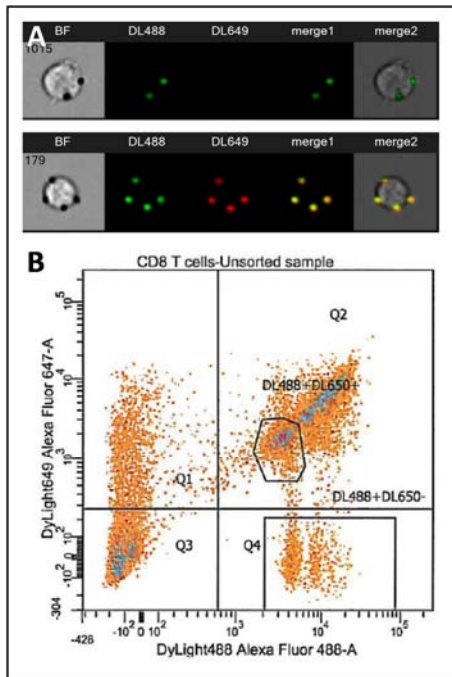


# T CELL LABELING AND TRACKING BY MRI IN MOUSE BRAIN DURING VIRAL INFECTION

Tatjana Atanasijevic<sup>1</sup>, Jasmin Herz<sup>1</sup>, Dorian McGavern<sup>1</sup>, and Alan Koretsky<sup>1</sup>  
<sup>1</sup>NINDS, NIH, Bethesda, MD, United States

**Target audience:** MRI researchers interested in cell labeling and tracking, immunologists interested in brain inflammation.

**Purpose:** Viral infection is a common cause of inflammatory disease in the central nervous system (CNS). Anti-viral T cells recruited from the periphery play a critical role in fighting CNS viral infections; however, this immune response is not always beneficial, as it can lead to inflammation and neuronal damage. Understanding trafficking of T cells within the CNS is critical for the design of strategies that augment protective immune responses while limiting collateral damage. The lymphocytic choriomeningitis virus (LCMV) model is an excellent system to study cytotoxic T lymphocyte (CTL) interactions in the CNS, as these cells are crucial for the development of the disease and are known to infiltrate the brains of the infected animals during the final stage of the disease (days 5 and 6 after intracerebral virus injection)<sup>1</sup>. MRI can be used to track the localization and movement of single cells *in vivo* to the level of detecting single cells if suitable quantities of iron-containing contrast agents can be incorporated within targeted cells. Micron sized iron oxide particles (MPIOs) typically contain 0.1-10 pg iron in an individual particle, allowing single cells labeled with only one or a few MPIOs to be detected by MRI<sup>2,3</sup>. In general, it is very difficult to promote uptake of iron-containing particles in T cells due to low phagocytic activity and a small cytoplasmic volume. When T cells have been successfully labeled it typically takes long incubation times of 12-48h<sup>4,5</sup> leading to possible degradation in cell functions. Here we have focused on development of strategies for labeling of T cells with commercially available 1  $\mu$ m sized MPIOs coated with streptavidin in short incubation times of 1-2h.



**Figure 1.** Internalization of MPIOs by CD8<sup>+</sup> T cells. (A) ImageStreamX image of a T cell that has internalized green, DL488<sup>+</sup> MPIOs (top row) or is surface labeled (bottom row) so the red DL649 antibody has access to the bead. (B) Sorting out T cells that have internalized MPIOs, Q4 gate, DL488<sup>+</sup>DL650<sup>+</sup> from the surface labeled cells, Q2 gate, DL488<sup>+</sup>DL650<sup>-</sup>.

adverse manner. The labeled single T cells can be detected *ex vivo* in the brains of LCMV-infected mice using high resolution MRI, which opens exciting possibilities for *in vivo* tracking of single T cells in inflammatory diseases of the CNS.

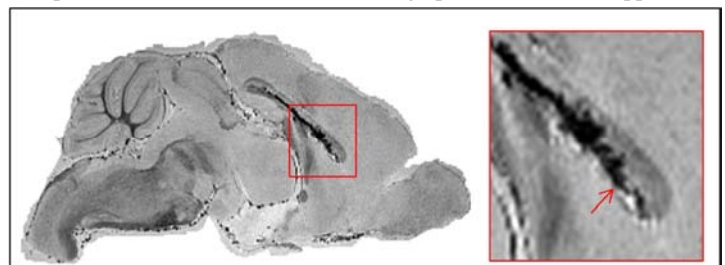
**Conclusion:** T cells can be labeled with MPIOs which are effective for tracking of single cells. Single T cell tracking by MRI in the brain will allow the study of mechanisms of CNS viral infections, as well as other inflammatory processes in the brain.

**References:** 1. Kim et al., *Nature* 457, 191-195, (2009), 2. Shapiro et al., *PNAS USA* 101,10901-10906, (2004), 3. Shapiro et al., *Magn Reson Med* 55, 242-249, (2006), 4. Arbab et al., *Transplantation* 76, 1123-1130, (2003), 5. Hu et al., *Magn Reson Med* 54, 1473-1479, (2005).

**Methods:** MPIOs were conjugated to CD3 antibodies, because cross-linking of the CD3 receptor is known to promote T cell receptor (TCR) internalization, and the binding is very fast. MPIOs were labeled with a fluorophore (DyLight 488) to simplify histological and flow cytometric analyses. An assay to quantitatively determine the internalization of MPIOs by flow cytometry based on the use of anti-streptavidin antibodies conjugated to a different fluorophore (DyLight 649) was developed. The antibody cannot penetrate the membrane on live T cells to bind internalized MPIOs, and, therefore, only binds particles located on the surface of the T cell (Fig. 1A). The described internalization assay also provides the ability to flow cytometrically sort internally labeled T cells from all of the other cells (Fig. 1B). T cells isolated from spleens of both healthy and LCMV-infected mice were incubated with CD3-coated MPIOs for 1h at 37°C. They were subsequently stained with anti-streptavidin antibody and analyzed for MPIO uptake. Labeled CTL were sorted and cells labeled on the inside (Q4 gate) were injected into the brains of mice, previously i.c. injected with LCMV, on day 5 after infection. On day 6, just before the onset of fatal seizures, the animals were perfused with fixative and scanned in a 14 T micro-MRI system (Bruker, Inc.) at isotropic resolution of 50  $\mu$ m using a 3D T<sub>2</sub>\*-weighted gradient-echo imaging pulse sequence (TR/TE=50/15ms, flip angle=12°, ns=20).

**Results:** The effectiveness of the labeling strategy is demonstrated by the image in Fig. 1A, taken by an instrument that combines flow cytometry and microscopy capabilities (Amnis, Inc.). The internalization of CD3-coated MPIOs was relatively low due to insisting on short, 1h, labeling times. Labeling efficiencies were 10-20% for naïve T cells and 5-10% for CTL, but still sufficient for purification of labeled T cells and for adoptive transfer. T cells labeled this way were evaluated using Annexin V and 7AAD staining with flow cytometry to determine the levels of apoptosis and cell death and no significant difference was observed between these and unlabeled control cells (data not shown). A set of different flow cytometric assays to address possible CTL activation due to CD3 targeting was conducted, but again, no significant difference from control cell populations was detected. *Ex vivo* MRI images (Fig. 2) demonstrated the feasibility of labeled T cell detection in the brains of LCMV-infected mice. The hypointense regions on these images are well correlated with the expected distribution of LCMV in the brain, i.e. they were mostly located in the meningeal and ventricular area.

**Discussion:** This study shows that it is possible for T cells to internalize MPIOs during fairly short time intervals. Moreover, the uptake of a small number of these large particles does not appear to significantly influence these cells in any



**Figure 2.** *Ex vivo* MRI detection at 50  $\mu$ m isotropic resolution of labeled T cells in brain tissue of LCMV-infected mice. Dark voxels on these images correspond to labeled CTLs. The right, zoomed in, panel displays their distribution in the lateral ventricle.