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# HIV dementia

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## P132

### A novel action of minocycline: infection in microglia and macrophages

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AIDS-dementia complex (ADC) is a neurological disorder caused by HIV-1 infection of microglia and macrophages and subsequent activation and degeneration of glial and neuronal components. Currently, HAART is unable to eradicate HIV-1 infection. Therefore, additional drugs with anti-viral and anti-inflammatory activity are needed. In this report, we demonstrate that minocycline (MC), a second generation tetracycline with proven safety and CNS penetration, potently inhibited HIV-1 replication in microglia and macrophages. Inhibition of infection by MC was observed dose-dependently with IC50 at 20 micrograms per milliliter. Reduction in p24 release by MC was sustained through the entire course of infection (up to 4 weeks), even when MC exposure was limited to the first 24 h of infection. MC was effective even at low viral MOI, and against R5- and X4R5-HIV, as well as HIV- and VSV-G-env pseudotyped reporter viruses. Astrocyte infection was also inhibited. These results are consistent with MC inhibiting entry as well as post-entry mechanisms of infection. Further studies indicated that MC inhibited HIV binding to microglia and subsequent activation of NF-kappaB and reactive oxygen species. Additionally, viral expression in chronically-infected U1 cells and the LTR-promoter activity in U38 cells were inhibited by MC. Together, our results demonstrate anti-viral and anti-inflammatory properties of MC in a broad range of target cells and have implications for the therapy of HIV infection.

## P133

### The HIV-1 protein Tat activates CXCR4 and induces the release of matrix metalloproteinases

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HIV-1 proteins are thought to contribute to the neurological manifestations of HIV-1 infection through actions on neurons and glia. In previous studies we have shown that the HIV-1 protein Tat induces the death of neurons by mechanisms that are dependent on the activation of a pertusis toxin-sensitive G-protein coupled receptor and the release of calcium from IP3-sensitive calcium pools. Inhibition of phospholipase C or IP3-receptors protected neurons from the toxic effects of Tat. We now provide evidence that the effects of Tat on calcium release and neuronal cell death are mediated by actions at the chemokine receptor CXCR4 and may involve the subsequent release of matrix metalloproteinases (MMPs). Inhibitory antibodies directed against extracellular domains of CXCR4 but not CCR5 prevented Tat-stimulated increases of calcium in neurons and astrocytes and significantly reduced neuronal cell death. One consequence of CXCR4 stimulation is the release of MMPs. When we added Tat to mixed neuron/astrocyte cultures we observed a significant increase in the release of MMP-1, 2 and 9 at time points preceding a significant increase in the death of neurons. MMP-1 applied onto isolated neurons resulted in a significant increase in the percentage of apoptotic neurons while the addition of MMP-9 significantly increased the percentage of necrotic neurons. Thus, Tat may induce neuronal dysfunction and death by activating CXCR4 and inducing the release of MMPs.

## P134

### Tat-producing C6 glioma cells induce local and transynaptic alterations in rat brain—implications for HIV dementia

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Background: HIV dementia is characterized by progressive motor, cognitive, and behavioral deficits, but the responsible virological and/or host agents have not been identified. Accumulating evidence suggests that the viral transactivating protein Tat may participate in the development of HIV dementia, and indeed, data from our labs has shown that Tat is potently neurotoxic and proinflammatory in cultured cells. Methods: To determine the consequences of Tat production in intact brain, we genetically engineered rat C6 glioma cells to stably produce Tat1-86 (or vector), and stereotaxically infused them into the striatum or hippocampus of adult male rats. Results: Animals injected with Tat-producing C6 cells, but not with vector cells or vehicle, into the striatum showed significant deficits in a rotorod task of motor performance, and additionally, striatal injections induced alterations in tyrosine hydroxylase, GFAP, and CD59 immunoreactivity in the ipsilateral substancia nigra. Likewise, injections of Tat-producing C6 cells, but not vector cells or vehicle, into the hilar region of the dorsal hippocampus increased the

expression of markers of neurotoxicity and inflammation in CA3 pyramidal cells and adjacent dentritic fields. Examination of patterns of Tat-immunoreactivity in animals injected with Tat-producing cells suggests that Tat is both secreted locally at the injection site, and is also transynaptically transported to adjacent brain regions. Conclusions: Our studies indicate that Tat production in rat brain can result in both behavioral and histopathological aberrations that model changes occurring in HIV encephalitis, suggesting that Tat may be an important participant in brain dysfunction in HIV dementia. These results also suggest that Tat can be transported synaptically in the brain, highlighting a potential role for Tat in specific synaptic dysfunction.

## P135

### Activation of NMDA receptor subtypes NR1/NR2B by macrophage secretory products: a possible neurotoxic pathway for HIV-1 dementia

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Background: Activation of N-methyl D-aspartate (NMDA) receptors is thought to play important roles in HIV-1 mediated neurotoxic activities. Recent reports suggest that neuronal death may be prevented by NMDA receptor antagonists. However, whether these receptors are engaged in a biologically relevant systems for HIV-1 dementia and play prominent roles for neuronal demise during disease is not yet known.

Objective: Therefore, we investigated whether secretory products produced during natural infection of human macrophages by HIV-1 could activate the NMDA receptor subtypes NR1/NR2B.

Methods: Human monocyte-derived macrophages (MDM) were infected with the macrophage tropic virus HIV-1ADA and/or stimulated with CD40 ligand (CD40L) to reflect the viral infection and immune activation that occurs in brain tissue of infected subjects with dementia. Supernatants were collected 7 days after viral inoculation (multiplicity of infection was 0.1) and applied to oocytes by pressure ejection. The mRNAs encoding NR1 and NR2B were mixed at a ratio of 1:3 and injected into oocytes. NMDA receptor conducted inward currents were recorded by two-electrode voltage clamping.

Results: Pressure ejection of HIV-1-infected and CD40L-stimulated MDM fluids produced significant inward currents in oocytes over-expressing NR1/2B ($30.2 \pm 5.1$ nA, $n = 30$, Mean $\pm$ S.E.), but not in uninjected cells. In contrast, application of uninfected and unstimulated MDM fluids induced currents of $4.5 \pm 0.5$ nA ($n = 7$). The differences were significant ($p < 0.05$). Infected or stimulated uninfected MDM showed intermediate responses. The supernatant-induced currents were dose-dependant and blocked by then NMDA receptor antagonist APV (0.05 mM, $p < 0.05$), but not by the non-NMDA receptor blocker CNQX (0.02 mM, $p > 0.05$). Addition of L-glutamate decarboxylase to MDM fluids did not change the MDM fluids-induced inward currents.

Conclusions: These data suggest that the secretory factors from HIV-1-infected MDM activate NMDA receptor subtypes and that this mechanism may contribute to the neuropathogenesis of HIV-1 infection.

## P136

### IDO: the keeper of NeuroAIDS?

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Indoleamine 2,3-dioxygenase (IDO), the first and rate limiting enzyme in tryptophan catabolism in extrahepatic tissues, can lead to neurotoxicity through the generation of quinolinic acid. Furthermore, IDO-induced local depletion of tryptophan can lead to immunosuppression and alterations in brain chemistry. Since central nervous system (CNS) quinolinic acid levels are linked to CNS dysfunction and pathology in HIV and SIV infection, we sought to determine the regulation of IDO both *in vitro* in microglia, and in the brains of SIV-infected rhesus monkeys. Two groups of juvenile rhesus monkeys were infected with SIVmac182. One group was treated with an anti-CD8 antibody during the acute stage of the disease to exercabate rapid disease progression (3–4 months to AIDS), whereas the other group was treated during a chronic stage of infection, and sacrificed at >18 months p.i. These two groups were compared to uninfected control monkeys. Using real-time RT-PCR, *in situ* hybridization and immunohistochemistry, we demonstrate that the expression of IDO occurs in the brains of SIV-infected monkeys, and that the levels are highest in animals with SIV encephalitis. Examination of SIV viral load, TNF-a and IFN-g mRNA levels revealed that the level of IDO expression correlates best with IFN-g expression. IDO mRNA is found within microglia nodules, which largely contain activated (MHC-II positive) cells of the macrophage lineage, as well as scattered LCA-positive lymphocytes. Individual cells expressing IFN-g can be found within the IDO-positive nodules. *In vitro* studies on monkey and mouse microglia reveal that IFN-g is the most potent stimulator of IDO expression, and further stimulation with CD40-L and TNF-a only reveal a slight augmenting effect on IFN-g stimulation. These findings demonstrate the link between IDO expression, IFN-g levels, and brain pathology in neuroAIDS, and suggest a link between IDO expression, T-cell activity and viral persistence in the brain during HIV/SIV infection.

## P137

### Transcriptional regulation by HIV, Tat, and gp120 in astrocytes

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Given the large number of astrocytes in the CNS, we hypothesized that they may have a key role in the production of cytokines and chemokines in response to the presence of HIV-1, which have been implicated in neurological complications associated with HIV-1 infection. To characterize the effects of HIV-1 infection or exposure to its proteins Tat and gp120 on astrocytic transcription, RPA and microarray analysis (immuno- and neuro-specific arrays) of astrocytes were performed. Human fetal astrocyte cultures were treated with Tat (aa 1-72) (125 nM) or gp120 (250 pM) for 6 hours or infected with the HIV strain NL4-3 for 3 days. RNA extracts were analyzed by RPA. Fold induction was calculated after standardizing. RPAs were run in triplicate and

Student's t-tests were performed ($p < 0.05$ considered significant). For microarrays, radiolabeled cDNA was synthesized and probed against 1153 oligos per array. Z-score normalization was performed (standard deviation >1.5 considered significant). Infection of astrocytes by HIV or treatment with Tat and gp120 has a profound affect on transcription. RPAs indicate that, in general, the only significant chemokine and cytokine induction occurred by infection with HIV or Tat treatment. Tat and HIV both induced IL-6 (3-7x). The Tat group showed induction of IL-1b (20x), IL-8 (250x), RANTES (16x), I-309 (16x) and IFN-g, MIP-1b, IP-10, and MCP-1 (3-10x). The immuno-array revealed 123 genes were modulated by Tat and HIV infection. Gp120 affected 148 genes. From the neuro-array, 459 genes were affected by Tat, 120 genes by gp120 and 353 genes by HIV infection. Across both arrays, only 8 genes were modulated by all 3 treatments. Interestingly, a differential effect was found between the exogenous treatments and infection. 5 genes were activated by the proteins, but not by infection. 13 genes were suppressed by the proteins, but activated by infection. Astrocytic dysfunction occurs in response to HIV infection or exposure to viral proteins. The pattern of chemokine and cytokine production suggests that these cells may play an important role in the pathogenesis of HIV dementia.

## P138

### HIV-1 Tat and morphine synergistically disrupt striatal astroglia function *in vitro*

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An increased incidence and severity of HIV encephalitis among opioid drug abusers suggests a relationship between HIV and drug abuse. We previously found synergistic neurotoxicity with morphine and HIV-1 Tat exposure in striatal cultures. However, because the cultures in these experiments contained mixed cell types, it was uncertain whether disruptions in glial function contributed to the neurotoxicity. Astroglia express mu-opioid receptors and provide neuronal trophic and metabolic support. To assess the effect of opioids and Tat on astroglia, mouse striatal cell cultures enriched in astroglia were continuously treated with morphine (500 nM or 1 mM) and/or Tat (1-72) (100 nM). Astroglial function was evaluated by measuring intracellular Ca2+ ([Ca2+]i) and mitochondrial membrane potential (MMP). Cell viability was assessed by ethidium homodimer exclusion (24 and 96 h). Combined morphine-Tat exposure synergistically destabilized [Ca2+]i at 1 h following treatment. Furthermore, MMP, measured by JC-1, was also destabilized at 2 and 4 h with combined treatments. The effects of morphine-Tat exposure were attenuated by opioid receptor antagonists. Despite changes in [Ca2+]i and MMP, astroglial viability was unaffected by combined exposure at 24 h. In contrast, significant astroglial death was noted at 96 h; however unlike cell homeostasis, the combined toxic effects of morphine and Tat were additive. This suggests potential intrinsic temporal differences in astroglial responsiveness to combined morphine-Tat exposure, which initially include losses in ion homeostasis and later include overt toxicity. Collectively, our results suggest that astroglia are a principal target for opioid-HIV interactions and that synergistic disruptions in astroglial function might secondarily result in astroglial or perhaps neuronal death. Support: NIH DA13559, DA13728, and NS39253.

## P139

### Characterization of SIV long terminal repeat cis-acting sequences that interact with CCAAT/ enhancer binding protein transcription factors

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Two CCAAT/enhancer binding protein (C/EBP) sites within the human immunodeficiency virus type 1 (HIV-1) long terminal repeat (LTR) are critically important for viral replication in cells of the monocyte/macrophage lineage. These cells are likely important in transportation of virus into the CNS, generation of neurotoxic viral proteins, dysregulating intra- and intercellular CNS signaling pathways, and producing infectious virus. To better understand HIV-1 disease in humans, a number of animal model systems, including the simian immunodeficiency virus (SIV)/macaque model, have been used to address basic questions concerning retroviral pathogenesis. To assess the importance of C/EBP binding sites in retroviral pathogenesis, studies have been initiated to identify and characterize SIV LTR sequences with affinity for members of the C/EBP transcription factor family. Using electrophoretic mobility shift (EMS) and footprint analyses, four SIV LTR sequences with affinity for human monocytic C/EBP alpha and beta have been identified. C/EBP upstream site 1 (US1), upstream site 2 (US2), downstream site 1 (DS1), and downstream site 2 (DS2) are located at nucleotides −102 to −88, −386 to −373, +131 to +144, and +265 to +280, respectively. These sites were examined using competition and supershift EMS analyses, which demonstrated that C/EBP DS1 and DS2 exhibit high affinity for C/EBP alpha and beta. By comparison, both US1 and US2 exhibit lower affinity for C/EBP and may interact with other transcription factors. C/EBP US1 lies immediately upstream of a series of Sp elements and partially overlaps an NF-kappa B site, suggesting that C/EBP factors bound to this site may be involved in complex interactions with Sp and NF-kappa B factors with important roles in monocytic differentiation and cellular activation. Numerous sequence variants of each site, derived from over 100 LTRs cataloged in the Genbank and Los Alamos National Laboratory sequence databases, have been identified and are being characterized for relative binding affinity. Future studies will examine the functional impact of the SIV C/EBP sites and their variants on viral gene expression and replication.

## P140

### Involvement of dopamine in the pathogenesis of HIV-induced Neuro-AIDS

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HIV-infection causes a variety of neurological symptoms referred to as Neuro-AIDS. Its symptoms include motor,

cognitive and behavioral deficits that are typical manifestations of subcortical dementias. To study the mechanism of HIV-induced neurodegeneration, we performed experiments with SIV-infected macaques. SIV-infected monkeys develop similar immunological (AIDS) and neurological symptoms (Neuro-AIDS) as observed in HIV-infected humans. Reduced levels of dopamine were observed already in the asymptomatic phase of SIV infection in various dopaminergic brain regions. However, substantia nigra, an area enriched with dopaminergic cell bodies, seemed to be spared. These findings indicate that a dysfunction in postsynaptic dopaminergic areas may cause reduced dopamine levels. To restore the dopamine deficiency in SIV-infected monkeys, we treated the animals with dopaminergic drugs, such as selegiline or L-DOPA. Both treatments increased dopamine levels to normal concentrations. However, treated animals showed higher virus load in the brain and developed vacuolization of the grey subcortical matter. Moreover, SIV-specific histopathologic lesions were increased. Selegiline treatment of infected monkeys also induced TNF-a expression in microglia. Our findings suggest that an increase in dopamine availability may lead to microglia activation resulting in enhanced viral replication and production of neurotoxic substances. To elucidate whether dopamine directly affects virus replication, we incubated persistently HIV-infected cells with dopamine. We found that dopamine activates HIV replication in a dose dependent manner. Addition of antioxidants such as glutathione or N-acetylcysteine abolished this effect, indicating that changes in the redox state mediate enhancement of HIV- replication by dopamine.

Our results suggest that dopamine-induced oxidative stress may be involved in the pathogenesis of Neuro-AIDS. As drugs of abuse increase dopamine availability, our findings might explain the increased incidence of HIV-encephalitis in this risk group and generate concerns about the safety of dopaminergic drugs in the clinical management of HIV-infected patients.

## P141

### Induction of HIV-1 envelope associated neuropathogenesis using a Sindbis virus expression vector

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Background: The HIV-1 envelope protein is known to activate signaling pathways resulting in the release of molecules with a neurotoxic action, ultimately culminating in neuronal degeneration or death. Cells of glial lineage in the brain are considered a major source of these neurotoxins.

Objective: To study HIV-1 envelope associated neuropathogenesis, using a recombinant Sindbis virus (SIN) expressing the envelope of HIV-1.

Methods and results: A recombinant Sindbis virus (SIN) expressing the envelope from the neurovirulent HIV-1 strain JR-FL was constructed, together with a control vector expressing the enhanced green fluorescent protein (EGFP). Both SIN vectors infected human neural cell lines, as well as human primary neural cells relevant to HIV-1 neuropathogenesis. Infection of human cholinergic neurons by the HIV-1 envelope expressing SIN vector resulted in increased cell death (13.8 +/− 1.6%) compared to the control vector (6.0 +/− 0.7%) ($p < 0.001$) after 24 hrs. In addition, conditioned media (CM) from primary human astrocytes infected for 24 hrs with the SIN vector expressing the HIV envelope induced extensive neuronal death (45.5 +/− 1.8%) *in vitro* compared to control (4.1 +/− 0.6%) ($p < 0.001$), while CM of human monocyte derived macrophages (MDM) infected with the HIV envelope expressing vector resulted in a moderate increase in neuronal death (7.6 +/− 1.1% vs. 5.0 +/− 0.2% ($p < 0.001$)). Moreover, implantation of the HIV-1 envelope containing vector into the brains of mice elicited a strong inflammatory response together with neuronal loss, which was not observed upon implantation of the EGFP expressing recombinant SIN vector or conditioned media of uninfected cells.

Conclusion: Our findings indicate that SIN expression vectors can be used for studying HIV-1 neuropathogenesis both *in vitro* and *in vivo* and suggest that astrocytes are an important source of neurotoxins as a result of HIV-1 envelope expression.

## P142

### Neuropathogenesis of lentiviral infection in macaques: roles of CXCR4 and CCR5 viruses and interleukin-4 in enhancing monocyte chemoattractant protein-1 production in macrophages

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Neurological disease associated with lentiviral infection occurs mainly as a consequence of primary viral replication or a combination of virus infection and replication of opportunistic pathogens in the central nervous system. Recent studies have shown that whereas the disease can be caused by CCR5 viruses alone, its induction by CXCR4 viruses occurred usually in association with infections caused by opportunistic pathogens and in the presence of a TH2 cytokine, interleukin (IL)-4. Further, CXCR4 virus-mediated neurological disease developed preferentially in rhesus comapred to pig-tailed macaques. Since macrophages are the target cells for lentiviral infection in the brain, and since macrophage chemoattractant protein (MCP)-1 is one of the major chemokines that is closley associated with AIDS dementia, we tested for correlations between MCP-1 production and virus tropism in macrophages from the two species of macaques. The studies showed that the higher susceptibility of rhesus macaques to CXCR4 virus-mediated encephalitis correlated with heightened production of virus and MCP-1 in cultured macrophages from this species and that these effects were further enhanced by treatment with IL-4. Interestingly, the latter effect was restricted to macrophages inoculated with CXCR4 viruses, but not with CCR5 viruses. Further corroboration of these *in vitro* findings was demonstrated by enhanced MCP-1 expression in the brain lesions and CSF of rhesus macaques infected with a CXCR4 virus.

## P143

### RNA preservation in post-mortem cerebral cortex of AIDS and control brains

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Gene expression in HIV-associated dementia (HAD) and HIV encephalitis may offer important clues to their pathophysiology and treatment. Because it is dependent on extraction of intact ribosomal RNA from post-mortem brains, we determined the relationship between post-mortem interval (PMI) and brain pathology with intact ribosomal RNA and 5′:3′ end ratios of actin and GAPDH mRNA, using commercial kits for RNA extraction, analysis and amplification and oligonucleotide microarrays. Temporal cortical samples were obtained in part from the National NeuroAIDS Tissue Consortium (MH59656 and MH59724) and the U Miami Brain and Tissue Bank (HD39117). Intact 18S and 28S ribosomal fractions, determined by RNA analysis chips were found in 7 of 8 controls (PMIs of $16.7 \pm 3.6$ hr) and 10 of 16 AIDS cases (PMIs of $10.3 \pm 6.4$ hr). Neuropathology included two with acute ischemic changes in hippocampal neurons. PMIs in cases with degraded RNA were 15 h in one control with a normal brain and between 3 and 20 hr (mean $8.1 \pm 6.5$) in 6 AIDS cases; their neuropathology included HIVE in two, CMV in one, Alzheimer type II astrocytosis in two and normal brain in one. Repeated dissections and RNA extractions in 6 cases reversed the results of the RNA integrity studies in 5. Microarray analysis of 5′:3′ ratios of actin or GAPDH was done in 12 cases with intact ribosomal RNA.

Results were deemed satisfactory (ratio between 1–2) in 5 cases whose PMIs averaged $15.3 \pm 6$ and unsatisfactory in 7 cases, whose PMIs averaged $8 \pm 3.7$ h. There was no obvious correlation between neuropathology and RNA degradation. Preliminary analysis of oligonucleotide microarrays found gene expression changes for factors that may participate in repair of tissue injury. These results show a poor correlation between PMI and intact ribosomal RNA and between intact ribosomal RNA and satisfactory hybridization of housekeeping genes. They also suggest that repeat dissection of the same tissue may yield positive results if the first isolation is unsatisfactory. Supported by RO1-NS39177.

## P144

### HIV-1 Tat protein-mediated degeneration of rat hippocampal neurons: possible role for oxidative damage

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HIV infection is associated with dementia in a significant number of AIDS patients. HIV-associated dementia complex is a serious disabling disease characterized by cognitive, behavioral and motor dysfunction. Neuronal dysfunction and cell death in patients with human immunodeficiency virus type 1 (HIV-1) infection may be mediated by HIV-1 proteins and products released from infected cells. In this study, we investigated neurotoxic effects of HIV-1 transactivating protein Tat in the hippocampus of newborn and adult rats and in primary culture of rat hippocampal neurons. Microinjection of Tat into the hippocampus of newborn Sprague-Dawley rat pups caused alterations in animal behavior. Following 8 months after operation, neuropathological evaluation demonstrated significant decrease of neuronal cell density in the hippocampus of Tat-injected animals. Tat-mediated neurodegeneration was also evident when adult rats recieved multi-site injections of Tat into the hippocampus. Tat (400 nM) was shown to be toxic to rat hippocampal neurons in primary culture and its toxicity was associated with increased protein carbonyl formation. results of our study suggest a role for oxidative stress and oxidative damage of hippocampal neurons in mechanism of tat-induced behavioral dysfunction. This work is supported in part by NIH grants DA 11337, DA 09160, DA 13137.

## P145

### Gender and HIV related neurological disease

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Objective: Prior reports have suggested that there may be a gender difference in nervous system effects of HIV infection. We present the current results from a longitudinal study addressing this question.

Methods: At baseline, thirty-six HIV+ females, forty-seven HIV-females, and fifty-one HIV+ males were compared for age, education, absolute CD4 cell count, and plasma/CSF HIV RNA. Subjects were evaluated by neurological and neuropsychological examination every year.

Results: As expected, both male and female HIV+ groups had poorer function on the neurological and neuropsychological examination than the HIV-group ($p < .05$). At entry, no gender differences were found in plasma (males 3.31 log cp/ml, females 2.99 log cp/ml) or CSF HIV RNA (males 1.57 log cp/ml, females 1.12 log cp/ml) viral load or neurological examination in infected subjects. No significant differences in neurological or neuropsychological functioning over time were noted between the HIV+ males and females ($p < .01$).

Conclusions: Prior retrospective chart reviews found increased incidence and prevalence of AIDS Dementia in women. This study reports the two and three year follow-up of a cohort developed to address this question in a prospective fashion. Our findings show little difference in neurological functioning between HIV+ females and males when controlling for relevant factors such as antiretroviral use. It is not clear why this refutes the earlier concerns about a more rapid progression in women. One possibility for the difference is that early reports were biased because women were at a more advanced stage in the disease course at HIV diagnosis when nervous system involvement is more likely.

## P146

### HIV-1 induces MCP-1 production in human fetal astrocytes: requirement for virus replication

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It has been demonstrated that astrocytes produce MCP-1 upon stimulation with exogenous HIV-1 protein Tat and that Tat protein levels in CSF of AIDS patients with dementia are elevated. We were interested to investigate direct role of HIV-1 in production of MCP-1, in astrocytes. In previous experiments we demonstrated that bypassing requirement for receptor specificity, astrocytes are productively infected by pseudotyped HIV-1. Utilizing this newly developed model of productive HIV- 1 infection, human astrocytes were infected and tested for MCP-1 production by ELISA. We demonstrate here that HIV-1 replication stimulates MCP-1 production to levels that are comparable to stimulation with exogenous IL-1beta or TNF-alpha. Virus replication peaked on Day 7 with MCP-1 production reaching highest levels on Day 10 post infection, at 300 ng/ml, and declining to background levels thereafter. Infection of astrocytes with native NL4-3, UV inactivated virus or cultures treated with ddC, failed to induce MCP-1 production. Treatment of cultures with 10 nM exogenous HIV-1 gp120 also failed to induce MCP- 1 secretion. However, transfection experiments with CMV-Tat DNA induced MCP secretion from astrocyte cultures demonstrating role of HIV-1 protein Tat. We conclude that MCP-1 production in productively HIV-1 infected astrocyte cultures requires active virus replication and that Tat may play a role in this induction.

## P147

### Stroke and traumatic brain injury in nursing home residents with HIV dementia

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Objective: To determine if co-morbidities of stroke or TBI are associated with demographic, health, and treatment characteristics for people with HIV dementia (HIVD). Methods: We compared profiles of nursing home residents with HIVD to profiles of residents with HIVD with stroke and/or traumatic brain injury (TBI). The profiles were created using admission assessments from the Minimum Data Set (MDS), which includes almost all nursing home residents in the United States. These profiles include demographic characteristics, cognitive patterns, ability to communicate, behavior, health conditions, disability, and treatments received. We analyzed 1,405 MDS admission assessments for residents with HIVD from June, 1998 through December 2000. Of these HIVD residents, 1,222 had neither stroke or TBI, 92 had both stroke and TBI, 79 had stroke without TBI, and 12 had TBI without stroke. Given the large number with both stroke and TBI, and due to sample size issues, we combined residents with stroke and/or TBI into one group and residents without TBI or stroke into another group for analyses. Results: Those without stroke/TBI were more likely to be male,1 younger,1 and black1 than those with stroke/TBI. There were no differences in cognitive performance,2 memory,2 or indicators of delirium2 between the two groups, although those without TBI/stroke were more likely to be understood1. More than half of those with TBI/stroke had delusions1 and half had hallucinations1. There were no differences in verbally abusive2 or socially inappropriate behaviors,2 although those without TBI/stroke were more likely to resist care3 and this behavior was not easily altered3. Those with stroke/TBI were much more physically dependent1, more likely to experience an acute episode1, and to be at end-stage disease1. Those with TBI/stroke were much more likely to receive alcohol/drug treatment,1 to be in an Alzheimer's/dementia unit,1 and to receive hospice care1. Conclusions: Stroke and TBI are frequent in nursing home residents with HIVD and have a significant impact on neurological outcome and health care needs.

1 < 0.001; 2 not significant (>0.05); 3 < 0.05.

## P148

### A case study of HIV encephalitis: the utility of neuropsychologic (NP), neuromedical (NM), and neuroimaging (NI) evaluations in detecting early HIV brain involvement

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Objective: To describe ante-mortem NP, NM, and NI and post-mortem neuropathologic findings in a dated HIV seroconverter who died with HIV encephalitis (HIV-E).

Methods: The subject tested HIV seronegative in 6/89, and seroconverted in 6/90. We performed 3 NM, 3 NP, and 2 NI assessments between seroconversion and death (2000). A comprehensive neuropathologic assessment was performed. All NP, NM, NI and post-mortem examinations followed standardized protocols, and were administered in a University research setting.

Results: In 1990, the subject took ZDV, his CD4 count was 33, and his HIV RNA levels were 4.3 log copies/mL in plasma (pRNA) and 2.3 in CSF (cRNA). Although NM and MRI findings were normal, NP results identified definite mild global impairment. By 1996, he was taking combination antiretroviral therapy (ART), remained immunosuppressed, and was disabled. The mental status was normal, but the NM exam indicated that he had diffuse motor slowing. NP results now revealed moderate-severe impairment consistent with HIV-associated dementia (HAD). NI findings confirmed moderate volume loss with diffuse white matter damage. In 1998, he continued ineffective ART, but did not complete his NM exam. NP testing revealed severe impairment. At his final ante-mortem visit (2000), he had stopped ART, his CD4 count was 14, pRNA 6.2 and cRNA 3.9. The NM exam indicated abnormal cerebellar signs and hyperreflexia. He was too ill to complete NP testing, but his performance on the HIV Dementia Scale was severely impaired. Autopsy (3 months later) revealed marked white matter loss, high brain viral load, extensive dendritic damage and multi-nucleated giant cells consistent with HIV-E.

Conclusions: In this HIV-infected patient who progressed from normal to severely impaired cognitive function over 10 years, NP evaluations indicated brain injury earlier than NM or NI examinations. NP testing may identify individuals at risk for future HAD, and inform treatment in early HIV disease.

## P149

### Neurocognitive function among older compared to younger HIV-1 seropositve individuals

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Introduction: Ten percent of AIDS cases have been reported in people over 50 years of age. Given prolonged survival with the advent of HAART, this number is expected to increase; yet fundamental neuro-epidemiological and neurocognitive characteristics of HIV dementia in older individuals have yet to be described.

Methods: Two groups of HIV-1 seropositive individuals are being enrolled: <40 years old and 50+ years old. Annual neurological and neuropsychological exams are being completed. Diagnosis of Minor Cognitive Motor Disorder (MCMD) and HIV-associated Dementia (HIV-D) (American Academy of Neurology 1991 criteria) and dementia severity (Memorial Sloan Kettering criteria) are assigned at consensus conference of 2 neuropsychologists and at least 2 physicians using age/education matched neuropsychological norms. These data are preliminary and based on the first 35 of 200 seropositive individuals enrolled.

Results: No individuals enrolled to date have an MSK of 3 or greater (see table). Eleven percent of younger individuals (2/18) compared to 41 percent of older individuals (7/17) have mild to moderate dementia. ($p = 0.0523$, fisher's exact test) A pattern of increasing degree of impairment is seen in the older compared to the younger group.

Conclusions: These preliminary data suggest increased rates of cognitive impairment and increased severity of impairment in older compared to younger HIV-1 seropositive individuals. These findings could have important implications regarding the impact on clinical care for an emerging population of older HIV-1 seropositive individuals. Additional data from subjects recruited over the next 4 months will be presented.

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| | *Younger group* | *Older group* |
|---|---|---|
| Number per group (n) | 18 | 17 |
| Age (yrs) | 35.8 +/− 3.5 | 59.5 +/− 5.7 |
| Education (yrs) | 13.5 +/− 2.8 | 15.2 +/− 2.2 |
| Impairment rating | | |
| No dementia (MSK = 0) | 8 | 3 |
| Equivocal dementia (MSK = 0.5) | 8 | 7 |
| Mild dementia (MSK = 1) | 2 | 5 |
| Moderate dementia (MSK = 2) | 0 | 2 |

## P150

### CSF viral load, event-related potentials and retroviral therapy

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Introduction: HIV-associated encephalopathy (HIVE) is a major problem and retroviral agents reduce CSF viral load (VL). Analysis of CSF viral load is not performed in routine compared to blood VL. Material and methods: In 30 patients suffering from HIVE since 5+/−3 years besides conventional CSF analysis CSFVL was estimated using branched-technology. Optically event-related potentials were performed according to already published methods. The patient collective consisted out of three groups without any retroviral therapy (n = 10), monotherapy using zidovudine (n = 10) and dual therapy with different agents. Clinical neurological examination in order to detect HIVE was performed in all patients. Results: Comparing patients with and without HIVE CSFVL was highly different, but barely failed statistical significance. CSF white cell count and blood VL was different for patients with and without HIVE ($p < 0.03$).CSFVL and blood VL was different for patients with retroviral agents compared to patients without ($p < 0.05$). CSFVL and blood VL showed a significant correlation ($p < 0.02$). Discussion: Altough some limitation is given by small patient collectives a significant therapeutic impact of retroviral therapy on the central nervous system was found. In patients suffering from HIVE CSFVL and resistance profile should be performed in order to detect the best combination therapy.

## P151

### Measurement of estradiol levels in women with HIV dementia

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Objectives: Estrogen may have a neuro-protective effect in dementing illnesses such as Alzheimer's disease and Parkinson's disease. *In vitro* studies also show that estrogen is neuroprotective against HIV proteins and drugs of abuse; and it also inhibits HIV replication in lymphocytes. Our long term goal is to determine the role of estrogen in the development on HIV dementia. In this preliminar study, we determined estradiol levels in women with HIV dementia.

Methods: Five HIV positive women with a CD4 count below 300 cell/$mm^3$ were studied. Neurological evaluation included the Memorial Sloan Kettering Scale (MSK)and Spanish Version of the HIV Dementia Scale (HDS-S). Blood samples for estradiol and progesterone levels were measured by radioimmunoassay in serum. Findings were analyzed taking into account patients menstrual cycle phase.

Results: Patients have similar age, CD4 count, and menarche. Three patients (#1, 3, 4) were at follicular phase (progesterone <1 pg/ml) and two (#2, 5) at midcycle or luteal phase (progesterone > 1 pg/ml). One patient in the follicular phase presented with the lowest estradiol level, 12.4 pg/ml (normal 10-100 pg/ml), and was the only patient with snout and

| *Patient* | *Age* | *CD4* | *Viral load* | *MSK* | *HDS-S* | *Menarche y/o* | *Estradiol pg/ml* | *Progesterone ng/ml* |
|---|---|---|---|---|---|---|---|---|
| 1 | 38 | 265 | 21,882 | 0 | 13 | 11 | 18.8 | 0.32 |
| 2 | 31 | 118 | 38,154 | 1 | 11 | 12 | 60.9 | 6.22 |
| 3 | 35 | 34 | >750,000 | 1 | 15 | ? | 12.4 | 0.33 |
| 4 | 32 | 54 | 37,076 | 0.5 | 9 | 13 | 31.8 | 0.41 |
| 5 | 36 | 2 | 187,412 | 0.5 | 12 | 11 | 67.2 | 2.8 |

highest viral load and mild dementia. Both patients at midcycle or luteal phase presented with low estradiol levels, 60.9 and 67.2 pg/ml (normal > 100 pg/ml). Amongst these two patients, the only one with the lowest estradiol also scored lower on the MSK and HDS scale. Thus nearly all patients studied had estradiol levels that were below or near the lower limit for normal.

Conclusion: No firm conclusions can be drawn from the small sample size however, estradiol levels were suppressed in this patient group with HIV dementia. The lowest level of estradiol was presented in the patient with the highest viral load. Close attention to gonadal dysfunction in the midcycle or luteal phase may be warrant in future studies.

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## P152

### Predictors of neuropsychological evolution in HIV-1 infected patients in Brazil

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Introduction: Dementia associated with the human immunodeficiency virus is characterized by cognitive, behavioral, and/or motor decline over weeks to months in HIV-1 infected people. However, there is still un uncompleted knowledge concerning to the pathophysiology of this condition and predictive factors associated with an increased risk for the subsequent HIV-1 dementia development.

Objective: This study aims a prospective investigation of the neurpsychological aspects of HIV-1 patients treated with different antiretroviral therapies and deals with the identification of clinical and laboratorial factors associated with neuropsychological evolution in HIV-1 positive subjects.

Methods: Patients included (n = 88) were all antiretroviral teraphy-naive, HIV-1 seropositive, either male or female patients, at least 18 years old with a pre-study CD4 cell count of 50-250 cells/$mm^3$. Information on age, gender, risk group, instruction, neurological and psychiatric history composed the questionary in the first evaluation. A clinical, laboratorial, neurological, psychopatological and a neuropsychological assessment were applied every six months, during 3 years. Data were analysed using descriptive and analytical methods to study patientes neuropsychological evolution before and after different anti-retroviral teraphies.

Results: Patients with previous HIV-1 related symptoms had a significant worse performance in neuropsychological tests. Alcohol use was associated with a worse neuropsychological performance. AZT use was associated with a better performance in logical memory test. Patients in professional activity had a significant better performance in neuropsychological tests during the time of this study. Distinct related factors had influence on neuropsychological evolution before and after anti-retroviral therapy.