Measures of Bone Marrow-Derived Monocyte Migration into Brain during HIV-1 Encephalitis: Implications for the Pathogenesis and Treatment of HIV-1 Infection of the Nervous System

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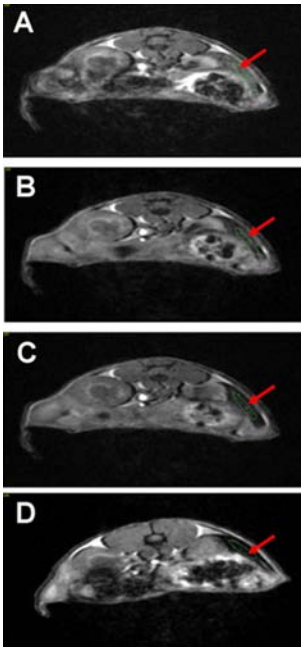

Figure 1. *Signal loss observed in the spleen. A. Preinjection, B. Three hours post injection, C. Six hours post injection, D. 24 hours post injection. Acquistion parameters are detailed in Materials and Methods.*

Introduction. The key pathogenic event in HIV-1-associated dementia is the migration of bone-derived monocytes into the brain and the spread of viral infection from the peripheral immune system into the nervous system. Indeed, the best measure of cognitive decline during progressive HIV-1 disease is not the absolute level of virus but rather the numbers of immune competent brain macrophages and microglia. A key technical challenge in studies of the neuropathogenesis of HIV-1 infection is real time detection of monocyte-macrophage migration into brain. This has not yet been demonstrated. We offer for the first time, to our knowledge, of quantitative measurements of superparmagnetic iron oxide (SPIO) labeling of bone marrow macrophages (BMM) from blood to brain during HIV-1 encephalitis (HIVE) induced in a murine immunodeficient model of human disease.

Methods. Severe combined immunodeficient (SCID) mice were injected with HIV-1_{ADA} -infected human monocyte-derived macrophages (MDM) into the caudate and putamen by stereotactic placement. After adoptive transfer of Feridex (Berlex Laboratory, Wayne, NJ) murine bone marrow derived macrophages (BMM), through the tail vein) in HIVE mice cell distributions into body and brain were assessed by T_2* weighted magnetic resonance imaging (MRI). Results were confirmed by histology. In replicate experiments, the distribution kinetics of BMM into liver, spleen and kidney were determined using short echo time T_2* MRI with measures preinjection and at 1, 3, 5, and 7 days post-injection. T_2* weighted MRI (7T) were acquired using a 25 mm birdcage resonator. MRI acquisition parameters: 3D GRE with TE=3 ms TR = 50 ms, Excitation pulse = 35°, NA=2, 30% echo, 35 x 25 x 50 mm FOV with resolution of 256 x 128 x 128 reconstructed to 256 x 256 x 128. Signal intensity was referenced to an external standard. BMM density was calculated in tissue at each time point as [SPIO] α $\ln(S_0) - \ln(S_l)$, where S_0 is the tissue signal intensity prior to injection of labeled BMM and S_l is the normalized tissue signal intensity at times after injection of labeled BMM. Migration of labeled BMM to the brain was confirmed in HIVE animals at days one, seven and 14 days by subimaging of the brain MRI, rigid model volume coregistration and subtraction of the preinjection MRI from each time point after histogram normalization using Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN). MRI were acquired using similar parameters to body acquisition except use of surface coil (12.5 x 15 mm) reception, 20mm x 20mm x 16mm FOV and 128x128x64 resolution. Experimental endpoints included labeled cell density measures in tissues of interest using Prussian blue staining of iron in histological sections.

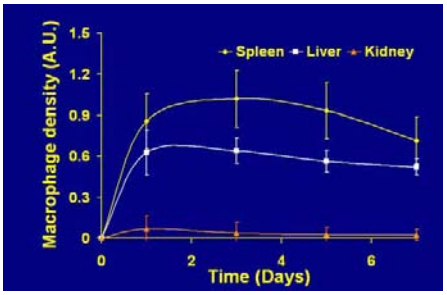

Figure 2. *Kinetics of MP accumulation in spleen, liver and kidney determined by signal loss in T_2* weighted MRI. N=6, mean ±S.E.M. are displayed for results one, three, five and seven days post injection.*

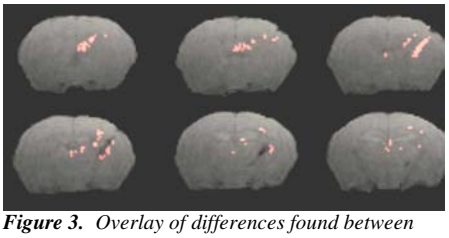

Figure 3. *Overlay of differences found between MRI at day seven after injection and preinjection MRI in slices near the injection of HIV infected human macrophages.*

Results. BMM were found in spleen, liver (Figs. 1,2) and brain concentrated in areas of HIV-1 infection (Fig 3). Histological analysis demonstrate that, on a tissue weight basis, spleen showed 3 to 5-fold greater BMM numbers than liver, demonstrating a saturation of signal loss at high labeled cell density in MRI analyses, but relative agreement was found between MRI and histological determinations of labeled cell distribution. BMM migration to the area of encephalitis is demonstrated on histology (Fig 4) showing that Feridex labeled cells, visualized by Prussian blue stain, migrated into the brain in the area of the HIV-1 infected human cells and astrocytosis.

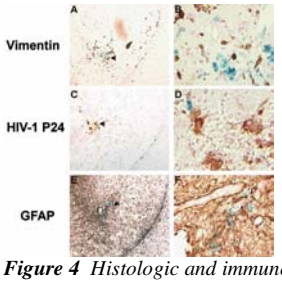

Figure 4 *Histologic and immuno-histological assessments of BMM migration into brain during HIVE. Relationships between Prussian blue-stained BMM and numbers of human cells (vimentin) virus infected cells (p24 antigens) and astrocytosis (glial fibrillary acid protein, GFAP are shown after 7 days.*

Conclusion. Magnetic labeling of macrophages allows quantitative tracking of distribution of BMM during HIVE over a period of 14 days. Although high density of cells make absolute quantitation difficult (due to saturation in the loss of signal) newer methods comparing signal loss in T_2* weighted MRI to histological determination of labeled cell density in spleen and liver can overcome this limitation. Detection of cells migrating into encephalitic brain from the bloodstream has been detected using a combination of T_2* weighted MRI and histology, concentrated in the area of the encephalitis. These methods assess both critical events in the pathogenesis of HIVE and HAD as well as providing a means to track cell-based drug delivery.

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