Multimodal Imaging of luciferase transgenic dendritic cells using magnetic resonance imaging (MRI) based tracking after allogeneic hematopoietic cell transplantation

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Introduction:

Insight into the trafficking pattern of dendritic cell (DC) populations into lymphoid and non-lymphoid tissues after allogeneic hematopoietic cell transplantation (aHCT) is critical to understand the complex processes that regulate graft-versus-host disease [1,2]. To generate a platform for bioluminescence imaging (BLI) and magnetic resonance imaging (MRI) based cell trafficking studies, we labeled luciferase transgenic DC with superparamagnetic iron oxide (SPIO) nanoparticles bound to a murine IgG antibody. The purpose of this study was to investigate the feasibility of imaging the migration and incorporation of magnetically-labelled sensitized DC-cells after allogenic hematopoietic cell transplantation (aHCT) using BLI and MRI as dual modality approach.

Materials and Methods:

Bone marrow transplants were performed as described previously [3]. Briefly, recipients were given 5 x 10^6 wt TCD-BM cells after lethal irradiation with 800 cGy (C57Bl/6➔Balb/c). For trafficking studies, 1x10^6 luciferase transgenic SPIO loaded CD11c$^+$ DCs were given intramuscular on d0. In vivo bioluminescence imaging (BLI) was performed as previously described [4]. Briefly, mice were injected intraperitoneally with luciferin (10µg/g bodyweight, intraperitoneally). Ten minutes later mice were imaged using an IVIS200 charge-coupled device (CCD) imaging system (Xenogen, Alameda, CA) for 5 minutes **(Fig.3).** Expansion was quantified in photons/second/cm^2. Imaging data were analyzed and quantified with Living Image Software (Xenogen) and IgorProCarbon (WaveMetrics, Lake Oswego, OR). DC were labelled with (10 pgFe/cell). Labelled DC were injected s.c. into the right proximal legs (1 million DC/footpad) of BALB-C mice. MR in vivo images were obtained prior to and at days 3, 8 & 11 after DC injection. MRI was done on a 94/20 Bruker BioSpec, (Bruker, Germany) system employing a quadrature whole body birdcage resonator (35mm inner diameter). The MRI–Protocol consisted of a Pilot-Scan, a T1-weighted FLASH sequence (TR/TE/FA: 350ms/5.4ms/40°) and a T2-weighted RARE sequence (TR/TEeff/FA: 3490ms/36ms/180°) with a field of view of 30mm x 30mm, a matrix of 256x256 pixel obtaining an in plane resolution of 117 x 117µm^2 for morphological reference. Slice thickness and distance was 1mm. Slice orientation was coronal in both sequences. The 3D FLASH sequence had a TE/TR of 2.94ms/20ms, a bandwidth of 55kHz, an echo position of 25%, a matrix of 256x256x96, a resolution of 105 x 105 x 104 µm, a t_{acq} of 12'17'' and an alpha of 10°. Prussian blue staining was performed to detect the iron positive cells in the cervical lymphnodes to confirm the BLI and MRI Imaging results **(Fig 1+2**.).

Results and Discussion:

Intracellular SPIO uptake was highly effective and did not impact viability, migratory of pDC and myDC. Bioluminescence imaging (BLI) allowed for displaying homing and expansion patterns of following aHCT. Locally injected DCs migrated to secondary lymphoid tissues and retained SPIO-IgG complexes as evidenced by histological analysis for iron particles and MRT following aHCT. Coupling SPIO nanoparticles to murine IgG antibody allowed for Fc receptor mediated endocytosis into pDC (Fig.1) and thereby provides a tool for tracking of this rare cell population after aHCT.

Based on the BLI/histology results we reasoned that it may be possible to track locally injected SPIO loaded CD11c$^+$ DCs. We found that SPIO labeled DCs could be detected after BMT at the injection site on day0. On consecutive days we found that DCs migrated throughout the muscle (**Fig. 3**). On day 8 we observed iron specific signal in the ipsilateral cervical lymph node (**Fig. 3**).

Conclusion:

Based on our findings BLI may be ideal for detecting regions of interest where luc$^+$ DCs accumulate while MRI is more suitable for displaying donor cell localization within their anatomical context at high resolution. This combined approach may allow a systematic analysis on the impact of immunosuppressive agents and tumor microenvironment on donor DC homing patterns after aHCT.

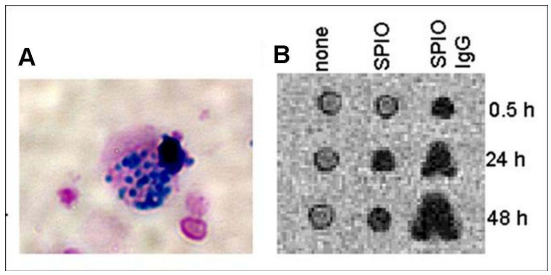

Figure 1:
A. Prussian blue stain for intracellular iron within DCs cultured with SPIO-IgG-FITC particles. Original magnification x1000.
B. MRI signal disruption within a sorted pDC cell population is displayed. Left panel: no SPIO, Signal disruption is significantly higher when SPIO-IgG as compared to SPIO are employed.

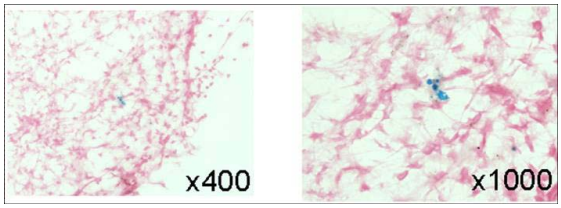

Figure 2 Detection of Fe within the cervical lymph node of an animal receiving local luc$^+$ SPIO$^+$ CD11c$^+$ cells in the right proximal leg. Original magnification x400 and x1000 as indicated.

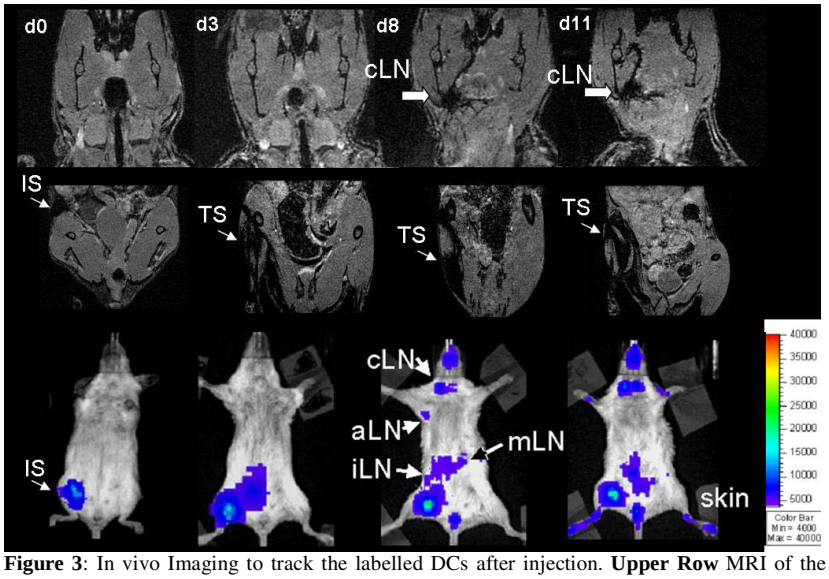

Figure 3: In vivo Imaging to track the labelled DCs after injection. **Upper Row** MRI of the cervical region, showing signal voids in the region of the cLN as indicated on d8+d11. **Middle row:** Signal void at the injection site (d0) and in the surrounding tissue (d3, 8, 11) as expected from the corresponding BLI. **Lower row:** Local injection of luc$^+$ SPIO$^+$ CD11c$^+$ cells in the right proximal leg on the day of BMT (C57B/6 ➔ Balb/c). Trafficking is monitored with BLI on the indicated days with: injection site= IS, cLN = cervical lymph node, aLN = axillary lymph node, iLN = inguinal lymph node, mLN = mesenteric lymph node, TS = Tissue.

References : **1**. Ahrens et al. Receptor-mediated endocytosis of iron-oxide particles provides efficient labeling of dendritic cells for in vivo MR imaging. Magn Reson Med. 2003;49:1006-1013. **2**. Shlomchik, W et al. Prevention of graft-versus-host disease by inactivation of host antigen-presenting cells. Science. 1999;285:412-415. **3**. Zeiser R et al. Inhibition of CD4+CD25+ regulatory T cell function by calcineurin dependent interleukin-2 production. Blood. 2006;108:390-399. **4**. Beilhack A et al. In vivo analyses of early events in acute graft-versus-host disease reveal sequential infiltration of T cell subsets. Blood. 2005;106:1113-1122.