^{19}F MRI for Visualization and Quantification of Cell Migration in a Diabetes Model

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1. Abstract

A novel ^{19}F based cell label that allows for both *in vivo* tracking and quantification of cell numbers, is applied in a murine diabetes model: Labeled diabetogenic T cells were tracked as they homed to the pancreas. We found that about 2% of the transferred cells reached the pancreas within 48 hours.

2. Introduction

The ability to track and quantify specific populations of cells *in vivo* -without ambiguity- would immensely help in the study of the etiology of various diseases. The technique could extended to cell therapeutics, including stem cell and immunotherapy.

A ^{19}F based cell label avoids the problem of the large ^{1}H background, while maintaining a sensitivity of almost 83% that of ^{1}H. Furthermore, quantification of cell numbers is more straightforward with ^{19}F labels.

We developed and applied a ^{19}F label to track pathogenic T cells in a murine autoimmune diabetes model. Importantly, we quantified pathogenic cells reaching the pancreas directly from the *in vivo* image data.

3. Results

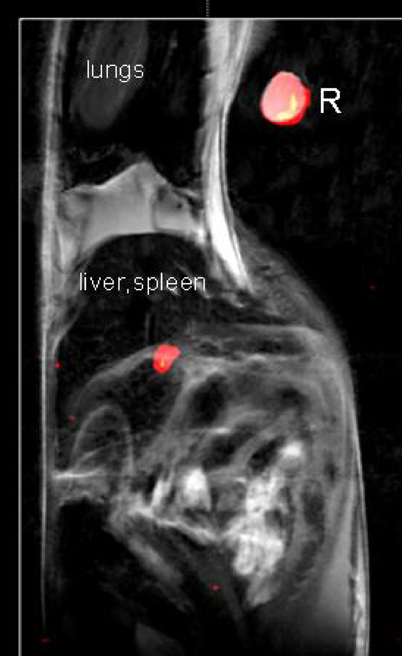

Fig. 1 *In vivo* MRI showing labeled T cells homing to the pancreas. The figure is a composite ^{19}F/^{1}H coronal slice through the mouse torso, where the ^{19}F is rendered in pseudo-color. ^{19}F-labeled, *in vitro* activated diabetogenic T cells, transferred i.p. 48 hours prior, home to the pancreas. The ^{19}F reference capillary (R) is placed next to the mouse. Based on this data, approximately 80,000 transferred cells homed to the pancreas after 48 hours.

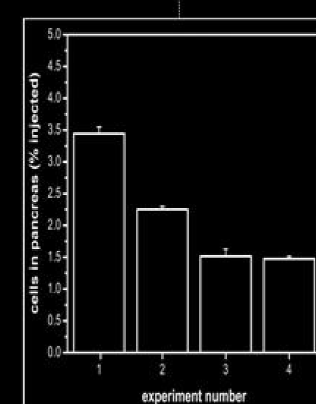

Fig. 2 *In vivo* quantification of T cells homing to the pancreas for n = 4 mice. The values represent the percentage of cells detected in the pancreas compared to the total number of i.p. transferred cells, ranging from 2-6 x 10^6 cells. See Methods for an explanation of the error bars.

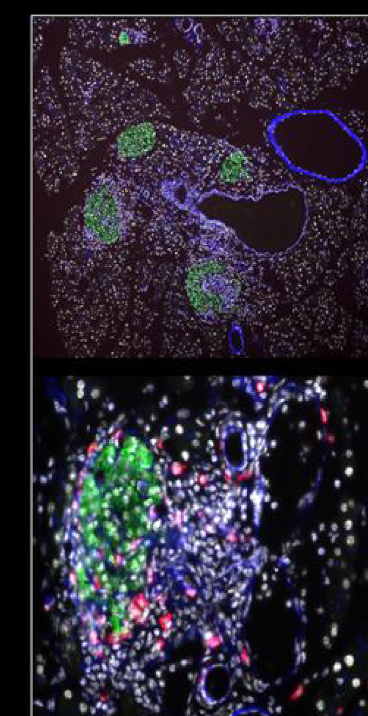

Fig. 3 Fluorescence micrographs of pancreatic tissue showing labeled T cells reaching the pancreas. The upper panel is at 100x, and the lower at 400x; T cells are stained red, insulin green, nuclei white and actin blue. The images show early insulitis, with the transferred T cells infiltrating into the islets, suggesting that labeling does not impair T cell trafficking, and confirming the MRI data.

4. Discussion

The *in vivo* quantification technique was confirmed in phantoms consisting of known cell densities in agarose.

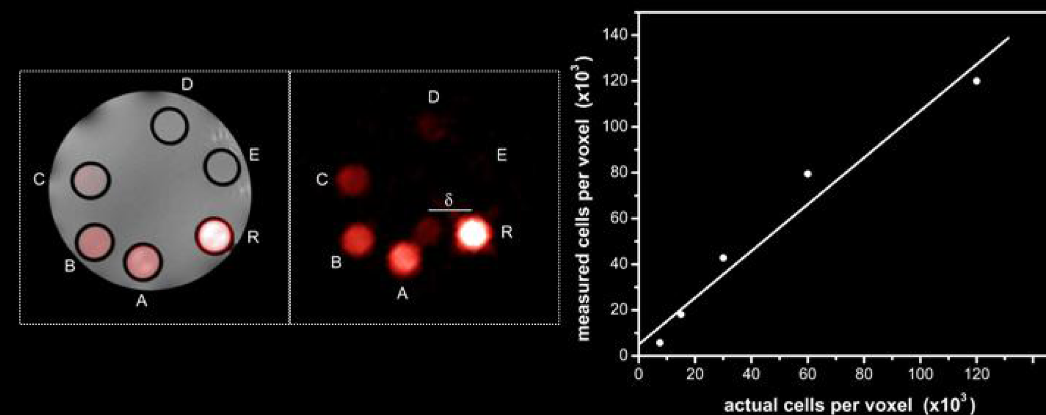

Fig. 4 Validation of T cell quantification using ^{19}F MRI. Shown is a composite ^{19}F/^{1}H (left) and intensity-rescaled ^{19}F image (center) through a phantom with known densities of labeled cells, where capillary A=12, B=6, C=3, D=1.5, and E=0.75 (x10^4) cells/voxel; R is a calibrated ^{19}F reference. δ represents the chemical shift artifact from highly concentrated tube, R. The final panel shows the actual versus MRI-measured cell numbers in the phantom (Pearson correlation coefficient = 0.98). The error bars for the ordinate are smaller than the data point symbol.

The quantification algorithm assumes that the number of fluorines per cell remains constant, although this value will change with cell division. However, this error is reduced by the use of activated T cells, and the relatively short time between cell transfer and imaging.

As control experiments, mice received either cell-free label, or nonfunctional, MHC-mismatched T cells. No ^{19}F was detected in the pancreas in these mice.

5. Conclusion

We have demonstrated the application of a novel ^{19}F label for the non-invasive tracking and quantification of a specific cell population *in vivo*. We found that 88000 ± 3600 cells, on average, reached the pancreas at 48 hrs. Although we applied the technique to tracking pathogenic T cells, it can readily be applied to different cell types or disease models, as well as cell therapeutics.

For more details, check out our upcoming paper in MRM.

Methods

Label synthesis

The ^{19}F label was an emulsion of perfluoro-PEG (Exfluor, Round Rock, Texas) and Pluronic L35 (Sigma-Aldrich, St. Louis, Missouri) at a 1:1 molar ratio.

Cell labeling and the murine diabetes model

T cells from the BDC2.5 transgenic mouse were purified and activated *in vitro*. The label, premixed with FuGENE 6 (Roche, Indianapolis, Indiana), was added to the cell suspension and incubated for 3 hours. Cells were washed and transferred, into NOD SCID mice which had received cyclophosphamide i.p. 24 hrs prior.

MRI

Mice were imaged using an 11.7 T, 89 mm vertical-bore micro-imaging system (Bruker), with a volume birdcage-type resonator tunable to either for ^{19}F or ^{1}H. ^{19}F images were acquired using a RARE sequence, TR/TE=1000/6.4 ms; ^{1}H images using a 2DFT spin-echo sequence with TR/TE=1200/22 ms. Eight 2mm-thick slices were acquired.

Cell quantification using MRI

The apparent number of labeled cells, C, was calculated from the *in vivo* MRI data, the external ^{19}F reference, and the measured F_c, which is [^{19}F]/cell on a per-slice basis. The real-valued noise magnitude, N, of the ^{19}F image was determined by calculating the standard deviation of voxel values near the image periphery. Next, the magnitude values were corrected to compensate for Rician-distributed noise. The average magnitude signal value, R, was then calculated in an ROI containing the ^{19}F reference, by interactively choosing a box containing the reference and automatically identifying voxels within it with magnitude >$2.5N$, thereby setting a confidence factor of >99% that the voxels scored contain actual ^{19}F signal. This automatically selected ROI was then dilated to account for partial volume effects. A parameter, r, the amount of ^{19}F per voxel in the reference, was also calculated. The total signal in the pancreas, P, was calculated in a similar manner. C was calculated using the relationship $C=(Pr)/(RF_c)$. The uncertainty in C was estimated by using the equation $\sigma(P)=N(2n)^{1/2}$, i.e., the standard deviation of P, and n is the number of voxels identified as having signal.

Acknowledgements

We acknowledge valuable assistance from Jelena M. Janjic, Deepak Kumar Kana Kadayakkara, Kevin Hitchens, Virgil Simplaceanu, Dewayne Falkner and Chris Navara. This work was funded in part by the National Institutes of Health (R01-EB003453, R01-EB004155, P01-HD047675 and P50-ES012359). The Pittsburgh NMR Center is supported by the National Institute of Biomedical Imaging and Bioengineering as a National Biomedical Research Resource Center (P41EB-001977).