Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model

M. Srinivas[1], P. A. Morel[2], L. A. Ernst[3], J. Janjic[1], D. Kadayakkara[1], D. H. Laidlaw[4], and E. T. Ahrens[1,5]

[1]Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Immunology, University of Pittsburgh, Pittsburgh, PA, United States, [3]Molecular Biosensor and Imaging Center, Carnegie Mellon University, Pittsburgh, PA, United States, [4]Computer Science, Brown University, Providence, Rhode Island, United States, [5]Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States

Introduction

We describe the development of a novel ^{19}F cell labeling technique for MRI and its application in a murine model of Type I diabetes (T1D). Importantly, the label allows for quantification of cell numbers directly from the *in vivo* image data. The use of a ^{19}F label precludes any background and is exquisitely specific for the labeled cells, while ^{1}H imaging provides the anatomical context [1]. The label is comprised of emulsified perfluoropolyether (PFPE) which is biologically inactive. Using the non-obese diabetic (NOD) mouse, an established model of T1D, we visualized the early homing of PFPE-labeled diabetogenic T cells to the pancreas. A novel computational algorithm provided accurate T cell quantification, with a sensitivity better than 28,000 cells per voxel *in vivo*. We observed that ~6% of the transferred cells homed to the pancreas after 48 hours. *In vitro* assays and histological analysis of fixed tissues confirmed the functionality of the labeled T cells, and ^{19}F NMR on excised organs was used to validate the quantification results.

Methods

The ^{19}F label was prepared by emulsifying PFPE to form nanoparticle suspensions with mean particle size ~105 nm in diameter. $CD4^+$ T cells ($4\text{-}6\times10^6$) from MHC-matched NOD BDC donor mice were purified, activated *in vitro*, labeled and transferred i.p. 48 hours before imaging. The recipient mouse was a cyclophosphamide-treated NOD SCID. For MRI, mice were anesthetized with ketamine/xylazine by continuous infusion during the imaging session via an i.p. catheter. Mice were intubated and connected to a mechanical ventilator delivering an O_2/NO_2 mixture. Imaging was performed using a ^{19}F/^{1}H volume birdcage resonator in an 11.7 T/89 mm vertical-bore micro-imaging system. ^{19}F images were acquired using a RARE spin-echo sequence with TR/TE=1000/6.4 ms, 64×32 image points, and 8 k-lines per excitation. ^{1}H images were acquired with a 2DFT spin-echo sequence with TR/TE=1200/22 ms and 512×256 image points. Eight contiguous, 2 mm-thick slices through the torso were acquired for both ^{19}F and ^{1}H with a FOV of 5×2.8 cm. All MRI excitations were respiratory-gated. The mice were sacrificed after imaging and the pancreas harvested for histology or NMR. The quantity of apparent labeled cells in regions of interest was calculated directly from the complex-valued *in vivo* MRI data set, an external ^{19}F reference, and the calculated ^{19}F content per cell determined from prior *in vitro* ^{19}F NMR measurements.

Results

Figure 1a shows a composite ^{19}F/^{1}H *in vivo* MR image of labeled diabetogenic T cell homing specifically to the pancreas. No ^{19}F signal was detected in the liver, spleen or other organs. T cells were injected 48 hours prior. Figure 1b is a negative control, where the animal received cell-free label only; the ^{19}F dose was equivalent to twice that of the transferred labeled cells. No ^{19}F was detected in or around the pancreas, but instead in the spleen and bladder. Figure 2 displays fluorescence micrographs of immunostained histological sections of pancreatic tissue from a NOD-SCID mouse that received labeled T cells. These data confirmed that the labeled T cells homed to the pancreas. SCID mice have no endogenous T cells. The histology shows early insulitis, with T cells infiltrating into the islets or around blood vessels. These data suggest that PFPE labeling does not impair T cell trafficking, consistent with the MRI data. Functionality of labeled T cells was also tested by *in vitro* migration and cytotoxicity assays (not shown). Figure 3 summarizes the quantification results for n=4 mice receiving labeled T cells and shows that approximately 6% of transferred cells homed to the pancreas after 48 hrs. The approximate cell numbers were confirmed via high resolution ^{19}F NMR spectroscopy of intact excised organs.

Discussion

We observed on the order of 5-7% of labeled transferred T cells in the pancreas using the MRI data. This is consistent with other studies [2] where approximately 6% of transferred NOD T cells reached the pancreatic lymph nodes and pancreas at 48 hours, as detected using FACS analyses. Thus, the majority of transferred cells remain in circulation, distributed within the i.p. cavity, or localized at concentrations too low to be detected in lymph nodes or other organs by MRI. Cells in circulation will not be detected using this technique. The absence of signal in the liver and spleen in the T1D model is significant because it implies that transferred cells were functional. The concentration of ^{19}F per cell affects cell number quantification, and was assumed to be constant 48 hrs after cell transfer. However, the activated T cells are unlikely to have gone through more than one division within 48 hrs, leading to a potential underestimate of cell numbers by a factor of two or less. This technology in its present form can be used to investigate detailed biological questions concerning the trafficking of T cells *in vivo*. Overall, the fluorine cell labeling technology is applicable to many cell types and diseases, and potentially for monitoring the trafficking of cellular therapeutics.

Acknowledgements

This work was funded in part by the National Institutes of Health (R01-EB003453, R01-EB004155, P01-HD047675, P41EB-001977 and P50-ES012359).

References

1. Ahrens E.T. et al. *Nat Biotechnol.* 2005 **8**:983-7.

2. Fabien N. et al. *J. Autoimmun.* 1995 **8**: 323-334.

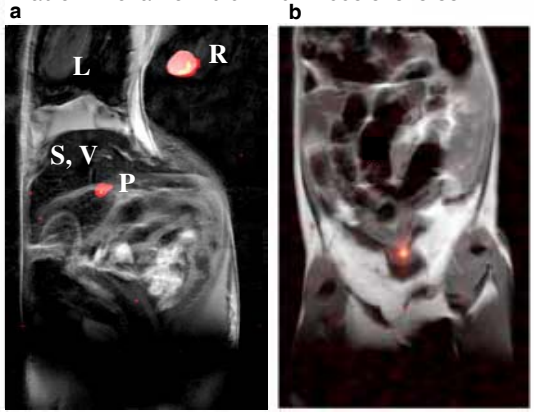

Fig. 1 *In vivo* composite ^{19}F/^{1}H images through the mouse torso. Here ^{19}F is rendered in pseudo-color and ^{1}H in grayscale. (a) shows the mouse receiving labeled T cells and (b) cell free label as a control. Indicated are the pancreas (P), lungs (L), external ^{19}F reference tube (R), and spleen and liver (S, V).

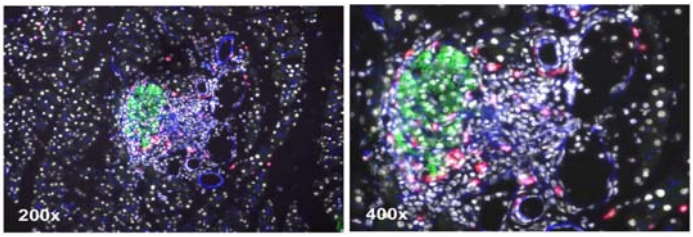

Fig. 2 Histological sections of the pancreas from a SCID mouse that received labeled T cells. Insulin is stained green, nuclei white, actin blue and T cells red.

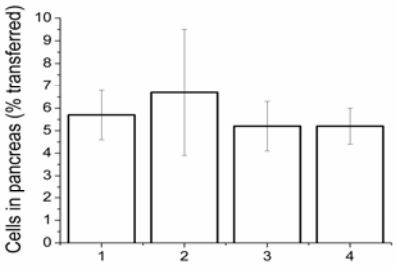

Fig. 3 Percentage of transferred cells that reached the pancreas in n=4 SCID mice.