^{1}H MRSI Revealed Influence of Genetic Background on Neuronal Behavior in Murine Model of HIV-1 Encephalitis

J. A. Nelson[1,2], S. Gorantla[2,3], H. Dou[2,3], M. Mellon[1], J. M. Bradley[1], D. Kibuule[1,2], H. E. Gendelman[2,3], M. D. Boska[1,2], L. Poluektova[2,3]

[1]Radiology, University of Nebraska Medical Center, Omaha, NE, United States, [2]Pharmacology and Experimental Neurosciences, University of Nebraska Medical Center, Omaha, NE, United States, [3]Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, Omaha, NE, United States

INTRODUCTION: Humanized murine models for human immunodeficiency virus type one (HIV-1) disease are critical if attenuation or prevention of disease in infected humans is to be achieved. Specifically, for HIV-1-associated dementia (HAD), a disease affecting nearly ten percent of infected persons, testing of adjunctive or neuroprotective therapies require such models. Disease is manifest during progressive immunosuppression and was previously mirrored in mice with severe combined immunodeficiency (SCID) where HIV-1 encephalitis followed stereotaxic injection of human infected monocytes into the subcortex (caudate and putamen). In order to address the role of specific immunodeficiencies in the induction of disease, we compared inflammatory responses and neuronal damage in C.B.-17/SCID mice (demonstrating truncation of DNA-dependent protein kinase, compromised neuronal repair mechanisms and functional natural killer (NK) cells) and, in contrast, Rag-2$^{-/-}\gamma_c^{-/-}$ mice (Rag) (absent recombinase-activated gene-2 and common cytokine receptor gamma chain without NK cells). The histologic, neuroimmunologic and ^{1}H MRSI metabolite differences were assessed in both models after induction of HIV-1 encephalitis (HIVE).

METHODS:

Histology: C.B.-17/SCID and Rag mice were injected in the subcortex with virus-infected human monocyte-derived macrophages (MDM) (n=36 for each group) and evaluated at 7 days post-injection for the presence of human cells (vimentin), levels of viral infection (HIV-1p24), astrocyte immunity [glial fibrillary acidic protein (GFAP)], microglial reactions [ionized calcium-binding adaptor molecule (Iba)-1] and neuronal viability through marker expression [microtubule associated protein-2 (MAP-2), neuron-specific nuclear protein (NeuN) and neurofilament (NF)]. Real time polymerase chain reaction (Q-PCR) was used to quantitatively evaluate expression of macrophage/microglia marker (Mac-1), GFAP, interleukin-1 (IL-1β), tumor necrosis factor alpha (TNF-α), Interleukin-6 and 10 (IL-6, IL-10), inducible nitric oxide (iNOS) and brain derived neurotrophic factor (BDNF). Flow cytometry of the injected and corresponding contralateral brain regions were assayed for mouse CD45low/high (residential vs. migrated macrophages) I-A^b, CD3 (total T-cells), DX-5 (NK cells) and CD14 (macrophages) antigens.

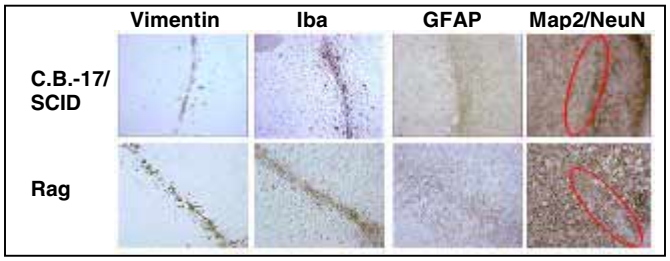
^{1}H MRSI: ^{1}H MRSI data were acquired 7 days post-injection (n=6 SCID; n=3 Rag). Four 1.0 microliter voxels were analyzed from each animal, two along the injection line (or area corresponding to the injection line) and two mirroring voxels from the contralateral side. Spectra were fit using AMARES (1) in the jMRUI 1.3 package (2). Spectra were obtained on a Bruker Biospec 7T/21 magnet operating at 300.41MHz with the following parameters: FOV=20mm, TE=33ms, TR=4 s, Averages=3, matrix size=24x24 encoding. Metabolite concentrations were normalized to creatine which was not significantly different between non-injected and HIVE mice in either model.

RESULTS:

Histology: Human cells were present along the injection line in both models (vimentin) (Figure 1). Compared to Rag mice, C.B.-17/SCID mice showed increases in microglia activation (Iba), astrocytosis (GFAP), neuronal damage (Map2/NeuN) (Figure 1), TNF-α and IL-1β, and enhanced chemo attraction of peripheral MDM in the injected hemisphere (p<0.05). C.B-17/SCID mice showed reduced MDM numbers in brain tissue (p<0.05). The levels of human MDM infection, expression of iNOS and BDNF were not different between groups.

^{1}H MRSI: C.B.-17/SCID showed decreased levels of N-acetyl aspartate/creatine (NAA/Cre), a neuronal marker, in both the injected and contralateral hemispheres when compared to non-injected C.B.-17/SCID mice (p<0.05); however, Rag mice did not show significant differences in NAA/Cre. Neither mouse model had significant differences in choline/creatine concentrations (Table 1).

Figure 1: Histological staining along the injection line at 10x magnification for representative C.B.-17/SCID and Rag mouse at 7 days post-injection.



DISCUSSION: C.B.-17/SCID murine HIVE model showed increased neuronal damage in the injection hemisphere [denoted by decreased NeuN/Map2 staining (Figure 1) and decreased NAA/Cre concentration (Table1)]. Diminished NAA/Cre levels were also observed in the contralateral hemisphere. This model had increased macrophage migration from the periphery (denoted by CD45 low/high) due to inflammation and a higher number of NK cells in the periphery than in Rag-2$^{-/-}\gamma_c^{-/-}$ mice. C.B.-17/SCID HIVE mice also had increased TNF-α and IL-1β. C.B.-17/SCID murine model has increased and widely distributed inflammatory responses with increased neuronal damage compared to the Rag-2$^{-/-}\gamma_c^{-/-}$ murine model and better mimics HIVE pathology.

Table 1: ***Comparison of N-acetyl aspartate/creatine (NAA/Cre) and choline/creatine concentrations for C.B.-17/SCID and Rag-2$^{-/-}\gamma c^{-/-}$ mice.***

	C.B.-17/SCID HIVE		C.B.-17/SCID non-injected		Rag HIVE		Rag non-injected	
Metabolite:	***Injected***	***Contralateral***	***Ipsilateral***	***Contralateral***	***Injected***	***Contralateral***	***Ipsilateral***	***Contralateral***
NAA/Cre	0.55±0.03*	0.57±0.04**	0.73±0.07	0.75±0.03	0.63±0.04	0.56±0.03	0.67±0.04	0.57±0.06
choline/creatine	0.24±0.01	0.24±0.01	0.19±0.01	0.26±0.01	0.17±0.005	0.21±0.01	0.16±0.01	0.20±0.01

*p<0.05 compared to ipsilateral hemisphere of non-injected mice within the same group.
**p<0.05 compared to contralateral hemisphere of non-injected mice within the same group.

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