In Vivo Detection, Tracking and Quantitation of SPIO-Labeled Dendritic Cells in the Mouse Lymphatic System

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Introduction: Metastasis is a leading cause of death due to cancer and tumour cells often utilize the lymphatic system for cellular trafficking to remote organs and tissues. The exquisite antigen processing and presenting capacity of dendritic cells (DC) together with their affinity for trafficking via the lymphatic system imbues DC with a powerful therapeutic potential. DC vaccine based cancer therapy in the clinic is feasible and non-toxic. Sensitive noninvasive imaging techniques are key to gain important information about the fate of DC vaccines *in vivo*. In this paper we demonstrate, for the first time, the ability to image the secondary lymphatic system in whole mouse body images and the ability to track and quantify iron labeled DC within the lymph nodes using an optimized MR microimaging system.

Methods: *Imaging*: Our microimaging technology is based around custom-designed ultra-high-performance gradient coils, adapted for use within whole-body clinical MRI scanners, but enabling an order-of-magnitude increase in peak gradient strength (500mT/m) and peak slew rate (3000T/m/s). This has enabled the use of the thermally demanding but signal-efficient 3D steady state free precession imaging sequence (or 3D FIESTA on GE MR scanners), to achieve very high spatial resolution in modest scan times while retaining high sensitivity to iron-loaded cells. Imaging was performed at 1.5 Tesla. Optimization of mouse body imaging included variations in timing parameters, bandwidth and implementation of RF phase cycling. 3DFIESTA images were acquired at a spatial resolution of 100x100x200 µm in ~ 40 min. *Experimental model*: EGFP+ mouse DC were labeled with Feridex (10 pgFe/cell). The effects of Feridex labelling on DC were tested using flow cytometry. Labeled DC were injected s.c. into the right hind footpads (10,000 and 1 million DC/footpad) of C57B16 mice (n=8). The same numbers of unlabeled DC were injected into the left footpad. *In vivo* images were obtained prior to and at days 2 & 3 after DC injection. In a separate group of 4 mice imaging was performed at days 2&9. *Quantification of image data:* 3D volumes were constructed in VGStudioMax 1.2™ (Volume Graphics GmbH). Lymph node and signal void volumes were manually segmented and measured by quantifying voxels matching specific criteria based on grey scale threshold value.

Results: Excellent whole body mouse 3DFIESTA images were obtained at 1.5T with minimal repetition times and phase cycling. Ten pairs of lymph nodes could be easily identified in various slices from the 3D data sets; including the popliteal, iliac, inguinal, renal, axial and brachial nodes (Figure 1). *Dendritic Cell Migration Model*: On day 2 after DC injection regions of signal loss were detected in the draining (popliteal) node on the right side (arrow in Fig 2. and 2C). Signal loss was not observed in the contralateral node (2B). Most importantly there was a greater degree of signal loss in the nodes of mice injected with 1 million DC (3A) compared with those receiving 10 fold fewer DC (3B). The volume of signal loss on day 2 was greater for mice injected with 1 million DC (versus 10,000) and the volume of signal loss increased from day 2 to day 3 for both 10,000 and 1 million DC injections (Fig.4A). Figure 4B shows that the volume of the draining lymph node increases significantly over time after the injection of DC. There was no significant difference measured in the lymph node volume for 1 million versus 10,000 DC injections. At 9 days post injection the region of signal loss had disappeared in 3/4 mice (not shown). Feridex, had little effect on DC morphology, and did not affect expression of cell surface MHC class II, CD86 and CD40, even at very high iron concentrations (not shown).

Discussion: Our results demonstrate that MR can be used to track DC migration *in vivo* and support the findings of three recently published papers (1-3). Our imaging results are most comparable to those of a paper by Baumjohann et al. (3); both studies used SPIO and examined the same migration pathway. In the Baumjohann paper *in situ* images clearly showed regions of signal loss within the nodes but were acquired in 12-16 hour scan times; *in vivo* images, acquired in reasonable scan times (45min), were presented after post processing in Photoshop to enhance the ability to visualize regions of signal loss. Here we show *in vivo*, high-resolution 3DFIESTA images acquired at 1.5T in practical scan times. This high quality image data permits the quantification of signal loss and lymph node volume changes over time. In these images we are able to detect signal loss due to the migration of DC *in vivo* after the injection of only 10,000 cells and to measure differences in signal loss generated after 1 million versus 10,000 injected DC migrate to the draining node. We are also able to quantify the morphologic changes in the popliteal node that are expected to occur with increased node cellularity after DC injection (4). This technique will allow studies of the dynamics and kinetics of DC migration *in vivo* and may provide much needed information for the development of more effective cancer vaccine therapies.

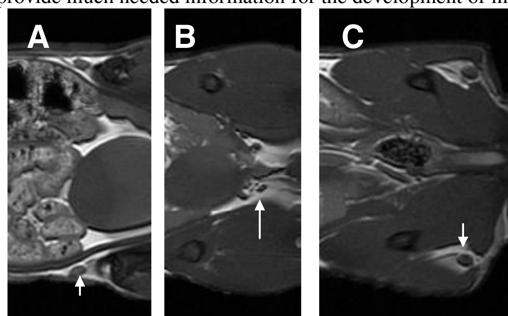

Figure 1. FIESTA image slices (cropped) from the same animal showing some of the various nodes (arrows) that can be visualized (a) inguinal, (b) iliac, (c) popliteal. 3D images were acquired in 40 minutes and have node SNR of ~ 50, node to fat CNR of ~ 65.

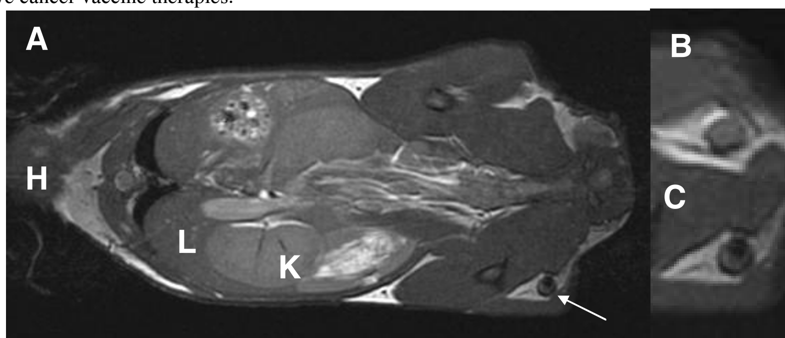

Figure 2. (A) FIESTA image of the mouse body showing the right popliteal lymph node (arrow) on day 3 after injection of Feridex-labelled DC into the footpad. (B&C) Enlarged and cropped images of nodes. (B) left popliteal node, left footpad was injected with 1 million unlabelled DC, (C) right popliteal node. H=head, K=kidney, L=liver.

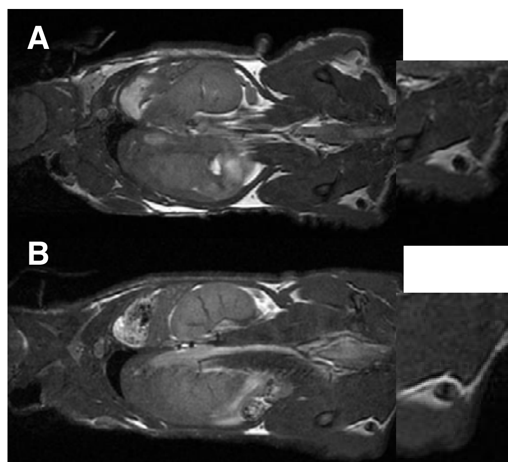

Figure 3. 1 million (A) and 10,000 (B) Feridex labeled DC into footpad. The degree of signal loss is greater in the node of the mouse injected with 1 million cells.

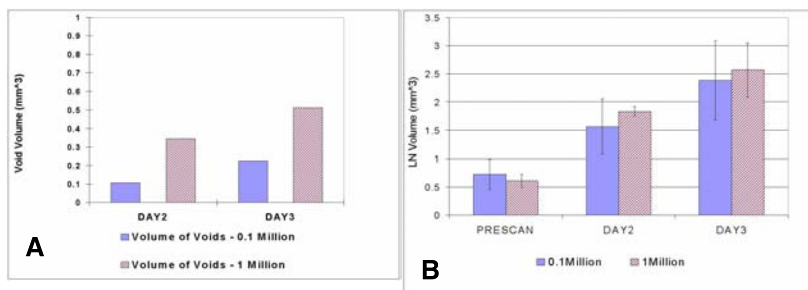

Figure 4. (A) Signal void volume within popliteal lymph nodes on days 2&3 post injection of 10,000 or 1 million SPIO-labeled DC. (B) Popliteal lymph node volumes measured prior to an on days 2&3 post injection of DC.

References

[1] Ahrens et al. **Nat Biotechnol** 2005, [2] de Vries et al. **Nat Biotechnol** 2005, [3] Baumjohann et al. **Eur J Immunol** 2006, [4] Martin-Fontecha et al. **J Exp Med**