In situ and in vivo magnetic resonance imaging of iron labelled dendritic cells in the mouse

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Introduction

Dendritic cells (DCs) play a critical role in the induction of adaptive immune responses. Upon microbial stimulation in the periphery, they gain access to the lymphatic system and migrate to the T cell zones of the draining lymph node (LN) where they prime antigen-specific naïve T cells. Several molecular and cellular approaches aim at the understanding of the migration process from the periphery to the T cell areas of the draining lymph nodes. However, most experimental settings are limited to non-physiological *in-vitro* assays or invasive *in vivo* models. In addition, as DCs are currently being tested as clinical vaccine against cancer, there is a strong need for non-invasive *in-vivo* imaging, i.e. monitoring of DC migration and biodistribution in the draining lymph nodes.

Due to its high spatiotemporal resolution and its feasibility for non-invasive *in-vivo* imaging, MRI has recently gained interest as a means of cellular tracking of cell-based therapies. Therefore, an efficient labelling method was adapted, based on two commercially available and clinically approved agents: small particles of iron oxide (SPIO), a contrast agent used in the clinic for liver imaging, and protamine sulfate, conventionally used in the clinic as antidote to heparin anticoagulation but also used as a transfection agent in molecular biology. Labelled DCs were injected into the foot pads of living mice, and the migration patterns into the draining lymph were analyzed by MRI *in situ* and *in vivo*.

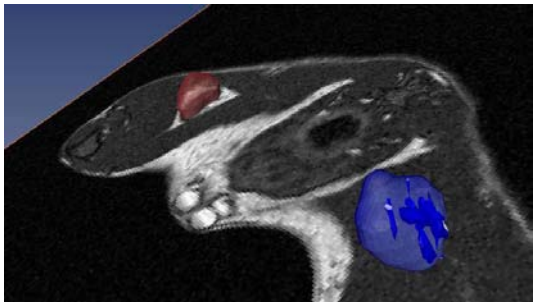
Materials and Methods

MRI was performed on a 4.7 T BRUKER Biospec scanner with a free bore of 40cm, equipped with an actively RF-decoupled coil system. Quadrature mouse volume coil was used as a receiver and transmitter coil. The scanning procedure started with the acquisition of T2 weighted spin echo coronal anatomical images for localisation of lymph node (slice thickness 1 mm, 16 slices, field of view 35 x 35 mm, matrix 256 x 128, TR = 2800 ms, TEef = 77 ms, 1 average) using a rapid acquisition relaxation enhanced sequence (RARE) with RARE factor equal 8. *In-vivo* axial images were acquired using MSME sequence (slice thickness 0.35 – 0.5 mm, 10 slices, field of view 30 x 30 mm, matrix 256 x 128, TR = 3000 ms, number of echoes 16, TE = 10 ms , 6 averages, scan time: 34min 30 sec). *In-situ* axial images were acquired using multi slice multi echo (MSME) sequence (slice thickness 0.35 mm, 10 slices, field of view 30 x 30 mm, matrix 256 x 256, TR = 3000 ms, TE = 10 ms, number of echoes 16, 30 - 50 averages, scan time: 6hours 20 min – 10 hours 40 min).

Results and Discussion

We combined clinically approved SPIO (Endorem) with protamine sulfate to achieve efficient labeling of murine bone marrow-derived DCs resulting in a resolution of 7.3 cells/voxel. The labeling showed no influence on the immunological properties of DCs, including surface marker expression, cell viability, and cell migration. Mature SPIO-DCs were injected into the foot pads of mice, and the migration into the draining lymph nodes was visualized by MRI. Distinct signal reduction patterns correlated with the biodistribution of SPIO-labeled DCs within Thy-1+ Tcell areas as confirmed by iron staining and immunohistochemistry. Injection of 1x10e6 SPIO-DCs resulted in the detection of 0.5-1x10e5 migrated DCs within the lymph node. DCs derived from CCR7-/- mice or SPIO alone did not reach the T cell areas.

One day after injection a MR signal loss could be detected in the lymph node corresponding to the side where labelled DC's were injected. This signal loss could be detected injecting $2x1*10^7$ down to $1*10^5$ labelled cells. Injection of Endorem in PBS into the food pad also led to a signal loss in the lymph node but at a spatially different substructure of the lymph node. Whereas the labelled DC's accumulate inside the T-cell area of the lymph node Endorem alone accumulates in the surrounding cortical region of the lymph node and is not able to enter the T-cell area. This is so because the transport into the T-cell area is an active, receptor mediated (CCR7) mechanism. With lower SNR the same imaging procedure was performed in living mices making this approach a useful tool to study migration of iron labelled DC's in vivo Fig1,2.



Conclusion

This study demonstrates that SPIO labelled DC's as well as their migration into the draining lymph nodes can be repetitively visualized not only *in-situ* but *in-vivo* by non-invasive MRI at high spatial resolution (140 µm in plane) and at a detectability around 7.33 cells/voxel.

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Fig 1. 3D visualisation of in vivo measurement after 36 hours after injection (right side with SPIO labeled cells is blue, left side with non labeled cells is red)

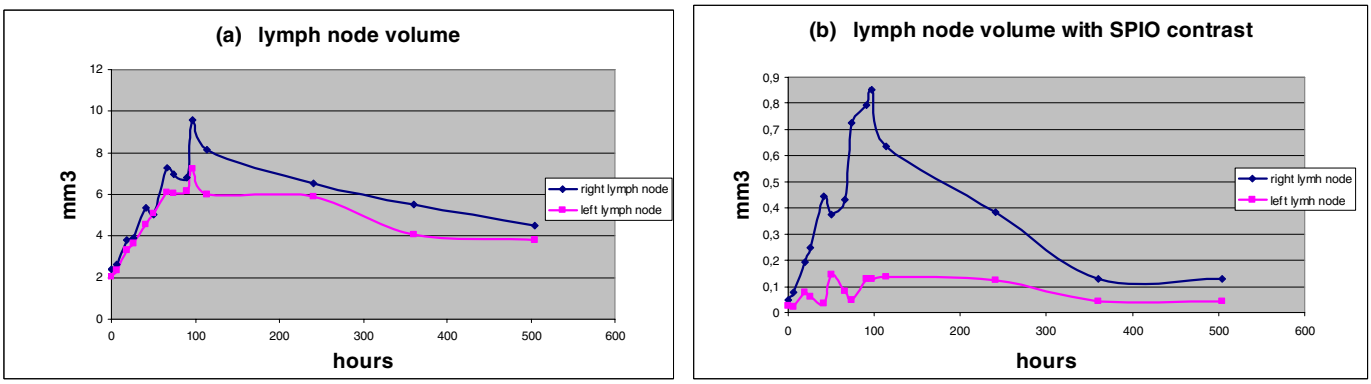

Fig 2. Increase of (a) lymph node volume and (b) volume with suppressed signal (contrast caused by presence of SPIO particles) after injection of SPIO labeled cells in right sides and non labeled cells in left side measured in vivo during 21 days after injection.