Cognitive, Behavior and Brain Microstructure Abnormalities Detected During Progressive HIV-1 Infection in Humanized Mice

Prasanta K Dash[1+], Michael Boska[2+], Sidra Akhter[1], Jaclyn Knibbe[1], Adrian Epstein[1], Robin High[3], Harris Gelbard[4], Larisa Poluektova[1], Howard E Gendelman[1] and Santhi Gorantla[1]

[+] Equal Contribution

[1] Department of Pharmacology and Expt. Neuroscience [2] Department of Radiology and [3]College of Public Health, University of Nebraska Medical Center, Omaha, NE,
[4]Dept. of Neurology and Center for Neural Development and Diseases, University of Rochester Medical Center, Rochester, NY, USA

Prasanta K Dash Ph. D
Post-Doctoral Research Associate
985880 Nebraska Medical Center
DRC 8020
Omaha, NE, 68198, USA
Email- prashantdash@gmail.com
Phone- 402-559-8925
Fax - 402-559-3744

Abstract

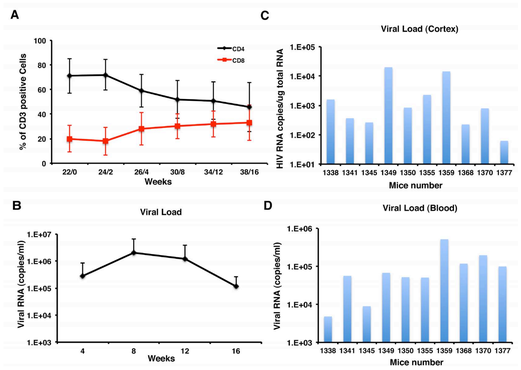
Background: We have reflected, in part, human neurological histopathology of progressive HIV-1 infection in humanized mice. To further investigate the significance of this model in representing human disease, we applied behavioral tests, histological characterization, and advanced neuroimaging tests to determine relationships between neurocognitive deficits, advanced viral infection, neuropathology, and brain metabolites. **Methods:** Humanized NOD/*scid*-IL-2Rg$_c^{null}$ mice transplanted at birth with human CD34$^+$ hematopoietic stem cells were infected with HIV-1$_{ADA}$ at 22 weeks of age. Infection was followed for 16 weeks. Mice were bled at 0, 4, 8 12 and 16 weeks post-infection (wpi) for human immune cell analysis by flow cytometry and viral load (VL) measurements. Animals were subjected to serial volume selective proton magnetic resonance spectroscopy (^{1}H MRS) and Diffusion Tensor Imaging (DTI) at pre- and 4, 8, 12 and 16 wpi to measure brain metabolites and changes in tissue architecture, respectively. Total RNA from cortex was isolated at the study end from both infected and control mice groups and HIV-1gag was quantified using Real-time quantitative PCR to look at the brain viral RNA level. Animals were evaluated one week prior to and 4 and 8 weeks following viral infection by open field activity (OFA) test as a measure of habituation in new environments, motor function and field exploratory behavior. At the study end, brains were analyzed immunohistochemically for neuronal, oligodendrocytes and glial inflammatory markers. **Results:** Mice showed sustained plasma viremia with concomitant reductions in CD4$^+$ T lymphocytes. Behavioral abnormalities included failure to habituate to a new environment and increased anxiety with HIV-1 infection. Systemic viral loads were associated with selective cortical N-acetyl aspartate, creatine and choline reductions. Diffusion tensor imaging (DTI) abnormalities in white matter fiber structure and in brain connectivity in cortical gray matter correlated with levels of HIV-1 infection. Post-mortem multispectral imaging microscopic analysis of brain tissue showed reduction in synaptophysin, neurofilament, glial antigens correlating with DTI and metabolite changes. **Conclusions:** These results demonstrate, for the first time, notable relationships between viral load, imaging abnormalities and brain region-specific neurodegeneration in a mouse model demonstrating neurocognitive deficits. These findings are clearly reflective of human disease and can be applied to future therapeutic interventions for neuroAIDS. .

Methods

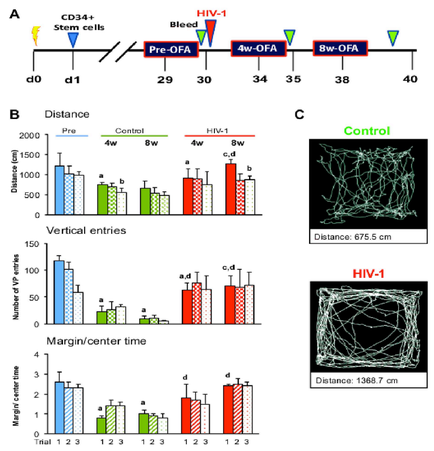
Mice: Humanized mice at 22 weeks of age were infected with HIV-1$_{ADA}$ and the CNS deficits assessed during progressive viral infection using ^{1}H MRS and DTI acquired at 0, 4, 8, 12 and 16 weeks after viral infection. Mice were bled on 2nd week and 4, 8, 12 and 16th weeks post HIV-infection and the sera and cortex was analyzed for HIV-1 RNA. Cell associated CD45, CD3, CD4, CD8, CD14 and CD19 were quantified by flow cytometry. **Behavior analysis:** Ten female mice were randomly allotted to either HIV-1 infected or control group (n=5 per group). OFA testing was done before infection and 4 and 8 weeks after infection in HIV-1 infected mice. Control mice were injected with PBS at the time of infection and tested for OFA at the same intervals. At each time point mice were tested for OFA using 20 min session for three consecutive days (3 trials). Mice were bled from sub-mandibular vein under isoflourane inhalation anesthesia after the OFA testing to collect blood for flow cytometry and viral load. Total distance travelled measured in the floor plane and vertical entries measured in the vertical plane reflect the exploratory and habituation behavior in a new environment. First five minute travel paths of control and HIV-1 infected mice from trial 3 of 8w time point is shown **Immunostaining:** Brain sections were immuno-stained for glial cells (MAG)astrocytes (GFAP), microglia (Iba1), neuronal cell bodies and processes (MAP2), pre-synaptic vesicles and intermediate filaments (Synaptophysin and NF). Quantitative fluorescence microscopy with an automated fluorescence microscope and multispectral imaging system (Nuance EX) was used for all quantification. Images were captured from each microscope field with a 20x objective.

Results

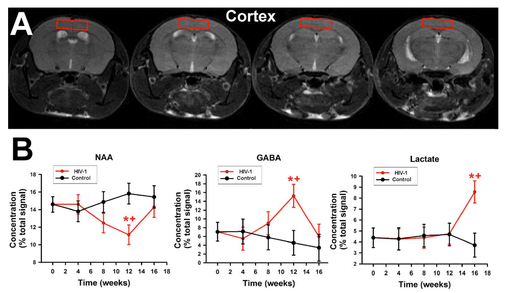
1. Immunological status and Viral load in HIV-1 infected Humanized Mice



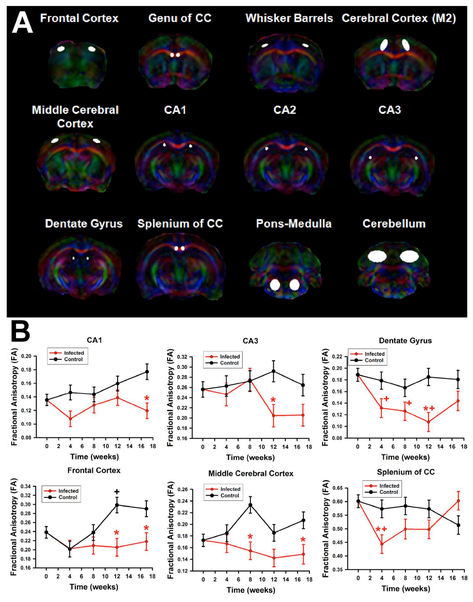
2. Effect of HIV-1 infection on open field behavior



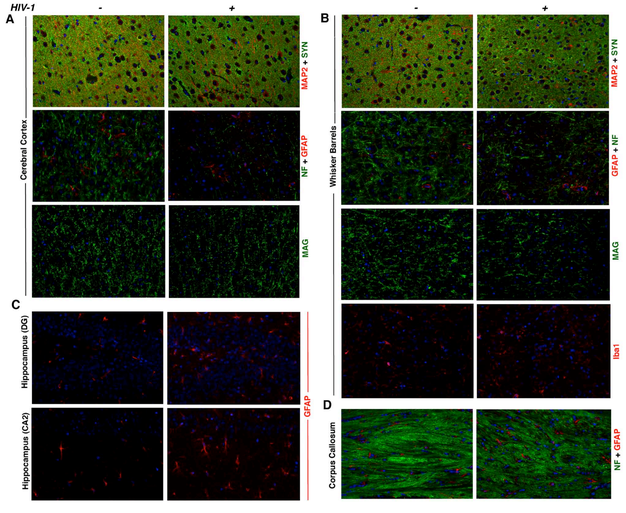
3. Metabolite levels from ^{1}HMRS scans of Mice over time



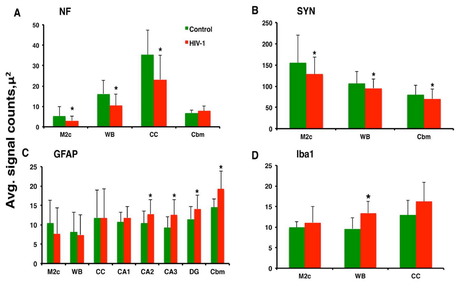
4. Results from DTI scans of Humanized Mice



5. Immunofluorescence labeling in different brain regions of Mice



6. Quantitative fluorescence microscopy of Neuronal and Glial markers of Humanized Mice



Conclusions

- Humanized mice show immune and virologic changes following HIV-1 infection with sustained viral load in periphery, but brain VLs may not be directly correlated to blood levels.
- Peripheral HIV-1 infection of mice resulted in memory loss and anxiety as analyzed by open field behavior testing.
- Metabolic consequences of HIV-1 infection were seen in the cerebral cortex as reduced NAA and increased GABA were seen at 12 weeks and reversed at 16 weeks, but increase in lactate at 16 wpi.
- The most consistent and notable changes were seen as reduced FA in hippocampus and cortical regions of HIV-1 infected mice compared to controls. FA reductions is dentate gyrus and splenium of corpus callosum regions were found to be reversible. We also observed transient alterations in increased diffusivity in white matter regions of infected mice compared to controls.
- We observed significantly decreased SYN expression in cortical regions and cerebellum. GFAP expressions for astrocytes was found to be increased in 3 regions of hippocampus and cerebellum. Microglial expression was increased in WB regions and in genu of corpus callosum. Significant reductions in NF- positive fibers were seen in cerebral cortex, WBs and corpus callosum of HIV-1 infected mice as compared to control animals. We also observed a decrease in the Myelin associated glycoprotein expression in the cerebral cortex and WBs of infected mice. The reduced presynaptic and neurofilament expression seen in the cortical and white matter regions of HIV-1 infected humanized mice indicate ongoing neurodegeneration potentially compatible with a dying-back axonapathy.

References

1. **Boska MD[+], Dash PK[+], Gendelman HE and Gorantla S et al., Brain Microstructure and Metabolites Mirror cognitive and behavior deficits during Chronic HIV-1 infection of Humanized Mice. (Manuscript submitted, (+ equal Contribution)**
2. **Dash PK, Gorantla S, Gendelman HE, et al. Loss of neuronal integrity during progressive HIV-1 infection of humanized mice. *Journal of Neuroscience*, Mar 2 2011;31(9):3148-3157.**