Cellular MR Imaging of Magnetically Labeled Encephalitogenic T-Cells in the Mouse Spinal Cord

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ABSTRACT: The purpose of this study is to detect and monitor encephalitogenic T-cells by MRI in the mouse model of multiple sclerosis. Experimental autoimmune encephalomyelitis (EAE) was induced in mice by adoptive transfer of myelin peptide specific T-cells intracellularly labeled with superparamagnetic iron-oxide nanoparticles (SPIO). The phenotype, proliferative and encephalitogenic properties of magnetically labeled cells were compared to non-labeled cells. MRI was performed on the spinal cord *in vivo* and by *ex vivo* MR microscopy (MRM) and hypointense lesions were visualized in labeled mouse cords. Histology confirmed that iron-labeled T-cells present in EAE lesions were detected on MRM.

INTRODUCTION: In EAE, T-cells activated against myelin peptides initiate an inflammatory demyelinating disease in the CNS that is similar to multiple sclerosis. To initiate EAE, T-cells are stimulated with specific myelin peptides, that subsequently differentiate into Th1 proinflammatory cells, and traffic to the CNS (1). Other immune cells are later recruited non-specifically to the lesion sites, and these consist of nonspecific T-cells, macrophages and B-cells. These cells contribute to the inflammation, demyelination and axonal loss. The trafficking patterns of encephalitogenic T-cells into an EAE lesion has not been described by MRM. Fluorescence studies using yellow fluorescent protein (YFP)- labeled T-cells have provided the only *in vivo* data we are aware of to date on trafficking in EAE (2). Trafficking patterns can show significant time points and locations of initiating steps in disease, which predate clinical signs, and are essential to understanding the disease pathophysiology.

METHODS: Animal studies were performed in accordance with NIH animal care and use guidelines. Lymph node (LN) cultures from proteolipid protein (PLP)-immunized mice were incubated with Feridex (Berlex, USA) complexed with Poly-L-lysine (PLL) (1:2000 dilution) (3). 20 million labeled or unlabeled LN culture cells were injected I.P. into recipient mice. *In vitro* studies were done to compare proliferation and cytokine production of magnetically labeled versus non-labeled cells. H&E, Luxol fast blue, and Prussian blue histology of the spinal cord were performed. *In vivo* MRI was performed at day 8-14 from transfer in a 7.0 T 39G/cm gradient Bruker system and 35 mm birdcage coil. Mice were anesthetized with 1.5% isofluorane and placed supine with physiological monitoring. 2D gradient echo images were acquired, slice thickness 250 to 300 microns, in-plane resolution 70-85 micron. TR 500-1000 ms, TE 4.3-5.5 ms. Mice were euthanized, perfused, and the spinal column fixed in formalin. *Ex vivo* MRM was performed in a 7T, 8.1 cm vertical bore Oxford magnet with 250 mm ID, 95G/cm gradients. Spinal cord(s) were imaged in Fomblin (Ausimount USA) in a 10 or 20 mm Bruker volume coil. 3D T2*W gradient echo images were acquired, TR/TE 240/4.3 to 280/5.4ms, 35x35x65 to 30x30x46 μm^3 resolution.

RESULTS AND DISCUSSION: Cell cultures from immunized donor mice LN were labeled by incubating with Feridex-PLL complexes (3) showed positive Prussian blue staining for iron. These LN cells showed strong proliferative responses to the immunogen PLP in vitro, indicating the labeled cell cultures respond to antigen comparably to unlabeled cells. FACS analysis showed similar numbers of cells to express the cytokine Interferon-γ, indicating both T-cell populations have differentiated into Th1 phenotype. After adoptive transfer of labeled or unlabeled cells, MRM was performed at the onset of disease symptoms. Discrete hypointense regions of variable size in the thoracic and lumbar cord were identifiable *in vivo* at resolutions of 75x75x250 to 300 microns. The hypointense appearance of the lesion was consistent with the presence of iron-labeled cells. Cords imaged *ex vivo* at 35x35x50 microns showed detailed distribution of hypointense lesions, with larger lesions correlating to regions seen *in vivo*, shown in **Fig.1**. Diffuse hypointensity in lumbar cord white matter and caudal nerves was imaged in another adoptive transfer mouse at disease onset. *Ex vivo* MR microscopy was performed and compared to histology (**Fig. 2**). The white matter is hypointense and uniform. Cellular infiltration in white matter and lumbar-sacral plexus nerves are shown by histology in the same region of the cord on H&E stain and sections contain numerous Prussian blue positive cells indicating the presence of iron.

CONCLUSIONS: These results indicate that SPIO-labeled encephalitogenic T-cells have comparable immunologic profiles to unlabeled cells, induce EAE disease, and can be detected for the first time by in vivo MRM in the mouse spinal cord. This method enables tracking timing and distribution of activated T-cells in a living system. Monitoring these cells can further our understanding of T-cell-mediated autoimmune CNS diseases and potentially visualize response to immunomodulatory or immune cell-based therapies.

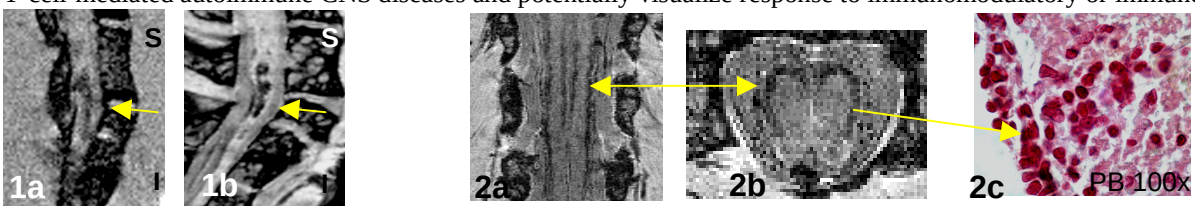

Fig.1: (a) *In vivo* and (b) *ex vivo* MRI in a labeled mouse lumbar spinal cord with EAE lesion. **Fig.2:** 3D MRM, 30x30x46 μm^3 of a labeled mouse with widespread cellular infiltrates in the lumbar cord. (a) Coronal and (b) axial views. (c) Histology, Prussian blue.

REFERENCES: 1. Kuchroo VK, *et al*. *Annu. Rev. Immunol.* **20**:101-123 (2002). 2. Costa GL, *et al*. *J. Immunol*167:2379-2387 (2001). 3. Frank JA, *et al*. *Radiology*, in press