



## Investigator in training

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### Increased vulnerability of ApoE4 neurons to chronic morphine and Tat exposure: protection by diosgenin and selegiline

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HIV infection continues to rise in drug abusing populations. However the combined effect of HIV and drugs of abuse have not been well studied due to the absence of relevant experimental human models. We established an *in vitro* chronic model of exposure of human neurons with morphine and HIV proteins. Astrocyte cell lines (C6-Tat, SVGA-Tat) were developed that were stably transfected with Tat1-86 driven by CMV promoter or a tetracycline inducible promoter. Cultures of human fetal neurons were exposed to the Tat expressing cells by coculture in transwells. Mitochondrial dysfunction was monitored by JC1 assay and expressed as % of control. A decrease in mitochondrial membrane potential activity occurred 12 h after coculture with 1  $\mu$ M morphine but significant changes ( $P < 0.05$ ) were noted at 72 h (C6Tat  $78\% \pm 5.94$ ; SVGATat  $86\% \pm 2.13$ ). Chronic treatment of neurons with 80 nM Tat 1-72 protein and 1  $\mu$ M morphine for 5 days also caused a decrease in mitochondrial potential activity ( $85\% \pm 2.45$ ;  $P < 0.05$ ). 24 hr withdrawal of morphine, Tat and diosgenin resulted in further increase in neurotoxicity ( $72\% \pm 2.15$ ;  $P < 0.05$ ). Pretreatment with diosgenin 10  $\mu$ M and selegiline 1  $\mu$ M protected against chronic Tat and morphine withdrawal toxicity ( $P < 0.05$ ). No protection was noted by Didox 100  $\mu$ M, Trimidox 100  $\mu$ M, Imidate 1  $\mu$ M, Ebselen 5  $\mu$ M, Euk8 100  $\mu$ M, Trolox 10  $\mu$ M. We also compared changes in mitochondrial potential following a 6 h synergistic Tat1-72 and morphine exposure in neurons from fetuses following genotyping for apolipoprotein E (ApoE). Cultures with genotype ApoE3/4 showed maximal toxicity ( $75\% \pm 5.30$ ;  $P < 0.05$ ) followed by Apo2/4 ( $87\% \pm 3.32$ ;  $p < 0.05$ ). Thus the ApoE4 allele may be associated with higher risk of neurotoxicity in drug abusers with HIV infection. Thus chronic opiate use particularly in genetically susceptible individuals may enhance the risk of dementia in HIV infected patients. Diosgenin and selegiline need to be further explored for their neuroprotective effects in this population.

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### NFBP, a novel human protein from glial cells that interacts the NF-kappaB subunits to modulate HIV-1 LTR

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Transcription of human neurotropic viruses including JCV and HIV-1 in CNS cells is modulated by a series of ubiquitous and inducible DNA binding transcription factors. NF-kappaB represents a family of inducible transcription factors whose activity is important for regulation of JCV and HIV-1 in glial cells. Results from our earlier studies show that the subunits of NF-kappaB such as p50 and p65 may interact with other cellular proteins and that interaction modifies their activities. In order to identify new potential NF-kappaB binding proteins in glial cells, we utilized a Yeast Two-Hybrid system. A segment of a gene with an open reading frame of 5.6 KB, which interacted, with the p50 subunit of NF-kappaB was isolated using a cDNA library from the human astrocytic cell line, U-87MG. This gene was called NF-kappaB binding protein (NFBP). Northern blot analysis shows NFBP cDNA hybridizes with RNA greater than 5 KB present in both glial and non-glial cells. FISH analysis identified the location of the gene on chromosome 10 q24.2-q25.1. Results from a series of transfection studies revealed the ability of NFBP to stimulate HIV-1 LTR in glial cells. This stimulation increased upon stimulation of NF-kappaB with PMA suggesting that NFBP may interact with NF-kappaB to increase transcription of HIV-1 LTR. Additional studies showed that NFBP interacts with p50 and p65 individually to increase transcription of HIV-1 LTR. Interestingly, expression of NF-kB with tat, a viral transactivator whose full activity upon LTR requires NF-kappaB, in glial cells caused in a substantial enhancement of the LTR transcription. The biological relevance of the interaction of NFBP and NF-kappaB and tat will be discussed.

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### Contributions of the cellular immune response to herpes simplex virus pathogenesis in the central nervous system

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Despite the restrictive nature of the blood-brain-barrier, circulating lymphocytes can infiltrate the central nervous system (CNS), particularly during CNS disease. The factors governing this infiltration following herpes simplex virus (HSV) infection have not been identified. To define the relationship between psychological stress (a known modulator of immune function), the recruitment of HSV-specific T cells into the CNS, and development of HSV encephalitis (HSE), a murine model of HSE was developed which examines primary and memory cytotoxic T lymphocyte (CTL) responses. Naive or rVVEsgB498-505-vaccinated C57BL/6 mice were subjected to restraint stress and intranasally infected with HSV-1. Naive, stressed mice exhibited increased disease symptoms and mortality compared to non-stressed controls. Elevated levels of HSV in the brain preceded the onset of mortality only in mice exposed to stress. A concomitant increase in CD8+ and CD4+ T cells in the brain was observed throughout infection. In controls, these CTL were gB498-505-specific, while CTL of stressed mice lacked this specificity. In contrast, both stressed and non-stressed mice possessing gB498-505-specific CTLm prior to HSV challenge did not have detectable levels of HSV in the brain and were protected against HSE, despite elevated levels of gB498-505-specific CTLm in the brain. Studies are in progress to determine the individual roles of CD8+ and CD4+ T cells, as well as the expression of adhesion molecules by these cells, in the development of HSE under stress and non-stress conditions. These findings suggest that protection against HSE is afforded when HSV-specific CD8+ T cells infiltrate the CNS in time to limit HSV spread, and that this protection is compromised by stress. Delayed infiltration of HSV-specific CTL into the brain may promote HSE in naive mice, while the presence of HSV-specific CTLm in the brain prior to HSV challenge may be protective, possibly by limiting HSV replication and spread within the CNS. Supported by National Institutes of Health Research Grant AI49719.

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### Detection of fractalkine levels in the cerebrospinal fluid of HIV-infected patients

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The CX3C chemokine fractalkine is suggested to play an important role in inflammatory brain diseases, e.g., by its chemotactic properties. To investigate the induction of fractalkine in chronic HIV encephalopathy, which is characterized by monocytic cell infiltration into the brain, fractalkine levels were determined in cerebrospinal fluid (CSF) and serum samples of HIV-infected patients with (n = 10, HIV-CNS) and without (n = 23, HIV-controls) HIV-induced CNS-diseases and HIV-negative control patients (n = 23, controls) by semiquantitative western blot analysis. Fractalkine serum concentrations did not differ between the

two groups of HIV-infected patients, but were elevated in HIV-infected patients with CNS complications compared to HIV-negative controls (HIV-CNS: mean  $26,156 \pm 3,238$  arbitrary units (AU)/ml; controls: mean  $8,877 \pm 1,530$  AU/ml). CSF fractalkine levels were significantly increased in both HIV-groups compared to control patients. Additionally, HIV-infected patients with CNS diseases had significantly higher levels compared to those without CNS complications (HIV-CNS: mean  $165.5 \pm 15.6$  AU/ml; HIV-controls: mean  $112.1 \pm 12.7$  AU/ml; controls: mean  $35.2 \pm 11.2$  AU/ml). Fractalkine levels did not correlate with CSF and serum viral load and other CSF parameters, but decreased in one patient with available follow-up CSF/serum samples upon initiation of antiretroviral therapy. Taken together intrathecal fractalkine release was observed in the majority of patients with HIV infection, even in those without CNS complications. The highest fractalkine levels were detected in CSF (and serum) samples of patients with HIV-induced CNS disorders. These results suggest a dysregulation of brain fractalkine expression during HIV-infection, which may participate in HIV-induced chronic CNS inflammation.

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### Effect of Borna disease virus infection on BDNF induced-synaptogenesis

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Borna disease virus (BDV), a non-segmented, negative-stranded RNA virus, causes central nervous system (CNS) diseases characterized by behavioral abnormalities in wide range of animals species. Seroepidemiological studies tend to show that BDV could be associated with human neuropsychiatric disorders such as schizophrenia. The persistence of BDV in the CNS is associated with damages to specific neuronal populations. Since the replication of BDV is non-cytolytic, the mechanisms underlying BDV neurotoxicity are not well understood. One hypothesis is that BDV infection alters the response of neurons to neurotrophic factors. Neurotrophins play instrumental roles in promoting neuronal survival and process outgrowth in the CNS. Neurotrophin signaling triggers a cascade of intracellular events, leading to adaptive biochemical responses. We have shown recently that PC12 cells infected with BDV become resistant to NGF-induced differentiation, a phenotype linked to defects in the MEK/ERK signaling cascade (Hans et al. *J Biol Chem* 276, pp 7258–7265). We have now used primary rat neuronal cultures to examine the effects of BDV infection on neuronal physiology with a more relevant host cell. We found that cultures of hippocampal neurons were highly susceptible to BDV replication and spread. Although the viral infection was non-cytopathic and did not cause overt damage to the cells, we found that the expression of molecules involved in neuroplasticity, such as the growth-associated protein-43, Synapsin, Synaptophysin and VAMP-2 were selectively blocked after infection. Moreover, BDV-infected neurons responded only weakly to the stimulation by neurotrophins BDNF and NT3, not only in terms of ERK signaling cascade activation, but also in synaptic remodeling. These findings may be of importance to explain the molecular bases of BDV-induced cognitive and neurodevelopmental alterations.

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### Gp120IIIB and NMDA neurotoxicity is reduced by FKN in human neurons

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HIV-1 related encephalopathy has been linked to both activation of microglia and the presence of apoptotic neurons. Understanding of neurotoxicity in this context is a major goal. A neuroprotective activity of the chemokine Fractalkine (FKN), a delta chemokine, was described when rat hippocampal neurons or microglial cells were exposed to gp120IIIB or Fas-ligand. Moreover FKN and its receptor CX3CR1 are expressed within the brain, and a FKN over-expression has been observed in HIV-1 infected human brain. The aim of this work was to study first the expression of FKN and its receptor in human fetal brain cells *ex vivo* and *in vitro*. Secondly, we investigated the effects of NMDA and gp120IIIB in the presence of FKN in human neuronoglia cells or human neuroblastoma cell line (SK-N-SH). Immunohistochemistry revealed that CX3CR1 was present in mostly all astrocytes (90%±5%), microglial cells (85%±5%) in purified cultures, while 30% to 50% neurons expressed CX3CR1 in mixed primary neuronoglia cells. FKN was also observed on 70%±10% astrocytes, 65%±10% microglia in purified cultures, and 25%±10% neurons clusters in mixed primary cultures. RT-PCR revealed FKN mRNA as a 209BP size transcripts and CX3CR1 mRNA as 408BP size transcripts in purified astrocytes, microglial cells, SK-N-SH cells and as expected in mixed primary cultures. A clear staining of CX3CR1 and FKN by a specific antibody for the ligand and the receptor was detected on neuroblasts in normal human fetal brain tissues. Adjunction of 50 nM of FKN simultaneously with either gp120IIIB or NMDA for 24 h reduces by 26% TUNEL positive cells in primary neuronoglia cell cultures. Using calcium flux assay, 50 nM FKN alone was able to reduce  $[Ca^{2+}]_i$  influx induced by NMDA by a dose dependent manner. The known potentiation of NMDA induced  $[Ca^{2+}]_i$  mobilization by gp120 is also decreased in presence of 50nM FKN.

In conclusion: (1) both human glial cells and neurons express FKN and its receptor (2) NMDA and gp120 neurotoxicity is decreased in presence of FKN (3) gp120 mediated neurotoxicity via NMDA could be reduced by the adjunction of FKN.

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### *In vitro* models of HIV-associated sensory neuropathies

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Objectives: To establish *in vitro* models of NRTI (Nucleoside Analogue Reverse Transcriptase Inhibitor) and gp120 neurotoxicity in primary DRG (Dorsal Root Ganglia) cultures; 2) to study mechanisms of NRTI and gp120 neurotoxicity in DRG neurons.

Background: HIV-associated sensory neuropathies represent the commonest neurological complication of HIV infection. They include Distal Symmetrical Polyneuropathy due to HIV infection per se, and Antiretroviral Toxic Neuropathy associated with the use of NRTIs. Presently, there are no established models of NRTI-induced sensory neuropathy, and moreover, pertaining to DSP, little is known about gp120 neurotoxicity in the peripheral nervous system (PNS).

Methods: Dissociated embryonic rat DRG neurons were plated on a Schwann cell monolayer. 1) Varying concentrations of ddC, ddI, d4T, AZT, gp120<sub>IIIB</sub> or vehicle control were added, and after 15 hours of incubation, the cells were fixed and immunostained for beta-tubulin. The total neuritic length and the number of neurites per neuron were measured in each culture using confocal microscopy. 2) Varying concentrations of ddC, gp120<sub>IIIB</sub> or vehicle control were added, and after 24 hours, the cells were fixed and immunostained for both cytochrome c and beta-tubulin 3) Varying concentrations of vehicle control, ddC and gp120<sub>IIIB</sub> +/- DEVD were added, and TUNEL staining performed after 36 hours.

Results: 1) Dose-dependent reduction of total neuritic length and number of neurites per neuron was observed for ddC, ddI and d4T, with ddC being the most potent and d4T being the least. In contrast, AZT did not cause morphological neurotoxicity. Gp120 also caused dose-dependent reduction of the above morphological parameters, with toxicity being seen at low (pg/ml) doses. 2) & 3) Gp120<sub>IIIB</sub> but not ddC resulted in dose-dependent cytochrome c release and TUNEL staining in DRG neurons. The gp120-induced TUNEL staining was ameliorated by DEVD.

Conclusion: We have shown that both gp120 and NRTIs cause neuritic toxicity in the PNS. Furthermore, Gp120 but not ddC causes dose-dependent apoptosis in DRG neurons.

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### A randomized placebo-controlled trial of 5% lidocaine gel for HIV-associated distal symmetric polyneuropathy

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**Objective:** To investigate the analgesic efficacy and safety of 5% lidocaine gel in the treatment of painful HIV associated distal sensory polyneuropathy (DSP).

**Background:** DSP is the most common neurologic complication in HIV infection. Pain, which is the most debilitating symptom of DSP, is difficult to treat. Lidoderm (R) (lidocaine 5% gel) was effective in alleviating neuropathic pain in an open-label study of HIV-associated DSP.

**Methods:** In a double-blind, placebo-controlled, crossover, multicentered study, 64 subjects were randomized to receive either Lidoderm (R) or vehicle gel for two weeks (treatment phase A). A washout period of two weeks was followed by a cross-over to the alternate agent for another two weeks (treatment phase B). The primary outcome measured was difference in pain on the modified Gracely scale between the two treatment groups. Secondary outcomes included differential effect of the first treatment, global pain relief and pain response by neurotoxin exposure.

**Results:** The average pain scores of those treated with lidocaine gel (1.12) and placebo (1.13) were not significantly different ( $p = 0.94$ ). Difference in pain scores between baseline week and washout period (lidocaine 0.03, placebo 0.08) was not different ( $p = 0.31$ ); neither was the difference between baseline week and second week of treatment phase A (lidocaine 0.11, placebo 0.09) ( $p = 0.74$ ). The global pain relief was also not significantly different between the two groups (lidocaine 2.25, placebo 2.23) ( $p = 0.72$ ). The pain responses of lidocaine gel treated subjects with current exposure to neurotoxic antiretrovirals (1.18) did not differ compared to those without (1.10) ( $p = 0.358$ ). There were no significant adverse effects.

**Conclusion:** Lidocaine 5% gel is a safe but ineffective agent in the treatment of pain in HIV-associated DSP.

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### HIV-1 infects human neuronal cultures

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HIV-1 infection of neurons in HIV-1 disease is both rare and controversial. HIV-1 DNA has been detected in neurons by in-situ PCR and more recently by laser capture microdissection (LCM) and PCR in HIV infected post-mortem brains by us and others. On this basis, we investigated whether human neurons *in vitro* were equally vulnerable to HIV-1 infection.

In this study, human neurons in culture were infected with  $\times 4$  and R5 HIV-1 strains (NL4-3, ADA, YU-2, 89.6) and pseudotype VSVG/NL4-3. Infection was monitored 1–21 days post-infection via p24 ELISA, PCR, ICC and Western Blotting.

We found that 20–70% of virus entered these cultures with 5–60% of cells expressing HIV-1 antigen 3–7 days post-infection. Infection with the VSVG pseudotype was associated with enhanced HIV-1 entry and replication compared to native viruses within these cultures. HIV-1 infection led to transient downregulation of calmodulin kinase II CamKII in neurons and glial glutamate transporter in astrocytes within these cultures.

In conclusion, HIV-1 is able to replicate in enriched human neuronal cultures culminating in cellular dysfunction of both neurons and astrocytes. Moreover, these results were confirmed *in vivo* by the use of LCM and PCR. HIV-1 gag DNA was detected in microglia, astrocytes and neurons in post-mortem brain tissue from adult and pediatric cases. We propose that HIV-1 infection of neurons may directly contribute to the neuronal damage described in HIV disease.