we demonstrate that DNA damage repair pathways are significantly dysregulated during the course of JCV infection affecting genomic content and leading to genomic instability. We observed an increased ploidy in the metaphase spreads prepared from JCV infected human adtrocytes. The frequency of appearance of polyploid cells correlated with the duration of infection. Higher rates of micronuclei and comet formation were observed in JCV infected cells compared to uninfected controls. Results from FACS analysis, during course of infection of astrocytes showed an accumulation in G2/M phase, and the number of apoptotic cells increased, as well as number of cells expressing phosphorylated Histone2A.X, indicating increased rate of DNA damage in infected cells. Increased levels of phosphorylated Histone2A.X also were detected in chromatin-associated extracts from JCV infected astrocytes and in immunocytochemical

analysis of JCV infected cells. Upregulation of Ku70, DNA-PKcs and Rad51 and downregulation of Artemis in protein extracts from JCV infected cells was detected by Western blotting. Fidelity of DNA DSB repair in in-vitro NHEJ assay and sequence analysis of the region of repaired DNA was significantly impaired in JCV infected human astrocytes. In chromatin associated protein extracts prepared from PML/AIDS brain tissue (3 cases), levels of expression of Artemis and Mre11 were dramatically reduced, but expression of Rad51 increased compared to HIV-positive, no pathology (3 cases) and HIV-negative control (3 cases) samples. Alteration of Artemis function, and changes in DNA repair pathways may be important for the life cycle of JCV and the pathogenesis of PML.

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P46

Regulation of non homologous end joining pathway of DNA repair by pura in astrocytes

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Pura is a ubiquitous nucleic acid-binding protein, which has been implicated in the control of eukaryotic gene transcription. Pura associates with DNA sequences positioned in close proximity to viral and cellular origins of replication suggesting a role for this protein in DNA replication. Initiation of transcription and replication requires alteration in the structure of duplex DNA, and the DNA unwinding activity of this single-stranded nucleic acid-binding protein was confirmed in previous studies. Here we demonstrate that the presence of Pura significantly affects DNA damage and DNA repair pathways through interfering with nonhomologous end joining (NHEJ) process. In metaphase spreads prepared from Pura−/− cells after cisplatin treatment, extensive fragmentation, chromosomal breakage, rejoining and complex chromosomal configurations were found, whereas in Pura+/+ cells chromosomal integrity was preserved. Higher rates of comet formation were observed in cells in the absence of Pura after treatment with the DNA DS break inducer bleomycin. FACS analysis demonstrated that, after synchronization and cisplatin treatment, 52.4% of Pura null cells expressed phospho-Histone 2A.X compare to only 12.8% of Pura-positive cells indicating an increased rate of DNA damage in Pura null cells. In chromatin associated extracts, prepared at various time of cisplatin treatment following synchronization, expression of phospho-Histone 2A.X was substantially higher in Pura null cells. Increased expression of phospho-Histone 2A.X in the same cells without cisplatin treatment may reflect high levels of basal DNA damage in the absence of Pura. Expression of Ku70 was reduced in the presence of Pura, but Mre11 was upregulated. Results from NHEJ assay demonstrated that the end-to-end joining activity of cells in the absence of Pura was higher than in Pura null cells. The reconstitution of full-length recombinant Pura in Pura−/− cell extracts inhibited end-joining. Sequence analysis of the products of DSB repair showed that, in the presence of Pura, although end to end joining activity was reduced, it occurred without errors. In the absence of Pura end-joining activity was higher but religation occurred with the loss of genetic material and was associated with increased exonuclease activity Taken together, these studies demonstrate that Pura is an important factor in regulation of DNA repair and possibly acts as a "guardian" of the genome in promoting the fidelity of DNA replication.

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P47

Regulation of complement factor 3 gene expression by HIV-1 NEF, C/EBP isoforms and p38 MAPK in astrocytic and promonocytic cell lines

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The complement system is an integral component of both the innate and adaptive immune responses in the defense against invading pathogens. However, uncontrolled complement biosynthesis and activation can be injurious to host tissues. HIV/SIV infection is known to induce the synthesis of the complement component C3 which could contribute to the pathogenesis of HIV in the CNS. Furthermore, HIV virions are resistant to complement mediated lysis. Studies have shown increased C3 deposition in astrocytes, neurons, microglia, infiltrating macrophages and multinuclear giant cells in the brains of SIV-infected rhesus macaques. Since dysregulation of complement synthesis may contribute to NeuroAIDS pathogenesis, we analyzed in detail the mechanism of transcriptional regulation of C3 expression in the astrocytic and promonocytic cell lines, U-373MG and U937 respectively. Our studies utilizing C3 promoter-Luciferase construct demonstrate that viral protein Nef induces C3 promoter. Furthermore, the C/EBP family of DNA binding transcription factors, C/EBP-beta, LAP, and C/EBP-delta stimulate expression of the C3 promoter, while LIP inhibits C3 promoter activity. Site-directed mutagenesis of the distal C/EBP site (bZIP1) in the C3 promoter significantly reduced C/EBP delta mediated activation while, mutation in the second, more proximal C/EBP consensus sequence (bZIP2) had a minimal effect. Our results also demonstrate that selective inhibition of p38

MAPK by SB202190-HCl or overexpression of a dominant negative p38 alpha mutant inhibits C/EBP-delta mediated C3 promoter activity. The data strongly suggest that p38 MAPK regulates C/EBP delta mediated C3 gene activation.

P48

siRNA targeting vaccinia virus double-stranded RNA binding protein [E3L] exert potent antiviral effects: Intersection with innate immune system

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The Vaccinia virus gene, E3L, encodes a double-stranded RNA [dsRNA]-binding protein. We hypothesized that, owing to the critical nature of dsRNA in triggering host innate antiviral responses, E3L-specific small-interfering RNAs siRNAs] should be effective antiviral agents against pox viruses, for which Vaccinia virus is an appropriate surrogate. In this study, we have utilized two human cell types, namely, HeLa and 293T, one which responds to interferon [IFN]-β and the other produces and responds to IFN-β, respectively. The antiviral effects were equally robust in HeLa and 293T cells. However, in the case of 293T cells, several distinct features were observed, when IFN-β is activated in these cells. Vaccinia virus replication was inhibited by 97% and 98% as compared to control infection in HeLa and 293T cells transfected with E3L-specific siRNAs, respectively. These studies demonstrate the utility of E3L-specific siRNAs as potent antiviral agents for small pox and related pox viruses.

P49

Detection of JC virus DNA sequences and viral protein expression in esophageal disorders and esophageal cancer

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In industrialized countries, the incidence of adenocarcinoma of the esophagus has risen dramatically in the last decades, at a rate of approximately 5% to 10% per year. Although chronic gastro-esophageal reflux is the usual underlying cause of the repetitive mucosal injury and also provides an abnormal environment during the healing process that predisposes to intestinal metaplasia, the etiology of adenocarcinomas of the esophagus remains unclear. The human polyomavirus JCV, which causes Progressive Multifocal Leukoencephalopathy, a fatal demyelinating disease frequently seen in HIV-1 infected patients, subclinically infects 85-90% of the population world-wide. JCV is oncogenic in experimental animals and is associated with human brain tumors. JCV has been found in epithelial cells of normal mucosa of the upper and lower gastrointestinal tract and recent studies have demonstrated the presence of viral DNA sequences and the expression of the early protein, T-Antigen in neoplastic cells of human colon carcinomas. We examined the presence of JCV DNA sequences and protein expression in normal and malignant human esophageal tissues. 70 well-characterized biopsies from patients with a spectrum of esophageal disorders were studied by PCR and immunohistochemistry. JC viral DNA was isolated from 11 of 13 normal esophageal biopsies (85%) and from 5 of 5 esophageal carcinomas (100%). By immunohistochemistry, JCV T-antigen was detected in 10 of 19 carcinomas (53%), Agnoprotein in 8 (42%), p53 tumor suppressor in 11 (58%) and ?-catenin in 4 (21%). None of 51 benign esophageal samples expressed viral proteins. Laser Capture Microdissection verified the presence and specificity of JCV DNA sequences. Beta-Catenin and p53 co-localized with JCV T-antigen in the nuclei of neoplastic cells. The results provide evidence for infection of gastrointestinal tract cells by JCV and suggest a potential role of JCV in the development of upper digestive tract carcinomas. Supported by Grants from the NIH awarded to LDV and KK.

P50

Characterization of the JCV VP1 loop mutations in CSF from HIV positive and negative patients with Progressive Multifocal Leukoencephalopathy.

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Background: Viral Protein 1 (VP1) is the major capside protein of the human Polyomavirus JC (JCV); its outer loops consist of the five most hydrophilic regions and correspond to the BC, DE, EF, GH and HI loops,

according to the analogous three dimensional structure of SV40 VP1. It is suggested that the external loops provide principal antigenic viral structures and cellular receptor binding sites, thus, VP1 loop mutations, characterized by the variations of affected amino acid residues, could modify the ability of the virus to interact with the receptor and, consequently, the infectivity of JCV.

The purpose of our study is to determine whether or not specific amino acid substitutions in VP1 outer loops of JCV, isolated from cerebrospinal Fluid of patients with different forms of Progressive Multifocal Leukoencephalopathy (PML), could be associated with the progression of the disease.

Methods: Semi-nested PCR was used to amplify a region of VP1 gene from CSF collected from 1 HIV+ Slow Progressing (SP) PML, 2 HIV+ Fast Progressing (FP) PML and 2 HIV negative SP PML patients. The amplified regions encompass for the five outer loops and were ligated to PCR 2.1 plasmid and used to transform competent INValphaF' cells. A total of 40 clones carrying partial VP1 gene sequences were sequenced using T7 primer and an AB 310 Genetic Analyzer (Applied Biosystems). We regarded, for each DNA sample, nucleotide mutations occurred in at least three independent clones and the determined amino acid substitutions were detected in reference to JCV Mad 1 genome.

Results: We analyzed a total of 40 clones (a mean of 8 clones for each patient) and a total of 100 VP1 nucleotide mutations were detected, both synonymous and non-synonymous, the latter determining a total of 34 different amino acid substitutions. The amino acid changes involved all the considered loops and mutations at BC and EF loops sequences were found in all the JCV isolates. In particular, in the CSF of the 3 SP PML patients, both HIV + and HIV-, four hot spots were detected: the residue 75, belonging to BC loop, where the Arg was substituted by a Lys, usually found in Archetype isolate; the residue 117, belonging to the inner part of the gene (beta-d), with a Ser -> Thr substitution, also characteristic of the Archetype isolate; the residue 167 (Asp -> Glu), belonging to EF loop and the residue 128 (Thr -> Ala), belonging to DE loop. In the CSF of a FP PML patient, nucleotide mutations at position 1664 and 2268 determined amino acid substitutions at residue 267 (Ser -> Phe, HI loop) and at residue 60 (Lys -> Stop codon, BC loop), already indicated in previous studies as associated with fast progression of PML.

Conclusions: taken together, the results showed strict homology of the polymorphisms detected in all the SP PML patients, both HIV+ and HIV-. Among the polymorphisms, mutation at Arg- 75 on the BC loop, that it was recently reported to be involved in recognition of cellular receptor and seems to result in nonviable virus, is highly frequent. We could hypothesize that the alteration at the defined hot spots determines a decrease in the viral activity and slow progression of the disease.

P51

Unraveling the mechanism of viral neuroinvasiveness using a genetics approach

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Neuroinvasiveness, the main feature of neurotropic viruses such as rabies virus (RV), refers to the ability to invade the nervous system from a peripheral site. To identify the viral and cellular factors responsible for the ability of an RV to enter the CNS from a peripheral site and to cause lethal neurological disease, we exchanged the genes encoding the different RV proteins and regulatory genomic sequences of a highly neuroinvasive RV strain with those of a highly attenuated non-neuroinvasive RV vaccine strain. Phenotypic analysis of chimeric RVs assembled from neuroinvasive and non-neuroinvasive RV strains demonstrated that, although RV pathogenicity is a multigenic trait involving different RV encoded proteins and transcriptional elements, the RV G plays a predominant role in the ability of an RV to invade the CNS from a peripheral site. Important features required for such a "neuroinvasive G protein" are its abilities to facilitate rapid virus entry into neurons and efficient virus spread. Analysis of chimeric RVs also revealed that the strength of interaction between the RV G and M protein plays a decisive role in viral assembly/budding and thereby contributing to the neuroinvasiveness of individual RV strains.

P52

Detection of the MSRV endogenous retrovirus in multiple sclerosis patients and healthy persons

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Multiple sclerosis (MS)-associated retrovirus (MSRV) is an endogenous retrovirus proposed as MS co-factor, that forms extracellular virions. We detected circulating MSRV in 100% of Sardinian patients with active MS, and MSRV presence in CSF paralleled MS clinical progression. The virus was evaluated by nested or real-time RT-PCR assays for MSRV/HERV-Wpol or env. MSRV viremia per se is not indicative of MS, since it is detected also in healthy donors and patients with other diseases. However, both percent MSRV positivity and mean copy numbers are widely different, with the highest values in patients with active MS, and the lowest in healthy persons. We examined three cohorts of healthy volunteers without known risk of MS: donors of a Blood Transfusion Unit (BD, N = 39), and operators of the Neurology (NEUR,N = 41) and Surgery

(SURG,N = 17) Units of our University. No significant differences were detected between SURG and BD, while 54% of NEUR were found MSRV(+) and highly viremic (NEUR vs BD: p = 0.0001): if confirmed in other cohorts, this might imply that MSRV could be a transmissible agent. We performed a blind observation for six years of early MS patients, that at study entry had similar mean age and EDSS score, but differed for MSRV presence in CSF. At follow-up,the MSRV(+) and MSRV(−) cohorts significantly differed for mean EDSS (p = 0.001),and annual relapse rate (p = 0.023). Two MSRV(+) patients, but none MSRV(−), entered the progressive phase (p = 0.009). We concluded that MSRV(+) CSF at MS onset is associated with disability accumulation, higher rate of clinical re-exacerbations and conversion into secondary progression.

P53

IFN-beta activates CUGBP1 resulting in increased expression of dominant-negative CEBP-beta and suppression of the SIV LTR

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IFN-beta, the type I IFN expressed during virus infection in the brain has been shown to inhibit active HIV replication in macrophages via a transcriptional mechanism involving expression of a truncated, dominant-negative form of the transcription factor C/EBP-beta (LIP). We have demonstrated that IFN-beta can also inhibit ongoing SIV replication in primary macrophages in vitro. Using our accelerated and consistent SIV model of HIV CNS disease, we have demonstrated that increased expression of IFN-beta and LIP coincided with downregulation of acute SIV replication in the brain. These results suggested that innate immune responses involving IFN-beta may contribute significantly to the mechanisms controlling acute SIV replication in the CNS.

The expression of different isoforms of C/EBP-beta (LIP and the wild type isoform LAP) is regulated by alternative translational initiation at downstream AUG start sites. Increased expression of LIP is regulated in part by an RNA binding protein referred to as CUG repeat binding protein (CUGBP1). It has been demonstrated that phosphorylation of CUGBP1 leads to increased binding of CUGBP1 to C/EBP-beta mRNA and the consequent increase in LIP expression.

In the studies presented here we demonstrate that the ability of IFN-beta to regulate transcription from the SIV LTR in cells of macrophage lineage involves activation of CUGBP1. We are studying the role IFN-beta signaling in the binding of CUGBP1 to C/EBP-beta mRNA, which is accompanied by enhanced expression of LIP. Our studies suggest that IFN-beta inhibits SIV replication at least in part by promoting LIP production through the activation of CUGBP1 and its binding to the GC-rich region of C/EBP-beta mRNA.

P54

MH2 domain of Smad3 reduces HIV-1 Tat-induction of cytokine secretion

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HIV-1 infection of the central nervous system (CNS) is associated with dysregulation of several important cytokines and chemokines, which are involved in inflammatory process. Earlier studies ascribed a critical role for Tat, a potent viral transcription activator, in this process by enhancing the expression of several immunomodulators including TGF? and MCP-1. Investigation of signaling pathways which are controlled by these cytokines led to identification of MH2 domain of Smad3, the downstream activator of TGF? pathway, as a modulator of MCP-1 promoter activity. The level of MCP-1 is increased in AIDS patients with neurologic problems, through recruitment of inflammatory cells, which can contribute to neuropathogenesis of AIDS. Therefore, we attempted to investigate the effect of MH2 on expression of MCP-1 and other immunolmodulators in CNS cells. By employing an adenovirus expression vector, we demonstrated that MH2 can decrease the levels of Tat-induced activation of MCP-1 and several other cytokines and chemokines in astrocytic cells. In addition, we showed that MH2 significantly reduced the activity of cytokines produced by cultures of adenovirus-MH2 transduced cells as measured by the transmigration of human PBMC cells. Thus, MH2 domain of Smad3 is a potential agent that may be developed as an inhibitor for the cytokine-mediated inflammatory responses in the brain and may have the potential to prevent transmigration of HIV-1-infected monocytes across the blood brain barrier in AIDS patients.

P55

Neuronal damage mediated by HIV-1 Tat involves deregulation of cytoskeletal functions

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HIV-1 Encephalopathy (HIVE) is characterized by deterioration of neuronal processes. While there is mounting evidence of neuronal toxicity and cell death induced by the HIV-1 transactivating factor Tat, the molecular events linked directly to its detrimental

effect on neuronal cells remain unclear. Our recent work using rat embryonic cortical neurons as well as immunohistochemical analysis of HIVE tissue samples showed that Tat causes rapid degradation of microtubule-associated protein 2 (MAP2) and the collapse of cytoskeletal filaments. The mechanism of Tat action on MAP2 stability involved Tat-mediated translocation of the proteasome to the site of microtubule filaments. While MAP2 is a well known structural component of the microtubules, emerging studies suggest an important function for this protein in signal transduction pathways. In the following study we investigated molecular mechanisms of neuronal damage upon Tat-mediated loss of MAP2. Treatment of rat embryonic cortical neurons with recombinant Tat1-72 resulted in a dose-dependent loss of MAP2 and down-regulation of the catalytic subunit of the cAMP-dependent protein kinase A (PKA). While MAP2 has been shown to be an anchoring protein for PKA, loss of MAP2 in neurons exposed to Tat would greatly affect PKA-dependent signal transduction pathways and neuron-specific targets.

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P56

HTLV-1 Tax induction of cell cycle arrest in human CD34+ cells: Implications for viral latency in human hematopoietic stem cells

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HTLV-1 is the etiologic agent of adult T-cell leukemia (ATL), an aggressive CD4+ malignancy. Although HTLV-2 is highly homologous to HTLV-1, infection with HTLV-2 has not been associated with lymphoproliferative disorders. Lentiviral mediated transduction of CD34+ cells with HTLV-1 Tax (Tax1) induced G0/G1 cell cycle arrest and resulted in the concomitant suppression of multilineage hematopoiesis in vitro. Tax1 induced transcriptional upregulation of the cdk inhibitors p21cip1/waf1 (p21) and p27kip1 (p27), and marked suppression of hematopoiesis in immature (CD34+/CD38−) hematopoietic progenitor cells (HPCs), in comparison to CD34+/CD38+ cells. Tax1 also markedly protected CD34+ cells from apoptosis. Notably, HTLV-1 infection of CD34+ cells also induces p21 and p27 expression and retards cell cycle progression. In contrast, HTLV-2 Tax (Tax2) did not detectably alter p21 or p27 gene expression, failed to induce cell cycle arrest, did not protect cells from apoptosis and did not suppress hematopoiesis in CD34+ cells. Tax1 also robustly repressed expression of the Survivin gene, which has recently been demonstrated to facilitate cell cycle progression in CD34+ cells. G0/G1 cell cycle arrest and transcriptional repression of Survivin defines two novel roles for Tax1 and suggests that this viral oncoprotein may uniquely function to facilitate HTLV-1 latency in CD34+ cells. A Tax2/Tax1 chimera encoding the C-terminal 53 amino acids of Tax1 fused to Tax2 (Tax221) displayed a phenotype in CD34+ cells similar to that of Tax1, suggesting that unique domains encoded within the C terminus of Tax1 may modulate cellular gene expression and may account for the phenotypes displayed in human hematopoietic progenitor and stem cells. The remarkable differences in the activities of Tax1 and Tax2 in CD34+ hematopoietic progenitor and stem cells may reflect the sharp differences observed in the pathogenesis resulting from infection with HTLV-1 in contrast with HTLV-2. The implications of infection of hematopoietic stem cells by HTLV and the establishment of retroviral latency in these pluripotent cells will be discussed.

P57

Transduction of HTLV-1 and -2 tax oncoproteins in primary human neuronal cells induces expression of proinflammatory cytokines and sensitization to apoptosis

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Infection with Human T-cell leukemia virus type 1 (HTLV-1) results in the development HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a non- fatal, chronic inflammatory disease of the central nervous system (CNS). Although the pathogenesis of HAM/TSP remains to be elucidated, previous evidence suggests that elevated levels of the pro-inflammatory cytokines TNF-alpha, IL-6 and IL-1 beta in the CNS are associated with neuropathogenesis. We have previously shown that the expression of HTLV-1 Tax (Tax1) is sufficient for the induction of pro-inflammatory cytokines in primary microglial cells. HTLV-2 is highly related to HTLV-1 and shares a high degree of sequence homology particularly in the Tax region, but infection with HTLV-2 is significantly less pathogenic. To investigate and compare the effects of Tax1 and Tax2 expression on the dysregulation of proinflammatory cytokines in primary neuroglial cells, lentivirus vectors were used to transduce primary human astrocytes and oligodendrocytes. Expression of Tax1 in primary human astrocytes and oligodendrocytes results in significantly more robust levels of pro-inflammatory cytokine induction in comparison to Tax2. Notably, expression of Tax1 sensitized primary human astrocytes to apoptosis mediated by serum withdrawal, in stark contrast

to astrocytes transduced with Tax2. The C-terminus of Tax1 has been shown to uniquely encode a PDZ binding motif (PBM) and this region has been implicated in the elevated transforming potential demonstrated by Tax1 as well as playing a role in the transcriptional transactivation of cellular genes. A Tax-2/Tax-1 chimera (Tax221), containing the C terminal 53 aa of Tax1 fused to Tax2, demonstrated a Tax1 phenotype, with respect to proinflammatory cytokine induction and sensitization of primary human astrocytes to apoptosis. This suggests that the C -terminus of Tax1 functions in the transcriptional transactivation of cellular pro-inflammatory cytokines and in accentuating serum withdrawal-mediated apoptosis in primary astrocytes. The differential cytokine induction patterns demonstrated by Tax1 and Tax2 and the sensitization to apoptosis may reflect differences relating to the heightened neuropathogenesis associated with HTLV-1 infection.

P58

CD163 expression by perivascular and parenchymal mononuclear phagocytes in CNS tissues suggests trafficking of HIV-1 infected monocytes/macrophages in HIVE

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Previously, we demonstrated two subsets of productively infected (HIV-1 p24+) mononuclear phagocytes (MP) in the CNS of patients with HIV encephalopathy (HIVE): 1) accumulating perivascular macrophages, multi-nucleated giant cells and nodules that are CD14+/CD16+/CD45+ and, 2) CD14-/CD16+/CD45- ramified microglia in white matter. CD14+/CD16+/CD45+ cells appeared to be cells that have recently entered the CNS from the periphery, however, the origin of the CD14-/CD16+/CD45- ramified microglia in the parenchyma remained unclear. While these cells were phenotypically microglia, the increased number of these cells with the concomitant absence of evidence for proliferation suggested that these ramified cells may be derived from perivascular macrophages through parenchymal invasion and phenotypic adaptation. CD163, a monocyte/macrophage specific scavenger receptor for hemoglobin-haptoglobin complex, is reported to be expressed in the CNS by perivascular macrophages but not by resident microglia in human brain. In the studies presented here, we utilize this disparity in the expression pattern of CD163 by brain macrophages to further characterize the MPs in the CNS in HIVE and in HIV-1 associated Progressive Multifocal Leukoencephalopathy (PML). Our results reveal CD163 positivity in cells with microglial morphology in HIVE, but not PML. Both parenchymal microglia and perivascular macrophages are CD163+ in HIVE. In contrast, only foamy macrophages associated with areas of demyelinating lesions were CD163+ in PML CNS tissues. Results of these studies, demonstrating accumulation of CD163+ MPs in the CNS provides strong evidence for the role of increased monocyte/macrophage trafficking in the pathogenesis of HIVE.

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P59

Increased CD163 expressing peripheral blood monocytes/macrophages are CD16+ and correlate with viral load and CNS invasion in SIVmac251 infected Rhesus macaques

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In autopsy tissues from HIV-1 infected individuals with encephalopathy (HIVE), we have demonstrated significant accumulation of CD163+ macrophages that gather perivascularly, within the brain parenchyma and comprise multinucleated giant cells and microglial nodules. Further, these cells are CD16+ and many harbor productive virus and appear to be the reservoir of productive HIV-1 infection in the CNS. As CD163 is reported to be expressed in the CNS by perivascular macrophages but not by resident microglia in normal brain, these studies provided strong evidence for the role of increased monocyte/macrophage trafficking in the pathogenesis of HIVE. In our present study we investigated uninfected and SIVmac251 infected Rhesus macaques with and without SIV encephalopathy (SIVE) for alterations in peripheral blood monocyte activation/maturation markers by flow cytometry and assessed how these changes might correlate with viral load and CNS disease. Like HIVE, accumulating perivascular macrophages and ramified microglia are CD163 positive in SIVE. Flow cytometry revealed an increase in CD163 mean fluorescence intensity (MFI) of monocytes/macrophages in circulation that correlates with viral load. Additionally, CD163hi/CD16+ cells are seen at a greater frequency in macaques with high viral loads, with an even greater frequency of CD163hi/CD16hi monocytes/macrophages. These studies provide additional support for the role of altered monocyte activation/differentiation in the peripheral blood to the

contribution of SIV neuropathogenesis and, potentially, HIVE.

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P60

Monocyte/macrophage trafficking and the development of HIV and SIV Encephalitis

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Dysregulation of monocyte proliferation, activation, and trafficking leads to invasion and accumulation of macrophages in CNS during the course of HIV/SIV infection. We have conducted phenotypic studies to characterize the mononuclear phagocytes present in CNS and peripheral tissues. Perivascular macrophages are CD14+ and CD45/LCA+, whereas parenchymal microglia are CD14- and CD45-. Both perivascular macrophages and parenchymal microglia express CD16 in HIVE, likely derived from the CD14+/CD16+ expanded monocyte subset in circulation or alternatively due to microglial activation in patients with AIDS and HIVE. To further investigate the dramatic increase in microglial accumulation, we have investigated CD163, a recently characterized monocyte/macrophage marker, reportedly absent on microglia, as a biomarker in NeuroAIDS. Immunophenotypic analysis was performed using human and macaque CNS tissue and also in blood monocytes during SIV infection of Rhesus macaques, some of which were also treated with morphine. Interestingly, ramified microglia express CD163 in HIVE, but not in normal brain tissues, nor in HIV/PML tissues in areas outside of demyelinating lesions. The percent fraction of CD163hi/CD16hi monocytes in circulation was increased and correlated with viral load $P = 0.0001$. Furthermore the mean fluorescence intensity of CD163 is also increased. CCR5 expression is also increased on monocytes with CD163hi phenotype. Immunohistochemical studies in human HIVE CNS tissues demonstrate colocalization of CD163 and HIV-1 p24 staining. These results identify the CD163hi/CD16hi subset as an expanded subpopulation of monocytes in circulation that is likely to be involved in the establishment of the CNS reservoir of HIV/SIV infection. These studies suggest the potential efficacy of therapeutic approaches directed toward monocyte/macrophage developmental and survival pathways.

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P61

Hepatitis C virus from post-mortem brain and liver tissues: A mutational analysis of RNA and amino acids sequences of the E1 protein region

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Background: Hepatitis C virus (HCV) RNA can be recovered from several tissues, including liver, peripheral blood mononuclear cells, and brain. Sequence analysis reveals that viral subpopulations preferentially accumulate in separate compartments. HCV's two envelope proteins, E1 and E2, are thought to mediate viral entry into cells and may contribute to cellular tropism.

Methods: In the current IRB-approved study, RNA encoding the first 126 amino acids of the HCV E1 envelope protein and a portion of the E1 signal sequence were analyzed in extracts of plasma and post mortem liver and brain specimens of 13 patients with HCV viremia: 10 HCV/HIV-coinfected and 3 HCV mono-infected. Tissue specimens from 17 individuals without detectable HCV RNA in plasma provided controls. An 80 base-long segment of the 5′ untranslated region (UTR) was analyzed in parallel.

Results: As assessed by polymerase chain reaction (PCR) amplification and direct sequencing, HCV RNA was recovered from liver of 10/10 co-infected and 3/3 mono-infected patients and 0/17 HCV aviremic patients ($p < .0001$). Of the patients with HCV RNA in liver, 6/10 co-infected patients had HCV in the brain. At the nucleotide level, the brain sequence differed from both the plasma and liver sequences in 2/6 patients; and at the protein level, the brain sequence differed from both the plasma and liver sequences in one patient. This patient evidently had a mixed infection, as the predominant HCV in the liver was genotype 1b, while in the plasma it was genotype 1a. The brain sequence could not be unambiguously determined, but the HCV RNA appeared to be a mixture of genotype 1a and 1b. E1 contains four putative N-linked glycosylation sites in the region under analysis. All four sites were preserved in all but one patient whose first site was mutated in plasma, liver, and brain extracts. Sequence analysis of the mono-infected patients is underway.

Conclusions: HCV RNA could be recovered from the majority of the post-mortem brain tissues of HIV/HCV co-infected patients with HCV viremia (6/10). Thus, accumulation of HCV in the brain appears to be a frequent event and could contribute to the fatigue and cognitive deficits often noted in HCV patients. Investigations are needed to determine the distribution and density of HCV in the central nervous system and to assess the role CNS invasion plays in pathogenesis and treatment failure.

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P62

Interaction between human T cell leukemia virus type 1 Tax and protein components of the cellular secretory pathway

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Tax protein has been shown to play an integral role in human T cell leukemia virus-1-induced diseases including adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Extracellular Tax has been detected in the cerebrospinal fluid of HAM/TSP patients, suggesting that cell-free Tax may be physiologically involved in the progression of neurologic disease. We have previously demonstrated the secretion of full-length Tax and its co-localization with the cytoplasmic organelles relevant to secretion. The present study elucidates the mechanism of Tax secretion. To identify intracellular Tax interacting proteins, we have used an antibody array spotted with antibodies directed against cellular secretory pathway proteins. Upon reaction with protein extracts from Tax-treated cells, antibody array analyses have suggested the interaction of Tax with SCAMP1, SCAMP2, SNAP23, and COPII; proteins that facilitate transport between the nucleus and cytoplasm, and between the endoplasmic reticulum, Golgi complex, and plasma membrane. Subsequently, we have confirmed these specific protein-protein interactions by co-immunoprecipitation and GST pull-down assay involving the use of a GST-Tax fusion protein bound to glutathione-coupled particles to affinity purify proteins that interact with Tax. Collectively, these studies have demonstrated the interaction of Tax with multiple proteins in the secretory pathway and that Tax may be secreted and act as an extracellular effector molecule.

P63

Mechanisms of minocycline induced suppression of SIV encephalitis

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Minocycline, a tetracyclic antibiotic, has been shown to decrease the severity and incidence of encephalitis in a rapid, rigorous SIV/macaque model of HIV-associated CNS disease. In this model, a shift in the balance of MAPK signaling in the brain is observed over the course of infection, with a trend to increased activation of p38 and JNK, classically neurodegenerative pathways, during terminal infection when macaques have AIDS and SIV encephalitis. Minocycline has been shown to inhibit the activation of p38 signaling in the context of other neurodegenerative diseases, and this may be key to its neuroprotective properties in the SIV macaque model.

To examine the mechanism of this modulation of the cell signaling environment by minocycline, we have established an in vitro system in which a nitric oxide donor is used to activate both p38 and JNK signaling in U937 monocytic cells. We have found that pretreatment of these cells with minocycline results in a dose dependent suppression of this activation. Through the use of specific inhibitors, we have found the mechanism of this suppression to be independent of the activation of antagonizing pathways that signal through PKC or ERK. Our findings indicate that minocycline acts upstream of p38 and JNK, suppressing the activity of ASK1, a MAPKKK capable of leading to the activation of both p38 and JNK. We are currently investigating how minocycline inhibits ASK1 phosphorylation of downstream MAPKKs, focusing on redox sensitive regulation mechanisms of ASK1 activation.

P64

Neuroinflammation in the CNS leading to seizures due to virus infection

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Seizures in humans are often associated with viral infections. Several animal models have been proposed to mirror some of the features of seizures due to viral infection. Central nervous system (CNS) herpes virus encephalitis of mice, rats and rabbits can induce seizures but most animals die due to the viral encephalitis. Borna virus infection of rats in some instances can lead to seizures due in part to a persistent Borna virus (CNS) infection. To investigate the contribution of virus versus innate immune responses that lead to seizures following an acute viral infection, we have explored Theiler's murine encephalitis virus (TMEV) infection of C57BL/6 mice. Infection of C57BL/6 mice has been reported to lead to viral clearance due to anti-viral CD8+ T cells. We have found that infection of C57BL/6 mice with the DA strain of TMEV (2,000 pfu) leads to seizures in about 50% of mice. Mice develop clonic seizures between day 3 and day 10 following infection. Seized mice have an impaired

righting reflex during this period as well as poor motor coordination as measured by rotor rod analyses versus unseized or uninfected animals. Seized mice also fail to gain weight following infection as compared to unseized or uninfected control mice. Infection of either IL-6 or TNF-alphaR knockout mice lead to a decreased incidence of seizures. No seizures were observed in infected BALB/c or SJL/J mice. Pathologic studies found that seized infected mice had more extensive damage and inflammation in the hippocampus versus unseized infected C57BL/6 mice. We hypothesize that an early innate immune response after TMEV infection in the CNS such as IL-6 and TNF-alpha production leads to seizure development. We are currently exploring this concept.

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P65

Reactivation of HHV-6 in natalizumab treated MS patients

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In a recent trial of the a4 integrin antagonist natalizumab for the treatment of multiple sclerosis (MS), three patients developed progressive multifocal leukoencephalopathy (PML). PML is a demyelinating disorder of the central nervous system (CNS) most often observed in immunocompromised individuals. PML is caused by reactivation of clinically latent JC polyomavirus, and it has been suggested that impaired immune surveillance due to natalizumab treatment may have contributed to the observed JCV reactivation. As HHV-6 has been suggested to play a role in MS and has also been detected in high frequency in PML white matter within and surrounding demyelinative lesions, we asked whether this virus could also have been reactivated during therapy. We examined matched serum and CSF samples from a limited set of MS patients treated with and without natalizumab for evidence of HHV-6 reactivation as assessed by presence of viral DNA sequences and anti-HHV-6 antibodies. In this small cohort of natalizumab treated MS patients, we could detect HHV-6A DNA in the CSF of a surprising number of patients (2 of 24), while no such sequences were found in the CSF of the non-natalizumab treated group. Analysis of anti-HHV-6 IgG in serum using a novel electrochemiluminescence assay demonstrated significantly elevated levels in patients on natalizumab compared to controls. Studies to define serum HHV-6-specific IgM responses and CSF IgM and IgG responses are ongoing. This novel assay will also allow for the analysis of HHV-6 antibody responses to different proteins of HHV-6. Further, we confirmed the presence of HHV-6 in PML lesions by immunohistochemistry. We suggest the possibility that following reactivation, HHV-6 may be able to transactivate other viruses such as JCV. Collectively, these results support the hypothesis that treatment with natalizumab may be associated with reduced immune surveillance resulting in reactivation of viruses associated with MS pathogenesis.

P66

An inducible Tat transgenic mouse model for the study of HIV Neurologic complications

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Background: The HIV protein Tat is actively secreted by HIV-infected cells and has long been considered to be important in HIV associated neurologic alterations. In the brain, Tat would not only be released from infected cells that have gained access to the brain parenchyma from the periphery, such as macrophages and lymphocytes, but also the resident cells susceptible to infection, astrocytes and microglia. Therefore, Tat is readily available to have both a direct and indirect effect on the nervous system. Tat has been demonstrated to be a potent neurotoxin when it is directly applied to neurons. Tat also can have profound effects on the function of the central nervous system indirectly through its potential to alter the cellular function and state of the resident cells. However, much of this work has been performed in vitro due to a paucity of animal models for the paradigm.

Objective: To develop an in vivo animal model for the study of Tat-induced neurologic alterations.

Methods: A 2 plasmid tetracycline-inducible system was chosen. The first construct placed the tetracycline reverse transactivator under the control of the glial fibrillary acidic protein (GFAP) promoter (Clonetech; pGFAP-rtTA). The second construct introduced Tat 1-86 (HIV-1 IIIB) under control of the tetracycline responsive element in pTREX (Clonetech; pTRE-Tat). Each of these constructs was introduced into mice by microinjection of fertilized eggs. The mice were then crossbred to generate mice (tgTat) that would express Tat in GFAP+ cells following treatment with doxycycline (Dox).

Results: Tat was found to be expressed specifically in the brains of Dox treated tgTat mice by RT-PCR and Western blotting. Immunostaining also revealed the presence of Tat in cultured astrocytes derived from tg-Tat mice following Dox treatment. While there was no gross neuroanatomical defects readily apparent, immunohistochemistry revealed Tat positive cells in the Dox treated mice, whereas the vehicle controls showed no evidence of Tat. Mass spectroscopy revealed a significant decrease in several cholesterol species and a significant increase in HNE-lysine and HNE-histidine suggestive of increased oxidative stress. Although we

found no significant alteration in neuronal number among the Tat expressing mice, these findings led us to examine neurogenesis. We found that Tat expressing tgTat mice had a reduced capacity for neurogenesis by BrdU incorporation. As for the indirect mechanisms by which Tat may impact brain function, a protein array analysis revealed that several cytokines and chemokines were highly elevated in the Tat expressing Dox treated mice.

Conclusions: The results to date with the tgTat mice indicate that there is a state induced in vivo by Tat that is not only detrimental to proper neuronal functioning and survival, by inducing inflammatory and oxidative states, but also impairs neurogenesis. Several studies are ongoing to further characterize these mice. However, our findings thus far indicate that this murine model serves well for studying the ramifications of low-level Tat expression in the central nervous system.

P67

Rapid induction of type I IFN responses in brain macrophages following SIV infection

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Invasion of the brain by HIV and SIV can occur early after infection although the development of HIV-associated cognitive disorders does not typically occur until after the onset of immunosuppression. Thus, mechanisms must exist in the CNS to control acute HIV/SIV replication. Using a rapid and consistent SIV/macaque model of HIV CNS disease, we provided the first molecular evidence to support the existence of one such mechanism and subsequently proposed a model of transcriptional SIV latency in the brain. We demonstrated that SIV RNA that is readily detectable in the brain during acute infection (10 days p.i.) is greatly reduced during asymptomatic infection (21 days p.i.), despite the persistence of stable levels of SIV DNA in the brain at both times, indicating that acute SIV replication in the brain is suppressed at least in part at a transcriptional level. Next, we demonstrated that the induction of innate immune responses involving IFNb and LIP (the dominant-negative isoform of the transcription factor C/EBPb) parallels the suppression of acute SIV replication in the brain. Finally, we demonstrated that IFNb induces expression of LIP and that wild-type C/EBPb (LAP) and LIP bind to the SIV LTR and activate or suppress LTR activity, respectively, by recruiting or suppressing recruitment of histone acetylase activity. Collectively these data provide compelling evidence, both in vitro and in the brain, to support the following model: SIV/HIV invades the CNS during acute infection, at which time LAP predominates at the LTR and together with chromatin remodeling events, mediates LTR-dependent transcription resulting in the production of full length viral RNA in the brain. Concomitantly, acute virus infection triggers the production of IFNb in the brain, which induces alternative translation of C/EBPb mRNA resulting in increased expression of LIP relative to LAP between 7 to 21 days p.i. At this time, LIP predominates at the LTR, chromatin remodeling events at the LTR subside and the production of full length viral RNA becomes undetectable. What remains unclear is when and how IFNb-mediated immune responses are induced by SIV infection in the brain. Here, we demonstrate that SIV infection of primary blood-derived macrophages induces expression of IFNb mRNA, hallmark of the type I IFN response. We further demonstrate that macrophages isolated from the brains of SIV-infected macaques during acute infection (as early as 4 days p.i.) express high levels of SIV RNA as well as IFNb and MxA mRNA. Since MxA mRNA is commonly used as a marker to confirm the presence of active type I IFN protein, we conclude that cells in these populations have already responded to the type I IFN secreted by the SIV-infected cells. These data demonstrate that brain macrophages are infected in this model as early as 4 days p.i. and that acutely infected macrophages participate in the rapid induction of IFNb-mediated responses to SIV invasion of the brain.

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P68

Rabies virus induces survival of sensory neurons in purified cultures of dorsal root ganglia

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Rabies Virus (RABV) regulates the antiviral response in the Central Nervous System (CNS) inhibiting neuronal apoptosis by means of up-regulation of Bcl-2 or inducing lymphocytes death through Fas/FasL complex pathway. Dorsal root ganglia (DRG) sensory neurons play an important role in the transport, spreading and pathogenesis of RABV, since it is well known that those neurons capture and transport the virus from the periphery to the CNS. In this work, we evaluated the expression of both apoptotic and anti-apoptotic proteins during the infection with the highly neurotropic strain of RABV, CVS (Challenge Standard Virus) harvested in mouse brain. Infected and non-infected DRG cultures of adult mice were purified using the mitosis inhibitor cytosine arabinoside and processed at different times post-infection by immunohystochemistry. The results showed a significant increase in the number of infected neurons expressing Bcl-2 and FasL, whereas the proportion of infected neurons expressing Fas diminished. These results suggests that RABV induces the expression of the anti-apoptotic protein

Bcl-2 to counteract the apoptotic effects of infection on the neurons and, at the same time regulates the expression of apoptotic proteins such as FasL that could induce death of non-neuronal cell populations like Schwann cells and/or other cells involved in the immune response.

P69

Synaptic injury in NeuroAIDS

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Our laboratory has investigated the molecular basis for synaptic injury in HIV-1 associated dementia (HAD) because data from neuropathologic, neuropsychologic, and neuroimaging studies support the hypothesis that synaptic connections and function is affected early in the course of neurodegeneration. The principal mechanism for this type of injury is thought to ultimately involve excitotoxic activation of glutamate receptors in post-synaptic neurons, but results from clinical trials with NMDA receptor antagonists such as memantine have been disappointing. One explanantion for this may be increased sensitivity to excitotoxicity in an inflammatory setting such as HAD may facilitate synaptic injury following more physiologic glutamate release. We have previously shown (Bellizzi et al., J. Clin. Inv., 115(11):3185-3192, 2005) that high-frequency synaptic stimulation that induces long-term potentiation (LTP) in hippocampal slices can instead trigger calcium- and caspase-dependent dendritic injury, with failure of LTP, in slices exposed to a stable analog (cPAF) of platelet activating factor (PAF), a phospholipid mediator produced in post-synaptic membranes during normal synaptic activity that facilitates release of presynaptic glutamate, and in HAD, can also be released from infiltrating HIV-1 infected mononuclear phagocytes in levels that promote neuronal demise. Patch clamp recordings from post-synaptic neurons show no differences in duration or extent of glutamate receptor activation during stimulation in cPAF-treated or control slices, suggesting that similar patterns of glutamatergic activity might induce physiologic plasticity or synaptic injury depending on the presence of inflammatory mediators. Furthermore, we found that dendritic injury could be prevented and LTP restored by pre-treatment with a mitochondrial ATP-sensitive K+ channel antagonist known to induce chemical preconditioning in a number of disease models. Blocking Ca2+ influx or post-synaptic caspase activation prevented structural injury but failed to restore LTP to cPAF-treated slices. These results suggest that blocking abnormal NMDA receptor activation might not be sufficient to protect against synaptic injury, and that preconditioning vulnerable neurons may be one way to prevent activity-dependent synaptic injury while preserving physiologic plasticity. In support of this, we have recently demonstrated that rhodamine 123 fluorescence increases dramatically within 60 seconds in cPAF-treated hippocampal slices relative to control, before returning to baseline several minutes later. These results suggest that one of the earliest events in this type of synaptic injury may be mitochondrial dysregulation of that Ca2+. Experiments are in progress to determine the cellular locus and signaling pathways involved in these phenomena in order to develop a more effective therapeutic strategy to block this type of insult.

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P70

Circuit-specific abnormalities of dopamine synapses are present in NNTC brain specimens with HIV encephalitis

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Dysfunction of dopaminergic (DAergic) circuitry produces neurocognitive impairment and drives behavior that increases the risk of HIV transmission. We examined DAergic synapses in the striatum and prefrontal cortex of people with neurocognitive impairment due to HIV encephalitis. Pre- and post-synaptic DAergic protein markers were measured using Western blotting and RT-PCR in six decedents with HIVE and HAD, and six uninfected people obtained from one repository of the National NeuroAIDS Tissue Consortium (NNTC). In striatal homogenates two presynaptic markers were abnormal. The dopamine reuptake carrier was increased and tyrosine hydroxylase was decreased. Postsynaptic dopamine receptor type 2 protein (D2R) was decreased while D3R was increased. Alternatively spliced variants of D2R both were abnormal. Abnormal striatal synapse proteins were most prominent in specimens with abundant HIV Gag mRNA, and were distributed uniformly in striatum. Prefrontal DAergic synapses also were abnormal, but the changes were not identical to those observed in striatum. These data provide biochemical evidence that DAergic tone is abnormal in specific DAergic circuits in people with abundant HIV replication in the brain.

P71

The expanding spectrum of zoster sine herpete

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Herpes zoster or shingles is characterized by radicular pain and rash usually restricted to 1–3 dermatomes

and reflects reactivation of latent varicella zoster virus (VZV) from ganglia anywhere along the neuraxis. VZV reactivation may also cause pain without rash (zoster sine herpete) as first suggested in zoster patients who had dermatomal distribution radicular pain in areas distinct from zoster; unfortunately, no virological studies were performed. Subsequent cases of zoster sine herpete, strictly defined as dermatomal distribution pain without antecedent rash, have provided serological, virological, imaging, electrophysiological and pathological evidence supporting the role of VZV in this disease entity. Furthermore, VZV reactivation without rash may produce other neurologic diseases as described below.

The first verification of zoster sine herpete was in a physician who developed acute trigeminal distribution pain without rash, associated with a four-fold rise in serum antibody specific to VZV. Definitive virological verification of zoster sine herpete came from PCR analysis of CSF from two men with prolonged thoracic-distribution radicular pain without rash. Amplifiable VZV, but not HSV DNA, was found in their CSFs and blood mononuclear cells (MNCs). An additional virologically confirmed case demonstrated electromyographic fibrillation potentials restricted to chronically painful thoracic roots. MRI of another patient with virologically verified active VZV infection revealed inflammation in ganglia and nerve roots corresponding to persistent pain. Two remarkably compelling cases of zoster sine herpete, verified pathologically and virologically, deserve mention. The first was a 77-year-old man with T cell lymphoma and no history of zoster rash who developed an acute fatal meningoradiculitis of cranial nerve roots and cauda equina, pathologically and virologically confirmed to be caused by VZV. The second was an immunocompetent adult who had experienced relentless trigeminal distribution pain for more than a year without any history of zoster rash; pathological analysis of a trigeminal ganglionic mass confirmed severe VZV ganglionitis. Testing for VZV DNA and anti-VZV IgG antibody has expanded the spectrum of zoster sine herpete. Numerous cases of unifocal and multifocal VZV vasculopathy have been verified virologically in the absence of zoster rash. Acute VZV meningoencephalitis, myelitis or cerebellar ataxia may also occur without rash. Prevalence estimates of the expanding spectrum of zoster sine herpete await virological analysis of additional patients with prolonged radicular pain or other neurological symptoms and signs. Analysis should include a test for anti-VZV IgG antibody and PCR to amplify VZV DNA in CSF, and examination of blood MNCs for VZV DNA. Finally, the nosological entity of zoster sine herpete has considerable implications for analysis and treatment of patients with postherpetic neuralgia. Overall, VZV reactivation from latency in ganglia may produce various neurologic disorders all caused by the same pathogen.

P72

HTLV-I Tax down-regulates expression regulatory T cell-specific Foxp3

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Recent studies from our group have demonstrated that the expression of regulatory T cell-specific transcription factor Foxp3 was significantly reduced in CD4+CD25+ T cells from patients with human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) compared to healthy individuals (Yamano et al., JCI 115:1361-1368, 2006). This was accompanied by a lack of suppressive function in HAM/TSP CD4+CD25+ T cells compared to healthy individuals. Furthermore, we demonstrated that forced expression of the HTLV-I transactivator protein Tax into healthy donor CD4+CD25+ T cells resulted in down-regulation of Foxp3 expression and loss of suppressive function.

The present study was aimed at determining the mechanisms by which HTLV-I Tax down-regulates Foxp3 expression in regulatory T cells. CD4+CD25+ T cells were isolated from healthy donors by magnetic beads and transfected by Amaxa with expression vectors encoding Tax, or well-characterized mutants deficient in NF-kB (M22) or ATF/CREB (M47 and K88A) transactivation. After 24 hrs, cells were analyzed for Foxp3 expression using real-time RT-PCR (TaqMan).

Data from four independent experiments demonstrate that while overexpression of Tax and M22 led to significantly reduced levels of Foxp3 mRNA ($P = 0.04$ and $P = 0.02$, respectively), overexpression of K88A did not significantly reduce Foxp3 mRNA levels ($P = 0.06$). Our results suggest that Tax transactivation of the ATF/CREB pathway may be important for the dysregulation of Foxp3 expression in patients with HAM/TSP. Ongoing studies will examine the effect of Tax and Tax mutants on cytokine production in CD4+CD25+ T cells as well as the suppressive activity of CD4+CD25+ T cells. These studies, along with analysis of the Foxp3 promoter region, will aid in elucidating mechanisms governing Foxp3 expression and allow the development novel therapeutics aimed at rescuing Foxp3 expression in various autoimmune disorders where Foxp3 expression is deficient.

P73

The pattern of VZV gene expression at variable periods following death in a rat model of ganglionic latency

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Following a primary infection by the human herpes virus Varicella Zoster Virus (VZV) (varicella), the virus remains in a latent form in the trigeminal and dorsal root ganglia (DRG) for the life of the host. After a variable interval, the virus can reactivate to produce herpes zoster (shingles). An important unresolved question of VZV in the human peripheral nervous system is the differentiation betweeen true latency and a low level persistence or sub-clinical reactivation. It would be very interesting to distinguish latency or reactivation as a consequence of manipulation, which is impossible to determine while working with human ganglionic tissue. A direct approach to this problem requires the use of animal models. We therefore used a rat model of ganglionic latency (Sadzot-Delvaux et al. 1990) to determine whether the time spent at 40°C post-mortem has an influence on the pattern of expression of two VZV genes ORF63, which is known to be abundantly expressed during latency, and ORF40 a late gene not expressed in latency. Rats which had been latently infected with VZV were sacrificed and the DRG analysed directly or after they had been left at 4°C for 24 and 48 hr after death. After 48 hours, 6/13 were positive by RNA in situ hybridisation for ORF63 and 2/17 for ORF 40 and 2/6 ganglia were positive by nested RT PCR for ORF 63 as compared to 0/5 positive for VZV ORF40. Our results provide no evidence for reactivation of VZV in the rat DRG 48hr after removal, and provide indirect support for the hypothesis that patterns of expression of VZV genes detected in human tissue at even 48 hours post mortem reflect the pattern of expression in ganglionic latency.

C. Sadzot-Delvaux, MP Merville-Louis, P Delrée, P Marc, J Piette, G Moonen & B Rentier. An in vivo model of Varicella-zoster virus latent infection of Dorsal root ganglia. *J NeuroSci Res*, 1990, 26: 83–89.

P74

HIV-1 Vpr enhances M-CSF production in primary monocytes: Role of C/EBPbeta

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Macrophage colony-stimulating factor (M-CSF or CSF-1) appears to play an important role in promoting and maintaining macrophage reservoirs of human immunodeficiency virus type 1 (HIV-1) through its effects on monocyte proliferation, differentiation, susceptibility to infection and increased survival. M-CSF could also play a role in HIV-associated central nervous system disorders; M-CSF levels are moreover elevated in the cerebrospinal fluid in HIV-1 infected patients with dementia. To investigate the role of viral and cellular regulators on M-CSF regulation, we first cloned the M-CSF promoter upstream the luciferase reporter gene. We analyzed the potential role of Vpr, Tat and Rev as well as C/EBPbeta in THP1 cells for M-CSF promoter enhancement in cotransfection study. Both Vpr and C/EBPbeta upregulate M-CSF promoter activity in this cell line. In primary human macrophages, Vpr also upregulates M-CSF promoter activity. ELISA assay of culture supernatants demonstrated a 15-fold increase in M-CSF production in response to ectopically expressed Vpr. In view of the importance of M-CSF in monocyte/macrophage differentiation and survival, the role of Vpr in M-CSF activation may be an important aspect in the pathogenesis of HIV infection, the maintenance of the macrophage/microglial reservoir of HIV infection, and the development of CNS manifestations of AIDS.

P75

Annexin 2 overexpression in 293T cells increases HIV-1 Gag mRNA and protein levels and facilitates viral release

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HIV encephalitis, the pathological substrate of the cognitive and motor syndrome known as the AIDS dementia complex, is defined by the presence of virus in the brain with accompanying neuronal death. Much of the neuronal injury is caused by toxic viral proteins and inflammatory mediators produced by HIV-infected macrophages that accumulate in perivascular regions of the encephalitic brain parenchyma. Unlike in T lymphocytes, where HIV buds from the plasma membrane, in macrophages the virus assembles at the limiting membrane of and buds into endosomal multivesicular bodies (MVBs). An interaction between the viral protein that mediates assembly, p55Gag, and the cellular protein Annexin 2 (Anx2) was recently shown to occur in monocyte-derived macrophages (MDM) (Ryzhova et al., 2006). This interaction occurs preferentially at endosomal membranes and is important for HIV replication in macrophages, as depletion of Anx2 reduces the production of infectious virions and is associated with aberrant processing of p55Gag. To investigate the effects of Anx2 gain-of-function on HIV assembly and budding, we have overexpressed Anx2 in 293T cells, which have very low basal levels of the protein and in which HIV normally buds from the plasma membrane. Immunofluoresence demonstrates that Anx2 overexpression leads to the formation of large intracellular vacuoles. Co-immunoprecipitation experiments confirm that Anx2 and p55Gag interact in

293T cells and that this interaction is associated with detergent-resistant membranes. Anx2 overexpression has a functional effect on HIV replication in 293T cells, as those cells expressing Anx2 release more virions and have higher intracellular levels of Gag protein and mRNA. We hypothesize that overexpression of Anx2 increases the number and/or stability of HIV-assembly platforms in 293Ts, leading to increased Gag stability and virus release.

P76

Temporal analyses of the neuropathogenesis of a West Nile virus infection in mice

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A West Nile virus (WNV) infection in humans can produce neurological symptoms including acute flaccid paralysis, encephalitis, meningitis and myelitis. To investigate the pathogenesis of WNV in the peripheral and the central nervous system (PNS and CNS), we used a murine footpad inoculation model of WNV infection. Survival curves of virus-infected animals of ages 4- to 6-weeks-old demonstrated age-dependent mortality where older animals (6-weeks-old) had a higher mortality rate compared to younger animals (4- and 5-weeks-old). The mice that survived the virus infection formed WNV-reactive antibodies, confirming viral infection and clearance. The localization of viral RNA (vRNA) and antigen in infected murine tissues was investigated using TaqMan and immunohistochemistry (IHC) respectively. During a nine day infection,vRNA levels in the spinal cord and brainstem fluctuated, suggesting early viral clearance from these tissues by days 3–4 p.i. with later re-introduction. Viral antigens detected using IHC were primarily observed in three main regions of the brain: cortex, hippocampus and brainstem. Additionally, the dorsal root ganglion neurons of the PNS stained positive for viral antigens. These data are consistent with multiple routes of neuroinvasion following a peripheral inoculation of virus and do not preclude the previous observation that virus-infected peripheral neurons can introduce virus into the CNS by a retrograde transport mechanism.

P77

Signature sequences within the HIV-1 LTR predictive of HIVD

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

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Herpes simplex virus type 1 and the type 4 allele of the apolipoprotein E gene in Alzheimer's disease

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Infectious agents are putative factors in several chronic diseases, especially the age-associated ones, heart disease and Alzheimer's disease (AD). Our aim is to elucidate the role of herpes simplex virus type 1 (HSV1) in AD, following our discovery that HSV1 resides latently in brain of many elderly people, and that in brain of APOE-e4 carriers, it confers a major risk of AD (Itzhaki et al., 1997; Lin et al., 1998). HSV1-APOE interaction in AD is strongly supported by our finding that APOE determines disease severity in disorders caused by diverse pathogens (Itzhaki et al., 2004). Further evidence for HSV1 presence in brain—and for its having replicated there, perhaps recurrently—is afforded by our detecting intrathecal antibodies to HSV1 (these are long-lived) in many elderly people (Wozniak et al., 2005) and, by in situ PCR, detecting HSV1 DNA in neurons and glial cells.

We recently discovered that HSV1 infection of cultured neural cells affects the degradation of amyloid

precursor protein (APP) (Shipley et al., 2005); also, it causes deposition of intracellular beta-amyloid 42. Further, the level of abnormally phosphorylated tau too increases in the infected cells. Preliminary experiments suggest that in brain of HSV1-infected mice, there is an accumulation of beta-amyloid 42.

Results of others, including epidemiological data, indicate that infection and stress cause cognitive decline, probably via cytokine entry into brain and consequent inflammation. We propose that latent HSV1 is reactivated by the inflammation which, in turn, is augnmented by the activated virus (cf. enhanced inflammation in mice with pre-clinical prion disease—Combrinck et al., 2002); damage is thereby increased—perhaps via beta-amyloid 42 production—especially in APOE-e4 carriers, leading eventually to AD.

Our amyloid data are the first to point to an actual cause of the abnormal deposition of beta-amyloid in brain of sporadic AD sufferers and, by implicating HSV1, they indicate the usage of antiviral agents for treatment, and perhaps of vaccination against HSV1 for prevention of AD. The latter possibility is supported by our finding that vaccination of mice against HSV1 is highly protective against establishment of viral latency in brain (Lin et al., 2001).

P79

Development of a small rodent model of HIV-associated sensory neuropathies

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Background: Peripheral neuropathy is the most common neurological complication of HIV (human immunodeficiency virus) infection affecting quality of life and effective use of treatment regimens. Recently we developed in vitro models of HIV-associated neuropathies (distal sensory polyneuropathy due to the HIV infection itself and antiretroviral toxic neuropathy due to some of the dideoxynucleoside analogues). We used these models to investigate mechanisms of neuronal injury and more importantly to screen for neuroprotective compounds. However, there are no reliable models of HIV-associated neuropathies in rodents for effective drug screening. In this study, we developed a mice model of HIV-associated sensory polyneuropathies.

Methods: We administered oral ddI in drinking water of transgenic mice expressing HIV viral envelope protein gp120 under the GFAP promoter, which allows expression in the glial cells. After 1 month, we harvested the distal footpad skins, intrinsic foot muscles, sciatic nerves and spinal columns for a detailed evaluation of the peripheral nervous system by immunohistochemical and ultrastructural analyses.

Results: Transgenic mice expressing gp120 in the glial cells, when administered ddI, developed a sensory neuropathy characterized by reduction in intraepidermal small sensory fibers and reduction in the number of unmyelinated fibers in the distal nerves. There was no significant neuronal loss proximally in the sciatic nerve or at the dorsal root ganglion level.

Conclusions: This animal model combines the neurotoxic effects of high levels of gp120 in the endoneurium and exposure to ddI, a dideoxynucleoside analogue known to cause peripheral neuropathy in patients. Using this animal model we will be able to study pathogenic mechanisms and contribution of each to the axonal degeneration in detail. Furthermore, we will use this animal model to screen for drugs that are promising as neuroprotective agents in our in vitro assays.

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P80

Evaluation of excitotoxicity in rabies virus-infected primary neuronal cultures and in experimental rabies in mice

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There has been recent speculation that glutamate receptors may be rabies virus receptors and that excitotoxicity may play an important role in the pathogenesis of rabies. A recent human survivor of rabies received therapy with the NMDA antagonist ketamine and other drugs. In order to determine the role of excitotoxicity in rabies virus infection in primary neuronal cultures and in vivo in an experimental mouse model, we have evaluated the effects of NMDA antagonists in mouse embryonic neuronal cultures derived from the cerebral cortex and hippocampus of 17-day-old fetuses infected with the CVS-11 strain of fixed rabies virus (CVS) and in CVS-infected 6-week-old ICR mice. CVS-infected cultures showed trypan blue exclusion, morphologic features of apoptosis, and increased expression of activated caspase-3 over 3 days post-infection, which is consistent with CVS-induced neuronal apoptosis. Stimulated increases of intracellular calcium were determined in fluo-3 AM loaded neurons using a micro-fluorometer. Up to 3 days post-infection CVS-infected cortical and hippocampal neurons did not show increased responsiveness to glutamate. The non-competitive NMDA antagonists, ketamine (3 μg/mL) and MK-801 (60 μM), were found to have no significant neuroprotective effect on cultured cortical or hippocampal neurons as assessed by trypan blue exclusion. Glutamate (100 nM) significantly increased trypan blue exclusion at

1–3 days post-infection and also significantly increased trypan blue exclusion in CVS-infected neuronal cultures. In contrast, the pan-caspase-3 inhibitor DEVD-CHO (25 μM) exerted a marked neuroprotective effect and significantly reduced trypan blue exclusion in CVS-infected cultures, and caspase inhibitors require further evaluation in in vivo studies. Ketamine (120 mg/kg/d) was also administered intraperitoneally in CVS-infected adult mice and produced no beneficial effect on the fatal clinical disease, it did not ameliorate the neuronal apoptosis in the brain versus control CVS-infected mice, and it did not inhibit viral spread in the CNS. We have found no supportive evidence that excitotoxicity plays an important role in rabies virus infection either in vitro or in vivo.

P81

DC-SIGN facilitates productive infection of monocyte-derived dendritic cells by human T cell leukemia virus type 1

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Human T cell leukemia virus type 1 (HTLV-1) has been identified as the etiologic agent of adult T cell leukemia (ATL) and the neurologic disorder, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Numerous studies of HAM/TSP patients have demonstrated the generation of an intense cytotoxic T cell (CTL) response with large numbers of CD8+ CTLs directed against the HTLV-1 oncoprotein Tax 11-19 peptide. Additional studies have demonstrated that patients diagnosed with HAM/TSP exhibit rapid activation and maturation of dendritic cells (DCs) while ATL patients exhibit a maturation defect in this cell lineage. In addition to T cells, HTLV-1 is known to infect DCs. HTLV-1 infection of DCs could alter general DC function or the specific processing and/or presentation of HTLV-1-specific peptides, potentially playing a major role in the course of HTLV-1-associated disease. In this regard, we have demonstrated that an important antigen receptor on DCs, DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin), a C-type lectin, serves as a receptor for HTLV-1 binding using a quantum dot-based fluorescent binding assay. We have also demonstrated that silencing of DC-SIGN inhibits the infection of DC in a DC/T cell co-infection system. Furthermore, expression of DC-SIGN in B cells enhances viral binding, entry and infection. These investigations, which consider the involvement of DC surface molecules in HTLV-1 pathogenesis, are among the first explorations of the intricate mechanisms that underlie the interactions of HTLV-1 with the DC compartment. Our results provide further insights into the participation of DCs in HTLV-1 pathogenesis and increase our understanding of adhesion pathways that regulate DC trafficking and interactions with T cells during the development of disease.

P82

Extracellular Tax protein of human T cell leukemia virus type 1 activates monocyte-derived dendritic cells and enhances their maturation, function, and antigen-specific T cell proliferation

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HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is characterized by the generation of an intense cytotoxic T cell (CTL) response with large numbers of CD8+ CTLs directed against oncoprotein Tax. Previous studies suggest that Tax may be available for immune recognition by dendritic cells (DCs), the most efficient antigen presenting cell population. In this study, we have shown that purified Tax protein efficiently bound and localized to the cell membrane of monocyte-derived dendritic cells (MDDCs) and was internalized within a few hours. After uptake, Tax induced expression of DC activation markers MHC class I and II, and costimulatory molecules (CD40, CD80, CD86, and CD54) as well as the DC maturation marker, CD83. Tax has also promoted the production of major immune-directing cytokines IL-12, TNF-alpha, and proinflammatory chemokines MIP-1alpha, MIP-1beta, and RANTES, which drive a Th1-type immune response. The inhibitors of NF-kappaB have abrogated Tax-induced secretion of cytokines/chemokines indicating a role for NF-kappaB signaling in the Tax-mediated immune response. Finally, Tax enhanced the allogenic and antigen-specific T cell proliferation capability of MDDCs. These results indicate that extracellular Tax may selectively target MDDCs, be taken up by these cells most likely by specialized pathways, and promote their maturation and antigen-presenting functions, driving a Th1-type immune response.

P83

E2F1 induces neuroglial cell death by activation of the calpain pathway in an in vitro model of HIV Encephalitis

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HIV Encephalitis (HIVE), the pathological substrate of HIV Associated Dementia (HAD) is characterized

by microgliosis, astrogliosis, neuronal loss and dendritic damage. Neuronal damage in HIVE has been linked to soluble factors released by HIV infected macrophage/microglia that infiltrate the central nervous system (CNS), not direct neuronal infection. However, the exact mechanism of neuronal death in HIVE remains unknown. Interestingly, the pro-apoptotic cell cycle protein, E2F1, exhibits increased immunoreactivity and altered subcellular distribution in several neurodegenerative diseases including Alzheimer's Disease, Parkinson's Disease, Amyotrophic Lateral Sclerosis and HIVE. However, in these diseases E2F1 localized predominantly to the cytoplasm, a localization distinct from its well-defined nuclear role as a transcription factor during apoptosis and cell cycle progression. Based on these observations we hypothesized that cytoplasmic E2F1 might also contribute to neuronal death in HIVE. We used an in vitro model of HIVE in which we treated primary rat neuroglial cultures with supernatants from human monocyte-derived-macrophages infected with a neurovirulent strain of HIV-1 (HIV M/M). Using a concentration of HIV M/M that produced 50% neuronal loss by 20 hours, we observed increased E2F1 expression, accompanied by increased calpain activation by western blotting. To determine if increased expression of cytoplasmic E2F1 increased calpain activity, we utilized adenovirus vectors to express a cytoplasm-localized E2F1 deletion mutant (E2F1 (180-437)). As predicted, we observed increased calpain activity and cell loss. Furthermore, we observed an increase in E2F1/14-3-3 complex formation in our HIV M/M exposed cultures; such complex formation has been associated with increased E2F1 protein stability. Based on our in vitro observations, we propose that increased E2F1 stability mediated by 14-3-3 binding and cytoplasmic complex formation may contribute to calpain activation and subsequent neuronal death in HIVE.

P84

HIV Tat induces protein degradation of RON, a receptor tyrosine kinase that regulates tissue inflammation

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Macrophages are the primary source of HIV in the brain and are suspected to play an important role in the development of neuropathologies associated with AIDS in part due to the dysregulated expression of inflammatory mediators. We have demonstrated that the receptor tyrosine kinase, RON, a critical regulator of macrophage function and inflammation, inhibits HIV transcription and hypothesize that HIV should have mechanisms by which to circumvent negative signals that inhibit HIV transcription in macrophages. Previous results have shown that RON is decreased in brains from AIDS patients providing circumstantial evidence to support this hypothesis. We present data demonstrating that HIV Tat promotes the proteosome-dependent degradation of RON. In addition to describing a novel activity of Tat, we suggest that this is one mechanism by which HIV infection directly promotes inflammation to create a microenvironment favorable for HIV replication. Furthermore, by targeting RON HIV would increase susceptibility to opportunistic infections and facilitate the progression of AIDS-associated diseases.

P85

Pura modulates Rev/RRE-mediated expression of HIV-1 intron-containing mRNA in astrocytes

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In order to produce proteins important for successful viral replication, HIV-1 generates over 30 mRNAs: unspliced, singly spliced, and doubly spliced. To bypass the retention of unspliced pre-mRNA in the nuclei HIV has evolved a Rev-regulated transport system, which promotes the export of the unspliced RNA into the cytoplasm. This process requires a 351nt RNA—Rev responsive element (RRE), which is located in the env gene and is present on unspliced and singly spliced RNAs. Pura, a single-stranded DNA and RNA-binding protein, is implicated in the transcriptional control of a number of cellular genes and viral promoters, including JCV and HIV. Pura associates with polyribosomes and also proteins and mRNAs colocalized to the kinesin-associated granules in dendrites. Here we report that human Pura has the ability to augment Rev/RRE-mediated expression of HIV-1 intron-containing mRNA. Results from experiments using a construct that contains 3′ half of HIV provirus placed distal to the LTR of HIV and CAT gene or an intronless (env deleted) construct demonstrated that Pura increased Rev mediated CAT gene expression and that the presence of intron with the env gene is important. Silencing of Pura resulted in the inhibition of CAT gene expression. Results from reporter assays utilizing a construct, where the luciferase gene was placed in the intron with or without RRE under control of CMV promoter demonstrated that Pura augments Rev mediated activation of luciferase gene expression. Northern blot analysis of total or cytoplasmic RNA using a probe for RRE, which detects unspliced form of RNA only, and for CAT fragment, which detects both, spliced and

unspliced RNA, showed that Purα increased levels of unspliced RNA in cytoplasm and augments the effect of Rev. Purα interacts with Rev protein in an RNA dependent manner. Nucleic acid binding domain of Purα is important for this interaction. Purα associates with the region of Rev spanning second multimerization domain M2 and AD/NES. Results from gel retardation assays demonstrated that Purα can directly bind to RRE RNA and increase Rev:RRE complexation. Immunoprecipitation of nuclear and cytoplasmic RNA:protein complexes following RNA extraction and RT-PCR showed that Purα is associated with RRE RNA in cytoplasm of cells. These findings suggest that Purα may synergize with Rev in transporting of unspliced RNA, probably targeting them to sites of translation.

P86

A novel quantum dot-based high throughput fluorescence assay for the quantitation of human T cell leukemia virus type 1 binding to host cells

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Quantum dots (QD) are fluorescent semiconductor nanocrystals comprised of a cadmium selenide core with a semiconductor shell of zinc sulfide coated with a polymer shell allowing particles to be conjugated to biological molecules while retaining the optical properties of the particle. They have narrow emission spectrums, high quantum yield, and good photostability. We have used these unique properties of QDs to develop a simple high-throughput binding assay to study the attachment of HTLV-1 to host cells. In order to enhance the end point detection limit, we have biotinylated HTLV-1 (biot-HTLV-1) and used streptavidin-coated QDs (strep-QD). Primary standardization was performed on Jurkat CD4+ T cells since this cell type is representative of the major target population infected with HTLV-1 in patients. A dose-dependent increase was observed in HTLV-1 binding to Jurkat cells indicating linearity of the assay at both 4° and 37°C. The specificity of the assay was confirmed by using increasing amounts of non-biotinylated virus to demonstrate competitive inhibition. We have also tested differential binding of HTLV-1 to various cell types of pathogenic relevance. T cells and dendritic cells (DCs) exhibited maximal affinity as compared to other cell types examined. Finally, we have screened a number of blocking antibodies directed against a putative HTLV-1 receptor on DCs referred to as DC-SIGN (dendritic cell specific ICAM-3 grabbing non-integrin). We were able to map DC-SIGN epitopes important for HTLV-1 binding as compared to those for HIV-1 binding. This novel, easy-to-perform, high throughput assay can be utilized to study the binding of any virus that can be biotinylated, for the small molecule inhibitor screening, epitope mapping, and for receptor studies.

P87

Cross-interaction between JCV agnoprotein and HIV-1 Tat modulates transcription of HIV-1 LTR in glial cells

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The human polyomavirus, JCV is the causative agent of the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML), which is commonly seen in AIDS patients. The bicistronic viral RNA, which is transcribed at the late phase of infection is responsible for expressing the viral capsid proteins and a small regulatory protein, Agnoprotein. Immunohistochemical analysis of brain tissue with AIDS/PML revealed the co- localization of the HIV-1 transactivator, Tat and JCV Agnoprotein in nucleus and cytoplasm of bizarre astrocytes. In accord with this observation, we detected the co-presence of Agnoprotein and Tat in human astrocytes upon infection with JCV and HIV-1, or in astrocytic cells expressing these proteins after transfection. Interestingly, results from infection of human astrocytes with HIV-1 and JCV showed a decrease in the level of HIV-1 replication in cells, which are co-infected with JCV. Conversely, a slight increase in the level of JCV replication was observed in the presence of HIV-1.The co-presence of JCV and HIV-1 in astrocytes prompted us to investigate the possible cross-interaction of Agnoprotein with Tat and its impact on HIV-1 gene transcription. Our results demonstrate that Agnoprotein through its N-terminal domain associates with Tat, and the interaction causes the suppression of Tat-mediated enhancement of HIV-1 promoter activity in these cells. Results from RNA and protein binding assays showed that Agnoprotein can inhibit the association of Tat with its target RNA sequence, TAR and with cyclin T1. Furthermore, Agnoprotein is able to interfere with cross-interaction of Tat with the p65 subunit of NF-kB and Sp1 whose functions are critical for Tat activation of the LTR. These observations unravel a new pathway for the molecular interaction of these two viruses in the biologically relevant cells in the brains of AIDS/PML patients.

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P88

Dendritic cell function and resistance to cutaneous HSV-1 infections

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The immune system plays a particularly important role in controlling HSV-1 infection in both the nervous system and periphery. Innate mechanisms, including early IFN production and natural killer (NK) cell activation, represent the first line of defense against infection, limiting viral replication and spread before the more specific acquired CD8 T cell immune response is activated and optimal viral clearance achieved. The innate and acquired immune responses are ultimately linked together during antigen presentation amidst the inflammatory cytokine milieu generated during the innate immune response in the draining lymph nodes. Of professional antigen-presenting cells (APCs), dendritic cells (DCs) are thought to be particularly important links between the innate and adaptive immune responses against many pathogens. The role of DCs as mediators of resistance to HSV-1 infection was investigated using CD11c-diphtheria toxin (DT) receptor-green fluorescent protein transgenic mice, in which DCs can be transiently depleted in vivo by treatment with low doses of DT. We show that ablation of DCs led to enhanced susceptibility to HSV-1 infection in the highly resistant C57BL/6 mouse strain. Specifically, we showed that the depletion of DCs lead to increased viral spread into the nervous system, resulting in an increased rate of morbidity and mortality. Furthermore, we showed that ablation of DCs impaired the optimal activation of NK cells, CD4+ and CD8+ T cells in response to HSV-1. These data demonstrated that DCs were essential not only in the optimal activation of the acquired T cell response to HSV-1, but that DCs were crucial for innate resistance to HSV-1 infection.

P89

Dorsal root ganglion inflammation and distal symmetrical polyneuropathy in advanced HIV

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Dorsal root ganglion (DRG) infiltrates of activated macrophages have been documented in HIV, and are hypothesized to play a role in the generation of distal symmetrical polyneuropathy (DSP). While DRG inflammation and distal nerve damage is highly prevalent at autopsy of individuals with AIDS, their relationship to clinically diagnosed DSP has not been exhaustively investigated. Accordingly, we examined 32 lumbosacral DRG obtained at autopsy from an advanced HIV cohort for the extent of activated macrophage infiltrates, and correlated this analysis with pre-mortem clinical findings. Individuals participating in the Manhattan HIV Brain Bank study underwent neurological evaluations prior to death; DSP was diagnosed if they displayed abnormalities in two or more of the following: ankle reflexes, vibratory or pinprick perception. Patients were classified as symptomatic if they described pain, paresthesia or numbness. Eighteen of the 32 patients displayed clinical signs of DSP by this definition. At autopsy, their lumbosacral DRG were routinely processed for paraffin histology, and semiquantitative immunohistochemistry was performed for CD68 expressing macrophages, as well as soluble HIV apoptotic factor (SHIVA), a CD68 macrophage product with known neuronotoxic properties. Quantitations were performed in the most severely affected portions of the DRGs. In a subset of 25 individuals, sural nerve morphometry was conducted to estimate myelinated fiber density. Bivariate analyses were conducted to determine whether the severity of macrophage infiltrates bore any relationship to signs, symptoms, or diagnosis of DSP or to myelinated fiber density. The percentage area of macrophage infiltrates was highly variable in this sample, ranging from 2.5% to 51.7% of the DRG. The extent of CD68 or SHIVA staining was unrelated to whether or not the patient carried a pre-mortem diagnosis of DSP. There tended to be smaller areas of macrophage infiltration in patients with positive symptoms or signs of DSP (for example, the mean CD68 staining area was 8.7 +/− 5.5 for those with positive symptoms and15.8 +/− 11.0 for those without, $p = .0925$, t test). SHIVA and CD68 staining were similarly unrelated to the myelinated fiber density of sural nerve ($r = .117$ and .155 for SHIVA and CD68, $p = .5773$ and .4581, respectively). We conclude that while abnormal DRG inflammation is prevalent in advanced HIV, there are likely to be other, as of yet undetermined, factors that contribute to the development of clinical manifestations of HIV-related DSP.

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P90

Issues in varicella-zoster virus (VZV) ganglionic latency

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Varicella-Zoster virus (VZV) is a pathogenic human herpes virus that causes varicella (chickenpox) as a primary infection following which it goes into latent form in trigeminal ganglia (TG) and dorsal root ganglia (DRG). After a variable period, which may be many

years, the virus reactivates, either spontaneously, or after one or more triggering events, to cause herpes zoster (shingles). In about 50% of individuals over the age of 65 yr, zoster is complicated by post-herpetic neuralgia (PHN) which is a major cause of morbidity, and often refractory to treatment. A better understanding of the biological and molecular basis of VZV latency is essential to develop better therapies for zoster and its numerous neurological complications. Such studies are hampered by the highly cell-associated nature of the virus and the absence of a very good animal model of VZV latency. However, valuable information has been obtained from studies using rat, guinea pig and simian models.

After a 10 yr period of controversy, it is now established beyond doubt that latent VZV is located predominantly in neurons in human TG and DRG, with only occasional satellite cells infected. There has been more consensus on the extent of viral gene expression in VZV latency with VZV ORFs 4,21,29, 62,63 and 66 shown to be expressed using different techniques. However, it is still not known for certain whether the true extent of viral gene expression is limited to these six genes. Other key issues that need to be resolved in this field are (a) the extent to which results obtained in animal models can be extrapolated to human latency, (b) the nature, if any, of the functional roles of the latency-associated VZV-encoded proteins in the different stages of latency, (c) whether genes shown to be essential for experimental latency establishment may also have a role in reactivation, and (d) whether VZV latency and reactivation are involved in the pathogenesis of PHN.

P91

Pro-inflammatory role of PGE2 in microglia

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Microglia play an essential role in innate immunity and in the activation of T cells in the CNS. The pro-inflammatory cytokine IL-23 is associated with autoimmune disorders including EAE, IBD and RA. IL-12 and IL-23 share the p40 subunit, complexed to p35 (IL-12) or to p19 (IL-23). Although the role of PGE2 in CNS is controversial, PGE2 is expressed at high levels in CNS trauma, infection, or neurodegenerative diseases. The purpose of the present study was to determine whether generation of microglia in the presence of PGE2 affects their responses to inflammatory conditions, particularly the production of IL-12/IL-23. Microglia were generated in the presence of GM-CSF(MG) or GM-CSF+PGE2 (pMG) and characterized following stimulation with LPS. MG differ from pMG in several respects. First, pMG show higher levels of CD80, CD86, CD40, and MHCII. Second, although there is no difference in the amounts of IL-6, TNF, IL-12p40, and IL-10 between MG and pMG, pMG secrete significantly higher levels of IL-23. In agreement with the protein data, MG and pMG show differences in the expression of p19 and p35 mRNA. P19 expression was significantly higher in pMG, whereas p35 levels were higher in MG, with no difference in the p40 expression. IL-23 was shown to play a role in the differentiation/survival of CD4+T cells producing IL-17 (Th IL-17) which have an essential role in autoimmune diseases, including MS. This let us to investigate the role of PGE2 in vivo in experimental autoimmune encephalomyelitis, an animal model for MS. SJL/J mice were immunized with PLP and inoculated with dendritic cells generated in the absence (DCs) or presence of PGE2 (pDC). Mice, receiving pDC develop clinical symptoms earlier, have more relapses, and produce higher levels of IL-17 and IFN-g. These observations imply that PGE2 released in inflammatory conditions may increase the expression and release of IL-23 by microglia, and contribute to autoimmune disease through the induction, survival or activation of Th IL-17 cells.

P92

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

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The human immunodeficiency virus type 1 (HIV-1) vpr gene is an evolutionarily conserved gene among the primate lentiviruses. Several functions are attributed to Vpr including its ability to cause cell death, cell cycle arrest, apoptosis, and DNA damage. The Vpr-domain responsible for DNA damage as well as the mechanism(s) through which Vpr induces this damage has not been determined. Using site directed mutagenesis, we identified the helical domain II within Vpr (aa 37-50) as the region responsible for the DNA damage. Surprisingly, Vpr (delta 37-50) failed to cause cell cycle arrest or apoptosis, but maintained its capability to activate HIV-1 LTR and to localize to the nucleus. In addition, our cytogenetic data indicated that helical domain II induced chromosomal aberrations, which mimicked those induced by cisplatin, an anti-cancer agent. This novel molecular mimickary function of Vpr might lead to its potential therapeutic use as a tumor suppressor.

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HIV-1 Vpr exhibits high affinity for LTR sequences commonly encountered during late stage disease and HIVD

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

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MHV-68 inoculation of neonatal mice causes persistent infection and inflammation of the CNS mimicking cerebral EBV infection

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The Epstein-Barr virus (EBV), a gamma-herpesvirus, causes various neurological symptoms ranging from facial palsy to encephalitis. The molecular pathogenesis of these complications has not been elucidated, efficient therapeutic regimens are not available. We established a model system for neurologic gamma-herpesvirus diseases by inoculating newborn BALB/c mice intranasally with MHV-68. All types of inflammation observed in children in response to EBV can be observed in MHV-68 infected mice.

For characterization of the pathogenesis the kinetics of the viral appearance in the CNS and the related inflammatory response were determined and linked to each other.

At different time points post infection (p.i.) solid organs and blood of infected (n = 30) and control mice (n = 7) were checked for viral load by PCR, for viral activity and localisation by IFA, and for cellular infiltrates by H.E. staining and immunhistochemistry. Six days p.i. the viral load in brain tissue exceeded viremia significantly indicating intracerebral viral replication. Viremia was maximal one week p.i. and decreased rapidly, maximal viral load in brain was gained on day 12 p.i. and remained positive for months. Cellular infiltrates were initially detected 9 days p.i. (T-lymphocytes), their extent increased 16-48 days p.i. and decreased again 100 days p.i. A strong positive correlation was observed between CNS viral load and severity of inflammation, particularly in the temporal lobe.

Taken together, MHV-68 leads to delayed, persistent CNS infection followed by inflammation patterns observed in patient with cerebral EBV infection. High viral loads in CNS are indicative for severe inflammation.

P95

TNF-alpha modulates NMDA receptor trafficking by increasing the ceramide content of neuronal membranes

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The trafficking of ionotropic glutamate receptors at the plasma membrane is thought to modulate synaptic strength and play important roles in plasticity and survival. While much attention has focused on the protein-protein interactions that guide the insertion and removal of receptors from the cell surface, the contribution of local lipid composition to these fusion and budding events have not been studied. In this study we used imaging, biochemical, and electrophysiological approaches to determine how alterations in the local lipid composition modulate NMDA receptor trafficking. The cytokine TNF-alpha rapidly (within 2 min.) increased the number of NMDA receptors

that clustered into lipid platforms and altered the sphingolipid-phospholipid distribution of neuronal membranes by increasing the ceramide and sphingomyelin content. Consistent with increased numbers of functional receptors at the cell surface, NMDA-mediated excitatory currents (EPSCs) and the number of large focal calcium bursts were increased in neurons treated with TNF-alpha. Pharmacological inhibition of neutral sphingomyelinase prevented TNF-alpha-induced increases in the number of NMDA receptor clusters, NMDA-mediated current and large focal calcium transients, suggesting an important role for ceramide in NMDA receptor trafficking. These findings suggest that the fusion of NMDA receptor-containing vesicles with the plasma membrane occurs at lipid platforms and is initiated by the catabolim of sphingomyelin to ceramide.

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P96

Disseminated simian varicella virus infection in an irradiated rhesus macaque (Macaca mulatta)

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We describe correlative clinicopathologic and virological abnormalities in a rhesus macaque (Macaca mulatta) which developed disseminated simian varicella virus (SVV) infection 105 days after X-ray irradiation. Twelve other monkeys in the colony were also irradiated, but none became ill or developed skin lesions, although anti-SVV IgM antibodies developed in three monkeys, indicative of subclinical infection. The collective findings are virtually identical to that described in humans with disseminated VZV infection and support the usefulness of studying SVV infection in primates as a model for human VZV infection, particularly attempts to induce virus reactivation by X-ray irradiation.

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Characterization of a bone marrow progenitor cell line to be used as a model for the study of susceptibility to HIV infection

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Human immunodeficiency virus type 1 (HIV-1) infection of the monocytic cell lineage is involved in the pathologic events associated with acquired immunodeficiency syndrome (AIDS) and HIV-1 dementia (HIVD). Interestingly, CD34+/CD38? progenitor cells within the bone marrow are refractile to HIV-1 infection, possibly due to their low level expression of HIV-1 co-receptors, CXCR4 and CCR5, which upon differentiation are up-regulated, increasing susceptibility to infection. Based on a number of considerations, the CD34+/CD38+ TF-1 erythromyeloid progenitor cell line was selected as a model to study the differentiation process of hematopoietic progenitor cells. Treatment of TF-1 cells with PMA results in the development of a macrophage-like morphology. In addition, the cell surface molecules CD34 and CD38 were down-regulated, while CD69 expression increased dramatically, indicating differentiation and activation, respectively. The HIV-1 co-receptor expression was considerably increased while CD4 was down-regulated. Following treatment with PMA, TF-1 cells secrete various cytokines that are able to stimulate undifferentiated TF-1 cells in the absence of PMA; these include IL-8, MIP-1alpha, MIP-1beta, and RANTES. TF-1 cells that were treated with conditioned media from PMA-treated cells exhibited a phenotype similar to that of PMA-treated cells with the exception of a lower level of CD4 down-regulation. The concurrent expression of the HIV-1 receptor and co-receptors may provide a window of opportunity for HIV-1 infection of this progenitor cell population. In summary, these studies suggest that the TF-1 cell line could serve as a model to study the susceptibility of bone marrow progenitor cells to HIV-1 infection.

P98

A study on HIV-1 viral load and dopamine concentration in different regions of post mortem human brain

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Background: Human immunodeficiency virus type 1 (HIV-1) is known to enter the central nervous system (CNS) shortly after infection and getting localized in different brain regions. It has been hypothesized that HIV-1 infection mediated neuropathogenesis may also adversely affect the central dopaminergic activity resulting in neurocognitive deficits. There is however,

scarcity of data on the distribution of viral load as well as dopamine in the brain of HIV-1+ individuals who had ART intervention during life. Since, neurocognitive and neuropsychological deficits continue to persist even after ART intervention, it is important to determine viral load and dopamine levels in different areas of the brain.

Objective: The main objective of this study was to investigate the relationship between central regional viral distribution, and the regional concentration of dopamine and its metabolite, homovanillic acid (HVA), and their relationship with neurocognitive status evaluated before death of patients with HIV/AIDS.

Methods: Using the Real-Time polymerase chain reaction (real-time PCR) technology with high sensitivity of detection (<5 copies of RNA), we measured HIV viral load (VL) in frontal cortex, basal ganglia, caudate nucleus, putamen, globus pallidus and substantia nigra of autopsied human brains of HIV-1+ (N = 36) who were evaluated for NP and neurocognitive functions before death, and in the same regions of HIV- control cases (N = 7). Dopamine and its metabolite, homovanillic acid (HVA) were measured in the same brain regions of all the cases using highly sensitive CoulArray HPLC-ECD system.

Results: HIV-1 viral load in different brain regions was found to range between non-detectable (ND) and 8.1 × 106 virus copies/g tissue. However, no specific pattern of HIV-1 viral load was found in all the cases included in this study. None of the samples of brain regions of HIV- negative individuals showed detectable viral load. Central dopamine concentration in different brain regions ranged between 50-9000 pg/g tissue and HVA between 6–2500 ng/g tissue. Neurocognitive impairment in HIV-1+ individuals ranged between 1-4 despite ART intervention during life.

Conclusion: This study demonstrates the presence of varying concentration of HIV-1 RNA in different brain regions of HIV-1+ individuals. Concentration of dopamine and HVA also had a wide range in different brain regions of both HIV+ and HIV- cases. These data are being evaluated to test the hypothesis that HIV-1 impacts the central dopamine levels which may result in the neurocognitive deficits observed in HIV-1 infection. (This study was supported by NIH grants #s RO1 NS43982, RO1 NS41205 and RO1 DA 13550).

P99

SIV-induced pathological and functional changes in the peripheral nervous system

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The most common form of HIV-induced neuropathy is Distal Sensory Polyneuropathy (DSP), which affects 30–35% of patients with AIDS. To develop rational therapeutics for affected individuals, it is important to understand the mechanism by which HIV causes peripheral neuropathy. One of the major questions that would direct therapeutic efforts is whether DSP starts with injury to the distal nerves or whether damage of the neuronal cell bodies in dorsal root ganglia occurs first, with subsequent degeneration of the distal portion of their axons. SIV-infected macaques develop lesions in the peripheral nervous system similar to those observed in HIV-infected patients with DSP, including infiltration of macrophages and loss of DRG sensory neurons. In this study we examined the relationship between dorsal root ganglia (DRG) changes and sensory nerve function and pathology by measuring C-fiber conduction velocities in the sural nerves and epidermal nerve fiber density in the skin of SIV-infected macaques and uninfected control macaques. SIV-infected macaques had significantly lower sensory C-fiber conduction velocity (CV) in the sural nerves than uninfected animals ($p = 0.01$). The extent of conduction velocity decline correlated strongly with the increase in macrophage infiltration in the DRG ($p = 0.006$). Significant declines in density of epidermal fibers also developed in SIV-infected macaques ($p = 0.01$), but were not correlated with changes in the CV. These findings suggest that primary injury to the neuronal cell body mediated by activated macrophages alters functional properties of sensory nerves in HIV-infected individuals.

P100

HLA-G expression in human neural cells after HSV-1 infection

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HLA-G is a non classical human MHC class I molecule. It may promote tolerance, leading to acceptance of the semi-allogeneic fetus and tumor immune escape. We show here that two viruses—herpes simplex virus type 1 (HSV-1), a neuronotropic virus inducing acute infection and neuron latency, and rabies virus (RABV), a neuronotropic virus triggering acute neuron infection—upregulate the neuronal expression of several HLA-G isoforms, including HLA-G1 and HLA-G5, the two main biologically active isoforms. RABV induces mostly HLA-G1 and HSV-1 mostly HLA-G3 and G5. HLA-G expression is upregulated in infected cells and neighboring uninfected cells. Soluble mediators, such as upregulate HLA-G expression in uninfected

cells. The membrane-bound??IFN-b HLA-G1 isoform was detected on the surface of cultured RABV-infected neurons but not on the surface of HSV-1-infected cells. Thus, neuronotropic viruses that escape the host immune response totally (RABV) or partially (HSV-1) regulate HLA-G expression on human neuronal cells differentially. HLA-G may therefore be involved in the escape of certain viruses from the immune response in the nervous system.

P101

Vitamin E prevents Nelfinavir-induced alterations in Notch processing in cerebral endothelial cells

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Cerebral endothelial cells (CEC) are continually exposed to a variety of blood-borne factors and also conduct dynamic signaling interactions with blood-borne mononuclear cells, astrocytes, microglia, oligodendrocytes and neurons. Maintaining proper signaling and angiogenic capacity becomes increasingly important during states of chronic neuro-inflammatory diseases such as HIV encephalitis during which normal signaling crosstalk among cells of the neurovascular unit may be disrupted. Disruptions may be due to viral pathogenesis, inflammatory factors or to chronic exposure to anti-retroviral medications. We hypothesize that "normal" CEC signaling and fitness may be affected by protease inhibitor (PI)-induced oxidative stress, and that these alterations affect normal g-secretase substrate processing and signaling which may decrease the cell's fitness level and ability to withstand challenge. Since changes in the expression and processing of g-secretase substrates can directly alter angiogenic related transcriptional signaling, the effect of PIs on Notch and APP protein levels was determined. In this study, HIV PIs were shown to induce the generation of ROS and to modify oxidative stress signaling, both of which responses were prevented by Vitamin E pre-treatment. Moreover, unlike other PIs tested, Nelfinavir had an affect on both g-secretase substrate processing and protein expression, but not on gene expression. Additionally, the localization of NICD and APP within CEC and angiogenic capacity was altered by Nelfinavir treatment, but Vitamin E pretreatment restored normal patterns. Increased understanding of the mechanisms of crosstalk between PIs and CEC in regulating BBB integrity is critical to manage the treatment of AIDS patients in the era of rapidly growing numbers of available anti-retroviral drugs.

P102

The role of astrocytes and microglia in viral neurotropism

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Recent work in our laboratory suggest a novel concept that astrocytes and microglia, the brain's local immune cells participate in determining the neurovirulence and neuropathogenesis of neurotropic viruses upon interaction with invading viruses. Neurotropic coronaviruses (such as MHV-A59) induce pro-inflammatory cytokine signaling from brain immune cells astrocytes and microglia, while a non-neurotropic virus (MHV-2), produces significantly lower levels of these cytokines. MHV-A59 produces acute encephalitis and chronic demyelination, while MHV-2 produces only mild meningitis without encephalitis or demyelination. To dissect the relative importance of pro-inflammatory signaling by astrocytes and microglia during encephalitis and demyelination we used micro-arrays, real-time PCR and ELISA to compare the in vivo and in vitro cytokine profile of a recombinant virus (Penn98-1), which lacks the ability to induce demyelination but retains all the other properties of MHV-A59 including encephalitis, with the profiles of MHV-A59 and MHV-2. Since the pro-inflammatory cytokine profile of Penn98-1 is significantly reduced in astrocytes but not in microglia compared to MHV-A59, and only during the demyelinating phase but not during acute encephalitis, we conclude that the property of pro-inflammatory signaling from astrocytes, is essential for demyelination but not encephalitis. Since the genomic difference between MHV-A59 and Penn98-1 is only in the S gene, we conclude that the S gene controls pro-inflammatory cytokine signaling in astrocytes. However the reduced pro-inflammatory signaling of microglia by MHV-2, which lacks also the ability to induce encephalitis, may suggest that pro-inflammatory microglial signaling is essential for encephalitis. The emerging paradigm of the correlation between specific cytokine signaling and CNS biologic properties will further our understanding of virus-induced encephalitis, demyelination, and immunologic reactions of brain immune cells.

P103

TLR3 and anti-viral response in astrocytes and microglia

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Toll-like receptors (TLRs) have emerged as crucial receptors on immune cells that mediate innate immunity against pathogens. TLRs signal through MyD88, an adaptor molecule containing the TLR-IL-1 receptor domain (TIR), to activate NF-kappaB and MAP kinases. In addition, TLR3 and TLR4 (as well as IL-1R) also signal through TRIF (TIR domain-containing adaptor-inducing IFNbeta) that activates the IFNbeta

gene (primary response), resulting in the stimulation of secondary response genes. To investigate the relationship between TLR/IL-1R expression/activation and antiviral responses in primary cultures of human astrocytes and microglia we used HIV-1 and cytomegalovirus as targets. We show that TLR3 ligand (polyinosinic-polycytidylic acid: poly IC), TLR4 ligand (LPS) and IL-1 are powerful activators of microglia and astrocytes modulating inflammatory and antiviral gene expression, but that signal transduction pathways involved in the activation of the genes and the consequences of the receptor-ligand interaction on the anti-viral immunity are different. We have made the novel observation that the TLR3 ligand poly IC limits HIV and CMV replication in microglia and astrocytes through mechanisms involving interferon regulatory factor 3 (IRF3), IFNbeta, indoleamine 2,3-dioxygenase and viperin/cig5, whereas IL-1, which triggers activation of similar sets of genes on microarray does not. We propose that TLR3 poses as a unique target for potential therapy to activate innate anti-viral immunity in vivo.

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P104

Extracellular Tax-protein of human T cell leukemia virus type 1 induces the secretion of Th1 cytokines and beta-chemokines from monocyte-derived dendritic cells

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HTLV-1-associated myelopathy/tropical spastic paraparesis, a neurological disorder, is characterized by highly stimulated immune response including elevated levels of inflammatory cytokines/chemokines, and oligoclonal expansion of Tax-specific CD8+ cytotoxic T lymphocytes in the peripheral blood and cerebrospinal fluid. Studies have shown that HTLV-1 transactivator protein Tax is available for immune recognition by antigen presenting cells such as dendritic cells, the most efficient APCs. In this study we have shown that the treatment of monocyte-derived dendritic cells (MDDCs) with purified Tax protein induces the secretion of Th1 cytokines (IL-12, and TNF-alpha) and beta-chemokines (MIP-Ialpha, MIP-Ibeta, and RANTES) in a dose- and time-dependent manner as quantitated by antigen-specific ELISA. The kinetics and dose response studies were also performed at mRNA level by real-time PCR. A significant correlation has been observed in the Tax-induced secretion of cytokines/chemokines at both RNA and protein levels. These studies suggest that HTLV-1 Tax protein can initiate immune response by activating DCs, the mechanism(s) of which is currently under investigation.

P105

Cytokine induction in the olfactory bulb of mice following rapid invasion of a human influenza virus

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Infection with influenza virus is associated with characteristic systemic symptoms such as fever, somnolence and fatigue. These symptoms are considered part of the acute phase response (APR) and are regulated in part by cytokines produced by the host. Influenza virus is generally assumed to be restricted to the respiratory tree, and its symptoms induced by cytokines produced in the respiratory system acting upon the brain. We have demonstrated viral replication intermediates in the olfactory bulb (OB) of influenza-infected mice as early as 4 h after intranasal inoculation. Consequently, we hypothesized that the presence of the virus will increase the production of cytokines like tumor necrosis factor alpha(TNFa) and interleukin (IL)-1β in this region of the brain. Mature C57BL/6 male mice were inoculated intranasally with a high dose of highly purified PR8 H1N1 influenza virus; boiled virus served as a control. Mice were anesthetized and perfused with 4% paraformaldehyde at 15 h post-infection and whole brains, including OBs separated from the cribriform plate, were collected. Brains were post-fixed, sunk in 20% sucrose and frozen prior to microtome sectioning. OB sections were stained for viral H1N1 antigen, TNFa and IL-1β or were double-labeled with TNFa and the neuronal marker NeuN. Sections were examined and photographed using light and confocal microscopy. Viral antigen was concentrated in the olfactory nerve and the glomerular layer (GL) of the OB. A significant increase in the number of TNFa immunoreactive (IR) cells was found after a quantitative analysis in the external plexiform layer of the OB. Double labeling with NeuN demonstrated that neurons in the OB were producing the TNFa. Quantitative analysis of IL-1β IR cells showed no significant difference in the number of IR cells between infected and control groups. The distribution of the IL-1β IR cells indicates that this cytokine was produced both in microglia and neurons of the OB. We conclude that there is an increase in the production of cytokines like TNFa in the olfactory bulb after intranasal inoculation with PR8 influenza virus, which has also been demonstrated in our laboratory with quantitative RT-PCR. These cytokines may be part of the initial signaling to the brain that produces the APR following influenza infection.

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P106

Cysteine residues on HIV Tat mediate neurotoxicity via interaction with NMDA NR1A and NR2A Subunits

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Background: HIV associated dementia (HIVD) remains a prevalent neurological disorder in post-HAART era. Although the pathogenesis of HIVD is still poorly understood, substantial evidence suggests that HIV Tat protein may induce neurodegeneration through the activation of NMDA receptor. Here we investigated the interaction of HIV Tat with NMDA NR1A and NR2A subunits and determined if cysteine residues on Tat mediate its neurotoxicity.

Methods: HEK293 cells transfected with NR1A and NR2A plasmids (gifted by Dr. Richard O'Brien in Johns Hopkins University) were used for toxicity assay of Tat protein secreted from astrocytic cells (SVGA) transfected with Tat. S-nitrosylation of Tat protein was measured by Biotin Switch method. MTT assay was used to determine the neurotoxicity of recombinant HIV Tat protein in rat primary neuronal culture.

Results: HEK293 cells were transiently transfected with pRK5-NR1A and pRK5-NR2A plasmids and the expression of NR1A and NR2A protein was confirmed by immunostaining and Western blot analysis. Their functional properties were confirmed by incubation with 300 uM NMDA which caused cell death. SVGA were transfected with plasmids containing either Tat1-72 or Tat1-101. The expression of Tat protein in the culture supernatant was confirmed by Western blot analysis. Culture supernatants containing either Tat1-72 or Tat 1-101 induced 50% ($p < 0.01$) cell death in HEK293 cells expressing NR1A and NR2A, while they did not induce significant cell death in HEK293 cells with mock transfection. The cell death caused by Tat could be blocked with NMDA receptor blockers (10 uM MK-801 or 500 uM APV). Recombinant Tat1-72 protein also induced 25% ($p < 0.01$) cell death in HEK293 cells expressing NR1A and NR2A. Recombinant Tat1-72 was treated with S-nitrosylglutathione and cysteine residues of Tat were modified by S-nitrosylation which was confirmed by the Biotin Switch assay. S-nitrosylation of recombinant Tat protein diminished its neurotoxicity in rat primary neuronal cultures.

Conclusion: HIV Tat protein may induce neurotoxicity by interaction with NMDA NR1A and NR2A subunits. Cysteine residues on Tat are important in mediating its neurotoxicity. Post-translational modification by S-nitrosylation may play an important role in the host defense system against HIV neurotoxicity.

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P107

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

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

P108

Regulation of the SIV LTR in macrophages

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HIV and SIV infect both CD4+ lymphocytes and monocytes/macrophages. While CD4+ lymphocytes are the major host cells infected in the peripheral blood and constitute the major peripheral reservoir for latent HIV and SIV, macrophages are the predominant cells infected in the CNS and lungs. Studies from our laboratory have previously demonstrated the

existence of a latent reservoir of SIV in the brain. More recently, we published strong evidence to support our hypothesis that the innate immune responses to virus infection that suppress acute virus replication in the brain also facilitate transcriptional latency of SIV. Specifically, we demonstrated that IFNb, which is induced in the brain very early after infection, both suppresses SIV LTR activity and induces expression of the dominant-negative isoform of C/EBPb (CCAAT/enhancer-binding protein b). To identify critical sequences that regulate the SIV LTR in macrophages, we have generated a set of SIV LTR reporter constructs and have cloned and expressed the human isoforms of wild-type C/EBPb (LAP) and the dominant-negative isoform of C/EBPb (LIP). An important distinction of the SIV LTR compared to the well-characterized HIV LTR is that the C/EBPb-binding site in the SIV core promoter (−84 to −100 bp) overlaps the NF-kB-binding site suggesting a complex regulatory mechanism at this site. We present data on the transcriptional activity of the SIV LTR when LAP and LIP are expressed in macrophages and assess the relative contribution of the NF-kB and C/EBPb binding sites in the SIV core promoter.

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P109

Effector memory CD8+ T lymphocytes control polyomavirus viremia, but not viruria, in HIV+ PML survivors

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The presence of JC virus (JCV)-specific CD8+ cytotoxic T lymphocytes (CTL) is associated with a better outcome in patients with progressive multifocal leukoencephalopathy (PML). In HLA A*0201+ individuals, these cells cross-recognize epitopes of the polyomavirus BK. We sought to determine the frequency and phenotype of CD8+ T lymphocytes specific for two A*0201-restricted JCV epitopes, VP1p36 and VP1p100, as well as their impact of JC and BK viremia and viruria.

We studied 32 individuals, divided in 3 groups: 1) healthy subjects (n = 15), 2) HIV+ (n = 8) and 3) HIV+ PML survivors (n = 9). We devised a method to estimate the frequency of JCV-specific T CD8+ lymphocytes in fresh blood using pre-enrichment followed by tetramer staining assay. We then characterized the expression of T cell differentiation markers CD45RA and CCR7 with 7 color flow cytometry to classify these cells into naïve, central memory, effector memory or terminally differentiated effector memory T cells. Real time QPCR was used to detect JCV and BKV DNA in the blood and urine.

The frequency of VP1p36-specific T CD8+ lymphocytes ranged from 0.001% to 0.22% and for VP1p100 from 0.0014% to 0.015% of all CD8+ T cells, with no significant difference between the three study groups. In HIV+PML patients, CD45RA- CCR7- effector memory CD8+ cells were the predominant subset, especially early after disease onset, while in healthy subjects and HIV+ without PML there was no prevalent subpopulation. JCV and BKV DNA were not detected in any of the plasma and PBMC, but were found in 10% and 13.3% urine samples, respectively, independently of the detection of JCV-specific lymphocytes.

In conclusion, JCV-specific CD8+ T lymphocytes are present in very low frequencies in the peripheral blood. In HIV+ PML patients who had a favorable clinical outcome, effector memory cells were the predominant subset. This cellular immune response efficiently cleared polyomaviruses from the blood but did not prevent their excretion in the urine. Immunotherapies aiming at increasing this population of cells may be valuable in the treatment of HIV+ individuals with PML.

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P110

A highly conserved HIV-1 clade B downstream C/EBP binding site affects C/EBP and Tat transactivation

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Previous studies have shown that at least one CCAAT enhancer binding protein (C/EBP) site upstream of the TATA box and full-length C/EBP beta protein is necessary for human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) activity in cells of the monocyte/macrophage lineage. However, no studies have been performed concerning C/EBP sites downstream (DS) of the start of transcription. Analyses of 115 clade B LTRs have indicated that there are three potential C/EBP sites within the downstream LTR. Electrophoretic mobility shift (EMS) analyses demonstrated that only one of the three sites (DS3, +158 to +172) was able to bind members of the C/EBP family. Analyses of 115 clade C, 21 clade A and 19 clade D LTRs indicated that this downstream site is highly

conserved among different HIV-1 clades, suggesting the presence of a functionally important cis-acting element. EMS analysis of naturally occurring variants within DS3 demonstrated altered C/EBP recruitment. To perform functional studies, the 6C variant was selected (G-to-C change at position 6) because it resulted in a knockout phenotype without creating an alternate binding site. Results from transfection studies utilizing the parental wild type LAI LTR (LTR-WT) containing the knock-out 6C variant (LTR-DS3-6C) demonstrated loss of binding to this site results in an approximate 50% decrease in basal LTR activity in the U-937 monocytic cell line. Full-length C/EBP beta induction of LTR-WT was reduced 3-fold by loss of C/EBP binding to DS3. Interestingly, Tat induction was decreased 4-fold, suggesting that an alteration in the structural integrity of this site may perturbate the interaction of Tat with the neighboring TAR element that may involve C/EBP/Tat interactions. Co-transfection assays demonstrated that full-length C/EBP synergistically enhanced Tat transcriptional activity with both LTR-WT and LTR-DS3-6C, with a higher induction level in LTR-WT. These observations suggest the highly conserved DS3 site is important for HIV-1 LTR transcription and may be a critical element required for HIV-1 activation.

P111

Human neurons express dsRNA receptors and can mount inflammatory, chemoattractive and antiviral responses in virus infection

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The capacity of neurons to mount an innate immune response to a viral infection remains largely unexplored. To gain insight into the biology of human neurons in the course of neuronotropic viral infections, human post-mitotic neurons, NT2-N, were infected with rabies virus (RABV). Affymetrix oligonucleotide micro arrays were used to analyze global changes in neuronal gene expression. With a two-fold cut-off, RABV increased transcription of 231 genes. Genes of immunity represent 24% of the number of the RABV-upregulated genes. The function of highly expressed genes of immunity by RABV almost exclusively included a strong interferon-beta (IFN-beta) response and an IFN-mediated gene response, such as IRF7 and 2′5′OAS and chemokines (CCL-5, CXCL10). RABV also upregulated the expression of IL-1alpha, IL-6, IL-8 and TNF-alpha mRNAs. The micro arrays results were confirmed by real time PCR, immunocytochemistry and ELISA. Human neurons also produce a strong IFN-beta response after synthetic dsRNA (Poly I:C) treatment, but not LPS. Human neurons were found to express transcripts of the dsRNA receptors: TLR-3, RIG-1, MDA-5 and PKR. Expression of these dsRNA receptors was increased after rabies virus infection, IFN-beta, Poly I:C but not after LPS treatment. Immunocytochemistry analyses indicated that human neurons express TLR-3 and that infection redistributed cytoplasmic TLR-3 localization. The sequence of the TLR3 gene of human neurons was obtained. It was strictly identical to those obtained in human dendritic cells, suggesting that TLR3 expressed by neurons is fully functional. Moreover, TLR3 expression was detected in neurons of human rabies cases by immunohistochemistry. These results highlight that human neurons in absence of glia have the intrinsic machinery to mount a strong IFN-beta response and to produce inflammatory and chemoattractive mediators in response to viral infection.

P112

Effect of Heroin and HIV-1 gp120 on chemokine and chemokine receptor regulation in brain microvascular endothelial cells.

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Opioid abuse has been postulated as a cofactor in the immunopathogenesis of human immunodeficiency virus (HIV-1) infection and AIDS. Chemokines and their receptors have been implicated in the pathogenesis of neuroAIDS. HIV-1 has been shown to infect brain microvascular endothelial cells (BMVEC) in vivo and in vitro resulting in the modulation of blood brain barrier permeability and neurodegeration. We hypothesize that heroin and HIV-1 viral protein, gp 120 can alter the chemokine balance in the brain, via the modulation of the chemokines and its specific receptors further exacerbating the neuropathogenesis process in HIV-1 infection. BMVEC were treated with heroin (10-7- 10-11M) or gp120 (10ng/ml) separately or in combination for 24 hr, RNA was extracted, reverse transcribed and the chemokine, RANTES and chemokine receptors, CCR5 and CXCR4 gene expression were quantitated using real time QPCR. Our results show that heroin treatment increased CCR5 and CXCR4 gene expression in a dose dependent manner, with a reciprocal downregulation of RANTES gene expression by BMVEC. Treatment with a combination of heroin and gp120 showed an additive increase in CCR5 and CXCR4 gene expression. These results suggest that heroin may act as a cofactor in neuropathogenesis of HIV-1 infection potentially by dysregulating the local

production of HIV-1 suppressing chemokines and their receptors.

P113

Simian varicella virus reactivation in cynomologous monkeys

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Simian varicella virus (SVV) infection of primates shares clinical, pathological, immunological and virological features with varicella zoster virus (VZV) infection of humans. Furthermore, like VZV, SVV becomes latent in ganglionic neurons. We established natural SVV infection in 7 Cynomologous monkeys by exposing them to monkeys that had been inoculated intratracheally with SVV. Six naturally-infected monkeys developed varicella rash within 2 weeks after exposure. SVV DNA, but not SVV RNA, was detected in multiple ganglia, but not in lung or liver in 2 of the monkeys 3 months post-infection (p.i). Six months p.i., 4 of the remaining 5 naturally-infected monkeys received a single dose (200 cGy) of X-irradiation followed by tacrolimus (80–300 mg/kg/day) and prednisone (1 mg/kg/day) for 4 months. The fifth non-immunosuppressed monkey served as a control. A varicella-like rash developed in one immunosuppressed monkey 5 weeks later. Monkeys were sacrificed at monthly intervals after immunosuppression. Ganglia and viscera were analyzed for SVV DNA, RNA and protein. SVV DNA was detected in at least in one ganglion from all 5 monkeys, in lung from 2/4 immunosuppressed animals, and in lung from the control monkey. SVV ORF 63 RNA was detected in ganglia from 2/4 immunosuppressed monkeys and in the control monkey. SVV ORFs 9 or 40 RNA were detected in ganglia from the same 2 immunosuppressed monkeys, but not in the control monkey. SVV glycoproteins were detected in the skin rash from the immunosuppressed monkey and in lung from all immunosuppressed monkeys and in the control monkey. SVV glycoproteins were also detected in monkey ganglia that contained SVV RNA. SVV latency can be established in Cynomologous monkeys. Immunosuppression reactivates latent SVV, but may not be the only stimulus that induces reactivation.

P114

Cytokine regulation of the ERVWE1 promoter activity in astrocytic cells

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Two components of the HERV-W family of human endogenous retroviruses (HERV) are activated in multiple sclerosis (MS) and proposed as disease (co)factors: MSRV (MS-associated RetroVirus, able to form extracellular virions), and syncytin (env protein encoded by the ERVWE1 locus, that mediates trophoblast fusion during pregnancy). So far no tools can distinguish these two elements intracellularly. MSRV/HERV-W env-specific mRNA and protein are induced in MS brains, CSF and blood. In cultured carrier cells MSRV expression is increased by MS-detrimental cytokines (IFNgamma, TNFalpha, IL-1beta, IL-6), and inhibited by MS-protective IFNbeta.

We studied the regulation of ERVWE1 promoter in astrocytic cells, since in trophoblasts its regulation is linked to trophoblasts-specific binding sites in the ERVW-1 promoter. Using transient transfection assays and constructs of different size, we showed that IFNgamma, TNFalpha, IL-1beta, and IL-6 up-regulate the ERVW-1 promoter activity, while IFNbeta down-regulates it. The regulation occurs within the cellular moiety of the promoter region. TNFalpha activation occurs through NF-kB responsive-elements within the promoter. Electromobility shift and ChIP assays showed that TNFalpha enhances the binding of the p65 subunit of NF-kB to its cognate site within the promoter, and that the effect is abolished by siRNA against p65. These findings show an additional role for p65, regulating ERVW-1 promoter and inducing syncytin in MS patients. The URE-HWLTR region on chromosome 7q21–22 was amplified from DNA of MS patients and controls and sequenced. The alignment revealed that all sequences are homologous and highly conserved, thus indicating that differential activation of transcription factors in patients and controls is related to the inflammatory microenvironment.

P115

Human T cell leukemia virus type 1 Tax induces the expression of murine dendritic cell markers associated with maturation and activation

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HTLV-1 is the etiologic agent of both adult T cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the genesis of HAM/TSP likely involves several steps,

the generation of a highly specific and effective population of Tax-specific CD8+ cytotoxic T lymphocytes (CTLs) that migrate to the central nervous system is likely of pivotal importance in this neuropathologic process. Presentation of Tax peptides by activated dendritic cells (DCs) to naïve CD8+ T cells likely plays an important role in the induction of a Tax-specific CTL response. Studies have suggested that Tax may be secreted from HTLV-1-infected cells and act as an extracellular cytokine. We report herein that treatment of a murine dendritic cell line JAWS II with purified Tax protein induces DC activation and maturation involving an increase in the production of CD80, CD83, and CD86 mRNA as demonstrated by microarray analyses and quantitative RT-PCR assessment. Additionally, treatment of JAWS II DCs with extracellular Tax decreases the ability of DCs to present an MHC class I-restricted peptide, indicating that Tax likely matures the DCs to the point where presentation of a secondary antigen is restricted. The implication of the experimental results is significant with respect to the generation of a Tax-specific CTL compartment and the eventual neurologic dysfunction observed in HAM/TSP.

P116

Inflammation caused by envelope of the multiple sclerosis RetroViral element

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The Multiple Sclerosis RetroViral element (MSRV) isolated from MS patient cerebrospinal fluid belongs to the Human Endogenous RetroVirus (HERV) W family which comprises about 2% of the human genome. MSRV envelope protein (ENV) may contribute to initiate and/or exacerbate MS. 1) ENV stimulates Peripheral Blood Mononuclear Cells (PBMC) which display polyclonal expansions of TCR Vbeta16 T cells supporting a possible superantigen activity. 2) ENV activates PBMC from MS patient blood to produce inflammatory cytokines IFN-g, IL-12p40 and IL-6, in relation with disease severity score. 3) ENV induces human monocytes and dendritic cells to produce inflammatory cytokines through engagement of CD14 and Toll Like Receptor 4 (TLR4). ENV can trigger a maturation of dendritic cells to support a Th1 type of T lymphocyte response. For in vivo validation in the Experimental Allergic Encephalitis (EAE), mice are injected with a combination of antigenic myelin peptide and adjuvant (Complete Freund) or ENV protein. Clinical score shows significant EAE symptoms both in mice injected with ENV or adjuvant, and no symptom in control mice. Anti-ENV antibodies block encephalitis when given together with ENV. Cultures of splenocytes from either ENV or adjuvant treated mice with the myelin antigen lead to IFN-g production, suggesting a T lymphocyte polarization towards the myelin antigen. In conclusion, ENV can promote in vivo inflammation leading to disease similar to EAE. Evaluation of myelin damage in brain and of immune responses from ENV injected mice will provide clue to validate if disease promoted by ENV is similar to conventional EAE model.

P117

Modulation of pro-inflammatory immune responses during herpes encephalitis

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Despite the advent of antiviral therapies, herpes simplex virus (HSV)-1 infection of the brain remains the leading cause of sporadic fatal encephalitis in immunocompetent individuals. Using a murine model of HSV-1 brain infection, our laboratory aims to elucidate the contribution of virus-induced immune responses to the progression of this disease. We have previously identified a vigorous cascade of pro-inflammatory immune responses in the brains of HSV-1-infected Balb/c mice beginning as early as 5 days post-infection. Production of potent mediators by HSV-1 infected microglia, such as CXCL10 and CCL2, lead to a significant T-cell infiltration within the first 7 days post infection. Based on these previous findings, we are currently investigating the contribution of this non-protective inflammatory response to the development of fatal HSV-1 brain disease. We are presently evaluating the effects of pro-inflammatory and anti-inflammatory mediator production at the site of infection on the progression of cytokine and chemokine production, T cell infiltration, viral replication and spread, and host survival. By over-expressing a pro-inflammatory chemokine (CXCL10), as well as an anti-inflammatory cytokine (IL-10), we aim to modulate the intensity of this neuro-immune response in order to assess its contribution to the development of HSV-1 encephalitis.

P118

Brain-Derived HIV-1 envelope glycoproteins with lower CD4-dependence and reduced sensitivity to a fusion inhibitor than their splenic counterparts

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Background: We previously described envelope glycoproteins of an HIV-1 isolate adapted in vitro for growth in microglia that acquired a highly fusogenic phenotype, lower CD4 dependence and higher CD4 affinity, as well as resistance to inhibition by anti-CD4 antibodies. Here, we investigated whether similar phenotypic changes are present in vivo.

Methods: Envelope clones from the brain and spleen of an HIV-1-infected individual with neurological disease were amplified, cloned, and sequenced. Amino acid changes in V1/V2, V3 and V4 variable regions of gp120, and in the carboxy-terminal heptad repeat domain (HR2) of gp41 ectodomain, were found. Functional clones were then used in cell-to-cell fusion assays to test for CD4 and co-receptor utilization, and for sensitivity to various antibodies and inhibitors.

Results: Phylogenetic analysis demonstrated clustering of sequences according to the tissue of origin, as expected. Both brain- and spleen-derived envelope clones mediated fusion in cells expressing both CD4 and CCR5, and brain envelopes also used CCR3 very efficiently as co-receptor. We found that brain envelopes had a lower CD4-dependence and higher CD4 affinity, since they were able to mediate fusion in the presence of low levels of CD4 on the target cell membrane, and they were significantly more resistant to blocking by anti-CD4 antibodies than spleen-derived envelopes. In contrast, we observed no difference in CCR5 utilization or in sensitivity to the CCR5 antagonist TAK-779. However, brain-derived envelopes were significantly more resistant than those from spleen to the fusion inhibitor T-1249, and concurrently showed slightly greater fusogenicity.

Conclusions: Our results confirm an increased affinity for CD4 of brain-derived envelopes that may have originated from in vivo adaptation to replication in microglial cells. More interestingly, we note the presence of envelopes significantly less sensitive to a fusion inhibitor in the brain versus spleen of an untreated, HIV-1-infected individual. Changes in the interaction with CD4 but not CCR5, and/or the specific polymorphism found in HR2 in these brain sequences, may be related with the observed phenotypic differences.

P119

High prevalence of JC virus urinary excretion in immunocompetent Portuguese patients

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Objectives: JC polyomavirus is ubiquitous in humans. Its infection seems to be acquired during childhood, mainly asymptomatically. After primary infection, JC Virus (JCV) is thought to remain latent in renal tissue. Reactivation could occur under certain conditions, and progeny is excreted in urine. Although JCV urinary excretion has been reported in 30–70% of adults worldwide, none of the performed studies have concerned the Portuguese population. In this order, the aim of the present study was to evaluate the prevalence and pattern of JCV urinary excretion in Portuguese individuals.

Methods: Urine samples from 109 immunocompetent individuals attending to several laboratories in the Portugal central region were collected and screened for the presence of JCV DNA. This patient group included 64 women and 45 men aged between 15 and 77 years old. After nucleic acid extraction viral DNA was submitted to topoisomerase I treatment. PCR amplification was performed using specific primers from the VP1 coding region of the JCV genome. Amplified products were visualized by agarose gel electrophoresis.

Results: Fifty four (49,5%) out of 109 urine samples screened revealed the presence of the expected fragment from JC Virus genome. From the studied group, thirty one (47,7%) out of 65 women and 23 (51,1%) out of 45 men excreted JCV in the urine. The prevalence of urinary excretion increases with age. A maximum value was observed for patients older than 70 years.

Conclusion: The present findings reveal that half of immunocompetent Portuguese population from the Portugal central region of excretes JCV in urine. In spite of this relatively high value, it is concordant with prevalence rates observed in other countries. Also in accordance with several reports, Portuguese excretion rate it's slightly higher among men than women. This study also supports the evidence that older patients present the highest rate of JCV excretion in urine.

P120

Pathogenic consequences of dysregulated immune responses to concomitant viral infections

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It is known that concomitant infections in humans are quite prevalent, with a prominent example of this being coinfection with Human Immunodeficiency virus (HIV) and one of the Hepatitis viruses (HBV or HCV), for which the clinical outcome is different from that of either infection alone. One can postulate that simultaneous immune challenges –including responses to infections, cancers, allergens and autoimmune diseases—may also result in a substantively different outcome than any single and isolated response. To date, however, most responses to

immunologic challenges are investigated using immunologically naïve mice with each pathogenic agent or immune response being evaluated individually. In order to further the understanding of the effects of polymicrobial infections and their influence on the host's subsequent immune response as a whole, we chose to look at coinfection of permissive mice with measles virus (MV) and lymphocytic choriomeningitis virus (LCMV). When introduced singly, adult immunocompetent mice are able to clear the infection and survive the challenge of either of these viruses. In this model, only CNS neurons can be infected with MV (due to a targeted expression of a MV receptor to this cell type), and the intra-peritoneal route of LCMV challenge restricts infection to the periphery without invasion of the CNS. Thus, the sites of viral infection and replication remain mutually exclusive. This compartmentalization allowed us to readily observe any immunopathological consequences that occurred in response to the dual infection of these mice. What we found was a significant correlation between dual infection and signs of illness, including notable weight loss. Also, upon immunohistochemical analysis of serial brain sections of these mice, in comparison to mock infected or singly infected mice, we observed that coinfection led to a significant increase in CD8+ T-cell infiltration, presumably of LCMV-specificity, despite a lack of LCMV presence within the CNS. Our results indicate that these CD8+ T-cells are trafficking into the CNS in an antigen-independent manner, likely mediated by chemokines. This study demonstrates that while induction of anti-microbial immune responses is antigen-dependent, recruitment appears to be antigen-independent. If so, this misrecruitment may result in pathological consequences for the host which is infected simultaneously with multiple pathogens.

P121

Protection of neuronal cytoskeletal antigen expression in HIV-1-exposed cultures of differentiating human neuroepithelial precursor cells

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Objective: To study the effect of HIV-1 on neurogenesis using differentiating cell populations derived from human neuroepithelial precursor cells (NEP).

Background: Early events in HIV-1-associated neuronal injury include dendritic alterations, aberrant neurofilament metabolism, and loss of synaptic integrity. The CNS may respond to neuronal injury by differentiating new neurons and glia from resident populations of multipotent NEP. Thus there is a theoretical basis for treating HIV-1 infection of the CNS by stimulating endogenous neurogenesis to achieve localized neuronal replenishment.

Design/Methods: Human fetal-derived NEP propagated as neurospheres were seeded onto adherent substrate to initiate differentiation. Adherent cultures were then differentiated to a selective phenotype by incubating them in one of two differentiation media. To produce a mixed astrocyte-neuronal population, cultures were incubated in Dulbecco's Modified Eagle's Medium (DMEM) and F12 nutrient mixture (DMEM/F12) supplemented with N2 supplement and 2.5% (v/v) fetal bovine serum (fbs). To produce a predominantly neuronal phenotype, cultures were incubated with Neurobasal medium (NB) supplemented with 10 ng/ml brain-derived neurotropic factor (BDNF) and 10ng/ml platelet-derived growth factor A/B (PDGF). The adherent NEP cultures were continuously exposed to macrophage-tropic HIV-1 (strain SF128A) or lymphotropic HIV-1 (strain SF2) diluted into culture medium at 50ng p24 per million cells. No macrophages or microglia were added. Cultures were then assayed for cytoskeletal antigen expression by quantitative immunoblots or indirect immunofluorescent staining (IFA) or for cell numbers by immunocytochemistry. Cultures were harvested for assay at days 5, 21, and 42 after initiation of differentiation and HIV-1 exposure.

Results: After 5 days, all NEP cultures differentiated into a mixed population of astrocytes, expressing glial fibrillary acidic protein (GFAP), and neurons, expressing nuclear Hu and cytoskeletal antigens from microtubule (beta-III-tubulin) or neurofilament (neurofilament-L). In both culture phenotypes, HIV-1 exposure trended to produce lower neuronal cytoskeletal antigen expression but higher GFAP antigen expression after 5 days. After 22 days, HIV-1-exposed cultures differentiated in MDM/fbs had 25-40% less neuronal beta-III-tubulin or neurofilament-L (NF-L) expression but equal amounts of GFAP expression as unexposed control cultures. After 22 days, HIV-1-exposed cultures differentiated in NB/BDNF/PDGF had equal or greater NF-L or beta-III-tubulin expression and equal amounts of GFAP antigen expression as unexposed controls. IFA showed filaments with patchy NF-L staining in the virus-exposed MDM/fbs cultures; filaments had more intense, continuous NF-L stain in the virus-exposed NB/BDNF/PDGF cultures. The decrease in neuronal cytoskeletal antigen expression after 22 days in MDM/fbs cultures exposed to HIV-1 was not due to a virus-specific decrease in neuronal or even total cell numbers. After 42 days of differentiation, virus-exposed cultures in DMEM/F12/fbs had 15–30% less NF-L expression than unexposed controls, while virus-exposed cultures in NB/BDNF/PDGF had equal NF-L expression as unexposed controls. In both culture phenotypes, GFAP expression in virus-exposed cultures was equal to or greater than that in unexposed control cultures.

Conclusions: When human fetal NEP are continuously exposed to lymphotrophic or macrophage-tropic

HIV-1, there is selective depression of cytoskeletal antigen expression in neurons but not astrocytes differentiated from these NEP. The 68 kDa neurofilament protein is particularly sensitive to this HIV-1 effect. Neurotrophic factors BDNF and PDGF protect neuronal antigen expression in differentiating and maturing neurons from the effects of prolonged HIV-1 exposure. In the absence of neurotrophic factors, HIV-1 antigen exposure does not block the genesis of neuronal lineage-committed cells from NEP, but HIV-1 exposure may impair neuronal process development. This in vitro effect suggests similarities to the dendritic simplification seen in the brains of cognitively impaired, HIV-1-infected patients.

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P122

Levels of neurotrophin receptors in the SIV encephalitic brain

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Despite the advent of HAART, HIV-associated dementia is still estimated to occur in 10% of patients that develop AIDS and less-severe symptoms of HIV encephalopathy (HIVE) continue to occur in up to 50% of patients. Effective therapy for HIV CNS disease remains elusive, in part due to our lack of understanding of the disease process in the brain. We have developed an accelerated, consistent SIV/macaque model of HIV CNS disease with strong parallels to HIV infection in which SIV-infected macaques develop moderate to marked encephalitis with microglial and astrocyte activation, astrogliosis, and neuronal loss. Brain derived neurotrophic factor (BDNF) has long been known to have a neuroprotective effect as well as a major role in neural development. Recent studies have also shown that BDNF is capable of triggering apoptosis, as well. This negative signaling pathway is propagated through the p75NTR. This receptor has been shown to bind nerve growth factor (NGF) and NT-3, as well as BDNF. It physically interacts with both the trk (tyrosine kinase) receptors A, B, and C, which are the positive receptors for NGF, BDNF and NT-3 respectively. While BDNF signaling through its primary receptor, trkB, probably has a neuroprotective effect, signaling through the p75NTR could be severely detrimental.

We hypothesize that the levels of neurotrophin receptors change with progression of SIV CNS disease towards pro-apoptotic pathways. Addtionally, drugs or treatments that maintain the positive (trkB) levels in the brain will be neuroprotective. To test this hypothesis, we measured levels of p75NTR and trkB in the parietal cortex of SIV-infected macaques. We found that the ratio of trkB to p75NTR decreases with increasing severity of encephalitis, suggesting a role for neurotrophin receptors in progression to HIV CNS disease.

P123

Heterologous desensitization of CXCR4 by CCR5 engagement: A protective mechanism against CXCR4-Mediated neurotoxicity of HIV-1 gp120 and SDF-1

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Infection with human immunodeficiency virus-1 (HIV-1) often causes neurological problems and can eventually lead to the developement of dementia. G protein-coupled chemokine receptors serve besides CD4 as coreceptors for the envelope protein gp120 of HIV-1. In transgenic mice expressing gp120 in the brain and in mixed neuronal glial cell cultures HIV-1 gp120 initiates a signaling cascade that leads to neuronal death. Using mixed neuronal/glial cultures from rats and mice genetically deficient in one or both HIV coreceptors with wild type as control, we found that depending on the viral strain, either CCR5 or CXCR4 or both mediate neurotoxicity of HIV/gp120 via a pathway involving p38 mitogen-activated protein kinase. On the other hand, we identified Akt/PKB as an essential component of CCR5-dependent neuroprotection that was provided by MIP-1beta and RANTES. Furthermore, these CCR5 ligands abrogated neuronal death initiated by SDF-1 acting on CXCR4. Coexpression of CXCR4 and CCR5 on both glia and neurons, and Ca2+ imaging experiments demonstrated that engagement of CCR5 prevented CXCR4-triggered increases of intracellular [Ca2+]i, and suggested that CCR5 ligands can protect neurons by modulating CXCR4-mediated toxicity through heterologous desensitization.

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P124

Dynamic role of cerebrospinal fluid in establishing early lentiviral neuropathogenesis and transfer of virus from brain to systemic tissues

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Lentiviruses such as human, simian and feline immunodeficiency virus (HIV, SIV, FIV) gain rapid access to the cerebrospinal fluid (CSF) and central

nervous system (CNS) where they are thought to establish a protected viral reservoir, encourage the evolution of viral variants and induce neuropathogenesis. Virus entry into the CSF is rapid but the functional significance of virus in the CSF/ventricular compartment is poorly understood. To examine the infectious interactions and fate of CSF virus we followed plasma, CSF and brain viral titers over time after introduction of a bolus of free or cell-associated feline immunodeficiency virus (FIV) into the lateral cerebral ventricle. Intracerebroventricular (i.c.v.) inoculation with cell-free FIV resulted in: 1) high levels of plasma FIV RNA which preceded the detection of viral RNA in the CSF, 2) high CSF FIV RNA levels with 50% of the animals showing an inversion of the CSF to plasma viral load (CSF>plasma), 3) a unique biphasic pattern of CSF viremia and 4) significant proviral DNA in the brain. Analysis of FIV envelope variation indicated that early CSF FIV was closely matched to plasma whereas the subsequent appearance of virus in the CSF did not match the plasma. Although the brain proviral burden was substantially enhanced by the i.c.v. infusion, the pattern of envelope variants in the brain (proviral DNA) generally failed to correlate with patterns seen in plasma RNA, CSF RNA or PBMC DNA. FIV-infected macrophages provoked an acute inflammatory response and suppressed the CD4+:CD8+ T cell ratio but did not produce a detectable viremia. The infection of CNS parenchyma and the high efficiency of transfer of infectious virus from the CSF to the periphery suggest that the CSF provides an important pathway for establishing a brain reservoir and for the efficient transfer of infectious virus from the brain to systemic organs.

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P125

Spatially distinct and functionally independent mechanisms of axonal degeneration in a model of HIV associated sensory neuropathy

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Background: Distal symmetric polyneuropathy is a frequent neurological complication in late stages of HIV infection. While a direct infection of the virus in sensory ganglion neurons is unlikely, HIV envelope protein, gp120 has been shown to induce apoptosis of neurons through a pathway involving Schwann cells. Since it is unknown if the axonal degeneration is a consequence of the neuronal death or a primary direct effect on the axons, we investigated gp120 induced axonal toxicity, using compartmentalized cultures of sensory neurons.

Methods: Embryonic sensory dorsal root ganglion neurons were plated in Campenot chambers, where axons grow in isolation from the cell bodies. Gp120 was added to the cell body compartment or to the side chamber, housing the axons, both in presence and absence of Schwann cells. Serially we monitored the axon length and examined apoptotic cell death after 72 hours. We also investigate if gp120 induced axonal degeneration is prevented by treatment of axons with zVAD, a caspase inhibitor. Axons exposed to gp120 were stained with antibodies against cytochrome-C and activated caspase III.

Results: Our results show that gp120 causes neuronal apoptosis and axonal degeneration through two, independent and spatially separated mechanisms of action: i) an indirect insult to cell bodies, requiring the presence of Schwann cells, results in neuronal apoptotic death and subsequent axonal degeneration; ii) a direct, local toxicity exerted on axons through activation of mitochondrial caspase pathway, that is independent of cell body. This local axonal toxicity is mediated through binding of gp120 to axonal chemokine receptors and can be prevented by chemokine receptor blockers.

Conclusion: In conclusion, we propose a novel pathway of axonal degeneration mediated by gp120 that is dependent on local activation of caspases in the axon. This observation suggests that axonal protection is a relevant therapeutic target for HIV-associated sensory neuropathy. Furthermore, chemokine receptors inhibitors, which are currently being developed as HIV entry inhibitor drugs, may also have a therapeutic role in HIV-associated peripheral neuropathies by preventing axonal degeneration.

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P126

JCV agnoprotein affects oligodendrocyte development and survival

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The late region of JCV encodes a small, highly basic protein known as agnoprotein. Agnoprotein has a critical role in the regulation of the viral life cycle and also modulates certain important host cell functions including cell cycle progression and DNA repair. A cell line that constitutively expresses agnoprotein was created from CG-4 cells. CG-4 cells are bipotential and can be induced to differentiate into oligodendrocytes or type 2 astrocytes depending upon culture conditions. Morphological analysis of cells during early stages of differentiation to oligodendrocytes showed a significant delay in formation of processes and low complexity of outgrowth network of cells expressing agnoprotein. Level of MBP mRNA in agnoprotein expressing cells induced to differentiate into oligodendrocytes decreased compared to agnonegative cells. Growth of

agnoprotein positive cells was impaired during differentiation. High level of DNA damage was detected by DNA fragmentation and Comet assays in agnopositive cells during differentiation into oligodendrocytes. We observed that withdrawal of mitogens during differentiation to oligodendrocytes resulted in a decrease of agnoprotein expression. No change in agnoprotein mRNA level was observed. Treatment of cells with an inhibitor of the 26S proteasome, MG132, restored agnoprotein level, as well as treatment of cells with staurosporine, which is an inhibitor of kinases, including PKC, PKA, CK2. Co-treatment of cells with MG132 and staurosporine resulted in the marked increase of levels of agnoprotein, suggesting that agnoprotein is a subject of degradation by the Ubiquitin-proteasome system and for this event phosphorylation may be important. Results from Immunoprecipitation of lysates with anti-agnoprotein antibody following Western blot analysis using anti-phospho-Serine antibody demonstrated an accumulation of Serine-phosphorylated-agnoprotein. MG132 treatment of CG-4 cells that constitutively express agnoprotein with mutation of Serines at positions 7, 11 and 15 and induced to differentiation to oligodendrocytes, showed that this mutated agnoprotein cannot be degraded through Ubiquitin-proteasome system. Presented observations and our previous reports indicating involvement of agnoprotein in regulation of cell cycle and DNA damage repair pathways suggest that agnoprotein may interfere with survival of myelin-forming cells and ability of the proteasome system to prevent the accumulation of potentially deleterious proteins.

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Diffusion tensor magnetic resonance imaging in multiple sclerosis patients

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Purpose: The purpose of this study was to develop a semi-automated method to investigate the fractional anisotropy (FA) changes in multiple sclerosis patients using voxelwise analysis and comparison to a normalized FA atlas using a standard neuroanatomical space.

Materials/Methods: Diffusion Tensor Imaging (DTI) was performed on 10 normal controls and 6 patients with MS lesions. We acquired DTI by collecting diffusion-weighted images using a spin-echo EPI sequence. Diffusion gradients were applied in six non-collinear directions with a b value of 1000 sec/mm2. Twenty 6mm axial slices covering the entire brain were imaged using a 1.5 T scanner (TR = 6000ms, TE = 100 ms, FOV = 240 mm, 98 × 128 & 4 acquisitions). FA images were then created using an in-house modified diffusion tensor toolbox of SPM99. The FA maps were then spatially normalized into the space of the SPM99 EPI template and smoothed. A normal FA atlas was created using the 10 normal subjects. Statistical maps were generated by voxelwise comparison of the FA map of each of the patients with the normal FA atlas in a two sample t-test (uncorrected p value of 0.05).

Results: The statistical maps showing regions of significant FA differences were compared with routine T1, T2, and fluid attenuated inversion recovery (FLAIR) images. There were numerous regions in the MS patient DTI images, with statistically significant ($p = 0.05$) FA changes. Also, there are several regions of FA changes throughout the brain in the normal appearing white matter of these pateints (NAWM) that were not visible on the routine MR images. Similar findings were seen on rest of the five MS cases analyzed as well. In two cases however there were lesions visible on MRI that did not show significant FA changes.

Conclusions & Discussion: Our results show that this semi-automated method significanlty improved the speed, accuracy and reproducibility of FA map analysis in MS patients. Also, this method demonstrated our ability to globally visualize FA changes as well as characterize quantitatively the extent of MS lesions and abnormal FA in NAWM. Such analysis of FA data using a standard anatomical atlas will greatly help in longitudinal follow-up of these MS patients. These preliminary results are very encouraging and warrant further investigation.

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Cognitive impairment in HIV correlates with peripheral blood mononuclear cell levels of SHIVA, a neuronotoxic protein

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Prior studies have suggested a link between circulating CD14+CD16+ macrophage subsets and HIV-associated cognitive dysfunction, although there is debate regarding a mechanistic basis for this association. Soluble HIV apoptotic (SHIVA) protein was cloned from an HIV-infected CD14+CD16+ macrophage cell line, and has been demonstrated to induce apoptosis in T cell and neuronal cell lines. Its presence in the brain tissues of patients with HIV encephalitis has been documented. In the present study, we undertook an analysis of the relationship between SHIVA levels in peripheral blood mononuclear cells (PBMC), cognitive functioning and cerebral metabolites measured with single-voxel proton magnetic

resonance spectroscopy (MRS) in 31 HIV-infected CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study participants. Individuals from the Mount Sinai subsite were chosen after completion of structural MR imaging that indicated an absence of focal brain lesions. A comprehensive battery of neuropsychologic tests was administered to all subjects, and CD4 counts, plasma and CSF HIV viral loads were determined. N-acetylaspartate (NAA) and choline (Cho) were used as measures of neuronal integrity and inflammation, respectively, and expressed as a ratio to creatine (Cr) for frontal gray and white matter and basal ganglia. Quantitation of SHIVA in PBMC was performed via real time PCR from extracted mRNA, and normalized to levels of PBMC G6PDH mRNA. SHIVA levels were expressed as a ratio of G6PDH/SHIVA; thus, smaller ratios represented larger proportions of SHIVA mRNA in PBMC. Sixteen of the 31 patients were cognitively intact, and 15 displayed impairments of variable severity. As predicted, SHIVA ratios were lower (i.e., SHIVA levels were higher) in cognitively impaired compared to unimpaired participants ($t[29] = 2.40$, $p = .0229$). In addition, lower SHIVA ratios were related to greater global neuropsychological deficit ($r = -.504$, $p = .0033$) and lower global neuropsychological t-score ($r = .506$, $p = .0032$). SHIVA ratios did not correlate with log serum or plasma HIV viral load, demographic or risk factors for HIV, or CD4 counts. Lower SHIVA ratios were associated with increased Cho/Cr in frontal white matter ($r = -.454$, $p = .0110$), but did not correlate with NAA/Cr in any region. These results support the association of SHIVA-expressing peripheral blood macrophages with HIV-associated cognitive impairment. The MRS data suggest that despite SHIVA's known neuronotoxic properties, the mechanism of association between its peripheral elevation and cognition may be predicated upon non-specific inflammatory perturbations in brain, and not selective neuronal toxicity.

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P129

Treatment of West Nile disease with humanized monoclonal antibody after the virus is in the brain

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People infected with West Nile virus (WNV) most often visit their physicians after showing symptoms suggestive of neurological infection. The question addressed in this study is if WNV-reactive antibody can improve disease signs in a hamster model after the virus is demonstrated to be in the brain. The hypothesis is based on the high activity of a humanized monoclonal antibody, hE16, in a mouse model when administered later in infection (Oliphant et al., Nat. Med. 11:522, 2005). In this study, virus was demonstrated to be in the brains of hamsters at 5 days post-viral injection (dpi) by cell culture assay, quantitative RT-PCR and immunohistochemical staining of WNV in neurons. Eighty percent of hamsters treated i.p. 5 dpi with 100 mg/kg of humanized monoclonal antibody, hE16, survived WNV disease, whereas, 37% of placebo-treated hamsters survived ($P < 0.001$). If administered at 2 dpi, 100% survived. We tested the hypothesis that hE16 is effective if delivered directly into the brain instead of by peripheral administration. The antibody was delivered into the brain 5 dpi using convection-enhanced delivery through a cannula implanted into the brain. The hE16 was detected in the CNS, but none was detected in the kidney. The survival of hE16-treated hamsters was 88% as compared to 22% of placebo-treated animals ($P < 0.001$). For additional proof, the majority of hamsters having WNV in their cerebrospinal fluid, a marker for CNS infection, were protected with hE16 administered i.p. at 5 dpi. This humanized monoclonal antibody, therefore, is a possible treatment for the post-exposure, WNV-infected humans that develop signs of neuroinvasive disease.

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P130

The immunophilin ligand GPI1046 protects DRG neurons from toxic effects of gp120 and dideoxynucleoside by alteration of calcium handling

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Sensory neuropathy is the most common neurological complication of HIV infection and there are currently no effective therapies for this painful neuropathy. The pathology of HIV-associated sensory neuropathy (HIV-SN) is characterized by prominent axonal degeneration and a modest loss of dorsal root ganglion (DRG) neurons. HIV-SN may involve toxic actions of the HIV coat protein gp120 and there is evidence for an increased incidence of toxic neuropathy in patients taking dideoxynucleoside (DDX) drugs. In this study we tested the effectiveness of a non-immunosuppressive immunophilin ligand to attenuate gp120 and DDX-induced DRG dysfunction. The immunophilin ligand GPI1046 protected cultured DRG neurons from axonal degeneration induced by gp120 and DDX. The amplitude of gp120-induced calcium transients was

attenuated in cultures pre-treated with GPI1046 suggesting that the protective effect of the immunophilin ligand may involve altered calcium handling. To determine how GPI1046 modified calcium handling in DRG neurons, we measured the total ER calcium load by releasing calcium from the ER using the sarco/endoplasmic reticulum calcium pump inhibitor thapsigargin. GPI1046 reduced the total calcium load in ER. Capacitive calcium entry was diminished in DRG neurons pre-treated with GPI1046 suggesting that GPI1046-associated ER depletion was due to an inadequate refilling of calcium stores. These findings suggest that non-immunosuppressive immunophilin ligands may be useful neuroprotective agents in HIV-SN.

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P131

Altered expression of breast cancer resistance protein (BCRP) during HIV-1 brain infection: Implications for neuropathogenesis

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Efflux mechanisms restrict the brain penetration of xenobiotics at the blood brain barrier (BBB). BCRP, transporter located at luminal membrane of brain microvascular endothelial cells (BMVEC) may serve as a gatekeeper for the xenobiotics entering brain, including antiretroviral drugs. To investigate BCRP expression during HIV-1 brain infection, we assessed BCRP distribution/level by immunohistochemistry and Western blot in autopsy brain tissues obtained from patients with HIV-1 encephalitis (HIVE, $n = 8$), HIV-1 positive patients without evidence of encephalitis (HIV+, $n = 7$), and seronegative age-matched controls (HIV-, $n = 6$). BMVEC demonstrated strong BCRP staining in most of the brains of HIV- controls or HIV+ samples without evidence of encephalitis, while HIVE featured decreased BCRP labeling of in endothelium paralleling macrophage infiltration. Microglia and perivascular macrophages were positive for BCRP in HIVE; however, few positive macrophages showed BCRP staining in HIV+ samples and none in HIV- controls. Western blot analysis performed in the same human brain tissues demonstrated 4.5-fold increase in CD68 (macrophage marker) and 18-fold increase in BCRP protein levels in HIVE ($p < 0.02$) as compared to HIV− controls. HIV+ brains showed augmented levels of CD68 (1.8 times) and BCRP (6 times, $p < 0.03$) as compared to controls. To confirm in vivo observations, we established FACS method for BCRP detection on primary human monocyte-derived macrophages (MDM) obtained from five seronegative donors. HIV-1 infection led to consistent two-fold increase of BCRP-positive MDM (20–30% vs. to 12–15% of uninfected MDM). HIV-1 infection resulted in ten-fold increase of BCRP levels in MDM assessed by Western blot in protein extracts derived from the cells analyzed by FACS. BCRP expression in human BMVEC (obtained from three donors) was down regulated after co-culture in HIV-1 infected MDM or exposure to TNF-alpha. In vitro and in vivo observations demonstrate up-regulation of BCRP expression in infected macrophages and down-regulation on BMVEC in the setting of HIVE. Since antiretroviral drugs are substrates for BCRP, these finding have important implications for the treatment of HIV-1 CNS infection.

P132

Survival defects and skewed memory phenotype of HIV-specific CD8+ T cells are detectable in early clinical stages of chronic HIV infection

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HIV-specific CD8+ T cells have been shown to be mainly of the CD45RA-CD62L- effector memory phenotype and are highly sensitive to spontaneous and CD95/Fas-induced apoptosis. In this study, we examined the memory phenotype (CD45RA-CD62L+ central memory CD8+ T cells, CD45RA-CD62L- and CD45RA+CD62L- effector memory CD8+ T cells) and apoptosis sensitivity (spontaneous and CD95-induced) of HIV-specific CD8+ T cells from 39 HIV-infected individuals to determine the clinical correlates of skewed memory phenotype and increased apoptosis sensitivity. No correlation was observed between percentages and absolute numbers of HIV-specific CD8+ T cell memory subpopulations and total CD4+ T cell counts, viral load and disease duration. Skewed phenotype and statistically significant differences in the ratio of CD45RA-CD62L- and CD45RA+CD62L- effector memory CD8+ T cells was found already in patients of clinical stage A1 when HIV-specific CD8+ T cells, CMV-specific CD8+ T cells and total CD8+ T cells were compared. That ratio was 10.8 for HIV-specific CD8+ T cells, 3.1 for CMV-specific CD8+ T cells and 1.8 for total CD8+ T cells. When HIV-specific CD8+ T cells were compared across clinical stages, higher absolute HIV-specific CD8+ T cells of both effector memory subpopulations were found in A1 compared to A2 and A3, however, the ratio

between the two effector memory populations was not different between these clinical stages. When spontaneous and CD95-induced apoptosis sensitivity was analyzed, no correlation was observed between apoptosis sensitivity and total CD8 count, viral load and disease duration. However, a statistically significant inverse correlation was found between the percentage of CD95-induced apoptosis and total CD4 counts. A statistically significant increase in apoptosis sensitivity for both spontaneous and CD95-induced was already found in clinical stage A1 when HIV-specific CD8+ T cells were compared to total CD8+ T cells and CMV- and EBV-specific CD8+ T cells and argues against chronic antigenic stimulation as a mechanism responsible for apoptosis sensitivity. Our data indicate that the skewed memory phenotype and increased apoptosis sensitivity of HIV-specific CD8+ T cells is already present at early stages of chronic HIV disease and does not correlate with disease duration and viral load.

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p65 associates with C/EBP-beta in vivo and modulate HIV-I gene expression

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HIV-1 transcription is a complex event and involves several cellular and viral proteins. Tat is an early protein in HIV infection released by infected cells, regulates the HIV-1 promoter partly through its interaction with TAR-RNA. Tat is known to interact with several DNA binding transcription factors including C/EBP-beta. Tat also participates transcription initiation and activates cellular genes in TAR independent manner. Previous in vitro studies revealed that p65 subunit of NF-kappaB and C/EBP-beta binds to HIV-LTR and play important role in HIV-1 transcription. Here we examined p65 and C/EBP-beta binding abilities to HIV-LTR in vivo, and we evaluate their effect on each other in the presence or absence of Tat. The ectopic expression of p65 along with Tat resulted in an increase of p65 binding to the LTR. This increase correlates with enhanced HIV-1 promoter activity. We also demonstrated using EMSA and ChIP assays, as well as siRNA directed against C/EBP-beta, that p65 binds more efficiently to the LTR in the presence of C/EBP-beta. Further, co-expression of C/EBP-beta and Tat leads to a decrease in p65 binding, which allows C/EBP-beta to bind more efficiently to the LTR. Inhibition of p65 expression by siRNA significantly decreases C/EBP-beta-binding and LTR expression. These observations confirmed the ability of p65 and C/EBP-beta to bind HIV-1 LTR in vitro and in vivo, and pinpoint the important role of both factors in regulating HIV-1 gene expression.

P134

Clinicopathologic correlates of Hepatitis C Virus in the brains of HIV-infected individuals: A pilot study

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Hepatitis C virus (HCV) has been detected in the brains of individuals with HIV infection; to date, the relative frequency of this occurrence and its clinicopathologic manifestations are unclear. We undertook a survey of 20 HIV-infected patients to determine: 1. If HCV RNA could be detected in post mortem samples of brain and liver; 2. If detection of brain HCV RNA correlated with pre mortem plasma HCV load; and 3. Whether any clinical or pathologic features could distinguish patients with brain HCV RNA from those without. Utilizing reverse transcriptase polymerase chain reaction (RT-PCR), HCV sequences were detected in the livers of 10 patients, all of whom had HCV detected in pre-mortem plasma samples. Frontal lobe HCV sequences were detected in 6 of the 10 co-infected patients. In these patients, plasma HCV load did not predict the presence of brain HCV; when liver HCV was detected, there was always plasma HCV load, and levels of plasma virus did not correlate with brain status. Complete neuropsychologic assessments were performed on 18 patients prior to demise; impairment in abstraction/executive functioning appeared worse with HCV, with mean t scores for patients without HCV infection 43.2 (7.3), for patients with HCV in liver but not brain 39.5 (9.0), and for patients with HCV in both brain and liver 33.2 (5.1) ($p = .0927$, ANOVA). When patients with brain HCV were contrasted to all patients without (regardless of liver status), their impairment in abstraction and executive functioning was significantly worse ($p = .0395$, student's t test). Neuropathologic analysis did not reveal any characteristic histology related to the detection of brain HCV. There was no evidence of brain HIV in any patient with concurrent HCV infection, either by PCR analysis or routine histology. While this pilot study is limited by small numbers of co-infected patients with detectable brain HCV, we conclude that in the setting of HIV: 1. brain HCV can commonly be detected when virus is present in liver; 2. The magnitude of HCV viremia does not predict the presence of brain sequences; and 3. There are significant differences in the neuropsychologic performance of patients with brain HCV compared to those without, however, a distinguishing neurohistology is not evident. Further studies are warranted to determine whether HCV clinical effects are predicated upon brain infection, or a function of co-morbid systemic disease.

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P135

Anti-varicella zoster virus (VZV) IgG antibody in cerebrospinal fluid is the virological test of choice to diagnose VZV vasculopathy

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Varicella zoster virus (VZV) vasculopathy is a serious neurological complication of VZV reactivation, which produces stroke secondary to viral infection of cerebral arteries. Accurate diagnosis is important since treatment with acyclovir can halt progression and prevent stroke. Unfortunately, the diagnosis of VZV vasculopathy is not always easy. Many patients do not have rash before experiencing transient ischemic attacks or stroke, and other vasculitides produce the same clinical and imaging abnormalities. Before PCR, the detection of anti-VZV IgG in CSF had been used to confirm the diagnosis of VZV vasculopathy. But when PCR proved to be so valuable to diagnose HSV encephalitis, clinicians used PCR, even to the exclusion of anti-VZV IgG antibody, to diagnose VZV vasculopathy. However, our laboratory receives calls from clinicians who describe zoster patients with typical clinical, CSF, imaging and angiographic features of VZV vasculopathy who do not have amplifiable VZV DNA in their CSF. Analysis of many of these cases detected anti-VZV IgG in these "VZV PCR negative" CSFs. This led us to compare the diagnostic value of detecting VZV DNA to anti-VZV IgG antibody in the CSF of subjects with clinical and laboratory evidence of VZV vasculopathy. Subjects with clinical and laboratory features of VZV vasculopathy must have tested for both VZV DNA and anti-VZV IgG antibody in CSF on the same day and found to be positive for either or both.

Of 19 subjects with virologically-confirmed VZV vasculopathy, 18 (95%) had anti-VZV IgG antibody and only 6 (32%) had VZV DNA in their CSF. Thus, in VZV vasculopathy, the diagnostic value of detecting VZV IgG in CSF is greater than that of PCR detection of VZV DNA ($p = 0.0013$). While a positive PCR for VZV DNA in CSF is helpful, a negative PCR does not exclude the diagnosis of VZV vasculopathy. Finally, because many patients with VZV vasculopathy, including those presented herein, do not fulfill all four typical clinical criteria (a history of zoster rash followed by neurological symptoms and signs of CNS disease, MRI/CAT abnormalities, angiographic changes and a CSF pleocytosis), we recommend that all patients with vasculitis of unknown etiology have their CSF tested for anti-VZV IgG antibody.

P136

Drugs of abuse increase IDO expression in HIV-1 infection

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Dendritic cells (DCs) are the first line of defense against HIV-1 infections. Increased kynurenine pathway metabolism has been implicated in HIV-1 neuropathogenesis and development of HIV-1 AIDS dementia (HAD). The rate-limiting enzyme for this pathway is indolamine 2, 3-dioxygenase (IDO) and its downstream product, quinolinic acid is a potent neurotoxin. We hypothesize that drugs of abuse like heroin and methamphetamine induce IDO that in turn helps to produce more quinolinic acid to cause neurotoxicity and/or peripheral immune tolerance. Blood monocyte derived dendritic cells (MDC) are isolated from normal subjects, HIV-1 infected subjects who are normal progressors (NP) and drug using NP (NP-DU). RNA was extracted, reverse transcribed and IDO gene expression was quantitated by real time QPCR using IDO specific primers. The results showed a significant increase ($p < 0.01$) in IDO gene expression in MDC from HIV-1 infected subjects compared to normal subjects. Further, IDO expression was significantly higher in NP-DU ($p < 0.004$) compared to drug non-using NP. The gene expression results were confirmed at the protein level by western blot and flow cytometric analyses. These results suggest that drugs of abuse may exacerbate HIV-1 infection probably by upregulating IDO expression in dendritic cells. A better understanding of role of IDO in immunosuppression and HIV-1 will help design novel therapeutic strategies using specific inhibitors and siRNA against the IDO gene in HIV-1 infected subjects.

P137

Simian immunodeficiency virus infection in simian fetal brain progenitor cells and microglial cells

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Human immunodeficiency virus type 1(HIV-1) infection of the central nervous system (CNS) often results in neurological diseases and dementia. It is necessary to investigate the underlying pathogenic mechanism

leading to CNS change to prevent the development of neuro-AIDS. It is generally believed that the brain lesions are not directly caused by cytopathic effects as a result of virus replication, but rather indirectly caused by HIV-specific products or soluble factors secreted by HIV-infected microglia/macrophages. The HIV-DNA was detected in astrocytes and neurons of the autopsied brain specimens by in situ PCR method. However, it is still unknown that the viruses latently infected in these cells were necessary to develop neuro-AIDS.

We analysed the neuropathogenesis of simian immunodeficiency virus (SIV) encephalitis, as an animal model of HIV encephalitis, using in vitro culture system. SIV also infects and replicates in macrophages/microglias much more than in neuronal/glial cells. It is difficult to determine whether the neuronal damages are due to the direct effect of SIV infection or due to the secondaly effects of infected macrophages or microglial cells. Thus we used the differentiated brain progenitor cells (BPCs) from embryonic brains of a Cynomolgus macaque (Macaca fascicularis) by a neurosphere assay as a neuronal/glial cell culture which contains neither macrophages, microglias, fibroblasts nor endothelial cells. We also developed the microglial culture from the same monkey. We analysed the infectivity of three kinds of SIV strains (SIV17E-Fr, SIV17SN, SIVmac239/316E) in each cell culture. SIV17/E-Fr and SIV17SN replicated in neuronal/glial cells much more than SIVmac 239/316E. All SIV strains infected microglias and had cytopathic effects. They inducted the several kinds of βchemokines (iMIP-1α, MIP-1β, RANTES, IL-10) and αchemokine(IP-10). We co-cultured SIV-infected neuronal/glial cells and uninfected microglial cells. The co-culture experiments showed that the SIV which had latently infected in neuronal/glial cells transferred to microglial cells and were significantly amplified. These results suggested that neuronal/glial cells functioned as reservers of SIV in the CNS.

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Characterization of HIV infected cells in infiltrates associated with CNS opportunistic infections

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Background: HIV-Clade C is the commonest HIV subtype in Africa and Asia, but it remains unknown if this clade can invade the brain and cause neuronal damage. Only anecdotal reports of HIV dementia exist in this population. Here, CNS opportunistic infections (OI) are common and frequently cause death. It remains unknown if OI may serve as a portal of HIV entry into the brain, putting patients at risk of developing HIV encephalitis and dementia.

Methods: Formalin fixed tissues from 15 patients (toxoplasma encephalitis- n = 5, tuberculous meningitis-n = 5 and cryptococcal meningitis- n = 5) who came to autopsy at the National Institute of Mental Health & Neurosciences, Bangalore, India were immunostained for HIV-p24, CD68 and GFAP. None had received antiretroviral therapy and all died within 6 months of onset of neurological symptoms.

Results: Cerebral toxoplasmosis had the largest amounts of HIV infected cells most prominent around the foci of toxoplasma encephalitis, perivascularly or in organized microglial nodules at sites distant from the focus of the OI. In tuberculosis, the HIV infected cells were most prominent in the meningeal infiltrate and noted to infiltrate the cerebral cortex in some patients. HIV infected cells were occasionally present in the choroid plexus, suggesting that this is the major portal of entry for these cells into the sub-arachanoid space. Patients with cryptococcal meningitis had the least amount of inflammatory infiltrates but also had HIV infected cells in the choroid plexus.

Conclusions: The finding of large amounts of viral antigen within the brain in conjunction with various OI suggests that HIV dementia may supervene once the OI are successful treated. On the other hand, effective treatment of OI may inhibit OI induced pathology and help limit viral burden. This will have important public health implications in addressing the HIV epidemic in developing countries.

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DENGUE virus infection modifies TNF-alpha and BCL-2 mRNA expression in infected SH-SY5Y neuroblastoma cells

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Dengue (DENV) virus infection could induce neurological manifestations, which are indistinguishable of typical viral encephalitis. It is little known the viral or host factors involved in nervous system compromise during severe cases of hemorrhagic dengue. In this work, human neuroblastoma cell lines was

infected with serotype 2 of DENV, infection proportions and cell viability using the MTT assay was analyzed. TNF-alpha and Bcl-2 mRNA expression levels was determinated using a real time PCR. Infection was shown using immunocytochemistry and plaque inducing assay with neuroblastoma infected cells seeded over susceptible monkey fibroblasts (indirect plaques). DENV infection causes a 40,4% of cell mortality at 48 h post infection (p.i.), without changes in TNF-alpha and Bcl-2 transcripts. But at 96 h p.i. there was a 24 fold increase in TNF-alpha mRNA and a 12 fold fall of Bcl-2 mRNA, findings possibly related with reported mortality. These results suggest that neuronal death and cytokine up-regulation could be a part of phenomena involved in dengue virus induced encephalitis.

P140

Replication of human polyomavirus JC in human brain microvascular endothelial cells: Possible mechanism for crossing the blood-brain-barrier

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Introduction: The pathogenesis of progressive multifocal leukoencephalopathy (PML) is poorly understood and how JCV crosses the blood-brain-barrier (BBB) remains unclear. We hypothesized that cell-free JCV during viremia crosses the BBB by infecting human brain microvascular endothelial (HBMVE) cells.

Method: An in-vitro BBB was developed to study the transmigration of cell-free JCV across the BBB by plating primary human brain cortical astrocytes (HBCA) and HBMVE cells on the lower and upper surfaces of cell culture inserts, respectively. The integrity of the in-vitro BBB model was determined by the FITC-BSA permeability assay before and after each transmigration experiment. HBMVE cells were cultured in-vitro, infected with JCV Mad 1A and were analyzed for early viral protein T-antigen (TAg) DNA and mRNA transcripts expression by quantitative real-time PCR.

Results: Approximately, 4% of the JCV inoculated in the upper chamber of the in-vitro BBB model was recovered from the lower chamber supernatant (LCS) during the first 7 days post-inoculation (p.i.). The rate of transmigration peaked on day 3, was lowest on day 5, and increased again on day 7 p.i. Moreover, the LCS of the JCV inoculated BBB model was infectious, based on infection of naïve primary human fetal glial (PHFG) cells. About 5% of JCV was attached or internalized into the HBMVE cells after 2 hr of infection. JCV efficiently replicated in HBMVE cells and TAg DNA level in HBMVE cells on day 20 post-infection (p.i.) was 884-fold higher than that on day 0. Similarly, TAg transcripts first appeared on day 3 p.i., and continued to increase rapidly thereafter until the end of the experiment (day 20 p.i.). Replication efficiency of JCV in HBMVE cells was very similar to the PHFG cells.

Conclusions: Our data suggest that JCV can infect and replicate in HBMVE cells and can cross the in-vitro BBB. To our knowledge, this is the first study demonstrating replication of JCV in primary HBMVE cells, a critical component of BBB. Understanding the mechanisms of transmigration of JCV across the BBB may help to develop therapeutic intervention for prevention of PML.

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PERSISTENT hijacking of brain proteasomes in HIV dementia: Data from National Neuro AIDS tissue consortium specimens

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Inflammation decreases the rate of protein degradation through the ubiquitin-proteasome complex. Immunoproteasome (IPS) complexes are induced that divert the substrate repertoire towards processing of peptides for antigen presentation in histocompatibility complexes (Class I HLA). Borrowing the proteasome apparatus for heightened antigen presentation persists until a pathogen has been eradicated. But infection of the brain with Human Immunodeficiency Virus (HIV-1) provokes a persistent inflammatory response that does not eradicate the pathogen. An abnormally persistent diversion of routine protein turnover can cause the accumulation of misfolded protein, and could exacerbate senile neurodegeneration. To determine if HIV-1 influences brain proteasome structure and function, we measured proteinase activities and subunit composition in prefrontal cortex of 153 HIV-infected people from the National NeuroAIDS Tissue Consortium Cohort. Subjects with neurocognitive impairment (HAD) or inflammation associated with HIV encephalitis (HIVE) had abnormal multicatalytic proteinase profiles. The concentration of catalytic beta subunits of the 20S proteasome was abnormal. Interferon-gamma (IFNg) inducible subunits of immunoproteasomes (IPS) were increased. 11S regulatory ("lid") complexes of IPSs increased in parallel. Several components of the 19S lid complex were decreased. The light chain of Class I HLA was increased, suggesting increased vigilance of immune surveillance, but heavy chains were not increased. IPS synthesis was correlated with abnormal concentrations of high molecular weight polyubiquitinylated conjugates, which suggests that altered protein turnover occurs. IPSs were localized to many cells that can present antigen, including neuronal perikarya, axons,

reactive astrocytes, microglial cells, endothelial cells, and oligodendrocytes. We conclude that brain HIV-1 infection hijacks the proteasome persistently, possibly for heightened antigen presentation.

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Early onset, late effect: The central nervous dopamine metabolism and its impact on neurophysiological deficits in AIDS patients

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Background: Since the beginning of the pandemia, the aspect of monoamine transmitter disturbances of the central nervous system (CNS) in HIV-1-positive patients has been intensively studied. Our aim was to evaluate the starting point and the initial mechanisms of these disturbances before there are any clinical signs or symptoms with respect to the prevention of late stage deficits in patients with advanced HIV-infection.

Methods: 81 male HIV-1-positive patients were enrolled in a prospective study. The patients were assigned to 6 groups according to the CDC classification, antiretroviral therapy and usage of psychotropic substances. All subjects underwent neurological examination, a neuropsycholgical test battery, cranial magnetic resonance imaging (MRI) and lumbar puncture.

Results: In early stage patients (CDC A1+2, B1+2) with elevated CSF viral load and signs of inflammation in CSF we found disturbances of the CNS dopamine (DA) metabolism, in terms of decreased dopamine, those patients are without any clinical signs of motor or affective disorders. Antiretroviral therapy did not reverse DA deregulation in cases with sustained inflammation and viral replication in the CSF. In untreated AIDS patients, elevated CSF protein levels correlated with decreased DA metabolites (hydroxvanillic acid). In treated AIDS (CDC B3, C1-3) patients, psychomotor speed decreased in correlation to elevated dopamine levels; subsequently dopamine metabolites were increasing with elevated CSF lactate levels.

Conclusions: A subset of patients seems to be susceptible to an early impairment of dopaminergic systems without showing any clinical signs, maybe due to efficient compensation mechanisms. This subset of early compromised patients may develop a late "burnout" of the compensatory mechanisms uninfluenced by ART and in worst cases possibly accelerated by toxic side-effects of ART itself. The study will be continued after these encouraging first results.

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Functional analysis of C/EBP transcription factor binding sites in the SIV LTR

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To examine functional properties of the CCAAT enhancer binding protein (C/EBP) transcription factor family with respect to human immunodeficiency virus type 1 (HIV-1) pathogenesis and neurologic disease, we have proceeded to characterize the simian immunodeficiency virus (SIV) LTR and the corresponding functional elements that interface with this critical family of regulatory factors. In this regard, studies have been initiated to identify and characterize SIV LTR sequences with affinity for members of the C/EBP family. Electrophorectic mobility shift (EMS) and competition analyses using U-937 monocytic nuclear extracts and purified C/EBP were performed, which demonstrated binding of C/EBP to four of the five potential C/EBP sites in the SIV LTR. The binding sites were designated C/EBP upstream site 1 (US1), upstream site 2 (US2), downstream site 1 (DS1), and downstream site 2 (DS2) located at nucleotide positions -102 to -88, -386 to -373, +131 to +144, and +265 to +280, respectively. Results indicated that C/EBP DS1 and DS2 exhibited relatively high affinity for C/EBP alpha and beta. By comparison, both US1 and US2 exhibited lower affinity for C/EBP, and DNase I footprint analyses confirmed these observations. Additional studies also indicate that US1 and US2 may interact with other transcription factors. Functional analysis of these sites utilizing transient transfection has demonstrated US2, DS1, and DS2 to be repressive in nature while US1 was shown to function as an activating element in cells on the monocytic lineage. Studies will continue to examine the functional impact of the SIV C/EBP sites and their variants on viral gene expression and replication in the SIV/macaque system.

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HIV-1 LTR activity is influenced by the differential recruitment of Sp transcription factors during monocytic differentiation

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Infection of cells of the monocyte/macrophage lineage by human immunodeficiency virus type 1 (HIV-1) has been shown to be important in the pathogenesis of the disease. Viral replication in this cell lineage is, in part, mediated by interactions between the HIV-1 long terminal repeat (LTR) and a variety of host cell and viral proteins. Consistent with this logic, basal

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

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HIV-1 Tat hyperpolarizes mitochondrial membrane potential in vulnerable neurons

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Infection with the Human Immunodeficiency Virus (HIV-1) initiates a series of events that can culminate in HIV Associated Dementia (HAD). HAD is a progressive neurological disorder that results in motor, cognitive and social dysfunction. Highly Active Anti-Retroviral Therapy (HAART) initially decreased the incidence but not prevalence of HAD, and temporary amelioration of symptoms has lead us to investigate the molecular basis for the reversible component of HAD. Tat is demonstrated to have both in vitro and in vivo neurotoxic effects, and may be a key initiator of neuronal dysfunction. Recent data from our lab has demonstrated that dosing of rodent cortical neurons with sub-lethal concentrations of Tat elicit mitochondrial hyperpolarization. Mitochondrial hyperpolarization is a recently described phenomenon whose mechanisms and consequences are not fully elucidated, but may have profound consequences for synaptic transmission and other energy-dependent processes. The present study investigates the mechanism(s) leading to mitochondrial hyperpolarization, focusing on several mitochondrial systems including: pH, electron transport chain (ETC) activity and ion retention. Using the cationic lipophilic dye Rhod123, we confirm the observation that rodent cortical neurons treated with Tat undergo mitochondrial hyperpolarization. In a similar experimental paradigm, use of the pH-sensitive fluorophore SNARF-1 reveals a decrease in internal mitochondrial pH with Tat treatment in concordance with hyperpolarization. Isolated mitochondrial protein was used to examine the individual complexes of the ETC. High concentrations of Tat (>1 ug/mL) decreased the functional activity of Complex III while in contrast, as little as 10 ng/mL of Tat decreased the activity of Complex IV. In order to measure the effects of Tat in a more physiologically relevant system, cortical neuronal respiration was measured using a Clark electrode. A significant decreased in neuronal respiration was observed using a dose of 100 ng/mL Tat, corroborating inhibition of complex IV in this in vitro system. Next, we investigated mitochondrial ion homeostasis. Using a mitochondrial targeted YFP-calmodulin construct, we measured Ca2+ concentrations and found that Tat treatment decreased intra-mitochondrial calcium concentration. We speculate that this decrease in calcium may contribute to mitochondrial hyperpolarization after exposure to Tat. Future studies will examine the molecular basis for the decrease in Ca2+ and investigate whether Tat affects the ability of NAD(P)H to participate in modulating mitochondrial membrane potential. In aggregate, these studies will provide us with better insight into how Tat subverts normal neuronal communication during energy-dependent processes.

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Roles for the N-Methyl-D-Aspartate receptor subtypes 2A and 2B, and the calcium activated protease calpain in HIV-induced hippocampal neurotoxicity

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Neuronal damage in HIV-1 infection in the brain is thought to occur at least in part through N-methyl-D-aspartate receptor (NMDAR) excitation initiated by soluble neurotoxins from HIV-infected brain macrophages and non-infected, activated glial cells (macrophages, astrocytes). Previous studies have implicated a number of HIV- and monocyte-derived macrophage (MDM)-associated neurotoxins that directly or indirectly activate neuronal NMDARs, including glutamate, quinolinic acid (QUIN), platelet activating factor (PAF), reactive oxygen species, NTox, Tat and gp120. Furthermore, brain regions enriched in NMDAR 2A and 2B subunits (NR2A, NR2B), such

as the hippocampus and basal ganglia, are particularly vulnerable to HIV-associated damage. Using cultured rat hippocampal cells and HIV-1-infected human monocyte-derived macrophages (HIV/MDM), we examined the role of NR2A and NR2B in HIV/MDM-induced hippocampal neuronal death. We also attempted to identify contributions of different excitotoxins to HIV/MDM neurotoxicity. We used the primary HIV-1 strain, Jago, derived from the cerebrospinal fluid of an individual with HIV-associated dementia and that robustly replicates in MDM. We found that i) susceptibility of hippocampal neurons to HIV/MDM varies according to the developmental expression patterns of NR2A and NR2B; ii) NMDAR activation by HIV/MDM results in neuronal calpain activation, which contributes to neuronal death; iii) selective antagonists of homomeric NR2B/NR2B- and heteromeric NR2A/NR2B-containing NMDARs as well as an inhibitor of calpain activity, afford neuroprotection against HIV/MDM; iv) HIV/MDM do not release elevated levels of QUIN, although inhibitors of the QUIN synthesis afforded neuroprotection against HIV/MDM; and v) enzymatic degradation of glutamate in HIV/MDM supernatants partially reduced neurotoxicity. These studies confirm that HIV/MDM excitotoxicity occurs through the release of glutamate as well as other excitotoxins (candidates include NTox), and they establish a link between HIV infection, neuronal NR2A and NR2B activation, and calpain-mediated hippocampal neuronal death. They further suggest a dominant role for NR2A and NR2B in determining neuronal susceptibility in HIV-infected brain. Antagonists of NR2A and NR2B subunits as well as calpain inhibitors offer attractive neuroprotective approaches against HIV in both developing and mature brain.

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The G-protein coupled receptor APJ mediates survival signaling in hippocampal neurons and protects against HIV-induced neurotoxicity

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G-protein couple receptors (GPCRs) are expressed in many cell types, including neurons, where they can promote either survival or death under certain conditions. Among the GPCRs, the chemokine receptors CCR5 and CX3CR1 (fractalkine receptor) have been shown to promote neuronal survival in in vitro models of gp120 neurotoxicity. We recently described expression of the unique GPCR APJ in cultured neurons (Choe et al. 2000. J. Neurovirology.) APJ is also strongly expressed in cardiac myocytes and in other tissues. Interestingly, the endogenous APJ peptide ligand, apelin, is found within the systemic circulation and it has been shown to modulate cardiac contractility and blood pressure changes in vivo. We have examined the ability of apelin to modulate neuronal cell signaling functions in vitro and determined apelin's ability to promote neuronal survival in our in vitro models of HIV-induced neurotoxicity. Our models utilize either primary rodent hippocampal neurons or human NT2.N neurons exposed to supernatants from HIV-infected monocyte derived macrophages (HIV/MDM.) We have demonstrated that 1) apelin is expressed in hippocampal neurons; 2) apelin induces phosphorylation and activation of the cell survival kinases AKT and Ras/ERK-1/2 in neurons; 3) apelin-mediated kinase activation is dependent upon Gαi linkage that is associated with very weak induction of calcium transients; and 4) apelin protects hippocampal neurons from specific N-methyl-D-aspartate receptor ligand excitotoxicity as well as from HIV/MDM excitotoxicity. Thus, apelin/APJ signaling might represent an important endogenous neuronal survival response in hippocampal neurons in vivo, which are a particularly vulnerable neuronal subpopulation in the HIV infected brain. Our results also suggest that apelin should be further investigated as a potential treatment modality against neurodegeneration.

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Analysis of Foxp3 protein expression in HTLV-I infection

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Infection with the human T lymphotropic virus type-I (HTLV-I) can be associated with the development of a number of inflammatory conditions including a chronic inflammatory myelopathy termed HTVL-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), although the majority of individuals remain asymptomatic. In addition to HAM/TSP, a number of other disorders are associated with HTLV-I including myositis, uveitis, sialadenitis and alveolitis, suggesting a systemic inflammatory disorder. The determinants of disease risk for developing inflammatory disorder associated with HTLV-I are not completely understood. However, the immunological abnormalities including persistent expansion of activated T cells, hypergammaglobulinemia and spontaneous lymphoproliferation of T cells from patients with HAM/TSP suggest there may be a generalized disorder of immune regulation.

Recently, dysfunction of CD4+CD25+ regulatory T cells (T reg) have been implicated in a number of human immune-mediated diseases including multiple sclerosis, inflammatory arthritis and diabetes. The CD4+CD25+ T reg comprise a distinct subset of T cells

that contribute to the maintenance of immunological self-tolerance. We asked whether a dysfunction of T reg might contribute to the development of inflammatory disease in HTLV-I infection. A major advance in the understanding of T reg has been the recent characterization of Foxp3, a member of the forkhead/winged-helix family of transcription factors that was shown to be a critical mediator of T reg maintenance and function. To quantitate Foxp3 protein expression, we developed a flow cytometric assay for detection of Foxp3. We then analyzed Foxp3 expression in HTLV-I infection by comparing Foxp3 expression in the peripheral blood of individuals with HAM/TSP to that of asymptomatic carriers of HTLV-I and healthy donors. Analysis showed that HTLV-I-associated myelopathy is associated with lower expression of Foxp3 compared to asymptomatic carriers of HTLV-I and healthy donors in the peripheral blood leukocytes. In individuals infected with HTLV-I, Foxp3 expression was inversely correlated to HTLV-I proviral load. These results suggest that impaired Foxp3 expression may contribute to the development of inflammatory disease in HTLV-I infection.

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Borna disease virus infection does not induce activation of microglia in vitro

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Neonatal Borna disease virus (BDV) infection of the rat brain is associated with microglial activation and damage to and death of certain neuronal populations. Since persistent BDV infection of neurons in vitro is non-cytolytic and non-cytopathic, activated microglia have been suggested to be responsible for neuronal cell death in vivo. However, this has not been conclusively demonstrated, and the mechanisms of activation of microglia in neonatally BDV-infected rat brain are unclear. To investigate these issues, activation of primary rat microglial cells was studied following exposure to purified BDV, to persistently BDV-infected rat neuronal cell lines, to BDV-infected primary cortical neurons and astrocytes, and to BDV-infected neurons rendered apoptotic by staurosporine. None of these treatments resulted in microglia activation as assessed by release of NO and TNF-alpha. In addition, no signs of toxicity were found in BDV-infected neurons co-cultured with microglia. In contrast, control treatments with LPS or the HIV-encoded Tat protein produced a dose-dependent activation of microglia, resulting in comparable toxicity in both BDV-infected and uninfected neurons. Taken together, our results indicate that microgliosis in vivo might not be directly related to BDV infection but may be triggered by yet unknown mechanisms.

The opinions expressed in this study may not necessarily reflect the official positions of the Food and Drug Administration of the USA, USDA and the Federal Government.

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Screening random peptide libraries with SSPE brain-derived recombinant antibodies identifies multiple epitopes in the C-terminal region of the measles virus nucleocapsid protein

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Chronic infectious and inflammatory diseases of the CNS are often characterized by a robust B cell response that manifests as increased intrathecal IgG synthesis and the presence of oligoclonal bands. Further, the oligoclonal IgG is directed against the agent that causes the disease. Using laser-capture microdissection and single-cell PCR to analyze the IgG variable regions of plasma cells from the brain of a patient with SSPE, we detected at least nine expanded plasma cell clones within the brain parenchyma. Importantly, five of the eight human IgG1 recombinant antibodies (rAbs) produced from the SSPE brain plasma cell clones recognized the measles virus (MV) nucelocapsid protein, confirming that the antibody response in SSPE brain primarily targets the agent that causes disease. To determine the feasibility of using peptide screening for antigen discovery, four MV-specific rAbs were used to probe a random phage-displayed peptide library to find out if they would bind to MV-specific protein epitopes. All four SSPE rAbs panned against the peptide library enriched phage-displayed peptide sequences that reacted specifically to their panning rAb by ELISA. A search of the Genbank protein database revealed homologies for peptides selected by three of the SSPE rAbs to different amino acid stretches contained within the C-terminal 65 amino acids of the MV nucleocapsid protein. The specificity of SSPE rAbs to these regions of the MV nucleocapsid protein was confirmed by binding to synthetic peptides or to short cDNA expression products. This strategy can readily be applied for antigen identification in CNS inflammatory diseases of unknown cause such as multiple sclerosis.

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CCAAT/enhancer-binding proteins modulate human T cell leukemia virus type 1 long terminal repeat activation

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CCAAT/enhancer-binding protein (C/EBP) basic region/leucine zipper (bZIP) transcription factors have been shown to form heterodimers with cAMP-responsive element binding protein 2 (CREB-2), a bZIP transcription factor involved in regulating basal and Tax-mediated transactivation of the human T cell leukemia virus type 1 (HTLV-1) long terminal repeat (LTR). The interaction between C/EBPbeta or C/EBPzeta with CREB-2 has been previously shown to down-regulate Tax-mediated transactivation of the HTLV-1 LTR. In cells of the monocyte/macrophage lineage (proposed to play a role in HTLV-1 pathogenesis as an accessory target cell population), several members of the C/EBP family including C/EBPbeta are expressed at high levels. Therefore, the functional impact of C/EBP factor expression on both basal and Tax-mediated transactivation of the HTLV-1 LTR was examined. Low-level basal activation of the HTLV-1 LTR was significantly enhanced by overexpression of C/EBPbeta, C/EBPdelta, or C/EBPepsilon, whereas transactivation of the HTLV-1 LTR by Tax was inhibited by overexpression of C/EBPalpha and C/EBPbeta, and to a lesser extent C/EBPdelta. Inhibition of Tax-mediated transactivation of the HTLV-1 LTR was co-activator-independent and did not require binding of C/EBP to the Tax-responsive elements of the viral promoter. Deletion of the C-terminal leucine zipper dimerization domain or the basic region DNA binding domain of C/EBPbeta blocked C/EBPbeta from inhibiting Tax-mediated transactivation of the HTLV-1 LTR, suggesting that C/EBP dimerization (likely to CREB-2) may be critical for down-regulating Tax-mediated transactivation of the HTLV-1 LTR. Therefore, endogenous expression of C/EBPalpha and C/EBPbeta (which are primarily limited to myeloid lineage cells) may be critical factors limiting viral gene expression in cells of monocyte/macrophage lineage.

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Involvement of the ubiquitin-proteasome system in HIV-1 Tat-mediated neuronal damage

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Ubiquitination is a rapid and reversible mechanism for the covalent modification of target proteins which modulate synaptic functions. Poly-ubiquitination of target proteins, however, is an irreversible modification that marks proteins for degradation. Ubiquitin is attached to a lysine residue on the target protein by a multi-step enzymatic process. E3 ubiquitin ligases selectively mediate conjugation of target proteins to ubiquitin. There are two main classes of E3 ligases, proteins with a HECT (homologous to E6-AP carboxyl terminus) catalytic domain and proteins with a zinc-binding RING finger (really interesting novel gene) adaptor domain. Nedd4 is a ubiquitin-protein ligase containing a calcium/lipid-binding domain, multiple WW domains and a C-terminal HECT domain. Nedd4 is known to be involved in the selective ubiquitination of some regulatory proteins in transcription and membrane transport. The HIV-1 transactivating factor Tat is among the factors involved in the pathogenesis of HIV-1 associated neuronal dysfunction. In this study, we demonstrated dendritic localization of Nedd4 in rat embryonic cortical neurons. We additionally found that treatment of rat cortical neurons with increasing concentrations of recombinant Tat resulted in down-regulation of Nedd4. As the process of ubiquitylation is involved in the internalization and vesicular trafficking of cell surface receptors, Tat-mediated deregulation of Nedd4 may alter proper levels of synaptic receptors possibly affecting neuronal function.

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Increased mitochondrial mass in apoptotic HIV-specific CD8+ T cells

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What governs the increased apoptosis sensitivity of HIV-specific CD8+ T cells is poorly understood. Here we report the possible involvement of mitochondria and filamentous actin in this process. We report here that significantly higher mitochondrial mass (MM) is found in HIV- ($77 \pm 7.2\%$, $n = 15$) compared to CMV-specific CD8+ T cells ($50 \pm 5.9\%$, $n = 13$, $p < 0.0002$). Furthermore, CD95/Fas-induced apoptosis was remarkably increased in MMHigh (41.4±4% annexin V+, $n = 9$) compared to MMLow ($8.2 \pm 1.7\%$ annexin V+, $n = 9$, $p < 0.0001$) CD8+ T cells from HIV+ patients. Proapoptotic HIV-specific CD8+ T

cells exhibited a CD38+ Bcl-2Low MMHigh phenotype. Mitochondria colocalized with CD95/Fas capping, very early during CD95/Fas-induced apoptosis of HIV-specific CD8+ T cells, suggesting their active role as an amplification step for this apoptosis and a potential involvement of the cytoskeleton in this process. Indeed, disruption of actin by cytochalasin D (cytD) dramatically suppressed CD95/Fas-induced apoptosis of HIV-specific CD8+ T cells ($40.8 \pm 5\%$ versus $21.3 \pm 4.6\%$ annexin V, $n = 13$, $p < 0.02$, in the absence or presence of cytD respectively). This effect was independent of memory and activation status of these cells. The data above reveal mitochondria and filamentous actin as potential mediators of HIV-specific CD8+ T cell apoptosis further clarifying the molecular mechanisms governing this process.

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Detection of JCV DNA sequences and Expression of T-Antigen in a glioblastoma multiforme with small cell primitive neuroectodermal-like component

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The human polyomavirus JCV, the etiological agent of the demyelinating disease Progressive Multifocal Leukoencephalopathy, has been implicated in the development of primitive neuroectodermal tumors and various neuroectodermal and glial-derived tumors, including Glioblastoma Multiforme. Here we describe the unique clinical case of a 54 year-old man who presented with headaches, hemiparesis and drowsiness. T1 and T2 magnetic resonance images revealed a large solid tumor with a cystic component located in the right temporal lobe, with extension into the parietal lobe. Histologically, the tumor was composed of two areas, a predominant component of large neoplastic cells with pleomorphic atypical nuclei and abundant eosinophilic cytoplasm, which by immunohistochemistry was robustly reactive for glial fibrillary acidic protein, compatible with a GBM, mixed with several foci of poorly differentiated tumoral cells with basophilic, slightly elongated nuclei and scant cytoplasm, which were negative for GFAP, but strongly immunoreactive for synaptophysin, phosphoneurofilaments and Class III-beta Tubulin, characteristic of a Primitive Neuroectodermal Tumor. Results from PCR amplification in laser capture micro-dissected cells from both areas of the tumor revealed the presence of DNA sequences corresponding to the early, late and control regions of the JCV genome. Expression of the oncogenic T-antigen was detected by immunohistochemistry in the nuclei of neoplastic cells and the cell cycle dysregulator Agnoprotein was robustly expressed in the cytoplasm of tumoral cells in both phenotypes. No evidence for viral capsid protein was found, ruling out productive infection in these cells, which harbor viral DNA. Double labeling immunohistochemistry demonstrated the co-localization of viral T-Antigen with the cell cycle regulatory protein p53 in the nuclei of neoplastic cells in both areas of the tumor, suggesting that T-antigen binds, sequesters and inactivates wild type p53. Sequencing of the amplified products demonstrated the presence of the Mad-1 strain of JCV with distinct point mutations in the control region of isolates from both, GBM and small cell components.

The presence of the same strain of JCV in a rare tumor which exhibits divergent lines of differentiation, and the expression of viral proteins T-antigen and Agnoprotein in both areas of the neoplasia reinforces the notion of JCV as a key player in malignant transformation at an early stage of tumorigenesis, and provides further evidence on the monoclonal origin of two concomitant tumors of different phenotypes in the same patient. The finding of multiple mutations in the JCV control region is novel and may indicate important adaptations for maintaining T-antigen and Agnoprotein expression during tumor development.

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Peroxisome Proliferator-Receptor gamma (PPAR gamma) agonist suppresses HIV-1 replication by inhibition of the nuclear factor kappa B (NF-kB) in vitro and in an animal model of HIV-1 encephalitis (HIVE)

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Poor penetration of anti-retroviral therapy across the blood brain barrier poses an impediment in control of HIV-1 infection of brain macrophages. The PPARs are a family of nuclear receptors that regulate important physiological functions in response to ligand binding. Of the three PPARs (alpha, beta, gamma), PPARgamma regulates pleiotropic anti-inflammatory pathways. We hypothesized that PPARgamma stimulation may suppress HIV-1 replication. First we investigated the effect of PPARgamma agonists on HIV-1 replication in human monocyte-derived macrophages (MDM). PPARgamma agonist treatment caused 20-fold decrease of virus infection in MDM. In parallel, we demonstrated that PPARgamma stimulation led to effective suppression of HIV-1 replication in blood lymphocytes and brain macrophages using a

murine model of HIVE (immunodeficient mice reconstituted with human lymphocytes and intracerebrally inoculated with HIV-1 infected MDM). Animals fed with PPARgamma agonist, rosiglitazone suppressed HIV-1 replication in intracerebrally inoculated human MDM and reduced viremia by 50% as compared with placebo group. Next, in vitro experiments addressed the mechanism underlying inhibitory effects of PPARgamma activation on suppression of HIV-1 replication. We showed that rosiglitazone pretreatment suppresses HIV-1 replication by repression of the transcriptional activity of nuclear factor kappa B (NF-kB). Ligand activation inhibited IkB phosphorylation that affected subsequent IkB degradation and release of NF-kB binding subunits required for NF-kB mediate induction of HIV-1 infection. PPARgamma activation also significantly reduced HIV-1 induced NF-kB DNA-binding ability. Furthermore, PPARgamma stimulation suppressed HIV-1 LTR activity in a dose—dependent manner. The specific PPARgamma antagonist, GW9662 abolished the effects of rosiglitazone. Our in vivo and in vitro findings underscore the ability of PPARgamma agonists to suppress HIV-1 replication offering new therapeutic intervention in brain and systemic infection.

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Human astrocytes provide an inflammatory environment to human neurons following Rabies virus infection

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We have already established that human neurons express TLRs and in the absence of glia can mount an innate immune response to dsRNA (C. Prehaud et al., 2005, J. Virology, 79, 20, 12893). In order to monitor the effect of the surrounding cellular environment in the neighbourhood of the infected neurons, we investigated the role of the astrocytes. This study was undertaken by taking advantage of the pluripotency of the embryonal human teratocarcinoma cell line Ntera-2clD/1 which could be differentiated either in pure neurons (NT2-N) or astrocytes (NT2-A) but also in mixed cultures of neurons and astrocytes (NT2-N/A). Thus following Rabies virus infection (CVS strain, 24 hours post-infection) of NT2-N/A, a transcritome analysis was carried out by using Affymetrix U133 plus 2 microarrays which covered the whole human genome. The up regulated genes (4257) were clusterized. The most represented cluster covered the genes involved in the immune response. In addition to the genes which expression was already increased in neurons—and which up regulation levels were considerably exacerbated in the NT2N/A compared to NT2-N cultures, new genes involved in the immune response were recruited. Among them, 83.6% of the genes are representative of the inflammatory process, and most of them were controlled by IFN-g and TNF-a. Gene expression modulation was double-checked by real time PCR. We established a pure human NT2-A astrocyte cell line according to the method described by Sandhu J.K. et al. (2002) to investigate the astrocyte response after rabies virus infection and after treatment with inflammatory cytokines such as TNF-a and IFN-g. We concluded that astrocytes which are poorly infected by Rabies virus in vivo provide a peculiar environment by stimulating genes involved in the inflammation response.

P157

Intranasal delivery of HIV-1 Tat up-regulates brain inflammatory mediators in mice

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Intranasal (IN) delivery of HIV-1 Tat in mice was investigated as a possible model for HIV-1 infection in the brain. Following IN administration, distribution of [125I]-labeled Tat in the brains of Swiss Webster mice was evaluated by autoradiography and gamma counting. [125I]-labeled Tat was detected at the highest concentrations in the olfactory bulb, cervical nodes and trigeminal nerve tract. In the brain, lower concentrations were detected in the hypothalamus, frontal and parietal cortex, and hippocampus. Having evidence that Tat was effectively delivered to and distributed in the brain, we compared differential gene expression in the frontal cortex of the Tat versus vehicle IN-treated APPsw transgenic mice. The mice were treated with 6 ug Tat (n = 4) or vehicle (n = 4) three times per week for 4 weeks. Total RNA was isolated from the frontal cortex and differential gene expression analysis was performed using the CodeLink 35K mouse gene microarrays. Gene ontology profiles indicated an inflammatory and apoptotic response. Three genes of interest in the Tat-treated mice that were significantly elevated in the microarrays were validated by RT-PCR. One gene was the Toll-like receptor 9 (TLR9), which was previously shown to mediate innate immunity and enhance HIV-1 gene expression. A second gene, FAS, was also significantly increased and plays a key role in neuroinflammation. Interestingly, FAS was reported to be elevated in the CSF of patients with AIDS dementia. Finally, the gene sestrin was significantly elevated and has been associated with oxidative stress, in particular amyloid beta-induced oxidative stress. We conclude that this IN Tat model of neuroinflammation may be useful to study HIV-1-induced neurodegeneration in the CNS.

P158

Inhibition of HIV-1 infection by PEHMB involves perturbation of the viral co-receptor CCR5

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Neurological disease associated with HIV-1 infection of the central nervous system (CNS) remains a pressing problem, even in the face of successes achieved using the latest chemotherapeutic approaches to the treatment of systemic disease. For this reason, the development of compounds effective against HIV-1, particularly CCR5-using strains that predominate within infected cells in the CNS, remains a high priority. Our efforts in this area have focused on compounds that appear to inhibit HIV-1 infection by interfering with interactions between the virus and cell surface proteins. Polyethylene hexamethylene biguanide (PEHMB) is characterized by low toxicity and notable activity against HIV-1 BaL, which uses CCR5 as a viral co-receptor. The potency of PEHMB in the presence of both virus and target cells led us to hypothesize that PEHMB interferes with viral binding and entry mechanisms. Results from flow cytometric analyses of HIV-1-susceptible cells exposed to PEHMB demonstrated increased CCR5 detection despite effective inhibition of HIV-1 BaL infection by PEHMB. We hypothesized that inhibition of HIV-1 BaL infection by PEHMB may involve perturbation of CCR5 conformation and/or localization within the plasma membrane. These changes interfere with interactions with HIV-1, while making CCR5 more accessible to detection by antibodies. Ongoing investigations are examining specific interactions between PEHMB and CCR5 that may cause changes in co-receptor availability, expression, internalization, or conformation that result in inhibition of HIV-1 infection. These studies are being used to facilitate the development of novel compounds that can be used safely in the CNS to inhibit HIV-1 infection.

P159

Antiviral treatment of postherpetic neuralgia

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Varicella zoster virus (VZV) is a highly neurotropic, exclusively human alphaherpesvirus that causes chickenpox (varicella). After primary infection, virus becomes latent in cranial nerve, dorsal root and autonomic ganglia along the entire neuraxis. Decades later, virus reactivation produces shingles (herpes zoster), characterized by pain and rash restricted to 1-3 dermatomes. While the rash usually disappears within 4–6 weeks, as many as one million Americans are affected by persistent dermatomal distribution pain after herpes zoster, so called postherpetic neuralgia (PHN). Studies of patients with PHN and zoster sine herpete (radicular pain without rash) support the notion that low-grade viral ganglionitis contributes to pain. If chronic pain reflects active infection, then antiviral therapy has the potential to help patients with PHN.

We conducted a prospective, open-label phase I/II clinical trial to determine if antiviral treatment helps reduce PHN pain. Fifteen patients with PHN suffering moderate to severe pain were treated with intravenous acyclovir for 14 days followed by oral valacyclovir for one month. Pain was measured using an 11-point Numeric Rating Scale for Pain (NRSP). Based on previous studies of neuropathic pain, a $\geq$2-point reduction in the NRSP scale was chosen as representing clinically meaningful improvement. Eight of 15 (53%) patients reported a $\geq$2-point decrease on the NRSP [95% confidence interval (CI), 27% to 79%]. The clinically significant reduction in pain reported by most of our patients after sequential acyclovir and valacyclovir treatment suggests a promising effect of antiviral treatment on PHN. Further investigation in a larger, randomized, double-blind, placebo-controlled trial to treat PHN with antiviral agents is warranted.

P160

Assessing the role of perforin in controlling acute cutaneous HSV-1 infections

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The role of perforin in the control of HSV-1 in the skin and peripheral nervous system was investigated. It was found that HSV-1 levels in the FP tissue and the dorsal root ganglia of perforin deficient mice (PKO) were comparable to those in C57BL/6 mice (B6) at all times tested. Furthermore, there was no increase in the levels of latent HSV-1 in sensory ganglia of PKO mice, as determined by the ability to reactivate latent virus from explanted tissues. This suggested that HSV-1 infection is efficiently controlled in the absence of perforin-mediated immune functions. However, animals receiving PKO immune lymphocytes by adoptive transfer had significantly higher levels of HSV-1 in the FP than animals receiving WT immune lymphocytes, suggesting a role for perforin in the control

of HSV-1 infection. Cytokine production and early cytokine mRNA transcription were comparable between PKO and B6 mice and the frequency of HSV specific CD8+ T cells was slightly elevated in PKO mice. This suggests that the loss in protective function by PKO lymphocytes is due to the lack of perforin, and not due to immune-dysregulation caused by the perforin mutation. These data demonstrate that perforin normally contributes to the control of HSV-1 infection, but its absence is compensated for in the PKO environment.

P161

The requirement for CD4+ T cells in the generation of HSV-1-specific CD8+ T cell responses

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The requirement for CD4+ T cells in the optimal response to viral pathogens is controversial, and dependent upon pathogen tissue and cell tropism characteristics. It is known that CD4+ T cells are required for the generation of CD8+ T cell mediated cytolytic activity measured in vitro, but their requirement for CD8+ T cell functions in vivo is not certain. In these studies, analysis of the CD8+ T cell response to cutaneous HSV-1 infection was performed in mice depleted of CD4+ T cells prior to infection, or in class II MHC knockout (MHC-II −/−) mice, which lack CD4+ T cell development in vivo. The results demonstrated that the absence of CD4+ T cells did not impact CD8+ T cell-mediated cytolytic function in vivo, but diminished IFN-gamma synthesis by these cells. It was also observed that the CD8+ CD44 high CD25+ T cell subpopulation was not present in the absence of CD4+ T cells. However, the CD8+ CD44 high CD25− T cell subpopulation was present, and expressed both cytolytic and cytokine synthesis functions, although the levels of IFN-gamma produced were lower than in the presence of CD4+ T cells. Taken together, these data suggest that CD4+ T cells impact the quality of the primary response to HSV-1, and may impact CD8+ T cell memory responses.

P162

Protein kinase CK2: A potential target in the development of novel therapeutics against West Nile virus

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The identification of cellular factors that are required to complete various steps of the West Nile Virus (WNV) life cycle may lead to the development of novel therapeutics. WNV is an emerging CNS-pathogen that is found throughout the United States. The contribution of individual gene products to the severity of WNV pathogenesis is not fully understood. Here we report that WNV capsid functions as a phosphoprotein and its phosphorylation status represents a critical step in the viral life cycle. The host cellular kinase, casein kinase II (CK2) is the kinase that phosphorylates capsid in vitro as well as in vivo. In WNV capsid, serine-36 within a consensus CK2 motif serves as the target phosphorylation site by CK2. CK2-specific inhibitors such as DRB and TBB inhibit the phosphorylation of capsid. We explored the role of CK2 subunits (CK(α) and CK(β)) in phosphorylating capsid by using siRNAs targeting human CK(α) and CK(β) transcripts, and found a strong interaction between capsid with a cellular CK subunit. Finally, we are able to attribute the phosphorylation status of the capsid to its ability to participate in WNV budding. Reduced viral titer under the treatment of CK2-specific inhibitors as well as CK2-specific siRNAs that specifically depletes CK(α) and CK(β) mRNA transcripts support this finding. Thus, the experimental results of this study provide valuable clues for the development of targeted therapeutic strategies.

P163

Sialoadhesin binds HIV-1 and is elevated in monocytes from subjects with high viral load

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The central nervous system (CNS) constitutes an important viral reservoir created by HIV-1-infected monocyte/macrophages transmigrating from the peripheral blood into the perivascular region. This reservoir is especially problematic because infected and activated monocyte/macrophages secrete inflammatory and neurotoxic factors causing neurodegeneration, culminating in cognitive impairment. Since it is widely accepted that high viral load (HVL) is a risk factor for AIDS and HIV-1-associated dementia (HAD), we examined the phenotype of monocytes from at-risk subjects. We performed global gene expression analysis of circulating CD14+ monocytes isolated from HIV-1-infected individuals with HVL (>10,000

RNA copies/ml). Of the many differentially expressed genes, we selected sialoadhesin (Sn; CD169) for further analysis because of its role in cell—cell adhesion. Compared to controls, Sn exhibited a 19-fold increase ($p < 0.05$) in subjects with HVL. Sialoadhesin is a large, extracellular protein with an N-terminal domain which binds sialic acid and was initially characterized as a macrophage-restricted protein. Flow cytometry and immunocytochemical analyses of CD14+ monocytes isolated from HIV-1-infected individuals with HVL confirmed the elevated Sn expression found in the array data. To examine the potential biological relevance of Sn expression, THP-1 cells were transfected with a CMV-SN contruct. In vitro, Sn-expressing THP-1 cells demonstrated a capacity to bind replication deficient HIV-1 (HIV-1ΔTat/Rev) in a robust and highly specific manner. HIV-1 binding was blocked by pre-treatment with an anti-sialoadhesin monoclonal antibody, which recognized the N-terminus, sialic acid binding region. Removal of sialic acid from HIV-1, by pre-treatment with sialidase, disrupted Sn binding. We speculate that HIV-1 bound to Sn may protect the virus from degradation and possibly function as a transport mechanism to ferry the virus to reservoirs beyond the suppressive control of HAART.

P164

PROTEOMIC analyses of heroin-induced differential protein expression by mature dendritic cells (MDC)

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Heroin use is postulated to act as a cofactor in the immunopathogenesis of HIV-1 infection. Blood monocyte derived dendritic cells (DC) are the first line of defense against HIV-1 infection and are the initial target of HIV-1 in injection drug users. Although evidence of immune dysfunctions has been reported in heroin users, the molecular basis of the immunopathogenesis of HIV-1 infection in heroin users has not been delineated. The role of heroin on the expression of the proteome of mature dendritic cells (MDC) has not been elucidated. We hypothesize that heroin dysregulates proteins that may facilitate the immunopathogenesis of HIV-1 infection in heroin users. Utilizing the proteomic method of difference gel electrophoresis (DIGE) combined with protein identification HPLC-MS/MS, we show that heroin significantly (Student's t-test) dysregulates the expression of a number of proteins in MDC such as gelsolin isoform a, moesin, annexin 5, endoplasmic reticulum protein 29 isoform 1, cathepsin S and fibrinogen beta chain. Several of these differentially expressed proteins were confirmed by western blot and their respective genes by real-time, quantitative PCR (Q-PCR) analysis. Identification of unique, heroin-induced proteins may help to develop novel markers for diagnostic, preventative and therapeutic targeting in heroin using subjects.

P165

Nerve growth factor and neurotrophin—3 activate cellular kinases which are involved in rabies antiviral effect

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Nerve Growth Factor and Neurotrophin 3 induce a modulation of rabies virus transcription and replication in adult mouse dorsal root ganglia cultures. Therefore, the aim of this study was to evaluate the cellular kinases involved in this anti-viral activity. The sensory neuron cultures were infected and treated with the specific kinases inhibitors LY294002, Gö-6983 and DRB (PI3K, PKC and Casein Kinase II inhibitors, respectively) and with Nerve Growth Factor and Neurotrophin-3. Viral antigen produced by cultures was evaluated at 15 hours post-infection (p.i) using a fluorometric Cell-ELISA and viral messenger RNA (mRNA) and genomic RNA (gRNA) were also quantified by a real time PCR using the neuronal marker UCH-L1 as housekeeping gene at 6 h p.i. We found changes in viral antigen and RNA amounts in inhibitors and NGF treated cultures, indicating a total or partial infection restoration respecting inhibited cultures (inhibition of neurotrophin inhibition). These data could suggest that rabies virus infection inhibition caused by neurotrophin treatment can be due by a deregulation of neuronal enzymatic machinery involved in the different steps of viral cycle.

P166

Identification of the JCV promoter GRS-binding protein as Egr-1

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JC virus (JCV) can emerge from latency resulting in the cytolytic destruction of glial cells in Progressive Multifocal Leukoencephalopathy (PML). The non-coding

regulatory region (NCCR) of JCV contains cis-acting transcriptional regulatory elements including a 23 base pair GGA/C rich sequence (GRS) near the JCV origin (5112 to +4) that was previously shown to bind a protein complex designated GBPi after glial cells are treated with activated T-cell supernatant, certain cytokines or TPA. We now report evidence that GBPi contains the early growth response protein Egr-1. The GRS-GBPi EMSA band from TPA-treated cells was excised from the EMSA gel after autoradiography and was found to contain Egr-1 by Western blotting. Other non-inducible EMSA bands from the same gel were negative for Egr-1 as was the GRS-GBPi gel shift region of the EMSA gel from untreated cells. Further the TPA-induced GBPi gel shift was abrogated by antibody to Egr-1. Ectopic expression of Egr-1 induced a gel-shift in the same position as that induced by TPA. A set of oligomers spanning the JCV NCCR failed to give a gel shift after Egr-1 transfection except for one that partially overlapped the 23 base pair oligomer and contained the GGA/C repeats. Co-expression of Egr-1 with JCV late promoter reporters stimulated transcription 8-fold. Primary astrocytes infected with JCV showed an induction of Egr-1 protein that was maximal 10 days post-infection. We conclude that Egr-1 binds to the GRS element of the JCV NCCR and may have a role in the viral life cycle.

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P167

BAG-3 protein suppresses HIV-1 promoter activity by binding NF-kB p65 in microglia and astrocytic cells

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Co-chaperone proteins that share the Bcl-2-associated athanogene (BAG) domain are involved in a number of cellular processes, including proliferation and apoptosis. Among BAG family members there is BAG3, also known as CAIR-1 or Bis, that binds to Hsp70, phospholipase C-gamma (PLC-gamma) and possibly other partners. High levels of BAG3 protein have been found in several disease models. We observed increased expression of BAG3 protein in the cytoplasm of reactive astrocytes in the cortex of a case of HIV encephalopathy by immunohistochemstry. To investigate the effect of increased levels of BAG3 protein on HIV-1 replication, we performed functional assays using a HIV-1 LTR (−120, +66) driven firefly luciferase vector. In particular, we analyzed the effect of overexpressed BAG3 on the ability of NF-(κ)B p65 and Tat to activate HIV-1 LTR promoter, in human astrocytic cells, U87MG and in human primary colture of microglia. We found that increased levels of BAG3 protein inhibit the binding of NF-(κ)B p65 to the (κ)B-binding motifs of the viral promoter, as assessed by EMSA and ChIP assays. Further analysis demonstrated that this effect is mediated by the direct binding of NF-(κ)B p65 to the full length BAG3 protein. Our results suggest a new mechanism to control HIV-1 replication in infected astroglial cells.

In fond memory of Arturo Leone.

P168

Knock-down of BAG3 in glioblastoma cells promotes apoptosis and sensitizes to camptothecin treatment.

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BAG-family proteins are expressed in a variety of human cancers and tumor cell lines and have been shown to regulate cell growth and block apoptosis. Among BAG-family members, BAG3 protein expression has been demonstrated to be important in B-CLL and ALL cells survival. Immunohistochemical analysis performed on several astrocytoma and in glioblastoma multiforme specimens showed higher levels of BAG3 protein compared to normal brain. To test the possible involvement of this protein in the growth and survival of glioblastoma cells, we used a specific siRNA sequence directed on BAG3 mRNA in rat glioblastoma cells, C6. In in vivo experiments, C6 cells were stereotactically implanted into the caudoputamen of immunocompetent rats and treated with siRNA for Bag3 or with a scrambled sequence. After three weeks of siRNAs adminitrations control animal showed larger tumors compared to siRNA BAG3 treated tumors, which also showed TUNEL positive nuclei. We also found in in vitro experiments that C6 cells, when treated with camptothecin, a chemotherapeutic drug used in glioblastoma disease, are more sensitive to apoptosis (measured as cleavage of caspase 3) in the presence of siRNA of BAG3. Together these findings suggest that BAG3- based reagents can represent a new tool for glioblastoma multiform therapy.

In fond memory of Arturo Leone.

P169

Altered kinetics of STAT activation in interferon gamma-stimulated neurons

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It is becoming increasingly evident that immune strategies used to combat viral infections are not generic, but rather may be tailored to result in rapid clearance of the pathogen with a minimization (in many cases) of deleterious effect on the infected host. For example, non-cytolytic mechanisms of viral clearance

are especially important in nonrenewable cell populations, such as neurons of the central nervous system (CNS). In characterizing the neuronal response to interferon gamma (IFNg), a pluripotent immune cytokine required for non-cytolytic clearance of multiple neurotropic viral infections, we found that primary neurons demonstrated muted and delayed signaling via the phosphorylation and nuclear localization of two of the STAT proteins (STAT1 and STAT3), as compared to primary fibroblasts. This may in part be due to the observed lack of constitutive STAT expression in neurons. Although the initial neuronal response is dampened as compared to that in fibroblasts, neurons continue to phosphorylate STAT1 well beyond the period of responsiveness observed in MEF, in response to a 30 minute pulse of IFNg. Interestingly, SOCS-1, a major component of the interferon negative feedback loop, is expressed at a higher constitutive level in untreated neurons as compared to fibroblasts. Furthermore, SOCS-1 is downregulated in neurons after IFNg treatment, whereas in fibroblasts it is upregulated. We thus hypothesize that the altered kinetics of STAT phosphorylation in neurons reflects a combination of a low level of constitutive STAT expression, coupled with modifications in the negative feedback loop of the IFNg signaling pathway. Finally, examination of five IFNg-responsive genes demonstrated a 3-4-fold higher level of responsiveness (for four of the genes) in treated MEF as compared to treated neurons, suggesting that altered signaling kinetics may provide a mechanism for the heterogeneity of cellular responses to IFNg. Our direct, quantitative comparison of STAT activation kinetics in neurons and fibroblasts sheds new light on the heterogeneity of cellular responses to IFNg.

P170

HIV-1 Tat prevents the de-phosphorylation of Sp1 by TCF-4 in astrocytes

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We previously examined the role of TCF-4 on transcription of the human immunodeficiency virus type 1 (HIV-1) promoter in human astrocytic cells. We found that TCF-4 affects HIV-1 promoter through the GC-rich domain (nt -80 to nt -68) (Wortman et al., 2002). Here, we have characterized the physical interaction and a functional consequence of TCF4-Sp1 contact. We showed that expression of TCF-4 in U-87MG, human astrocytic cells decreased the basal and Sp1-mediated transcription of the HIV-1 promoter. Results from the glutathione S-transferase pull-down assay as well as the combined immunoprecipitation and Western blot analysis of protein extract from U-87MG cells revealed an interaction of Sp1 with TCF-4.Using in vitro protein chromatography, we mapped the region in Sp1 that contacts TCF-4 to amino acids 266 to 350. We also found that in cell-free extracts, TCF-4 prevented double-stranded DNA-dependent protein kinase (DNA-PK)-mediated Sp1 phosphorylation. Surprisingly TCF-4 failed to decrease Sp1-mediated transcription of HIV-1 LTR and Sp1 phosphorylation in cells expressing HIV-1 Tat. Results from IP/Western demonstrated that TCF-4 lost its ability to interact with Sp1 but not Tat in Tat-transfected cells. Taken together, these findings suggest that activity at the HIV-1 promoter is influenced by phosphorylation of Sp1 which is affected by Tat and DNA-PK. Interaction between TCF-4, Sp1 and/or Tat may determine the level of viral gene transcription in human astrocytic cells.

P171

A monoclonal antibody against HIV Tat attenuates neurotoxicity via glutamate receptors

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HIV dementia is a neurodegenerative disease for which there is no available neuroprotective therapy. Its pathogenesis is not well understood. However, viral proteins, such as Tat, the HIV transactivator protein, have been implicated as agents of neurotoxicity via multiple mechanisms, including effects on glutamate receptors.

We coincubated the HIV-1 Tat protein with a monoclonal antibody against the N-terminal of Tat, in human neuronal cultures. The antibody significantly ($P < 0.01$) attenuated the neurotoxicity caused by Tat alone, as measured by mitochondrial membrane potential. This protection occurred despite previous observations that peptides from the N-terminal of Tat are nontoxic. We thus hypothesized that the antibodies may have a more indirect neuroprotective function, preventing excitotoxicity at glutamate receptors.

Thus, we coincubated Tat and the anti-Tat antibody with known glutamate receptor agonists, NMDA and kainate, in our cultures. The Tat-antibody combination attenuated the toxicity seen with either agent alone, as effectively as kynurenic acid, a glutamate receptor antagonist. Neither Tat nor antibody alone blocked the excitotoxic effect, nor did an unrelated antigen-antibody combination.

To evaluate the specificity of the protective effect to the N-terminal region, we also coincubated Tat with polyclonal antibodies made against the N-terminal, C-terminal, and whole Tat. Preliminary results suggest protection with all of these antibodies as well.

The Tat-antibody complex appears to prevent excitotoxic effects at glutamate receptors. Host immune responses may be directed against neurotoxic properties of viral proteins and thus may influence host susceptibility to the effects of these proteins, such as onset of HIV dementia. Additionally, our observations may have important implications for development of therapeutic vaccines for HIV dementia, and more broadly for treatment of many neurological diseases.

P172

Programmed cell death and expression of apoptotic genes in PBMC from multiple sclerosis patients

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Recently we demonstrated that in Multiple Sclerosis (MS) a deregulation of programmed cell death (PCD) or apoptosis plays a key role in inducing or maintaining auto-reactive immune phenomenon leading to the development of demyelinating lesions.

In the present study we investigated the PCD of myelin basic protein (MBP) -specific T lymphocytes in 47 Relapsing-remitting (RR) MS patients, 29 with acute (AMS) and 18 with stable MS (SMS), together with 30 Healthy Controls (HC).

In particular, we analyzed by flow cytometry CD4+ and CD8+ apoptotic and CD3+ proliferating cell percentage, and by RT PCR the expression of different anti (FLIP, XIAP, Bcl-2) and pro (BID,APAF-1) apoptotic genes in sorted CD4+ and CD8+ T cells, previously stimulated with MBP peptides. Differences were analyzed by t-Student and Mann-Whitney tests. The percentage of apoptotic MBP specific CD4+ and CD8+ T cells decreased in AMS compared to SMS ($p < 0.05$) and HC ($p < 0.05$). Conversely, an higher proliferation index of MBP specific T cells was found in AMS and HC compared to SMS ($p < 0.05$). An higher expression, even thought no statistically significant, of both anti and pro apoptotic genes was shown in RRMS compared to HC; in addition, a significant increase of anti apoptotic genes FLIP, XIAP and Bcl-2 was observed in MBP- specific sorted CD8+ cells of AMS compared to HC.

The data obtained evidence a specific activation of immune system against MBP in patients with AMS and in HC, but the increase of PCD in HC switch off the MBP specific T cell immune response. Conversely in AMS, the decrease of apoptotic MBP specific T cell seems to be involved in the immune mediated destruction of myelin sheath.

P173

Human polyomavirus, JC virus (JCV), late regulatory agnoprotein is targeted by a serine/threonine phosphatase, PP2A

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Viral regulatory proteins play critical roles in different stages of the infection cycle. Recent studies have shown that JCV late regulatory agnoprotein protein plays an important role in viral gene regulation, replication and perhaps in viral encapsidation. In addition, this protein was also shown to alter cell cycle progression in which it appears that cells stably expressing agnoprotein largely accumulate at G2/M phase transition state. Furthermore, this protein contains several posttranslational modification sites including phosphorylation. Mutational analysis of agnoprotein phosphorylation sites, where Ser7, Ser11 and Thr21 were singly or combinatorially converted into Ala, resulted in a phenotype that is unable to sustain the viral infection cycle. Subsequent analysis of the released viral particles from mutant virus-infected cells by electron microscopy, Western, and Southern blotting showed that although the released mutant virions are morphologically indistinguishable from wild-type, they are deficient in DNA content which may account for their defective propagation. Since agnoprotein is phosphorylated by a Ser/Thr kinase PKC, we reasoned that perhaps the phospharylated sites are subject to regulation by a Ser/Thr phosphatase, protein phosphatase 2 (PP2A). Our results indeed showed that agnoprotein is targeted and dephosphorylated by this phosphatase. We also demonstrated that agnoprotein physically interacts with this enzyme and its interaction domain is localized to the middle portion of the protein. We are currently investigating the functional significance of this interaction with respect to viral infection cycle and cell cycle progression.

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Rictor/mTOR complex is activated in JC virus small t antigen expressing cells

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The early genome of JC virus (JCV) encodes two tumor antigens due to alternative splicing of its early transcripts, including large T antigen (LT-Ag) and small

t antigen (Sm t-Ag), although recent reports indicate that the same region also encodes three additional peptides, T'(135), T'(136), and T'(165). LT-Ag is known to play a major role in cell transformation and cell proliferation in tissue culture and animal model systems. However, the role of JCV Sm t-Ag in such processes remains unknown. Functional studies with simian virus 40 (SV40) Sm t-Ag showed that it enhances the ability of SV40 LT-Ag to transform rodent and human cells. Here, we investigated the effect of Sm t-Ag on both cell cycle progression and growth promoting pathways. To analyze this effect, we established several stable cell lines including U-87MG and NIH3T3, expressing Sm t-Ag, and followed their cell cycle progression. Sm t-Ag positive clones were arrested at G0/G1 phase of cell cycle by serum starvation and released upon serum stimulation. Results showed that the Sm t-Ag positive cells entered the S phase relatively faster than controls. This was accompanied by the earlier expression of G1/S phase-specific cyclins and cyclin-dependent kinases, including cyclin E and cyclin E-dependent kinase 2 (Cdk2). In order to understand the molecular mechanisms underlying Sm t-Ag-induced cell cycle progression, we analyzed several growth promoting pathways, including the pathway which involves mammalian target of rapamycin (mTOR). Results showed that Rictor/mTOR complex is relatively more active in Sm t-Ag positive cells, resulting in a higher phosphorylation of a down stream effector molecule, Akt at Ser473. In addition, p27kip1, an inhibitor of cdc2/cyclin E complex and a target of Akt, was found to be mostly retained in cytoplasm rather than being translocated into nucleus in Sm t-Ag positive cells. Collectively, these results suggest that Sm t-Ag promotes cell cycle progression, at least in part, through the activation of one of the growth promoting pathways known as the mammalian target of rapamycin (mTOR).

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P175

Induction of toll like receptor signalling in venezuelan equine encephalitis virus infection in mouse brain

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Venezuelan Equine Encephalitis Virus (VEEV), an alphavirus in the family Togaviridae, causes a highly virulent CNS disease in horses which is then transmitted to humans by mosquito bite. VEEV is highly infectious through aerosols and has been weaponized making it a potential bio-warfare agent. Outbreaks of VEEV have been reported throughout much of tropical America. Toll-Like Receptors (TLRs) are ligand binding molecules with a family of at least 11-12 members. They recognize conserved microbial sequences and induce specific biological response in the form of pro inflammatory cytokine induction. Inflammatory response following VEEV presence in the brains of mice is considered to be detrimental to host and contribute to the severity of the disease. In this study, we investigated the TLRs and its signaling pathway specific genes expression in VEEV infected mice brain. 3–5 weeks old mice were infected with a neurovirulent strain of VEEV, V3000. Brains were harvested from the infected mice at 48, 72 and 96 hour post infection. Total RNA was isolated and oligo array specific for Toll-Like Receptor Signaling Pathway was performed. VEEV infection resulted in upregulation of TLR 3 and several chemokines inflammatory cytokines and Interferon and interferon regulatory factors and genes involved in signal transduction such as MCP-1, IP10, Btk, Il12a, Il12b, Inf b1, Irf-1, Irf 7, Fos, Jun, Myd88, Nfkb1, Nfkbia, Prkra, Cd14 and Cd86. These results suggest the TLR3 and its associated genes may play a key role in virus recognition and an inflammatory response against VEEV.

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Expression of the E2F1 transcriptional targets, Cdc2 and PUMA, in primary neurons treated with HIV-gp120 and in the brain of HIV/HAD patients

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Alterations of the Rb/E2F1 pathway have been involved in neurodegenerative disorders including neuroAIDS. In order to test the hypothesis that an up-regulation of E2F1 activity is involved in HIV neuropathogenesis, we have been studying E2F1 expression and transcriptional activity in primary neurons exposed to HIV-gp120 and in human brain tissue samples from HIV+ patients with or without neurological deficits. Our data show that gp120 induces a time-dependent increase of the E2F1 protein levels in rat primary neurons. In these neurons, E2F1 is predominantly (if not exclusively) localized to the nucleus, and gene reporter assays indicate that gp120 promotes transcription of E2F1-dependent genes. In line with these observations, treatment of neurons with gp120 also increases E2F1 transcriptional targets, namely cdc2 and Puma. Importantly, primary neurons derived from E2F1 deficient mice were resistant to gp120-induced neurotoxicity, and showed no changes in cdc2 and Puma protein levels following gp120 treatment. Finally, the studies with brain tissue samples showed increased expression of E2F1 in the nucleus of neurons of HAD patients, but not in the HIV+ patients without dementia or in control (i.e. HIV negative) patients. These data suggest that aberrant

expression of E2F1 transcriptional targets in postmitotic neurons is one of the mechanisms involved in the apoptotic neuronal cell death observed in HIV neuropathology.

P177

Tropism of encephalitis-causing viruses in the central nervous system of rodents

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Hamster and mouse models for encephalitides, such as West Nile Virus (WNV), Western Equine Encephalitis Virus (WEEV) and Venezuelan Equine Encephalitis Virus (VEEV) were used in this study to identify infected neuronal or glial cells of the central nervous system (CNS) in the course of infection. We subcutaneously infected adult mice and hamsters using $10^{5.3}$ tissue culture infectious doses of WNV (New York strain) and intraperitoneal infection of WEEV (California strain) at $10^{1.5}$ CCID50/0.1 ml to adult hamsters and intranasal administration of VEEV (TC-83 vaccine strain) at 5×10^5 CCID50/0.05 ml to adult C3H/HeN mice. Tissues were fixed by cardiac perfusion using 4% paraformaldehyde and stained with antibodies specific for WNV E protein, WEEV protein, VEEV protein, Neuron Specific Enolase (NSE) and Glial Fibrillary Acidic Protein (GFAP) in paraffin-embedded serial tissue sections by immunofluorescence. The images were captured using the confocal microscope (Bio-Rad, MRC 1020). We observed the presence of WNV antigen in CNS tissues of mice and hamsters as early as 3 and 5 dpi, respectively. A strong WNV-specific staining was observed in the cytoplasm of neurons from spinal cord, cerebellum, cerebral cortex and midbrain of these rodents. The WNV-specific staining co-localized with NSE, but not with astroglial markers. The lack of tropism by WNV for astrocytes was also confirmed in primary murine astrocyte cultures. On the contrary, VEEV-infected cells were immuno-positive for GFAP as well NSE, suggesting VEE infects both neuronal and glial cells. A strong cytoplasmic staining of WEEV was observed in the cortex of hamster, which was co-localized with NSE. Immunostaining for all the viral proteins were sporadic. These data suggest that differential cellular staining of WNV, WEEV, and VEEV may account for some of the differences in the diseases caused between these viruses, and that further studies of tropism and cellular signals will elucidate mechanisms of pathogenesis.

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P178

Neurotoxic and neurodegenerative characteristics of factors released by astrocytes activated by conditioned media from HIV-1 isolates

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HIV-associated dementia (HAD) is a clinical manifestation of HIV that affects up to 20% of infected adults. A correlation between the severity of dementia and the production of neurotoxins has been demonstrated in HAD. Astrocytes, the most numerous cell type in the brain are non-productively infected by HIV-1, but have been shown to participate in the inactivation of neurotoxins with a rapid progression to dementia. Reactive astrogliosis resulting from activation of astrocytes by proinflammatory cytokines and chemokines has been hypothesized as a factor that contributes to neuronal damage through the dysregulation of the astrocyte-neuron networks. Further the toxins released by astrocytes may overstimulate neurons leading to the formation of free radicals and excitotoxicity. Astrocytes were incubated with conditioned media from infected U937 monocytoid and Supt1 cell lines infected with HIV-1 JRFL and SF-162 viral strains respectively. At specific time points supernatants were collected and cytokine expression assayed. Immunohistochemistry was also done on the cells. Our results showed significant release of MCP-1, MCSF, and IL-4. TNF-alpha, TNF-beta, EGF, and IGF were also released. Suppression of TARC, SDF-1, and IFN-gamma was observed. Our results show that astrocytes can release inflammatory cytokines and chemokines upon stimulation with conditioned media, without the need for the virus. Further, the chemokines and cytokines released were similar for the two viral strains studied. This similarity in response if applicable to primary viral strains of different subtypes will be significant in the design of therapeutic agents targeting specific pathways involved in the inflammatory response. We also looked at the downregulatory effects of MH-2 domain of SMAD2 on HIV-1 Tat transactivation of HIV-LTR and MCP-1 promoters. Using Adeno-MH2 constructs we were able to downregulate Tat induction of cytokines and chemokines, especially MCP-1.

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P179

The HSV-2 protein ICP10PK upregulates/activates the function/survival related proteins Rap1 and adenylate cyclase in neuronal cultures

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The HSV-2 protein ICP10 PK is neuroprotective (Perkins et al, J Virol. 76:1435, 2002). DNA array analyses comparing mock infected cells to cells infected with HSV-2 or an ICP10PK deleted mutant (deltaPK) identified 14 transcripts that were upregulated by HSV-2 but not deltaPK (mean difference > 5 fold) including Rap1 and adenylate cyclase. Rap1 is a Ras like GTPase that is involved in neuronal plasticity, calcium channel upregulation, excitability control and LTP. Adenylate cyclase generates c-AMP causing activation of PKA and CREB, which are involved in neurotrophin-mediated survival. RT-PCR and/or immunoblotting confirmed that Rap1 and adenylate cyclase were upregulated in primary hippocampal cultures by HSV-2 or the revertant virus [HSV-2(R)], but not deltaPK. Rap1 upregulation was seen at 30 min, and was still visible at 24 hrs p.i. Immunocomplex kinase assays indicated that B-Raf kinase was activated in HSV-2 but not deltaPK infected cultures, and pull-down experiments confirmed that Rap-1 bound the activated B-Raf. Adenylate cyclase levels were increased and CREB was activated (phosphorylated) in neuronally differentiated PC12 cells that constitutively express ICP10PK, but not in parental PC12 cells or PC12 cells that express a kinase negative ICP10PK mutant. CREB activation was decreased with the PKA inhibitor H89. The data indicate that ICP10PK upregulates Rap1 and adenylate cyclase in neuronal cells contributing to their survival and function.

P180

Mutagenesis of the La crosse virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide

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The La Crosse Virus (LACV) M segment encodes two glycoproteins (Gn and Gc), and plays a critical role in the neuropathogenesis of LACV infection as the primary determinant of neuroinvasion. A recent study from our group used a panel of recombinant M segment constructs to demonstrate that a region corresponding to the membrane proximal two-thirds of Gc, amino acids 860–1442, is critical in mediating LACV fusion and entry. Furthermore, computational analysis identified structural similarities between a portion of this region, amino acids 970-1350, and the E1 fusion protein of two alphaviruses: Sindbis virus and Semliki Forrest virus (SFV). These data suggested that the LACV Gc, like the alphavirus E1 and flavivirus E, functions as a type II fusion protein.

Within the region 970–1350, a 22 amino acid hydrophobic segment (1066–1087) is predicted to correlate structurally with the fusion peptides of type II fusion proteins. We performed site directed mutagenesis of key amino acids in this 22-amino acid segment and determined the functional consequences of these mutations on fusion and entry with cell-to-cell fusion and pseudotype transduction assays. Several mutations within this hydrophobic domain affected glycoprotein expression to some extent, but all mutations either shifted the pH threshold of fusion below that of the wild type protein, reduced fusion efficiency, or abrogated cell-to-cell fusion and pseudotype entry altogether. A mutation at position 1066 (W1066A) was particularly informative, since it did not affect glycoprotein expression, yet abolished fusion and entry. These results, coupled with the computational modeling, suggest that the LACV Gc functions as a type II fusion protein, and support a role for the region, Gc 1066–1087 as a fusion peptide.

P181

The role of non-structural protein NSs in La Crosse virus pathogenesis

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La Crosse virus (LAC), a negative stranded segmented RNA virus, is a member of the California serogroup of Bunyaviruses and an important cause of pediatric encephalitis and aseptic meningitis in the United States. The structural proteins of the LAC genome (L,GC/GN and S) have essential, well-defined roles in virus pathogenesis. However, the contributions of the nonessential, nonstructural proteins, Nsm and S(NSs), to viral pathogenesis are poorly understood. The NSs of other bunyaviruses, including Bunyamwera virus and Rift Valley Fever Virus have been suggested to play crucial roles in countering host immunity as interferon antagonists. Recently, we reported that the LAC NSs is a supressor of RNAi, a function shared by the influenza virus NS1, vaccinia virus E3L, and the Tomato Spotted Wilt virus NSs. In addition, 293T and Hela cells transfected with contructs containing the LAC NSs induced apoptosis as measured by Tunnel, Annexin V/propidium iodide staining and detection of cleaved PARP and active caspase 3, while a construct expressing the S alone (stop codon inserted at NSs condon 22 to truncate NSs translation) did not. The induction of apoptosis by LAC NSs is consistent with a report suggesting a homology between the LAC NSs and Reaper, a Drosophila protein associated with apoptosis. Collectively, these data suggest that the bunyavirus NSs influences viral replication by neutralizing cellular mechanisms designed to defend cells from infection with RNA viruses. Further, possible mechanisms by which the LAC NSs inhibits RNAi may include the sequestration of small dsRNAs, as has been described for the NS1 of influenza.

P182

Evaluation and characterization of neuroprotective compounds for HIV-dementia

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There are severe neurological complications that arise from HIV infection, ranging from peripheral sensory neuropathy to cognitive decline and dementia. The HIV proteins secreted from infected macrophages and astrocytes, gp120 and Tat, are cytotoxic to the surrounding neuronal cells. Mechanistically, this neurotoxicity may be mediated via HIV protein-induced oxidative stress. Recently, we have established a high throughput functional assay in primary rat hippocampal cultures to quantitate neurotoxicity following oxidative damage. We have screened over 2000 compounds for protective efficacy at a single dose (10 uM) and have generated a short list of potential neuroprotective compounds. We have verified the neuroprotective activities of these compounds and determined their potency in dose response studies. A few representative compounds from this list include the antipsychotic, trifluoperazine, estrogens, such as beta-estradiol cypionate and the antioxidant resveratrol. Initial testing of these compounds demonstrates neuroprotective efficacy in the low micromolar range. The drug-like nature of these compounds is being evaluated, as are blood brain barrier permeability and target tissue penetration. Thus, compounds like these and their derivatives, may provide new avenues by which neuroprotective treatment(s) may be derived. This approach may generate new classes of compounds to be developed preclinically toward the treatment of patients with HIV-Dementia.

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P183

Neuroimmunophilin ligands as therapy for HIV-mediated neurodegeneration

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HIV infection can result in severe complications in the nervous system. Even with the advent and implementation of highly active antiretroviral therapy (HAART) to diminish HIV viral load, the occurance of HIV sensory distal neuropathy(HIV-SN) and HIV-dementia (HIV-D) presists. As effective HAART therapy allows HIV patients to live longer, a larger proportion of patients experience some form of neuropathy and cognitive deficit. 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. Identification and development of therapeutic strategies to protect against this neuronal damage and the ensuing clinical deficits is a major unmet medical need. Neuroprotective compounds, such as neuroimmunophilin ligands (NIL), may provide a treatment to HIV neuropathy and dementia. These compounds are currently being evaluated in human clinical trials for neuroprotection in Parkinson's Disease and peripheral nerve injury. We wished to evaluate whether these compounds can protect vulnerable neuronal cells from the toxic assault of HIV Tat and gp120. We found that a prototypic NIL, GPI 1046, can dose dependently protect neurons in culture from HIV Tat and gp120-induced toxicities, with nanomolar potency. Nanomolar GPI 1046 also attenuated endoplasmic reticulum calcium release induced by gp120 and Tat. In addition, this compound displays antiretroviral activity, as it blocks HIV replication in lymphocytes and Tat-induced LTR transactivation in astrocytes. The neuroprotective and antiretroviral effects of this compound suggests that it may provide therapy to patients with HIV-SN and HIV-D.

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P184

The neurotropism of herpes simplex virus type 1 in brain organ cultures

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HSV-1 is an important cause of severe morbidity and mortality, including encephalitis. The mechanisms of HSV-1 penetration into the brain and its predilection to infect certain neuronal regions are unknown. In order to analyze the factors that render the central nervous system (CNS) susceptible for HSV-1 infection and to identify the cell types that are infected we have established an ex vivo system of mice organotypic brain slices. Whole mice brains were dissected, glued to Teflon disc and covered with 5% low melting agarose. Tissues were infected with 104–106 PFU of virus for 2 hours. The slices were then fixed, washed and either X-gal stained or examined for florescence. Different HSV-1 mutants, adenovirus, and vaccinia mutants, all containing reporter genes under control of exogenous promoters, were used. Neonate tissues showed restricted infection confined to meningeal, periventricular and cortical brain regions. The hippocampus was the primary parenchymatous structure that was also infected. Infection was localized to early progenitor and ependymal cells. Increasing viral inoculum enlarged the intensity and the infected territory, but the distinctive pattern of infection was maintained and

differed from that observed with adenovirus and vaccinia. Neonate brain tissues were much more permissive for HSV-1 infection than adult mice brain tissues. These findings suggest that HSV-1 infects selectively only certain cell types and that the non-permissiveness of the mature brain tissue can be overcome by viral amounts. Since HSV-1 encephalitis is mainly a disorder of the mature brain, studies are underway to delineate additional parameters that render the adult brain susceptible for HSV-1 infection.

P185

Evidence that the immediate early gene product, ICP4, is necessary for HSV-1 DNA circularization in infected cells

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In this study, we demonstrated that the ICP4 protein is involved in the process of the endless formation of infecting linear HSV-1 genome through the following evidences: (1) The ICP4 mutant virus d120 genome failed to form the endless conformation when it infected in Vero cells, whereas, a significant portion (approximate 50%) of the genomes of wild-type strain KOS formed the endless conformation after infection on Vero cells in the presence of 400 μg/ml of phosphonacetic acid (PAA) which is a DNA replication inhibitor,; (2) A significant portion (approximate 60%) of the genomes of ICP4 mutant d120 formed the endless conformation after infection in its complementary cells (E5), which expressed the ICP4 protein after d120 mutant virus infection; (3) When ICP4 proteins were provided by a DNA replication defected DNA polymerase mutant HP66, the genomes of mutant d120 could form the endless conformation in Vero cells. Western analysis using antibody specific to the ICP4 protein shows that although the d120 virions, purified from the d120-infected E5 cells, contained ICP4 proteins, the majority of ICP4 proteins associated with the d120 virions are in the 40 KD truncated form with a small fraction of the full-length 175 KD ICP4 protein. These d120 virion associated ICP4 proteins were unable to mediate the endless formation after infection in E5 cells when the expression of the ICP4 protein from E5 cells was inhibited by 200 μg/ml of cycloheximide. Collectively, this data suggest that the ICP4 protein has an important, previously unreported, role in mediating the endless formation of genome upon infection and that this function can be provided in trans.

P186

Astrocyte toll-like receptor 3 in the innate anti-viral immune response

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Toll-like receptor 3 (TLR3) is a viral nucleic acid sentinel activated by dsRNA within intracellular vesicles. To explore the spectrum of genes induced in human astrocytes by TLR3 we used a microarray approach and poly I:C (pIC) as ligand. One of the genes highly induced was indoleamine 2, 3 dioxygenase (IDO), a multifunctional enzyme implicated in the pathogenesis of SIV/HIV encephalitis. We show that astrocyte IDO is induced by pIC and IFNbeta, in addition to IFNgamma, and that IDO is suppressed under the conditions of inducible nitric oxide synthase expression, such as stimulation with IL-1. TLR3 ligation induced activation of transcription factors, NF-kappaB, Stat1, as well as C/EBPbeta, which have been shown to promote human IDO transcription. Repeated stimulation of astrocytes with pIC increased the expression of TLR3 and the levels of pIC-inducible genes including IDO, indicating a positive feedback loop established in the TLR3 signaling pathway. To determine whether TLR3 or IL-1R signaling in astrocytes induces an anti-viral state, we tested VSVg-env pseudotyped HIV-1. HIV-1 expression was inhibited in astrocytes treated with pIC and this effect was partially reversed by L-tryptophan. In contrast, IL-1 had no effect. TLR3 ligation also suppressed intracellular replication of human cytomegalovirus in astrocytes. Together, these results indicate that TLR3 is expressed and functional in human astrocytes and that astrocyte tryptophan metabolism may play a role in the innate antiviral immunity in the CNS. (Supported by NIH MH55477, NS040137, T32 NS007098, and P30 AI051519).

P187

Inhibition of mixed lineage kinase (MLK)-3 prevents human immunodeficiency virus-1 Tat-mediated neurotoxicity and monocyte activation

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The Human Immunodeficiency Virus Type 1 (HIV-1) gene products Tat and gp120 are toxic to neurons and can activate cells of myeloid origin–properties which are thought to contribute to the clinical manifestations of HIV-1 associated dementia (HAD). To investigate the intracellular signaling mechanisms involved in these events, the effect of Tat and gp120 on mixed-lineage kinase (MLK)-3 activation was examined. Tat and gp120 were shown to induce autophosphorylation of MLK3 in primary rat neurons; this was abolished

by the addition of an inhibitor of MLK3 (CEP1347). CEP1347 also enhanced survival of both rat and human neurons, and inhibited the activation of human monocytes, following exposure to Tat and gp120. Furthermore, over-expression of wild-type MLK3 led to the induction of neuronal death, while expression of a dominant negative MLK3 mutant protected neurons from the toxic effects of Tat. MLK3-dependent downstream signaling events were implicated in the neuroprotective and monocyte deactivating pathways triggered by CEP1347. Thus, the inhibition of p38 MAPK and JNK protected neurons from Tat-induced apoptosis, while the inhibition of p38 MAPK, but not of JNK, was sufficient to prevent Tat and gp120-mediated activation of monocytes. These results suggest that the normal function of MLK3 is compromised by HIV-1 neurotoxins (Tat, gp120), resulting in the activation of downstream signaling events that result in neuronal death and monocyte activation (with release of inflammatory cytokines). In aggregate, our data define MLK3 as a promising therapeutic target for intervention in HAD.

P188

Evidence for involvement of NFBP in processing of rRNA

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NF-kappaB Binding Protein (NFBP) is an RNA binding protein identified due to its ability to bind to NF-kappaB subunits, p50 and p65. NF-kappa B proteins are a family of inducible transcription factors which activate many cellular genes involved in apoptosis, the immune response and cellular proliferation. Additionally, viruses such as HIV-1 utilize NF-kappaB to activate viral transcription. Our previous work demonstrated that NFBP is involved in activation of HIV-1 LTR via its interactions with the cellular NF-kappaB complex and with the viral protein, tat. In order to investigate the role of NFBP in a normal cellular environment, we searched for homologous proteins from other species and found that NFBP shares 24% amino acid similarity to RRP5, a protein involved in processing of ribosomal RNA (rRNA) in yeast. In humans, rRNA is initially transcribed as a single 47S precursor which is modified by the addition of 2′-O-methyl ribose moieties, pseudouridines, and methyl groups, followed by cleavage at several sites to produce the mature 28S, 18S and 5.8S rRNAs. Cleavage of the rRNA precursor to generate the 18S rRNA is mediated by a ribonucleoprotein (RNP) complex termed the processome containing U3, a box C/D snoRNA, and at least 28 cellular proteins. We detected endogenous NFBP in the nucleolus, where it colocalizes with and binds to the U3 snoRNA. Furthermore, by employing siRNA techniques to target NFBP, we demonstrate that NFBP is essential for several early cleavage events necessary for maturation of 18S rRNA. Our results demonstrate that NFBP is a vital component of rRNA processing in human cells.

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P189

Expression of anti-apoptotic survivin in glial cells of progressive multifocal leukoencephalopathy and activation of the survivin promoter in JCV infected cells

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Progressive Multifocal Leukoencephalopathy (PML) is a sub-acute and fatal disease of the Central Nervous System, result of the productive infection of glial cells, particularly oligodendrocytes, by the opportunistic JC Virus. JCV is a human neurotropic virus, prevalent in the adult population world wide, with approximately 85 to 90% of the population exhibiting hemmaglutinating antibodies. Primary infection is sub-clinical and the virus remains in latent state presumably in the kidney, until its reactivation under immunosuppressive conditions, particularly in AIDS patients, to result in the lytic destruction of oligodendrocytes and in the development of PML. The lytic destruction of oligodendrocytes and the activation of astrocytes in response to the injury caused by JCV infection results in the characteristic histopathological landmarks of PML; extensive and confluent areas of myelin loss, in which numerous bizarre reactive astrocytes with atypical and pleomorphic nuclei, and enlarged oligodendrocytes harboring intra-nuclear eosinophilic inclusion bodies, which represent the site of active viral replication While apoptosis is a host defense mechanism to dispose of senescent or damaged cells, including virally infected cells, certain viruses have the ability to de-regulate apoptotic pathways in order to complete their vital cycles. One of these pathways involves a novel protein, Survivin, a member of the inhibitor of apoptosis gene family, which is abundantly expressed during development in embryonic proliferating tissues, but should be absent in terminally differentiated cells.

Immunohistochemical experiments performed in a collection of 20 brain samples from patients with AIDS related PML and 2 cases of non-AIDS PML, revealed increased expression of Survivin in bizarre reactive astrocytes and in the intra-nuclear inclusion bodies of JCV infected oligodendrocytes. In addition, JCV infected primary astrocytic cell cultures demonstrated enhanced expression of Survivin by Westen Blot and immunocytochemistry compared to non-infected cultures, which lack expression of Survivin. Finally, in order to determine the activation of the Survivin promoter by JCV T-Antigen we performed Luciferase

assays in human primary astrocytes co-transfected with either 1066 kb or 622 kb Survivin promoter constructs driving the Luciferase reporter gene, and two different amounts of a T-Antigen construct. Ectopic production of T-Antigen resulted in significant activation of the Survivin promoter transcription. Furthermore, it appears that activation of the Survivin promoter is directly proportional to the amount of T-Antigen present in the cells.

This is the first time that expression of the anti-apoptotic Survivin is demonstrated in clinical samples of PML and in JCV infected glial cell cultures.

Based on these observations we hypothesize that upon its reactivation, JCV attempts to prevent cells from entering the apoptosis pathway by activating the Survivin gene, which results in enhanced levels of Survivin expression. The presence of Survivin will in turn disrupt the apoptotic machinery and preventing infected cells from committing suicide, therefore allowing JCV to successfully complete its lytic cycle and resulting in the development of PML.

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P190

Theiler's murine encephalomyelitis virus subgroup-specific growth on BHK-21 cells is regulated by amino acid 57 within the leader protein

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Theiler's murine encephalomyelitis virus (TMEV) belongs to the genus Cardiovirus of the family Picornaviridae. TMEV strains are divided into two subgroups on the basis of their different biological activities. GDVII strain and other members of the GDVII subgroup cause acute and fatal polioencephalomyelitis in mice. In contrast, DA strain and other members of the TO or DA subgroup persists in the spinal cords of susceptible mouse strains and causes demyelination. GDVII strain grows well on BHK-21 cells at a titer that is 10-fold higher than DA strain.

When the sequence of GDVII and DA strains are compared, the leader protein (L), which is located at the most N-terminal of the polyprotein, has only 85% homology at the amino acid (AA) level, although the capsid proteins are over 94% homologous. The AA at the position 57 of L (L57) is proline in the case of GDVII strain, whereas it is serine in the case of DA strain. Thus, we focused on L57 as a determinant AA of subgroup-specific biological activities because proline is a critical determinant of protein structure while serine can be phosphorylated at times leading to the regulation AA of enzyme activities and protein-protein interaction.

Site-directed mutagenesis studies of L57 demonstrated that a single AA regulates the subgroup-specific virus growth of TMEV strains on BHK-21 cells. Further study also demonstrated that L57 regulates viral RNA encapsidation although it does not affect the synthesis of viral proteins and the assembly of virus intermediates: 5S protomer, 14S pentamer and 75S procapsids. The regulation of TMEV RNA encapsidation by L57 may be a key factor in TMEV subgroup-specific virus growth on BHK-21 cells.

P191

Rabies virus (RV) interaction with dynein light Rabies virus (RV) interaction with Dynein light chain LC8 is an important factor for the infectivity of virions, neuroinvasiveness, and pathogenicity

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Street Rabies Virus (RV) is a highly neurotropic and neuroinvasive virus that initiates its life cycle in peripheral neurons and is subsequently transported retrogradely into the central nervous system (CNS). Recent studies have discovered a binding motif in the RV phosphoprotein (P) for the dynein light chain LC8, which has been proposed to be involved in retrograde axonal transport. However, structural and thermodynamic analysis of LC8 and its targets exclude a direct involvement of a RV P-LC8 interaction to utilize the dynein transport system. Using reverse genetics, recombinant RVs lacking the LC8 binding domain were constructed. Our data show that infection with wild-type RV up-regulates LC8 2-5 fold but cellular LC8 levels remained unchanged when infected with a RV not containing the LC8 binding site. In addition, the absence of the LC8 binding site in RV P does greatly affects viral growth in neuronal cells, whereas the growth pattern on non-neuronal cells is unchanged. Detailed biochemical studies revealed that LC8 is an important factor for infectivity of RV virions in neurons but doesn't affect apoptosis or nNOS as previously suggested. Biological relevance of these findings was gathered by in vitro experiments. The results indicate that intranasal infection of adult mice with a RV without the LC8 binding motive partially attenuates RV but completely abolished disease after peripheral intramuscular inoculation. A delay of onset of rabies was also observed in immunodeficient mice.

P192

PEHMB may inhibit HIV-1 infection independently of the functions of the chemokine receptor CXCR4

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Molecules that inhibit HIV-1 infection by disrupting interactions with the viral co-receptor CXCR4 may

also interfere with normal chemokine receptor (CCR) functions. Within the central nervous system (CNS), CXCR4 and its ligand SDF-1 participate in neurogenesis, neuronal survival, and axonal pathfinding; inhibition of CXCR4 by a CCR inhibitor may adversely affect normal neuronal functions. For this reason, CCR inhibitors developed for use in the CNS should specifically inhibit HIV-1 infection while permitting normal CCR functions. Our efforts in this area have focused on compounds that appear to inhibit HIV-1 infection by interfering with interactions between the virus and cell surface proteins. Polyethylene hexamethylene biguanide (PEHMB) is characterized by low toxicity and considerable activity against HIV-1 IIIB, which uses CXCR4 as a viral co-receptor. The particular potency of PEHMB in the presence of both virus and target cells led us to hypothesize that PEHMB may interfere with viral binding and entry mechanisms. Flow cytometric analyses of HIV-1-susceptible cells exposed to PEHMB demonstrated that CXCR4 detection was decreased in an epitope-specific manner, indicating that CXCR4 remained on the cell surface despite its inability to support HIV-1 infection. These findings suggested that CXCR4-dependent inhibition of HIV-1 infection by PEHMB may not affect the normal functions of CXCR4 as a CCR. Ongoing investigations will explore the mechanism of PEHMB activity and its impact on normal CXCR4 functions in immune and neuroglial cells. These studies will facilitate the development of novel compounds that can be used safely in the CNS to inhibit HIV-1 infection.

P193

Molecular cloning and functional characterization of rare human polyomavirus, BKV, VP1 genotypes in polyomavirus nephropathy (PVN)

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The ubiquitous human polyomaviruses, JCV and BKV, are the primary etiologic agents of progressive multifocal leukoencephalopathy (PML) and polyomavirus nephropathy (PVN) respectively, the former being caused by JCV reactivation in the brain and the latter by BKV reactivation in renal allografts. PVN occurs in approximately 5% of patients undergoing renal transplantation though risk factors for developing the disease are not well established. BKV reactivation is controlled by the host immune response which may be impaired due to immunosuppressive therapy to prevent graft loss as well as inherent differences between donors and recipients. Unlike JCV, which has one predominant subtype, four BKV subtypes with characteristic amino acid sequences at residues 61-83 of the major capsid protein, VP1, have been previously identified. This region of VP1 overlaps with the serotyping region and differences in its sequence may influence virus-receptor interaction. To determine whether a particular subtype is associated with PVN, this region of the VP1 gene was subcloned and sequenced from PVN patients, their donors, and normal healthy individuals. The uncommon subtype IV and polymorphic genotypes were detected with much higher frequency in both donors and recipients when compared to normal healthy individuals. Several of the most prevalent polymorphisms were introduced onto the background of the wild-type strain (Type I) and viral stocks were prepared by transfection/infection. To establish whether the presence of rare or polymorphic genotypes affects the structure of the VP1 region, purified viral clones were tested in parallel by hemagglutination assay (HA) and quantitative-PCR (Q-PCR). In addition, the viruses were analyzed by Western blotting which revealed differences in migration under denaturing conditions suggesting that these polymorphisms alter the structure of VP1. Further analysis is needed to understand whether polymorphisms of VP1 alter the life cycle of BKV or may represent an advantage for BKV in escaping the host immune surveillance.

P194

Divergent effects of Tat and 17beta-estradiol on proteasome activity and HIV-1 replication in human astrocytes and monocytes

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As AIDS is now the third leading cause of death among women ages 25–44, understanding the role that female sex hormones might play in HIV-1 infection is especially critical. Recent data from our lab shows that estrogen has complex actions on the multicatalytic proteasome, which clears oxidized and damaged proteins from cells and has been shown to be an important aspect of cellular responses to viral infection. Indeed, the proteasome is critically important to nearly all steps of the HIV viral life cycle, and can regulate HIV-1 replication. To determine how the proteasome might regulate HIV replication in the brain, we measured the effects of the HIV-1 viral protein Tat on proteasome activity in human monocytes and astrocytes. Specifically, we examined the dose (50–200 nM)-and time-dependent (6–48 h) effects of Tat1-72, Tat1-86 and Tat 1-101 and mutant Tatd31-61 on proteasome activity in monocytic

THP1 cells, astrocytic SVGA cells, and primary human astrocytes. Data indicate that Tat1-72, Tat1-86, and Tat 1-101, but not Tatd31-61, significantly decreased proteasome activity in astrocytes but not THP1 cells. Proteasome activity in astrocytes was not decreased by inhibitors of lysosomal proteolysis (NH4Cl; 1 mM) or by treatment with 1 nM 17beta-estradiol. To next determine the effects of estrogen and proteasome inhibition on HIV-1 replication, we measured HIV-1 infection in human SVGA and THP-1 cells under conditions of estrogen treatment or proteasome inhibition. Interestingly, 17beta-estradiol significantly attenuated HIV infection (H9/HIV-1 IIIB) in SVGA cells, but was not effective against HIV-1 IIIB infection in THP-1 cells. Taken together, these data clearly indicate that the effects of Tat and host factors, including 17beta-estradiol, on HIV-1 regulation are cell type-specific, and further suggest that estrogens could act to attenuate HIV-1 replication in astrocytes.

P195

Clade C HIV-1 encephalitis in SCID mice results in less cognitive dysfunction than clade B

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The mechanism of pathogenesis underlying HIV associated dementia (HAD) or the viral determinants responsible are poorly understood. The incidence of HAD in India is reportedly significantly lower than in the U.S. (1-2% vs. 15-30%). We previously showed that the differential incidence of HAD correlates with subtype differences in the chemokine motif of HIV-1 Tat protein. While the clade B Tat contains a chemokine motif and displays monocyte chemotactic function, the clade C Tat lacks both.

In order to further define these potential differences in clade C vs B neurovirulence we have employed a model of HIV encephalitis in SCID mice. Human monocytes are infected with HIV-1ADA, HIV-1Indie, an Indian clade C isolate, or left uninfected (controls). HIV-1ADA and HIV-1Indie viruses displayed variable infectivities. In order to achieve similar levels of monocyte infection, we employed a 1:100 ratio of clade C to ADA virus which led to 78% and 70% infection (as assessed by immunocytochemistry) respectively at the end of 14 days of infection. HIV-1ADA infected, HIV-1Indie infected or control human monocytes (10,000) were then injected intracerebrally into weanling C57BL SCID mice. Four days after IC inoculation of monocytes these mice ($n = 6$ for each group) were monitored for cognitive function using a water-escape radial arm maze (RAM).

Confirming prior findings, HIV-1ADA infected mice made significantly more errors on the RAM compared to controls. This was seen for two different working memory measures, with largest group differences at the most demanding memory load. Interestingly, the clade C infected group exhibited cognitive performance at an intermediate level between the two other groups, resulting in a lack of siginificant differences in errors for the clade C vs. control and clade C vs. HIV-1ADA group comparisons. We plan to extend and replicate these findings in further investigations.

These preliminary results suggest that clade C HIV brain infection results in less cognitive dysfunction in SCID mice than clabe B (ADA) HIV. This is remarkably consistent with the clinical observations outlined above. We are currently examining the histopathological findings in SCID brains of clade C, ADA and control mice as well as brain viral load by realtime PCR. Furthermore, neuroimaging studies will be performed to confirm the neuronal loss in the appropriate parts of the brain. These studies are likely to provide further insights into the viral mechanisms of neurovirulence in HAD, as well as functional cognitive consequences of the pathological hallmarks seen in this disorder.

P196

Synergistic action of IGF-IR inhibition and GSK-3beta activation compromises growth and survival of JCV T-antigen positive mouse medulloblastoma cells in anchorage independence

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Medulloblastomas are malignant, WHO grade IV cerebellar tumors of the childhood. The large T-antigen from human polyomavirus JC (JCV T-antigen) is suspected to play a role in malignant transformation, and has been detected in neoplastic cells from human medulloblastoma biopsies. Previously, we have demonstrated that JCV T-antigen requires the presence of a functional insulin-like growth factor I receptor (IGF-IR) for transformation of fibroblasts and for survival of medulloblastoma cell lines, and that IGF-IR is over-expressed and constitutively phosphorylated in medulloblastoma clinical samples. Our results show that pyrrolo[2,3-d]pyrimidine (NVP-AEW541) designed to inhibit IGF-IR tyrosine kinase activity, attenuated growth and survival of JCV T-antigen expressing mouse medulloblastoma cell line (BsB8) in monolayer and suspension culture conditions. BsB8 cells were much more sensitive to NVP-AEW541 when cultured in suspension. Quantitatively, 20% decrease in cell number was observed in the presence of 0.1 uM NVP-AEW541, and over 80% of BsB8 cells were

eliminated with 1uM NVP-AEW541. Although monolayer cultures of BsB8 cells were more resistant, cell proliferation was completely blocked at 0.5 uM and some apoptotic death occurred at 1uM NVP-AEW541. These inhibitory effects on cell growth and cell survival were accompanied by dose dependent inhibition of IGF-IR, IRS-1, Akt, and Erks phosphorylation. Elevated sensitivity of BsB8 cells to NVP-AEW541 in suspension was accompanied by the loss of GSK3beta phosphorylation (activation) and subsequent apoptotic cell death. The medulloblastoma cells were partially rescued by the GSK3beta inhibitor LiCl, and were additionally sensitized to the NVP-AEW541 treatment by GSK3beta activator, sodium nitroprusside.

In summary, our data indicate that the impairment of IGF-IR function in synergy with the activation of GSK-3beta could be utilized against detrimental spread of medulloblastoma via cerebrospinal pathway in which medulloblastoma cells are exposed, at least temporarily, to anchorage independence. Such a possibility is presently tested in our model of mouse medulloblastoma.

P197

Longitudinal neuropsychological evaluations are sensitive to HIV effects in the era of HAART The Hawaii Aging with HIV Cohort

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Background: Most studies rely on cross-sectional data to determine correlates to cognitive dysfunction in people infected with HIV. While cost effective, deterioration over time may be a more sensitive measure of HIV neuropathogenesis.

Methods: The Hawaii Aging with HIV Cohort has enrolled 159 older (50+ years old) compared to 128 younger (<40 years old) HIV-1-infected individuals who complete annual neuropsychological examination characterizing the neuroepidemiology of aging with HIV. Two seronegative comparative groups matched by age, gender, education, and ethnicity are simultaneously enrolled and followed annually. In this analysis, we compared longitudinal neuropsychological performance by serostatus using mixed models and all individuals who had completed 3 annual visits and were free of illicit drug use and stroke (108 younger HIV+, 98 younger non-HIV, 129 older HIV+, and 106 older non-HIV).

Results: All groups were well-matched on key demographic variables, although estimated full scale IQ differed somewhat between groups (105 for younger HIV+, 110 for younger non-HIV, 111 for older HIV+, and 114 for older non-HIV). Annual rate of change in motor skills/motor speed, visual memory, and word list learning distinguished seropositive from seronegative individuals in this cohort. In contrast, only working memory distinguished seropositives from seronegatives at baseline evaluation.

Discussion: In the era of HAART and among individuals well-matched for all variables except serostatus, a longitudinal assessment rather than cross-sectional assessment of neuropsychological function appears to more powerfully capture HIV effects. This finding could have implications for designing studies to determine factors contributing to cognitive deterioration in the current era.

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Neuropsychological testing among subtype CRF01_AE HIV-1 in Bangkok

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Background: Little is known regarding clade-specific neurovirulence of HIV-1. Some reports suggest that virus subtypes have differing propensity to cause neurocognitive complications. In Thailand, early studies did not identify HIV-1-associated dementia (HAD). We now present data demonstrating neuropsychological testing abnormalities in patients infected with subtype CRF01_AE and selected for presence (n = 15) or absence (n = 15) of HAD.

Methods: ARV-naïve subjects with and without neurologist-identified HAD completed the WHO-NIMH neurocognitive battery. HAD and non-HAD

participants were matched by gender, age, education, and CD4 count. 30 age, education, and gender matched controls were also evaluated. HIV-1 subtype was determined by serological testing.

Results: Neuropsychological testing abnormalities were identified in most cognitive domains among HAD participants compared to HIV-negative controls. By contrast, only one test of verbal fluency distinguished non-HAD HIV-1-infected individuals from controls. All cases were confirmed to be infected with sub-type CRF01_AE.

Discussion: This study demonstrates neuropsychological abnormalities among HAD patients infected with sub-type CRF01_AE in Bangkok.

This work confirms the presence of HAD among individuals infected with this subtype. Future work is aimed at identifying change in neuropsychological testing with ARV treatment.

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Absence of JC virus antigens in Indian children with medulloblastoma

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The human polyoma virus, JC virus (JCV), replicates predominantly in the oligodendrocytes,the myelin producing cells in the central nervous system and results in fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML) especially in immunosuppressed patients with AIDS. Several investigators have also documented presence of the viral genome, early and late antigens in a variety of brain tumors particularly in medulloblastomas, gliomas, ependymomas etc. Reports also indicate presence of JCV in patients with colon cancer. The T antigen of JCV has been postulated to have oncogenic potential as substantiated by animal experiments.

Although JCV infects 80% of the population, there are scant epidemiological studies regarding JCV from India. There are also reports of low prevalence of PML in patients with AIDS from India and Africa. The present study was undertaken to investigate if Indian children with medulloblastomas also show evidence of JCV. Twenty-two consecutive cases of medulloblastomas were investigated for presence of T antigen and agnoprotein in biopsy specimens by immunohistochemistry. Out of the 22 patients, four had desmoplastic tumors while the rest had classical tumors. All children were below 10 years of age. Antibody to agnoprotein antigen raised in rabbit and a monoclonal antibody raised in mouse against SV40 T antigen that cross-reacts with JCV T antigen, were generous gifts from Dr. Kamel Khalili. Antigen retrieval was done according to Del Valle et al. (2002). The secondary antibody and avidin biotin detection system (Envision system/HRP kit) were purchased from DAKO Cytomation Denmark. Sections from an Indian patient with PML (courtesy, Dr Shankar, NIMHANS, Bangalore, India) were used as positive controls. Results indicate that while PML tissues showed consistent immunostaining both with antibody to T antigen and agnoprotein antibody, none of the tumors showed any positive staining for JC viral antigens. An earlier study by Engels et al from Delhi India (2002), also failed to substantiate presence of JCV antigens in Indian patients with brain tumors. It appears that there may be geographic variations in the prevalence of JCV in brain tumors and some additional causative agents or cofactors may be operating in Indian patients. An epidemiological survey of JCV in healthy Indian population may yield some valuable information.

P200

The rabies virus is captured only by large sensory neurons in vivo

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Rabies virus (RABV) is a highly neurotropic virus producing lethal encephalitis. In a rabid accident when the virus becomes inoculated into the periphery of the host, it may or may not be replicated and then captured by autonomic, motor or sensory neuron fibers. RABV captured by sensory fibers is retrogradely transported by fast axonal transport towards the soma located in the dorsal root ganglia (DRG) and from there to the central nervous system (CNS). Moreover, neurons in the DRG can be classified into subpopulations according to morphological, physiological and biochemical characteristics. Previous reports from our group have shown that the virus prefers certain neuron subtypes that can be mainly identified by their diameter. For this reason, the objective of this work was to do the morphological characterisation of the neuronal subpopulation of DRG most susceptible to infection in vivo. Adult mouse were inoculated in footpad with RABV (mouse brain-haversted CVS strain) and the animals were sacrificed at different times. The virus was detected by immunoperoxidase and the morphometric analysis was done with an ImagePC software. The neuronal populations was divided in large neurons ($\geq$35 μm) which correspond to 46% of the total neurons; intermediate neurons (between 21 to 35 μm) which correspond to 52% and finally smaller neurons ($<$20 μm) which correspond to 2% of the total neurons. The large neurons were always more susceptible to the infection. RABV preference for large-sized neurons (mainly innervating muscle and joints) could explain part of the neuropathogeny disease, these neurons display specify metabolic and biochemical conditions that they

promote the capture and transport of the virus from the peripheral tissue to the nervous system.

P201

Impairment of adult hippocampal neurogenesis by methamphetamine and HIV-Tat protein

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HIV infected patients who abuse drugs, including methamphetamine (METH), have an accelerated and more severe neurocognitive dysfunction compared with non-drug-abusing patients. Therefore, it is of paramount importance to determine the neurological substrate underlying cognitive dysfunction in METH users who are infected with HIV. Formation of new neurons from neural progenitor cells (NPCs) continues through adulthood. Adult neurogenesis in the hippocampal dentate gyrus (DG) may play an important role in the long-term maintenance of cognitive function. We hypothesized that both METH use and HIV infection may result in dysfunction of DG NPCs, thereby causing impairment of cognitive function.

In a systematic study to ascertain the effects of METH and Tat on neurogenesis in the adult hippocampal DG in vivo, we injected mice intraperitoneally with BrdU, a thymidine analog that is incorporated into proliferating cells, which was followed by immunohistochemical detection of BrdU positive cells. In an acute toxic dosing model (METH 40 mg/kg i.p.) we observed a reduction by 25% in proliferating DG NPCs in mice ($p < 0.01$). We also found that transgenic mice expressing the HIV viral transactivator protein Tat under the control of a glial fibrillary acidic protein (GFAP) promoter, exhibited a 50% decrease in proliferating NPCs as assessed by BrdU incorporation ($p < 0.001$).

To further characterize the effects of METH and Tat on neurogenesis, we developed an in vitro system in which proliferation and differentiation can be reproducibly controlled. Using this system, we observed that METH, but not Tat, when directly applied to adult rat NPCs, caused a dose-dependent reduction (at concentrations of 250 μM and higher) in the proliferating capacity of NPCs suggesting that while METH has a direct effect on NPCs, Tat effects are likely indirectly mediated. At higher concentrations (500 μM and above) METH caused apoptosis of NPCs as detected by TUNEL staining ($p < 0.01$). These changes in NPC function and survival upon METH exposure correlated with the development of markers of oxidative and nitrosative stress. In particular, the overall burden of protein nitrotyrosination was increased in METH-exposed NPCs and several proteins of approximate molecular mass of 26 kD, 30 kD, and 32 kD were preferentially nitrotyrosinated in response to METH. Future studies will identify specific NPC proteins that are modified by METH-induced oxidative and nitrosative stress and determine if Tat mediates its effects on neurogenesis indirectly, through interactions with glial cells.

We conclude that both METH exposure and expression of the HIV Tat protein cause a decrease in proliferating NPCs in the adult hippocampal dentate gyrus. METH exerts direct effects on NPC survival and proliferation, in part by causing oxidative and nitrosative stress. Tat, on the other hand, may alter NPC function and survival through effects on glial cells. Impaired neurogenesis in the settings of HIV infection and METH abuse may contribute to cognitive dysfunction.

P202

Immune Reconstitution Inflammatory Syndrome in the CNS of HIV infected patients

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Background: Immune reconstitution inflammatory syndrome (IRIS) is a recently recognized syndrome that results from a paradoxical deterioration in clinical symptoms or the appearance of inflammatory lesions following initiation of antiretroviral therapy in patients with HIV infection. It carries a high degree of morbidity and mortality and specific treatments for IRIS have not been identified.

Case reports: We describe three patients with quite distinct clinical syndromes of CNS IRIS who presented as diagnostic dilemmas. The first patient with cryptococcal meningitis had an acute deterioration requiring emergent intervention. The second patient with PML had a subacute presentation but did not require any changes in treatment. The third patient with HIV encephalitis with massive infiltration of T cells of predominantly CD8 subtype on brain biopsy. She had a more protracted course and showed only a transient response to steroid therapy. The most important common feature of these three patients was the onset of neurological problems in the setting of improving viral load and CD4 cell counts. The evolution and outcome of these clinical situations was variable. Steroid therapy was used to modify the clinical profile and manage the progression of mass effect and brainstem edema associated with the IRIS in the patient with cryptococcal meningitis. The therapy resulted in complete resolution of the lesion in the cerebellum but developed VZV encephalitis while on steroid therapy. The patient with PML showed spontaneous resolution of the IRIS but was left with residual deficits. This patient despite showing some signs of clinical

worsening at the time of development of IRIS was not treated with steroids because at the time the enhancing lesions were not considered life threatening and since subsequently the patient started showing spontaneous improvement, such intervention was not necessary. The patient with the dementing syndrome showed a significant improvement with steroids and change in antiretroviral regimen although she had residual cognitive impairment.

Conclusions: While immune reconstitution remains a therapeutic goal in patients with HIV infection, ironically, the very same phenomenon may result in tissue destruction leading to worsening of the disease. This is more apparent in the brain where this phenomenon can be fatal. Distinguishing IRIS from worsening of the underlying infection is important so that appropriate treatment can be initiated. Although some clinical features may help in the diagnosis, the clinical criteria are far from satisfactory, and biomakers need to be developed than can reliably recognize the immune response. Similarly, clinical trials are needed to establish the utility of immunomodulatory or anti-inflammatory agents and more specific agents need to be developed.

P203

A new mouse model of HIV infection: Application to study of HIV neuropathogenesis

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We created a model of HIV-1 infection of conventional mice for investigation of viral replication, control, and pathogenesis. The coding region of gp120 in HIV-1/NL4-3 or HIV-1/NDK was replaced with that of gp80 from ecotropic murine leukemia virus, creating chimeric retroviruses EcoHIV and EcoNDK with exclusive tropism to rodent cells. Adult, immunocompetent mice were readily susceptible to infection by a single i.v. or i.p. inoculation of EcoHIV or EcoNDK as shown by detection of virus in splenic lymphocytes, peritoneal macrophages, and the brain. Virus burden in vivo was assessed quantitatively by QPCR assays for genomic DNA, genomic and spliced RNA, and p24 ELISA. At the peak of infection, an estimated 1%-3% of spleen cells and up to 10% of peritoneal macrophages carried HIV DNA; virus burden in the brain was lower. The virus produced in animals was infectious, as shown by passage in culture, and immunogenic, as shown by induction of antibodies to HIV-1 Gag and Tat. In young adult mice, EcoNDK entered the brain within 3–4 weeks of infection and it induced expression of infection response genes, MCP-1, STAT1, IL-1beta, and complement component C3 in brain tissue. Long-term pathogenic and behavioral effects of EcoHIV infection in various strains of mice are under investigation. EcoHIV infection of mice forms a useful model of HIV-1 infection of human beings for convenient and safe investigation of HIV-1 pathogenesis, therapy, and potentially vaccines.

P204

Platelet decline predicts SIV and HIV-induced CNS disease

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As the prevalence of Human Immunodeficiency Virus (HIV)-induced central nervous system (CNS) disease continues to rise despite highly active anti-retroviral treatment (HAART), there is a critical need for identifying biomarkers that predict HIV CNS disease. We recently demonstrated that platelet decline predicted development of encephalitis in a cohort of 47 Simian Immunodeficiency Virus (SIV) infected macaques. Macaques that ultimately developed moderate to severe encephalitis demonstrated a greater decline in circulating platelet counts beginning as early as day 28 post-infection. Univariate analysis performed on platelet values obtained at day 56 post-inoculation demonstrated that SIV-infected macaques with the greatest decline in platelet numbers were 18 times more likely to develop SIV CNS disease than SIV-infected animals with minimal to no decline in circulating platelet counts. Results indicate that platelet decline is likely due to a peripheral destruction of circulating platelets although animals with moderate to severe encephalitis also demonstrated reduced numbers of megakaryocytes. We have extended this work to examine the predictive role of platelet decline in a cohort of HIV infected individuals from the North-East AIDS Dementia consortium. Preliminary results indicate that a moderate to large decline in circulating platelet numbers occurring 6–12 months prior to diagnosis of dementia is associated with a two-fold increased risk of dementia when controlling for factors such as viral load, antiretroviral therapy, presence of coincident AIDS defining illness, age, and duration of HIV infection. This association between decline in platelets and the development of neurologic disease suggests that monitoring platelet decline in HIV-infected individuals may serve as a predictive marker for clinical progression to HIV-induced CNS disease and provide clues to the role of hematopoietic growth factors in the pathogenesis of HIV CNS disease. Identifying those HIV-infected individuals at risk for CNS disease during asymptomatic stages of infection would promote interventive therapy to prevent neuronal damage and loss.

P205

Vitamin A deficiency effects on proinflammatory cytokines and Mu opioid receptor expression in the HIV-1 transgenic rat

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Background: Previous studies in vitro have shown that retinoids and opioids may interact to increase or suppress proinflammatory activity in mononuclear cells in vitro in association with similar or reciprocal changes in opioid receptor expression. In these studies, the potential effects of vitamin A deficiency on proinflammatory cytokine and mu opioid receptor (MOR) expression by T cells in vivo were examined using the HIV-1 transgenic (Tg) rat model.

Methods: T cells in whole blood samples from Tg and non-Tg rats on either a vitamin A deficient or normal diet were examined for expression of IFN-(γ), TNF-(α), and mu opioid receptor expression before and after activation with phytohemagglutinin (PHA).

Results: In vitamin A deficient wild-type and transgenic rats, activation of T cells in whole blood resulted in increased intracellular localization of IFN-(γ) and MOR and increased secretion of IFN-(γ) and TNF-(α). The highest levels of TNF-(α) secretion and MOR expression was observed for transgenic rats on the vitamin A deficient diet.

Conclusions: These data demonstrate that effects of retinoid metabolism on opioid receptor expression can be observed in vivo as well as in vitro and may underlie aspects of the immune dysregulation that occurs with HIV-1 infection, including individuals with neurological disease.

P206

Calpain/Cdk5 pathway is activated in in vitro model of HIV encephalitis

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In the pre-HARRT era, about 15∼25% of HIV infected individuals develop HIV associated dementia (HAD), which is characterized by neuronal and dendritic damage, neuronal loss, microgliosis and astrogliosis in the central nervous system. Although neurons are damaged in HIV infected patients, HIV does not infect neurons directly. Instead neuronal damage is mediated by neurotoxic factors from infiltrating, activated and/or infected macrophage/microglia. Neuroinflammation and the associated oxidative stress seen in patients with HAD is also seen in other neurodegenerative diseases including Alzheimer disease and Parkinson disease. Recently, a unique member of cyclin dependent kinase family, CDK5, has been implicated in neuronal loss in several neurodegenerative diseases. CDK5 requires association with one of its regulatory partners which in the brain are p35 and the p35 cleavage product, p25. Production of p25 from p35 is mediated by calpain cleavage and increases Cdk5 kinase activity over the p35:CDK5 complex. We hypothesized that increased CDK5 activity contributes to neuronal damage in HAD. To test this hypothesis, we used an in vitro model of HIV encephalitis, in which primary rat cortical neurons are treated with a primary, neurovirulent HIV-1 isolate (Jago) derived from the CSF of an individual with HIV-associated dementia. In our model system, we have observed a dose-dependent increase in calpain activity in response to Jago treatment. Calpain activity peaked at 8 hours after Jago treatment and was coincident with peak p25 generation. Inhibition of calpain led to a reduction in p25 production and provided protection from Jago-induced death. Further inhibition of CDK5 activity also provided significant neuronal protection for Jago toxicity. Our results suggested that calpain/cdk5 pathway mediates neuronal death in our in vitro model of HIV encephalitis. The MEF-2 transcription factor which promotes survival has recently been shown to be inactivated by CDK5 phosphorylation. Future studies will determine if CDK5 phosphorylation of MEF2 contributes to neuronal death in our model system.

P207

Interaction of the tumor suppressor proteins NF2 and p53 in a JCV T-antigen induced transgenic mouse model of neurofibromatosis

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The human polyomavirus, JC virus (JCV) infects greater than 80% of the human population and maintains a low level persistent infection in immunocompetent individuals. Under immunosuppressive conditions, the virus may become reactivated resulting in the fatal demyelinating disease, progressive multifocal leukoencephalopathy (PML). Studies in transgenic mice expressing the viral oncoprotein, T-antigen, under the control of natural JCV promoter sequences showed the development a range of neural origin tumors. Furthermore, studies of human neural and non-neural tumors have detected the presence of JCV DNA and expression of the viral proteins, T-antigen and Agnoprotein in tumor cells. We previously developed transgenic mice expressing JCV T-antigen under the control of the Mad-4 promoter of JCV which

consequently developed malignant peripheral nerve sheath tumors (mpnst) characteristic of neurofibromatosis. In tumor tissues, T-antigen binds to and inactivates wild-type p53, keeping p53 levels very high. NF2 is a tumor repressor protein found to be inactivated in neurofibromatosis type 2. As T-antigen has a propensity for interacting with and inactivate tumor suppressor proteins, an interaction between T-antigen and NF2 was hypothesized and then determined. In fact, our previous immunoprecipitation data suggested that p53, T-antigen, and NF2 could form a ternary complex in the mpnst cells. We also observed that over-expression of p53 resulted in down-regulation of T-antigen and NF2 though this down-regulation was tempered somewhat by the simultaneous presence of both T-antigen and NF2. Further studies will determine the mechanism by which p53 may effect NF2 function and will evaluate the potential for NF2 to regulate p53 function. The effect of JCV T-antigen on these pathways will be elucidated in parallel in order to shed light on JCV T-antigen mediated mechanisms of cellular transformation.

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P208

Molecular characterization of IRS-1–Integrin–TNF-alpha functional interplay in the context of neuronal damage in HIV–associated dementia

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HIV associated dementia (HAD) is a severe neurological disorder of the central nervous system, which develops in AIDS patients. Among numerous viral and cellular factors contributing to HAD, tumor necrosis factor alpha (TNF-alpha) released in the brain by activated and/or infected macrophages/macroglia is suspected to compromise stability of neuronal processes. Previously, we have demonstrated that the IGF-IR—IRS-1 signaling -induced damage. Since TNF-alpha triggers?axis protects neurons from TNF-alpha IRS-1 serine phosphorylation, and serine phosphorylated IRS-1 binds integrins, we asked how these molecules events affect neuronal stability. Here we confirmed that differentiated PC12/GR15 neurons, which express high levels of IGF-IR and IRS-1, are well protected from TNF-alpha –induced neuronal damage. We have also found that alpha1beta1-integrin, as well as serine phosphorylated IRS-1 are located in membrane rafts of differentiated PC12/GR15 cells. In this subcellular compartment, IRS-1 co-precipitated with alpha1beta1-integrin, however this protein—protein interaction was inhibited by IGF-I stimulation. We have demonstrated the presence of IRS-1 and alpha1beta1-integrin complexes by double immunocytofluorescence, and confirmed the binding by GST-pull down assay, which demonstrated that the domain responsible for alpha1beta1-integrin binding is located within the central portion of IRS-1, between aa 462 and 740. Presently, we are testing how the binding between alpha1beta1 integrin and the IRS-1 truncation mutant affects the stability of neuronal processes, and whether this -mediated degeneration?protein –protein interaction is responsible for TNF-alpha of neuronal processes observed in our HAD in vitro model.

P209

T cell activation induces toxicity in neural progenitor cells: A supplemental mechanism for neurodegenerative disorders?

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Background: Recent studies have shown that proliferation and differentiation of neural progenitor cells (NPC) may be altered following pathological insults in adult brains which may contribute to brain atrophy. Inflammatory infiltrates including T cells are present in the brain of patients with neurodegenerative disorders such as multiple sclerosis (MS) and HIV-dementia.

Objectives: To determine if activated T cells release soluble factors that induce toxic effects on NPC and to determine the mechanisms underlying such effects.

Methods: Supernatants (sups) from restive or CD3/CD28 polyclonally activated human CD8+ T cells were collected to treat (at 1:20 dilution) primary human embryonic NPC cultures. NPC proliferation was monitored by BrdU incorporation assay after 24 hrs of treatment. NPC differentiation was determined by immunostaining with anti-βIII tubulin (a neuronal marker), after 7 days of treatment. Granzyme B (GrB) released in T cell sups was detected by ELISA. NPC were treated with by either GrB immunodepleted activated T sups or recombinant GrB (4 nM) with or without pretreatment with pertussis toxin (PTX, a Gi protein inhibitor, 100 ng/ml) and Margtoxin (MgTX, Kv1.3 potassium channel blocker, 10 nM).

Results: Activated T cell sups (AcT) reduced both NPC proliferation and neuronal differentiation compared to control sups ($p < 0.05$). Production of GrB in AcT was higher than in restive sups ($p < 0.01$). Immunodepletion of GrB attenuated AcT-induced inhibition of NPC proliferation and differentiation ($p < 0.05$). Recombinant GrB also inhibited NPC proliferation and differentiation compared to control ($p < 0.05$). Recombinant GrB treatment also reduced cyclic

AMP level in NPC ($p < 0.01$). Pretreatment with PTX and MgTX both attenuated GrB-induced toxic effect on NPC ($p < 0.05$), indicating a Gi-coupled receptor and potassium channel Kv1.3 are involved in GrB effects on NPC.

Conclusions: GrB released by activated T cells may inhibit NPC proliferation and neuronal differentiation, indicating a novel pathological mechanism for brain atrophy in inflammation-related neurodegenerative disorders such as HIV-dementia and MS. GrB-induced toxicity in NPC may be mediated at least partially by PTX-sensitive G-coupled receptor and Kv1.3 potassium channel and these observations provide potential targets for therapeutic intervention in neurodegenerative disorders.

P210

Genotypic and phenotypic analyses of brain-derived HIV-1 envelope glycoproteins

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Numerous studies have genotypically characterized HIV-1 envelopes amplified directly from brain tissue or from isolates recovered from brain tissue of HIV-1-infected individuals. However, very few studies have addressed the phenotypic characterization of HIV-1 envelopes from brain. Therefore, very limited information is available regarding potential differences in sensitivity to multiple types of entry inhibitors that may be acquired through the continuous viral replication in the brain and adaptation to the brain environment. For these reasons, we used envelope-mediated cell-to-cell fusion and envelope-pseudotyped luciferase reporter transduction assays to evaluate the sensitivity of brain-derived envelopes to various types of HIV-1 inhibitors that target the entry process. We have found that envelope clones from individual brains/brain derived isolates may show a remarkable range in sensitivity to the fusion inhibitor T-1249, while sensitivity to other inhibitors such as anti-CD4 antibodies or small molecules that function as CCR5 antagonists is more consistent among these clones. However, envelopes clones that are able to use additional co-receptors such as CCR3 require the presence of inhibitors again both co-receptors in order to suppress fusion or infection. Compared with the sensitivity of envelope clones derived from peripheral isolates, brain clones are more likely to have lower sensitivity to fusion inhibitors, which may be related with increased fusogenicity and potentially with an increase in neurovirulence. The identification of genetic viral determinants for the phenotypes observed in functional assays is being investigated.

P211

Alteration in the lipid composition of normal appearing brain tissue in multiple sclerosis patients

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A primary neuropathology in multiple sclerosis (MS) is manifest by the breakdown of myelin. There are however, an increasing number of reports that suggest biochemical and biophysical irregularities in "normal" appearing brain tissue of MS patients. In this study we used an electrospray ionization tandem mass spectrometer to quantify the lipid and sterol content of MS and non-MS brain. Cortical brain tissue samples were obtained from the Rocky Mountain MS Brain Bank and were categorized as either normal tissue (no evident brain disease; $n = 9$), tissue adjacent to an MS lesion containing lymphocytic infiltrates ($n = 12$) or tissue adjacent to an MS lesion containing no lymphocytic infiltrates ($n = 11$). All tissues were normal in appearance with no evidence of glial scaring. The tissues were dissected into white and grey matter and lipids were extracted for analysis by electrospray ionization tandem mass spectrometry. In the white matter tracks of MS patients with evidence of lymphocytic infiltrates, we found evidence of abnormal sphingolipid levels of the long chain sphingomyelins C22:0 and C24:0. Interestingly, sphingomyelin levels were similar to control levels in MS patients without lymphocyte infiltrates. The long chain ceramides C22:0, C24:0 and C24:1 were decreased in both groups of MS patients compared with control patients. There was also evidence of selective changes in the phospholipid content of normal appearing white matter with decreases in one species of phosphatidylserine (C18:1) and phosphatidylethanolamine apparent in both groups of MS patients compared with control white matter. Sterol levels were altered in the white matter of all MS patients with decreases in total cholesterol (primarily the dimeric, trimeric forms) and 25-hydroxycholesterol. The reactive aldehyde 4-hydroxynonenal (4HNE), an indicator of cellular stress, was increased in the white matter of all multiple sclerosis patients sampled.

In the grey matter of MS patients we found a graded decrease in the levels of sphingomyelin C16:0, C18:0, C22:0 and C24:0 with greater decreases in tissues from MS brain with evidence of lymphocyte infiltrates compared to MS brain with no evidence of lymphocyte infiltrates. The long chain ceramides C24:0 and C24:1 were decreased in the grey matter of all MS patients compared with control grey matter. There were however, no apparent alterations in sterol metabolism and no evidence of lipid peroxidation as the levels of 4HNE were similar in the grey matter of MS and control patients. From these findings we conclude that disruption in the lipid biochemistry of "normal" appearing brain in MS patients may contribute to neural

dysfunction by altering membrane fluidity, electrostatic adhesion, transmembrane potential and membrane curvature.

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P212

Changes in the content of local lipid domains during NMDA receptor trafficking

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The trafficking of ionotropic glutamate receptors modifies synaptic integrity by controlling the number of receptors at the surface of dendritic spines. While the protein-protein interactions involved in glutamate-receptor trafficking are an active area of research, the roles of protein-lipid interactions in receptor trafficking have been more elusive to identify. To study how local lipid domains are modified during NMDA receptor trafficking we used a model system where NMDA receptors are rapidly inserted to the cell surface following a brief stimulation with the cytokine TNF. In cultured rat cortical cells exposed to TNF for two minutes, there was nearly twice the number of NMDA receptors per square micron at the cell surface (1.820+0.296 microns2) as compared with untreated cultures (0.973+ 0.164 microns2). When we pre-incubated neurons with an inhibitor of vessicle fusion (0.05% sodium azide for 30 minutes) prior to TNF application, the number of surface expressed NMDA receptors was similar to untreated cultures (0.908+ 0.365 microns2), suggesting that TNF mobilized a pool of vesicle bound receptors to fuse with the plasma membrane. To better understand the change in local lipid content that is required for this fusion event we stimulated neurons for 2 minutes with TNF, rapidly harvested and sonicated the cells, and isolated the NR1 subunits of NMDA receptors on an affinity column. The lipids bound to NR1 were then chloroform extracted and analyzed by electrospay tandem mass spectroscopy. On average each extraction yielded 20 to 50 lipid molecules per NR1 subunit representing a 150 nanometer2 patch of membrane surrounding the receptor. These local domains were found to have a lipid composition distinct from the rest of the plasma membrane and this local lipid composition was altered by TNF(α). Specifically, the NR1-bound lipid domains isolated after TNF exposure were enriched in the phosolipids phosphatidylserine and phosphatidylinositol, the sphingolipid ceramide C24:0 and the fatty acid cholesterol derivative linoleate C18:2. Because phosphatidylserine is one of the few lipids to have a formal charge at physiologic pH, we hypothesized that an increased amount of phosphatidylserine in the local lipid content surrounding NMDA receptors would increase the surface charge and decrease the local membrane potential. This change in transmembrane potential would increase the mean open probability of the receptor and would slow channel closure. Indeed, excitatory post synaptic currents (EPSCs) were enhanced in TNF treated brain slices. Together our findings suggest that the local composition of lipids bound to the NMDA receptor is modified during trafficking of the receptor to the cell surface, and that the lipid microdomain surrounding the NMDA receptor may itself modify the kinetics of channel function.

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P213

Analysis of a mutant p53 protein arising in a medulloblastoma from a mouse transgenic for the JC virus early region

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The human JC virus, JCV, is a neurotropic polyomavirus that causes progressive multifocal leukoencephalopathy (PML) in humans and is highly oncogenic in experimental animals. JCV has been reported to be associated with some human brain tumors including medulloblastomas suggesting a possible role for JCV in neoplasia, although this remains unclear. Transgenic mice with JCV T-antigen develop cerebellar tumors resembling human medulloblastomas. Previously, we reported that a JCV-induced mouse medulloblastoma contained two distinct cell subpopulations, one was T-antigen positive and the other negative. In T-negative clones, a novel mutant p53 was detected with an in-frame deletion (p53mt). We have now compared p53mt to wild-type p53 (p53wt) in p53-null cells. Unlike p53wt, p53mt had lost the ability to transcriptionally transactivate p21/Waf1. It had also partially lost its ability to bind to T-antigen. Cell fractionation experiments and immunocytochemistry showed that p53mt localized to the nucleus and the cytoplasm unlike p53wt which localized almost exclusively to the nucleus. Unlike mutant p53 from many human cancers, p53mt did not show a gain of function or dominant negative phenotype. Adenovirus expressing p53wt but not p53mt inhibited cell growth and induced G1 arrest and apoptosis of p53-null cells. Thus during the course of tumor evolution in the JCV

T-antigen-transgenic mouse medulloblastoma model, a mutation occurred that inactivated the function of the p53 tumor suppressor protein allowing tumor progression even in the absence of continued T-antigen expression. These data support a hit-and-run model for JCV tumorigenesis.

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P214

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

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Although the incidence of HIV-associated dementia (HAD) has decreased since the advent of highly active anti-retroviral therapy (HAART), the prevalence of HAD is increasing. Neuronal degeneration in HAD is thought to be caused by soluble factors released by CNS-resident, HIV-infected and/or activated macrophages and microglia. Although many molecules have neurotoxic potential, primary rat mixed neuronal/glial cultures treated with HIV-infected, monocyte-derived macrophage supernatants (HIV-MDM) exhibit a dose-dependent neuronal death that is blocked (>95%) by the NMDA antagonist MK801 (10 uM). However, our studies indicate that only 34% of the toxicity of HIV-MDM supernatants is due to their glutamate content, suggesting the involvement of additional HIV-MDM excitotoxins or the modulation of excitotoxin production by astrocytes in these mixed cultures. The astrocytic cystine/glutamate antiporter (xCT) is a key regulator of glutamate homeostasis, and can be modulated by the integrated stress response (ISR). Antiporter xCT activity results in increased cystine uptake (for glutathione production) and increased glutamate release from astrocytes. We explored HIV-MDM-mediated ISR activation in rat astrocyte cultures and found increased levels of the ISR target genes ATF4, BIP, and CHOP, as well as the endogenous oxidative-stress response protein Nrf2 by Western blotting of astrocytes exposed to HIV-MDM. We then assessed the ability of the ISR initiator thapsigargin to influence xCT activity in astrocytes. Thapsigargin augmented the activity and expression of xCT thereby increasing cystine uptake, and, presumably, glutamate export. Thapsigargin also inhibited the scavenging of extracellular glutamate by the astrocytic excitatory amino acid transporters (EAATs), similar to effects of TNF-alpha. These findings suggest HIV-MDM-released factors may trigger a net efflux of glutamate from astrocytes due to ISR-induced increases in xCT-mediated glutamate export and concurrent decreases in EAAT-mediated glutamate import. Taken together, these data suggest that induction of the ISR in astrocytes by HIV-MDM may induce elevation of extracellular glutamate levels, which could lead to neuronal dysfunction and death. These findings support a role for astrocytes in HIV-MDM mediated neuronal dysfunction and loss and implicate the ISR in mediating altered astrocyte glutamate homeostasis.

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P215

The CCL2 promoter of pigtailed macaques: Identification of nucleotide variation and their contribution to the development of CNS disease

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Development of HIV and SIV central nervous system (CNS) disease has been associated with elevated expression levels of chemokine ligand 2 (CCL2) in both the brain and cerebrospinal fluid (CSF). Recent work has shown that a -2578 guanine polymorphism in the human CCL2 promoter predisposes HIV-infected individuals to develop HIV-associated cognitive disorders (HACD) suggesting a positive correlation between elevated CCL2 expression and HIV neuropathogenesis. However, the ability to establish a definitive correlation between this polymorphism and CNS disease remains a challenge since several studies have indicated that the rate of neurological deterioration correlates with immunosuppression. To dissociate the development of CNS disease from immunosupression we examined the CCL2 promoter in macaques utilized in a rapid and consistent SIV model of HIV CNS disease. To date, all macaques in this model develop AIDS, but only 69% develop moderate/severe CNS lesions. Furthermore, macaques with moderate/severe CNS lesions have a statistically significant increase in CSF CCL2 levels and ratios of CSF:plasma CCL2 compared to macaques with no/mild CNS lesions. The CCL2 promoter of 29 pigtailed macaques was sequenced to identify nucleotide variation that may regulate CCL2 transcription and correlate with CCL2 levels in vivo. Variation within the population was further examined to correlate specific genetic variation with the incidence and severity of SIV CNS disease. Sequence alignment revealed that pigtailed macaques are 94% identical to humans at the CCL2 promoter and contain 39 sites of variation. We have identified no correlation between genetic variation in the CCL2 promoter and CCL2 levels in vivo or the incidence and severity of SIV CNS disease. These data suggest that, in this SIV model, determinants of SIV neuropathogenesis may be distinct from CCL2 promoter variation.

P216

IGF-I protects differentiated PC12 neurons from high glucose and TNF-alpha–mediated accumulation of reactive oxygen species (ROS)

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In HIV patients, both the virus itself and the use of antiviral medications contribute to metabolic abnormalities. In particular, initiation of protease inhibitors–based anti retroviral therapy is closely associated with the development of insulin resistance in 25–60%, and the development of new onset of diabetes in 6-7% of HIV-1 seropositive individuals. Since this apparent metabolic complication was associated with a significant increase of dementia, we have initiated series of experiments to evaluate possible synergy between diabetes and HIV infection, and its effect on neuronal injury. Our results indicate that both high glucose (25 mM for 16 hours) and prolonged exposure to TNF-alpha (50 nM for 3–5 days) elevated the accumulation of reactive oxygen species (ROS) in differentiated neuronal cultures of PC12/GR15 cells. The accumulated ROS were found in neuronal processes and in perinuclear cytoplasm, and resulted in a gradual loss of neuronal extensions. We have also found a synergy between TNF-alpha and high glucose in the process of ROS accumulation and neuronal damage. In the presence of IGF-I (50 ng/ml), however, differentiated PC12/GR15 neurons did not accumulate ROS above the control level, and were partially protected from neuronal degeneration triggered by a simultaneous action of high glucose and TNF-alpha. Our preliminary screening of the signaling pathways points at the p66shc—FOXO3a signaling axis as a possible mechanism by which IGF-IR exerts its anti-ROS function. In summary, our data indicate that high glucose and TNF-alpha may contribute to ROS –mediated neuronal damage, and confirm our previous observation with mesangial cells, which show that IGF-I antioxidant properties may be mediated by the p66shc—FOXO3a signaling pathway and could play a significant role in neuroprotection.

P217

HHV-6 reactivation in patients with encephalitis of unknown origin

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Encephalitis is a devastating neurologic condition with significant morbidity and mortality. Over 100 different infectious agents are associated with encephalitis, yet little is known about risk factors, long-term prognostic indicators and optimal clinical management. The California Encephalitis Project (CEP) was initiated in 1998 to provide rapid, state-of-the-art diagnostic testing for human encephalitis to better understand encephalitis in humans, including risk exposures, symptomatology, and clinical features. Recently, several cases of HHV-6 associated encephalitis have been reported in immunocompetent adults identified through the CEP. HHV-6 is a neurotropic virus that has been associated with a number of neurological disorders including meningitis/encephalitis, seizures, and multiple sclerosis. Particularly, a number of studies have suggested that reactivation of HHV-6 may be associated with neurological complications post bone marrow transplant. To evaluate whether infection or reactivation of HHV-6 could, in part, play a role in the pathoetiology of encephalitis, we examined cerebrospinal fluids (CSF) samples from patients who enrolled in the CEP, for evidence of cell-free HHV-6 viral DNA using PCR amplification. HHV-6 DNA sequences to multiple regions of the virus were amplified from a subset of individuals tested. Sequence analysis demonstrated homology to HHV-6 variant A or B in the PCR positive samples. Analysis of HHV-6-specific IgG and IgM in CSF using a highly sensitive novel electrochemiluminescence assay that use antibody-tagged labels that emit light when electrochemically stimulated is ongoing. Preliminary results demonstrated elevated anti-HHV-6 IgG in the CSF of approximately 38% of individuals tested suggesting possible reactivation of HHV-6. This assay has been adapted to detect HHV-6A p41/38 early antigen from supernatants of U1102 infected SupT1 cells. Of interest, is the observation that HHV-6A specific p41/28 antigen was demonstrated in serum from one encephalitis patient with PCR amplifiable HHV-6 sequences in CSF and brain (biopsy). Collectively, these results suggest that HHV-6 may be an under appreciated viral etiological agent in patients with encephalitis.

P218

The receptor tyrosine kinase RON represses HIV transcription in monocytes/macrophages by multiple-mechanisms

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It is critical to understand the molecular events that establish and maintain latency if strategies to purge HIV from latent reservoirs will be devised. However, such studies have been hampered by the rarity and inaccessibility of latently infected cells. We

hypothesize that signals which influence cell activation, survival, proliferation and maturation will have a direct impact on HIV transcription including extinguishing HIV transcription and establishing latency. To gain insights into signals that repress HIV transcription, we have explored mechanisms by which the receptor tyrosine kinase RON, a critical regulator of inflammation and tissue resident macrophage function, extinguishes HIV transcription. These studies extend the original observation that RON represses HIV transcription by using chromatin immunoprecipitation experiments to demonstrate that RON signaling alters recruitment of NF-kB to the HIV long terminal repeat, inhibits transcription elongation by establishing a paused RNA polymerase II complex and alters chromatin remodeling. These results show that multiple biochemical pathways are required for establishing latency in macrophages and suggest models for how cellular reservoirs for HIV are maintained in different tissues including the brain.

P219

Distinct CD8+ T cell subpopulations in response to acute HSV-1 infection

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Class I MHC-restricted CD8+ T cells play a crucial role in the control of HSV-1 within both the primary mucocutaneous tissues where infection is initiated, and within the peripheral nervous system (PNS) where HSV-1 targets sensory neurons and enters into a latent state of infection from which it may reactivate. It is now known, using murine models of infection, that HSV-1-specific CD8+ T cells alone are sufficient to limit HSV-1 replication in the skin, and that they also take up long-term residence within the sensory ganglia, presumably to be available to control HSV-1 as it reactivates from latency. The lack of a functional T cell response renders mice highly susceptible to infection, which results in the entry of HSV-1 into the central nervous system (CNS), infection of motor neurons, paralysis and death. The purpose of the studies reported here were to utilize flow cytometric analysis to determine the characteristics of the HSV-1-specific CD8+ T cell response, identified by binding of class I MHC tetrameric reagents specific for the T cell receptor specific for the immunodominant epitope recognized in C57BL/6 (B6) mice, during the initial phase of infection. Two subpopulations of CD8+ T cells were found: subpopulation 1, expressing the CD8+ CD44hi CD25+ CD62Llo phenotype, which resemble a "classical" effector T cell; and subpopulation 2, expressing the CD8+ CD44hi CD25- CD62Llo phenotype, which appear to be an alternative effector T cell. These studies will help determine the role of each subpopulation, their relationship to each other, and their contribution to long-term immunological memory.

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Neuroprotective function of Ginkgo biloba extract EGb761 against HIV-1 Tat-induced neurotoxicity: Involvement of glial fibrillary acidic protein

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HIV-1 infection of the central nervous system often results in a variety of neurological diseases including HIV-associated dementia. HIV-1 Tat protein has been shown to be an important contributing factor to HIV-associated neuropathogenesis. Despite the fact that several hypotheses have been proposed for Tat neurotoxicity, it is clear that the underlying molecular mechanisms are still evolving. Our recent studies have linked Tat expression to astrocytosis characterized by up-regulated expression of glial fibrillary acidic protein (GFAP), astrocyte dysfunction, and neuron death. These findings raise for the first time the possibility that Tat neurotoxicity is directly initiated by astrocytosis. The goal of the current study was to determine whether pharmaceutical intervention to block Tat-induced GFAP expression would alleviate Tat neurotoxicity. Using the inducible brain-targeted Tat transgenic mouse model, we found that Ginkgo biloba extract EGb 761, which is widely used to treat various neurodegenerative diseases, protected the mouse brain from Tat expression-induced neuropathologies. We then showed that EGb761 down-modulated GFAP expression at both mRNA and protein levels without any effects on astrocyte proliferation and survival. We also showed that the down-modulatory effects of EGb761 on GFAP was mediated by two major transcription factors AP1 and NF-(κ)B that are located within the GFAP promoter. To further ascertain the neuroprotective function of EGb761 against Tat neurotoxicity, we are currently crossbreeding the Tat transgenic mice with GFAP knockout mice and wish to determine whether Tat-induced neuropathologies would be significantly affected by the absence of GFAP gene expression. These studies may not only shed new lights on molecular mechanisms of Tat neurotoxicity, but also provide a new treating modality for neurodegenerative diseases involving astrocytosis.