Chronic low-level HIV-1 tat expression promotes a neurodegenerative phenotype

Alfred C. Chin\textsuperscript{1}, Alex M. Dickens\textsuperscript{1}, Amanda L. Trout\textsuperscript{1}, Jacqueline Lovett\textsuperscript{1}, Joelle Dorskind\textsuperscript{1}, Norman J. Haughey\textsuperscript{1}

\textsuperscript{1}Richard T. Johnson Division of Neuroimmunology and Neurological Infections, Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21287, US

Introduction: HIV associated neurological disorders (HAND) continue to be a significant cause of morbidity, and are associated with increased mortality in HIV infected individuals. Although the precise mechanisms for these neurological complications remains unclear, MRI studies have revealed age-related changes in brain volume of HIV-infected subjects despite stable cART. It has long been postulated that low level production of non-structural proteins from Nef/Nef-like genes may contribute to neurological damage. However, this hypothesis has not been experimentally tested. In this study we took advantage of a leaky Tetracycline-inducible promoter in the rtTA-Tat transgenic mouse to determine that very low level Tat production is associated with neuronal damage over time.

Methods: Animals: 11-12 month old (n = 14) gial fibrillary acidic protein (GFAP)-driven doxycycline-inducible HIV-1 Tat transgenic mice were used for this study. Induced mice (rtTA-Tat/Dox, n = 7) received doxycycline for 21 days to induce tat gene expression. Non-induced mice (rtTA-Tat, n = 7) received sucrose in water as a vehicle control. The control group were aged matched mice (rtTA/Dox, n=7) expressing the rtTA promoter, but not induced tat gene. rtTA/Dox mice received doxycycline for 21 days.

MRI: Brain volume measures (n = 4 per group) were performed using T2-weighted MRI images obtained by an 11.7 T horizontal bore magnet. Regions of interest were drawn to determine volumetric measures of brain regions and cortical thicknesses.

Biochemical analyses: Tat and cytokine mRNA levels were measured by qRT-PCR. Protein expression levels of β(III)-Tubulin, synaptophysin, and PSD95 were measured by Western blot to determine neuronal and synaptic integrity. Immunohistochemistry using GFAP was performed to quantify astrocyte expression and density.

Mass spectrometry: Sphingolipid concentrations were measured using high-pressure liquid chromatography coupled electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS). The following classes of molecules were identified: dihydroyceramide, ceramide, monoacyl-, diacyl- and sphingomyelin (C16:0-C20:1).

Conclusions:

- Very low-level chronic expression of the HIV-1 protein Tat was associated with ventricular enlargement, cortical thinning, dendritic simplification, induction of IL-1β in hippocampus, alterations in bioactive lipid content, and reactive astrogliosis.

- As cART is insufficient to prevent post-integration transcription of non-structural HIV-1 proteins, these findings suggest that chronic low-level production of Tat may contribute to neurological damage in HIV-infected individuals on cART.