

Development of an *in vivo* functional assay to monitor the effect of SPIO labeling on murine dendritic cells used for cell therapy in MRI

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Introduction Dendritic cells (DCs) have been used as cellular therapy for both cancer vaccination and induction of transplant tolerance.^{1,2,3,4} The migration of DCs from the periphery to lymph nodes (LNs) is critical in that DCs must migrate to the LN to interact with T cells and induce their effect. Imaging this migration *in vivo* using non-invasive, whole body imaging modalities such as Magnetic Resonance Imaging (MRI) provides insightful information that can be combined to provide temporal, anatomical and quantitative data, as well as provide an indication of the successful delivery of the therapy. The contrast agents used to track the cells, in this case the superparamagnetic iron oxide (SPIO) Endorem®, have the potential of being harmful to cellular function and viability. As studies to date rely only on *in vitro* functional assays, this study focuses on the development of an *in vivo* assay to monitor dendritic cell function while also using a reliable group of assays for monitoring label uptake, viability, phenotype and *in vitro* function. The *in vivo* assay monitors the ability of SPIO labeled and unlabeled DCs to both migrate from the periphery to the popliteal LN and initiate the proliferation of CFSE-labeled OT-II CD4⁺ T cells. The migration of the SPIO labeled DCs is monitored by sequential MRI on days 0, 1, 2, and 4 and the function of the injected DCs is monitored by the ability of the DCs to initiate T cell proliferation in the popliteal LN, as monitored by CFSE-dilution in FACS.

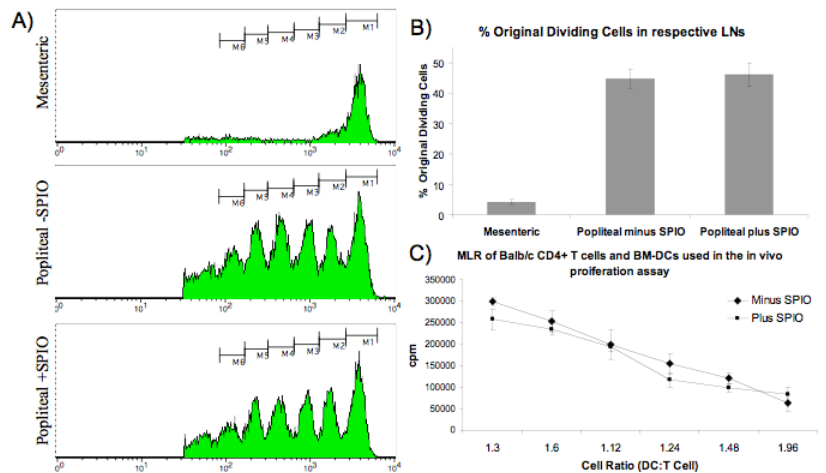


Figure 1 *In vivo* functional assay. (A) Representative example of T cell proliferation in the control mesenteric LN and the popliteal LNs both SPIO labeled and unlabeled. (B) The percent of original dividing cells in respective lymph nodes shown as an average of 3 experiments +/- SD. (C) MLR of same DCs used in the *in vivo* proliferation assay

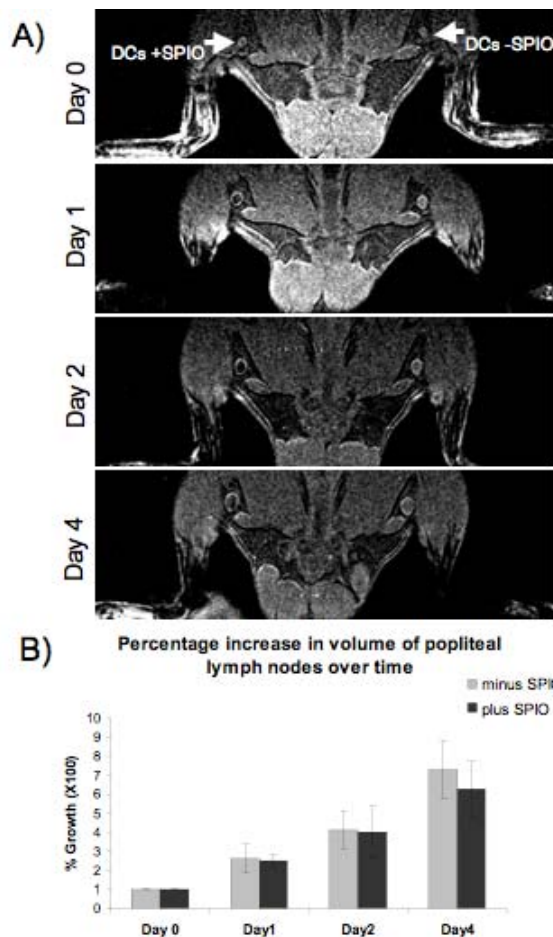


Figure 2 (A) Gradient-echo images of LNs where DCs +/-SPIO were injected in the right leg and DCs-SPIO were injected in the left leg. (B) Percentage increase in volume of popliteal lymph nodes over 4 days after the injection of SPIO +/- DCs.

Methods Bone marrow derived dendritic cells (BM-DCs) are made by isolating monocytes from C57Bl/6 bone marrow using negative selection and subsequent treatment with GM-CSF for 7 days before. BM-DCs were incubated with 100µg/ml of Endorem® for 4 hours. Cell viability was measured by trypan blue and AnnexinV/PI staining using FACS. Phenotype and maturation of labeled and unlabeled cells was monitored by FACS analysis of various surface markers (MHC-I, MHC-II, CD11c, CD40, CD80, CD86 and CD54). *In vitro* function was monitored by OT-II proliferation assays using whole OVA protein and OVA peptide, as well as mixed lymphocyte reactions (MLRs) using BALB/c CD4⁺ T cells. For the *in vivo* functional assay, 1*10⁶ LPS-treated, OVA peptide pulsed BM-DCs +/- SPIO are injected s.c. in the heel the day after the intravenous injection of 4*10⁶ CFSE-labeled OT-II CD4⁺ T cells. On Day 4 post DC injection, mesenteric and popliteal LNs were harvested, digested and analyzed of FACS. Mice were imaged sequentially by MRI on Days 0, 1, 2, and 4. MRI was performed on a 3T scanner (Philips Achieva) utilizing a 47mm Philips microscopy coil. Data was acquired using a gradient echo sequence with: flip angle=25°; FOV=45x30x8mm; matrix=296x292; slice thickness=0.5mm TE/TR=4.6ms/155ms.

Results The labeling procedure described labels BM-DCs with ~8pg iron/cell. SPIO labeled cells showed no decrease in viability as measured by trypan blue and AnnexinV/PI staining when compared to unlabeled cells. Phenotype and maturation of labeled and unlabeled cells measured by FACS analysis of surface markers also showed no changes. For the *in vivo* functional assay, FACS analysis of CFSE-labeled OT-II CD4⁺ T cells in mesenteric LN shows no T cell proliferation while popliteal LNs that had been injected with SPIO labeled or unlabeled DCs show similar amounts of proliferation - about 45% of original cells dividing. Sequential imaging on Days 0, 1, 2, and 4 shows that BM-DCs arrive in the popliteal lymph node within 24 hours and have an immediate effect on LN volume.

Conclusions The labeling procedure described here allows for the labeling of BM-DCs with SPIO in which there is no effect on the ability of the DCs to migrate and initiate T cell proliferation, as shown in the *in vivo* proliferation assay. Using MRI, the increase in volume of the LNs can be monitored and SPIO labeling has no effect on volume increase. Also, MRI shows that SPIO labeled DCs traffic to the LN within 24 hours.

References (1) Baumjohann et al. *Eur J Immunol* 2006, (2) de Vries et al. *Nat Biotechnol* 2005, (3) Reichardt et al. *J Immunol* 2008, (4) Roelen et al. *Transplantation* 2003.