Optimization of dendritic cell labeling for MR tracking after vaccination in cancer patients

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Introduction

Dendritic cells (DCs) are the professional antigen presenting cells of the immune system. Recent clinical studies indicate that mature DCs are effective cancer vaccines when loaded with tumor antigens[1]. To induce an effective immune response, these cells should not only express high levels of antigen-presenting and co-stimulatory molecules but also migrate to the lymph nodes (LN) to interact with naïve T cells. Monitoring cellular migration in vivo is important to improve the efficacy of DC-based therapies[2]. Recently we demonstrated that DCs labeled with superparamagnetic iron oxide (SPIO) can be imaged and tracked in melanoma patients after intranodal injection[3]. Here we demonstrate in detail that DCs can be efficiently labeled with SPIO without affecting DC function and that low numbers of SPIO-labeled DC can be imaged in vitro on a 3T MR whole body scanner using clinically applicable parameters.

Materials and Methods

Autologous DCs were cultured from monocytes obtained from peripheral blood[3] and labeled with the clinically approved SPIO formulation Endorem® by adding various amounts of SPIO to the DC culture one day before adding the maturation factors. Iron contents of DCs were determined by Prussian Blue staining and by measuring the average amount of iron per cell using a Ferrozin-based spectrophotometric iron assay[4]. The phenotype, T cell stimulatory function and migratory behavior on fibronectin-coated plates[5] were determined of both SPIO-labeled and unlabeled DCs. MR imaging of phantoms, containing SPIO-labeled DC in 6% gelatin between to layers of 8% gelatin, was performed at 3T (Siemens Magnetom Trio), using a body phased array coil. Samples were imaged using T2*-w gradient echo (GRE; TR 800 ms, TSE 15 ms, flip angle 35°; resolution $0.5 \times 0.5 \times 5.5$ mm^3) sequences.

Results and Discussion

DCs were effectively labeled with SPIO by adding the particles to the immature DC culture one day before maturation (Fig. 1A). The iron content per cell increased in a linear fashion with increasing concentrations of SPIO in culture (Fig. 1B). As at the highest concentration of SPIO (400 μg/ml) the cell viability was decreased, 200 μg/ml of SPIO was chosen as the optimal dose. At this concentration, phenotypic characterization of DC by flowcytometry for DC maturation markers showed that SPIO did not influence DC differentiation and maturation. Furthermore, both the allogeneic and autologous T cell stimulatory capacity of DCs was not changed by the addition of SPIO. Moreover, the migratory capacity of SPIO-labeled cells was still intact (Fig. 1C). Cell densities of 100/μl could still be observed in GRE images of phantoms (Fig. 1D). Using a threshold of 50% signal decrease in a GRE image, which is appropriate for in vivo conditions and signal to noise, we estimate to be able to detect 10^3 DCs/μl if present throughout some adjacent voxels. With a typical in vivo spatial resolution of $0.5 \times 0.5 \times 3.5$ mm this means we can detect 0.9×10^3 DCs/voxel if present in ~4 adjacent voxels. By using a local surface coil for signal reception the spatial resolution can be increased to $0.5 \times 0.5 \times 0.5$ mm pushing the detection limit down to 0.1×10^3 DCs/voxel in 4 voxels.

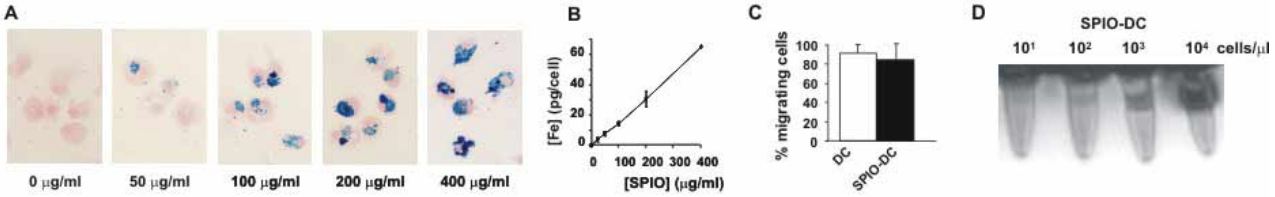

Figure 1: A. Prussian Blue staining of DCs cultured with increasing concentrations of SPIO (0-400 μg/ml). B. Average amount of iron oxide per cell for DC cultures with increasing amounts of SPIO. C. Percentage of migrating DC on fibronectin after labeling with SPIO compared to unlabeled cells. D. GRE image of phantoms containing increasing numbers of DCs cultured with 200 μg/ml (~25.5 pg/cell of iron oxide) imaged on a 3T whole body scanner. Tubes contain 10^1-10^4 SPIO-labeled DC/μl in a volume of 100 μl.

Conclusions

DC labeling with SPIO was efficient and did not affect DC function and migration at a concentrations of 200 μg/ml in the medium. Therefore, labeling DC with SPIO particles is a clinically safe method (without the need of adjunct transfection agents) for monitoring cell migration of cell vaccines. Low densities of SPIO-labeled DC can be imaged on the 3T spectrometer, demonstrating its potential for in vivo tracking of SPIO-labeled cells at a therapeutic dose.

References

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