^{19}F MRI tracking of dendritic cells in a novel migration assay

F. Bonetto[1], M. Srinivas[1], B. Weigelin[2], L. Cruz Ricondo[1], A. Heerschap[3], C. Figdor[1], and I. de Vries[1]
[1]Tumor Immunology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [2]Cell Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands, [3]Radiology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands

Introduction

Dendritic cells (DCs) are the professional antigen-presenting cells of the immune system. They constantly sample peripheral tissues of the body for pathogens, migrating to lymph nodes to activate T cells. Their decisive role in inducing immunity formed the rationale for DC immunotherapy: DCs loaded with tumor antigens are injected into cancer patients to stimulate T cells to eradicate tumors. However, and mainly due to an inefficient migration rate post-vaccination, success has been limited. DC migration can also be affected by several factors, such as the chemokine milieu at the injection site. In order to study these conditions in vitro, a suitable assay is required. The *aim of this study* is to introduce a novel ^{19}F MRI-based, quantitative assay to measure cell migration in varying chemokine environments in a 3D scaffold specially designed to mimic biological tissue.

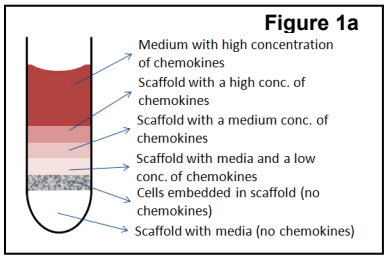
Methods

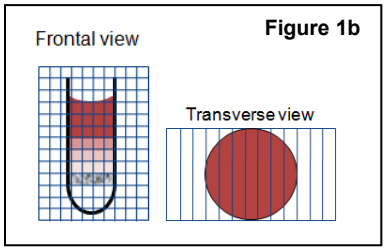
Human DCs were generated from peripheral blood mononuclear cells and matured as previously described [1]. For ^{19}F-labeling, a perfluorcarbon label was added. After maturation, cells were harvested, and viability determined by tryphan blue exclusion. Maturation marker expression by labeled DCs was determined by flow cytometry. The presence of the label within the cells was checked by microscopy.

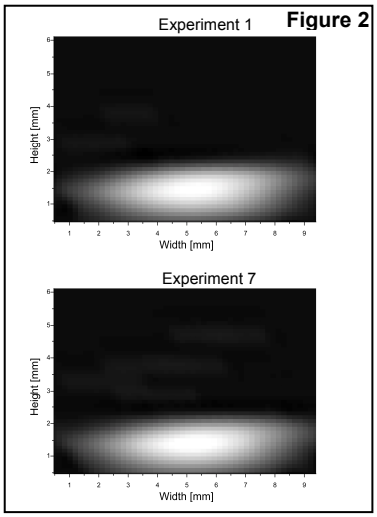
For migration assays (see Fig. 1), a fixed number of cells were embedded in the scaffold and a chemokine gradient was created with CCL21 (R&D Systems Inc.). A gel layer without chemokines was set below the cell layer as a control. The sample was placed vertically in the MRI scanner and only upward migration was considered to exclude any movement due to gravity. All experiments were performed on a 7T horizontal bore MR-system with a ^{1}H/^{19}F volume coil. ^{1}H 2D spin echo images and chemical shift spectroscopy (CSI) were used to track and quantify migration of 1-15x10^{6} cells. ^{1}H images were acquired with TR/TE=1000/22 ms and 0.125×0.125×10 mm^{3} resolution. A 0.47×0.47×10 mm^{3} matrix size with TR/TE=400/2.94ms was used for ^{19}F CSI (see Fig. 1b). Eight ^{19}F-CSI experiments (1.5 hours each) were sequentially performed in order to keep track of the migrating cells.

Results

Already after one hour (reference experiment) about 50×10^{4} ^{19}F labeled cells were found above the cell layer of 5x10^{6} DCs (see Fig. 2 and 3). The cell number in the migration region increased in the following experiments until 10 hrs after the start of the experiment. About 100×10^{4} (2% of total) more cells were found in experiment 7 relative to experiment 1. Migration seemed to stop thereafter (see Fig. 3). No cells were found below the starting cell layer during the whole study, indicating that cells moved upwards due to the chemokine gradient.







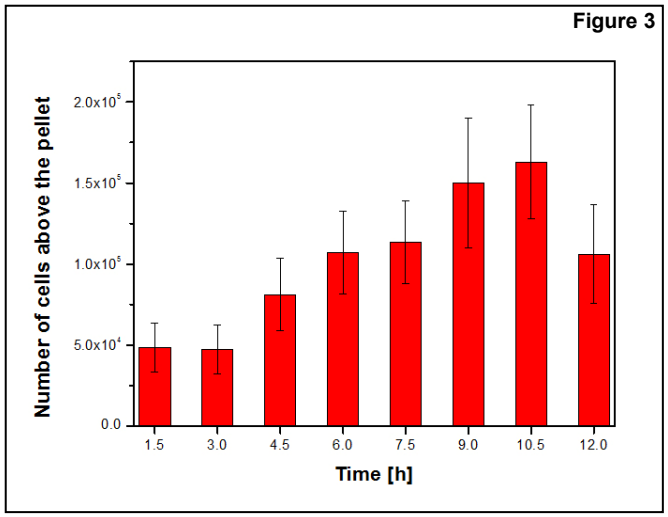


Fig. 1*: Scheme of the experimental setup showing the different scaffold layers used in the migration assay (Fig. 1a). The ^{19}F-CSI matrix grid and the slice thickness are schematically shown in Fig. 1b.* ***Fig. 2****: ^{19}F-CSI cell maps for experiments 1st and 7th when a 5×10^{6}DCs were inserted.* ***Fig. 3****: Evolution of the number of cells above the cell layer. This result is clearly showing that cells move upwards. Cell migration apparently stops after 10 hours.*

Discussion and Conclusions

In the present study we showed that ^{19}F-CSI can be used to track and quantify cell migration in opaque scaffold assays. The number of cells of which the starting cell layer was composed of was similar to the typical 15 million cells bolus injection in patients [2] and the migration rates were measured to be in the order of 2%, similar to clinical results [2]. Thus, this assay seems suitable to simulate in vivo clinical conditions, giving the option to analyze the problems involved in DC migration. The longitudinal study showed that cell migration stopped after about 10 hours. Possible causes for this behavior are cell death, for example due to lack of oxygen and nutrients and/or a progressive chemokine gradient reduction.

We have demonstrated the utility of the novel assay to study cell migration using MRI. In particular, the assay can also be applied to opaque tissue samples.

References

[1] P. Verdijk, T. W. Scheenen, et al.. "Sensitivity of magnetic resonance imaging of dendritic cells for in vivo tracking of cellular cancer vaccines." Int J Cancer **120**(5): 978-84 (2007).

[2] I. J. de Vries, W. J. Lesterhuis et. al.. "Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy" Nat. Biotechnol. **23**(11), 1407-1412 (2005).

Acknowledgements

This work was partially supported by NWO (VISTA grant) and ZONMW (911-06-021) for investments.