Measuring Dendritic Cell Migration to Lymph Nodes

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Introduction: The immune system plays a crucial role in preventing cancer. Cancer vaccines are currently undergoing intense investigation as an approach to providing immunotherapy for the treatment of many forms of cancer. Dendritic cells (DC) are potentially very effective therapeutic candidates for the treatment of cancer. In DC cancer therapy, the patients' own DC are activated (with self tumor antigen or known tumor antigen), and then injected back into the patient. Primed DC migrate to lymph nodes where they encounter lymphoctyes and mount an immune response. Many fundamental questions about how to best prepare and administer DC are unanswered. At present it is not possible to quantitate the delivery and migration of DC-based vaccines in human subjects without biopsy or excision of the target lymphoid tissue. Ultimately, this information will lead to improved efficacy of DC-based immunotherapies. Imaging the migration of DC, under different experimental conditions, may be the key to gaining sensitive and specific information about the fate of DC and ultimately lead to improved efficacy of DC-based immunotherapies.
To the best of our knowledge there are only two groups who have demonstrated the ability to track DC *in vivo* in experimental models[1,2] The first, and only, demonstration of MRI cell tracking in humans was performed in the Netherlands in 2005. This study showed that MRI using iron oxides for cell tracking was clinically safe, and superior to scintigraphy.[3] In this paper we show the ability to track DC in vivo in mice and to measure changes in lymph node volume and the degree of signal loss within the nodes over time with cell infiltration and cell death. These measurements correlate very well with DC infiltration measured by confocal microscopy.

Methods: DC were isolated from the bone marrow of 8-12 week old, enhanced green fluorescent protein (EGFP) C57Bl/6 mice. EGFP DC (0.1 or 1.0 $\times 10^6$), Feridex-labeled or control, were adoptively transferred into anesthetized syngeneic mice by subcutaneous injection into the hind-footpad. MR imaging was performed using a 1.5T GE CV/i MR scanner using a custom-built gradient coil and solenoid RF coil. Mice were scanned using the 3D fast imaging pulse sequence employing steady state acquisition (3D FIESTA or 3D SSFP) (TR/TE = 8.0/4.0, flip angle = 30º) at a resolution of 200µm³ isotropic over a 5cm FOV with 2 NEX and employing RF phase cycling. Acquisition time was less than 25 min. Mice were imaged 24-48 hours prior to adoptive transfer of DC and at 48 and 72 hours after DC injection. For quantitation of EGFP DC migration, the popliteal lymph nodes were removed, fixed with 4% formalin and cryoprotected. Lymph nodes were embedded in OCT compound and the entire node was cut into 16µm sections. Slides were examined using a Zeiss 510 LSM META-NLO 2 photon confocal microscope. Images of the whole lymph node were compiled using a 25× objective and the tiling function of LSM 510 software (Zeiss, version 4.6).

Results: Excellent whole body mouse 3DFIESTA images were obtained at 1.5T. DC can be labeled with enough Feridex (mean 10pgFe/cell) to allow their detection after in vivo migration from the footpad to the popliteal lymph node (Fig1). Feridex did not affect DC morphology or maturation; the expression of cell surface markers for MHC class II, CD86, and CD40 were not changed (not shown). An increase in the volume of the popliteal node, and an increase in the signal void volume, caused by DC infiltration, could be measured from the image data and were significantly different for different numbers of DC injected (Fig2). EGFP fluorescence was significantly greater, by 10-fold, in mice following injection of 1×10^6 DC compared with 0.1×10^6 DC. EGFP+ DC could be identified and quantified from confocal images (Fig 3). The distribution of EGFP fluorescence in draining popliteal lymph nodes is consistent with MR images. Analysis using the Spearman Rank Correlation technique demonstrates a strong positive correlation between amount of EGFP fluorescence in draining lymph nodes and MR image signal void volume (Rs = 0.8875) and the fractional signal loss (Rs = 0.6975).

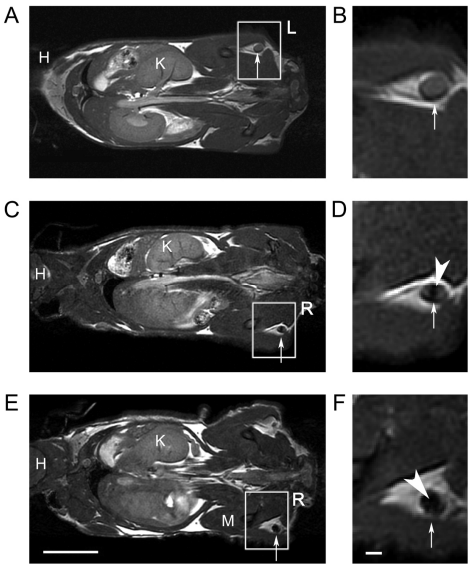

Figure 1. Coronal FIESTA images of the mouse body highlighting the popliteal lymph nodes (arrows) after injection of 1×10^6 unlabeled DC into the left footpad (A&B), and 0.1×10^6 (C&D) or 1×10^6 (E&F) Feridex-labeled DC into the right footpad. (B,D&F) are enlarged and cropped images of the nodes. Arrowhead indicates location of signal loss. H-head, K-kidney, M-muscle. Scale bars are 1cm for A,C&E and 1mm B,D&F. There is no apparent signal loss in the left node (unlabeled DC in footpad). The degree of signal loss was always greater with injections of 1×10^6 labeled DC compared with 0.1×10^6.

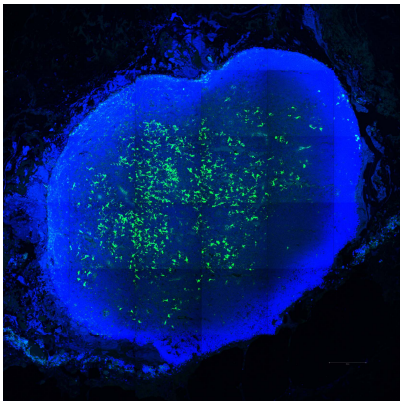

Figure 3. Confocal image showing a lymph node section with EGFP+ DC in green and counterstained with DAPI. From a mouse injected with 1×10^6 labeled DC.

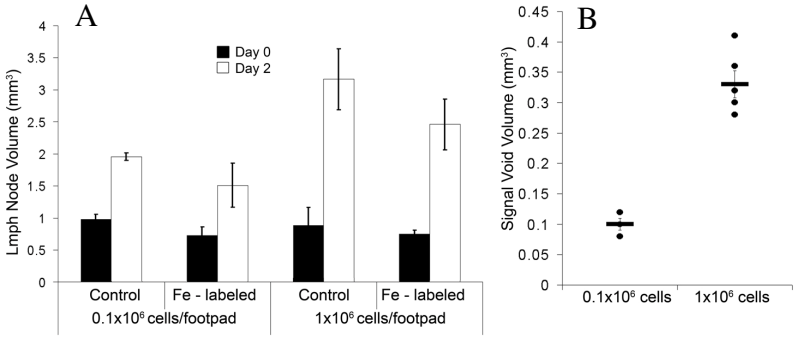

Figure 2. (A) Popliteal lymph node volumes measured prior (black bar) to and on day 2 (white bar) post injection of DC. (B) Signal void volume within popliteal lymph node on day 2 post injection of 100,000 or 1 million labeled DC. Data is mean +/- standard error (n = 6)

Discussion: *In vitro*-generated DC can be labeled with Feridex at a level which is sufficient to permit MR detection of DC that have migrated to draining lymph nodes. The lymph node volume, signal void volume and fractional signal loss correlate with the delivery and migration of *in vitro*-generated DC.

References [1] Ahrens et al. **Nat Biotechnol** 2005, [2] Baumjohann et al. **Eur J Immunol** 2006, [3] de Vries et al. **Nat Biotechnol** 2005.