Maximizing dendritic cell migration after vaccination therapy

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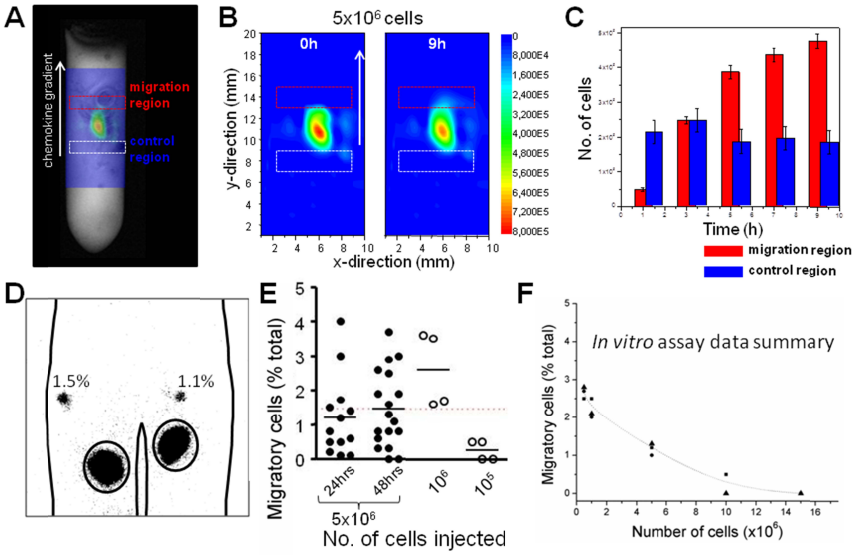
Introduction

Cellular therapy is key component of the recent paradigm shift in medicine, where a patient's own resources are harnessed towards a cure. For example, autologous antigen-presenting cells, principally dendritic cells (DCs), from a cancer patient are used to stimulate their immune system against the disease. Although about 20 clinical trials involving intradermally delivered DCs have been carried out, no more than 4% of the cells have reproducibility reached the target lymph nodes. However, the few cells reaching the lymph nodes are able to interact with and activate lymphocytes [1]. Optimization of this migration has proved difficult, in large part because there was no suitable in vitro assay to accommodate the large cell numbers typically used in such trials (millions of cells). We have recently developed such an assay [paper in revision], based on scale migration of DCs in a collagen scaffold using ^{19}F imaging to measure cell migration. The 3D scaffold mimics biological tissue. The assay can handle opaque tissue samples, which is a major strength compared to traditional microscopy-based migration assessments. Here we further validate the assay results with clinical DC migration data. In particular, we find that bolus injections of over 1 million cells result in extensive cell death and therefore reduced migration.

Methods

Human DCs were generated from peripheral blood mononuclear cells, and labeled with ^{111}In for scintigraphy in melanoma patients where indicated [2]. For ^{19}F-labeling, non-emulsion based perfluorocarbon nanoparticles were added [3]. For migration assays, a known number of cells was embedded in the scaffold with a superimposed chemokine gradient (A). Alternatively, cells were injected directly in tissue samples (B). Samples were placed vertically and only upward migration was considered, thus excluding nonspecific movement. All experiments were performed on a 7T horizontal bore, temperature-regulated MR-system with a ^{1}H/^{19}F volume coil. ^{1}H 2D spin echo images and MR spectroscopic imaging were used to track and quantify migration of 0.5-15x10^{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 MRSI (B). Eight ^{19}F-MRSI experiments (1.5 hours each) were sequentially performed.

Results and Discussion



^{19}F MRI is inherently quantitative [4]. Our ^{19}F labeling has previously been shown to be well-tolerated by DCs [3]. Migration occurred only with lower cell numbers, and always only in the migration layer. An example of data with 5 million cells in a tissue sample is shown (B). Migration did not occur in the control regions, for all cell numbers (C; red bars indicate cells in the migration and blue in the control regions respectively). The in vitro migration data show a strong correlation between the total cell number and their migration (F). We found that decreasing the number of cells in the cell layer resulted in a higher percentage of migration. Moreover, we found no difference in migration rates whether we used tissue samples or the gel scaffold.

When ^{111}In-labeled DCs were injected intradermally in melanoma patients and subsequently imaged using scintigraphy, we found that between 1-4% of the total cells reached the draining lymph nodes (D,E). Furthermore, the number of migratory cells increased as the total number of cells dropped from 5 to 0.1 million. This is in line with our assay results (F). However, the discrepancy at the lowest cell number is due to the low sensitivity of clinical scintigraphy, which was not able to detect the small numbers of migratory cells when only 0.5 million cells were transferred to the patient.

This low sensitivity of clinical imaging techniques is one key reason why an in vitro assay is necessary to optimize cell migration. Furthermore, this also greatly reduces costs and logistic issues. Previous migration assays suffered from being restricted by cell numbers, often only up to 10^{5} cells per assay, and/or required transparent samples. Our assay allows us to use the large cell numbers used in the clinic, together with opaque samples, including tissue. Thus, these assays could be carried out using human cells in human tissue. The technique is also inherently quantitative. The assay can readily be modified to study the effect of different chemokines or other factors.

Conclusion

Cell migration is challenging to measure quantitatively in vivo, however its optimization is necessary for the success of DC vaccination therapy. The therapy is expensive, and it is not feasible to study DC migration in patients using current technology in a sufficiently sensitive manner. Thus, our ^{19}F MRI-based migration assay can act as an in vitro substitute. We have shown that the in vitro results match patient data, and can be done in tissue samples. Thus this novel assay can be developed to predict and optimize DC migration in vitro, in a cheap and efficient manner. Finally, these data suggest that the typical cell numbers used in trials (generally millions or tens of millions of cells) can be too large for effective bolus injections.

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