Using a 19F MRI Tracer Agent for In Vivo Tracking of Human Dendritic Cell Vaccines

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Introduction

The inability to non-invasively track clinically relevant cell types after in vivo administration has been a significant roadblock to the acceptance of cellular therapies. Dendritic cells (DCs), the "professional" antigen presenting cells of the immune system, are increasingly being used as cellular vaccines in the setting of cancer and infectious diseases. Here we *ex vivo* labeled human DCs using a novel, commercially available fluorine (^{19}F) –based MRI tracer agent to visualize these transferred cells *in vivo by* ^{19}F MRI. In this study we show for the first time that clinically relevant human DCs can be effectively labeled in vitro with a ^{19}F tracer without impacting cell health, phenotype, or function. Moreover, the ^{19}F-labeled human DCs are capable of being detected by ^{19}F MRI 8 hours post injection into NOD-SCID mice, demonstrating utility and possible clinical application of this method.

Methods

Human monocyte derived immature DCs were generated in the presence of IL-4 (500 IU/ml) and GM-CSF (500 IU/ml) for 5 days, and maturated by exposure to TNF-α, IL-1β, IL-6, and PGE2 for 48 hours as previously described (1,2). Two different PFPE tracer reagents (CS1000 or CS1280+, Celsense, Inc., Pittsburgh PA) were used to label the DCs. These reagents were added to day 5 cultures, and the cells were then harvested, washed, and characterized on day 7. The ^{19}F labeling efficiency was determined by NMR of a fraction of the cells. Cell viability and phenotype were determined by microscopic analysis as well as flow cytometry. The IL-12p70 producing capacity of the DCs was tested following CD40L stimulation by measuring protein concentration in culture supernatants by ELISA (Endogen, Woburn, MA). The migratory responsiveness of the DCs to CCL21 (6Ckine; R&D systems, Minneapolis, MN) was examined using 96 well migration chambers (NeuroProbe, Gaithersburg, MD). To determine T cell stimulatory capacity, proliferation assays were performed using allogeneic T cells as responders (allo-MLR). The Cell-Titer Glow Assay (Promega, Madison, WI) was used to determine cell counts for both migration and proliferation assays. To determine anatomical locations of transplanted cells, ^{1}H images were acquired. Following conventional ^{1}H image acquisition, spin-density-weighted ^{19}F images were acquired using a Bruker AVANCE 7 Tesla system with a re-tuned RF coil. ^{19}F images were collected, and labeled cells were rendered in pseudo-color and overlaid onto the registered ^{1}H images.

Results and Discussion

While previous studies have demonstrated the utilization of ^{19}F tracer agents as tools for *in vivo* imaging of animal cells and immortalized cell lines (3,4), this is the first report demonstrating the utility of ^{19}F tracer agents in labeling clinically relevant human immune cells for *in vivo* tracking. This study illustrates that the ^{19}F tracer agents tested had no deleterious effect on cell function or viability while providing a high-resolution image of migratory cells following injection into NOD-SCID mice. This study also offers *in vivo* functional confirmation that transplanted ^{19}F-labeled human DCs can indeed migrate to draining lymph nodes in response to chemokines of a murine host. This novel animal model may now provide researchers with a valuable tool for pre-clinical *in vivo* human DC studies.

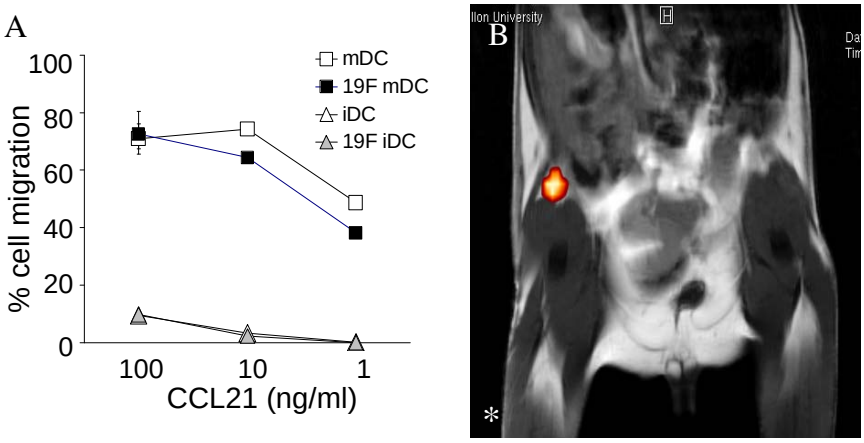

Fig 1. Migration of ^{19}F-labeled human DCs. (A) *In vitro* analysis of the migratory responsiveness of immature DCs (iDC) and mature DCs (mDC) to the lymph node associated chemokine CCL21. (B) MRI of a NOD-SCID mouse demonstrating *In vivo* migration of human mature DC. Images were acquired 8h post-injection of ^{19}F-labeled human DCs ($2x10^6$). The DCs were injected into the quadriceps at the location indicated with the asterisks (*). The anatomical 1H image is grayscale and the ^{19}F image is rendered in hot-iron scale showing the accumulation of perfluorocarbon-labeled human mature DCs

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References

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