Quantification of cell trafficking in vivo using magnetically sensitive histograms

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Introduction: Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cancer cell vaccines can elicit potent anti-cancer immune responses[1] and are currently used clinically to boost patient's immune response. Paracrine production of GM-CSF by irradiated tumor cells recruits dendritic cells (DC) that capture tumor antigens released by cancer vaccines. However, to induce an effective immune response, DCs must not only be activated but also migrate to the lymph nodes where they prime antigen-specific naïve T cells. Quantifying this cellular migration following antigen capture *in vivo* is critical for evaluating strategies to improve the efficacy of GM-CSF based tumor vaccines. Here we describe a non-invasive *in vivo* cell transfer labeling method, which relies upon DC capture of dying, Feridex® labeled, cancer cells and their contents. Furthermore, using a novel method, based upon histograms that display magnetic sensitivity, we assessed the feasibility of quantifying antigen capture and trafficking of DCs to draining lymph nodes.

Materials and Methods: B16 melanoma cells and a GM-CSF producing "bystander cell" (B78H1/GM-CSF) were irradiated ($1x10^4$ and $5X10^3$ rads, respectively) and labeled with SPIO (Feridex) by overnight incubation. Iron uptake was determined by Prussian Blue as well as by Ferrozine assay. $1X10^6$ labeled B16 cells along with $1X10^5$ labeled B78H1/GM-CSF cells were injected into the footpads of mice in a total volume of 25 ul. MR imaging was performed on a Bruker 9.4T horizontal bore animal scanner. A T2-w coronal spin echo image was acquired for localization of the draining popliteal lymph nodes (slice thickness 1 mm, 24 slices, field of view 56 X 24 mm, matrix 128 X 128, TR = 3000 ms, TE = 50 ms, 1 average) using a rapid acquisition relaxation enhanced sequence (RARE) with a RARE factor of 8. Axial images were acquired using a multi-gradient echo (MGE) sequence (slice thickness 0.350 mm, 8 slices, field of view 24 X 24 mm, matrix 240 X 240, TR = 1000 ms, TE = 4 ms, number of echoes = 4, averages = 3, gauss512 fat suppression) as well as a RARE sequence (slice thickness 0.350 mm, 8 slices, field of view 24 X 24 mm, matrix 240 X 240, TR = 2000 ms, TE = 60 ms, 3 averages, Rare factor 8). RARE images were used for an accurate anatomical determination of lymph node size while MGE images were used to resolve SPIO labeled DCs. For each MR image, we manually selected a region of interest (ROI) for each popliteal lymph node using the SE image. These ROIs were then copied onto the MGE image of the corresponding slice and a pixel intensity histogram was created. To control for minor variations in absolute signal intensity between individual scans, we calibrated low-signal areas on adjacent muscle tissue in each slice and determined our low signal threshold based up minimum pixel intensity in this muscle tissue. We summated pixels falling below this minimum pixel intensity for all slices and plotted these values as "black pixels." Single-cell suspensions were made from popliteal lymph nodes after the MR imaging and pooled by prior "black pixel" grouping. Cells were then run over magnetically activated cell sorting (MACS) columns for SPIO-positive cell selection and counted with a microscope and hemocytometer.

Results and Discussion: Immunosurveillant DCs were effectively labeled indirectly (*in vivo* cell-cell transfer of SPIO), and arrived in the popliteal lymph node as soon as 3 days following tumor vaccination. Using a combination of SE and MGE images (figures 1a and 1b) we were able to construct magnetically sensitive histograms, shown in figure 1c & 1d which were used to evaluate the amount of antigen taken up by DCs at the vaccine site and transported to the draining popliteal lymph node (black pixels in figure 1e). These black pixel values were correlated with cell counts from our magnetically positive cell populations found within each black pixel grouping (figure 1e). The number of sorted, magnetically positive DCs was found to correlate exceptionally well with our black pixel counts throughout multiple experiments, thus confirming our ability to quantify, *in vivo*, dendritic cell trafficking to lymph nodes. In this study, we demonstrate the sensitivity of our cell transfer labeling technique in distinguishing high versus low levels of antigen delivery by dendritic cells. We also display the vast biological variation present within the vaccine setting and believe we have developed a tool that will prove useful in identifying this variability. Additionally, we have previously reported evidence that MRI tracking of magnetically labeled DCs may be sensitive enough to evaluate the efficacy of potential immune modulators[2]. As Feridex is clinically approved and DC MRI tracking has been introduced in the clinic[3], this method may be readily clinically translatable as a quantitative means to determine vaccine activity in patients with and without immune adjuvants.

[1]Dranoff et al., Proc Natl Acad Sci USA 1993 (90) 3539
[2]Long et al., Proc. ISMRM 2007, 3636
[3]de Vries et al., Nat Biotechnol 2005 (23) 1407-13

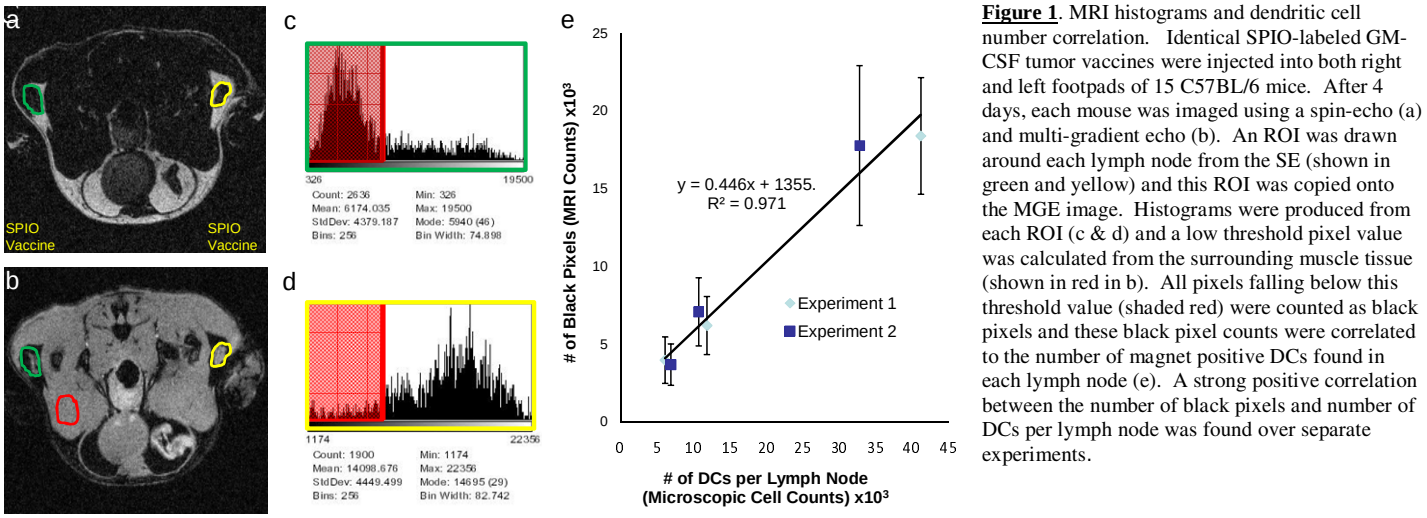

Figure 1. MRI histograms and dendritic cell number correlation. Identical SPIO-labeled GM-CSF tumor vaccines were injected into both right and left footpads of 15 C57BL/6 mice. After 4 days, each mouse was imaged using a spin-echo (a) and multi-gradient echo (b). An ROI was drawn around each lymph node from the SE (shown in green and yellow) and this ROI was copied onto the MGE image. Histograms were produced from each ROI (c & d) and a low threshold pixel value was calculated from the surrounding muscle tissue (shown in red in b). All pixels falling below this threshold value (shaded red) were counted as black pixels and these black pixel counts were correlated to the number of magnet positive DCs found in each lymph node (e). A strong positive correlation between the number of black pixels and number of DCs per lymph node was found over separate experiments.