Using MRI to track SPIO-labeled effector and regulatory immune cells in a cancer model

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Introduction: Superparamagnetic iron oxide (SPIO) has been used for a number of years to label a variety of immune cell types for in vivo detection with MRI [1]. Much of the work in this field has been concentrated on labeling dendritic cells (DCs) [2], macrophages [3], and with more limited success, effector T cells (i.e. CD8+ cells) [4]. However, a growing area of interest in cancer research is the behaviour of regulatory cells such as myeloid derived suppressor cells (MDSCs) [5] and regulatory T cells [6]. These regulatory cells suppress both inherent and induced immune responses (i.e. via treatments such as immunotherapies) resulting in the underperformance of many anti-cancer treatments. Our current work investigated the migration patterns of three types of SPIO-labeled cells in a mouse tumor model following intravenous tail-vein injection. In order to assess the effector and regulatory cell response, in particular in response to vaccination, we evaluated the migration patterns of suppressor MDSCs and Tregs, as well as that of active cytotoxic T cells in a mouse C3-HPV16 cervical cancer model. The therapeutic cancer vaccine, DepoVax™ (DPX)-0907 [5], has previously been shown using flow cytometry to reduce the proliferation of suppressor cells in vaccinated mice. In this study we monitored changes in suppressor cell migration patterns in response to mice vaccinated with DepoVax. The two main objectives of this study were 1) to demonstrate reliable homing of regulatory and effector cells to tumors and lymph nodes (LNs), and 2) to observe changes in these patterns in response to cancer immunotherapy.

Methods: Female C57BL/6 mice (4-6 weeks old) underwent C3 tumor cell implantation on Day 0, with $5x10^5$ cells implanted subcutaneously (s.c.) into the left flank. A subset of these mice was then terminated approximately 28 days post-implantation and the lymph nodes and spleens were removed for cell isolation. Treg and CD8 effector T cells were isolated from lymph nodes using T cell panning (CD8 cells were also primed with antigen *in vitro*), and MDSCs were isolated from spleens using MACS isolation (Miltenyi-Biotec, Cologne, Germany). Cells were labeled using ~35nm SPIO-Rhodamine B (Biopal, Worchester, MA) at 0.1mg/ml for 24hrs. On Day 14 post-implantation, 8 mice received DepoVax™ with 5µg R9F (4 per cell experiment for Treg and MDSC). Vaccine formulations were delivered via a single 50µL s.c. contralateral immunization (right flank). MRI scans were performed beginning approximately 28 days post tumor implantation (two weeks post-vaccination) and again on days 1, 2, 3, 6 and 9 post-cell injection (n=8 for Tregs, n=8 for MDSCs, n=4 for CD8s). All data were acquired on a 3T magnet equipped with 21 cm ID gradient coil (Magnex Scientific, Oxford, UK) interfaced with a Varian DD Console (Agilent, Santa Clara, CA). A 30mm ID quadrature transmit/receive RF coil (Doty Scientific, Col., SC), was used to image tumors, vaccination sites, and left & right inguinal lymph nodes simultaneously. Sagittal images were obtained using a 3D true-FISP (bSSFP) sequence (TR/TE = 8/4 ms, flip angle = 30°, 38.4x25.5x25.5 mm FOV with 256x170x170 matrix centered on the torso, $150µm^3$ isotropic resolution, 4 signal averages and 4 frequencies). Total scan time was 64 minutes per animal.

	Iron concentration (per cell)	Number of Cells injected
CD8	**~3-5 pg**	**~10 million**
Treg	**<1 pg**	**~1-2 million**
MDSC	**~25-30 pg**	**~3-5 million**

Table 1 – Iron Concentration and number of cells injected for all 3 cell types.

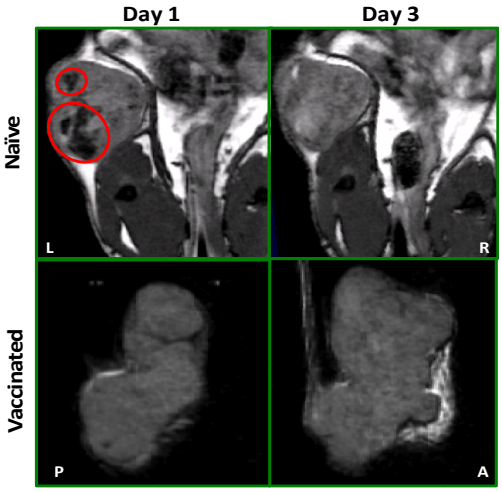

Figure 1 – MR images of SPIO-labeled MDSCs. For naïve mice, these cells have migrated to the tumor on day 1, but have cleared from the site by day 3. However, for vaccinated mice, no iron-loaded cells appear to have migrated to the tumor (image is zoomed to tumor for clear visualization).

Results & Discussion: UV/VIS spectrophotometry revealed highly variable labeling efficiency for the different immune cells (see Table 1). MDSCs, which are large immature monocytes, were highly effective at passive ingestion of SPIO, whereas CD8s were less effective, and Tregs were the least effective at passive SPIO labeling with cells having less than 1 pg iron/cell. Cell viability was reasonably consistent at 80-90%.

For the in vivo migration of the labeled cells, different numbers of injected SPIO-cells were used based on cell yield from the isolation techniques combined with the labeling efficiency (Table 1). A minimum of 10 million injected SPIO-CD8 cells was required per mouse for migration to tumors to be visible (data not shown). Any fewer cells resulted in difficulty seeing labeled cells at tumors, and higher numbers of cells are both very impractical and often resulted in the formation of cell clots. All mice injected with CD8 cells (n=4) exhibited evidence of cell migration to tumors.

Since MDSCs had much higher labeling efficiency, between 3-5 million cells per injection was sufficient for consistent viewing of migration to tumor sites (Figure 1) in all mice. Although Tregs have the least amount of iron per cell, due to the difficulty in obtaining sufficient quantities of pure CD4+CD25+FoxP3+ cells, only 1-2 million cells were injected per mouse. However, this was sufficient for viewing migration of the Tregs to both the tumor site as well as several different lymph nodes throughout the mouse, particularly the inguinal lymph nodes (Figure 2). Tregs appear to home to tumors with very high efficiency, probably leading to immune suppression within the tumor microenvironment.

MDSC migration also significantly varied with treatment. In all four non-vaccinated mice, SPIO-MDSCs were visible at the tumor site (Figure 1), however, none of the vaccinated mice (0/4) showed evidence of SPIO-MDSCs having migrated to the tumor. Study of Treg migration in response to vaccination is ongoing.

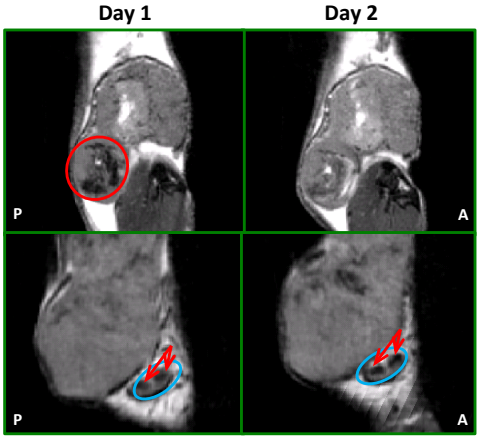

Figure 2 – MR images of SPIO-labeled Tregs. These cells have migrated to both the tumor (upper panel) and draining inguinal lymph node (lower panel) on day 1. Cells have mostly cleared from the tumor by day 2, but remain in the LN for much longer (not shown).

Conclusions: We successfully tracked the migration and clearance of MDSC, Treg and CD8 immune cells to tumor sites in a cervical cancer model. This is the first demonstration using MRI to track both MDSC and Treg cells, and differences in migration of MDSCs in response to immunotherapy were observed. MRI has the potential to be a valuable tool for understanding the behaviour of regulatory cells, which are a major area of interest in cancer research. By understanding the effects that various immunotherapies have on tumor progression and in vivo recruitment of these cells at the preclinical level, it will be possible to undertake more focused efforts on developing novel anti-cancer treatments and studying their effects on immune cells.

References: [1] Long & Bulte. *Expert Opin Biol Ther* 2009;**9**:293-306. [2] Dekaban et al. *J Immunother* 2009;**32**:240-251. [3] Heyn et al. *Magn Reson Med* 2006;**55**:23-29. [4] Kircher et al. *Cancer Research* 2003;**63**:6838-6846. [5] Karkada et al. *J Immunother* 2010;**33**:250-261. [6] Zou. Nat Rev Immunol 2006;**6**:295-307.