Published in final edited form as:

Magn Reson Med. 2010 November ; 64(5): 1510–1519. doi:10.1002/mrm.22519.

Longitudinal Tracking of Human Dendritic Cells in Murine Models Using Magnetic Resonance Imaging

Karen C. Briley-Saebo^{1,2,*}, Marylene Leboeuf², Stephen Dickson¹, Venkatesh Mani¹, Zahi A. Fayad¹, A. Karolina Palucka^{2,3}, Jacques Banchereau^{2,3}, and Miriam Merad²

¹Department of Radiology, Mount Sinai School of Medicine, New York, New York, USA

²Department of Gene and Cell Medicine, Immunology Institute, Mount Sinai School of Medicine, New York, New York, USA.

³Baylor Institute for Immunology Research, Baylor Research Institute, Dallas, Texas, USA

Abstract

Ex vivo generated dendritic cells are currently used to induce therapeutic immunity in solid tumors. Effective immune response requires dendritic cells to home and remain in lymphoid organs to allow for adequate interaction with T lymphocytes. The aim of the current study was to detect and track Feridex labeled human dendritic cells in murine models using magnetic resonance imaging. Human dendritic cells were incubated with Feridex and the effect of labeling on dendritic cells immune function was evaluated. Ex vivo dendritic cell phantoms were used to estimate sensitivity of the magnetic resonance methods and in vivo homing was evaluated after intravenous or subcutaneous injection. R2*-maps of liver, spleen, and draining lymph nodes were obtained and inductively coupled plasma mass spectrometry or relaxometry methods were used to quantify the Feridex tissue concentrations. Correlations between in vivo R2* values and iron content were then determined. Feridex labeling did not affect dendritic cell maturation or function. Phantom results indicated that it was possible to detect 125 dendritic cells within a given slice. Strong correlation between in vivo R2* values and iron deposition was observed. Importantly, Feridex-labeled dendritic cells were detected in the spleen for up to 2 weeks postintravenous injection. This study suggests that magnetic resonance imaging may be used to longitudinally track Feridex-labeled human dendritic cells for up to 2 weeks after injection.

Keywords

cell tracking; iron oxides; dendritic cells

INTRODUCTION

Dendritic cells (DCs) are immune cells that are critical for the induction of adaptive immune response (1). DCs can be generated ex vivo from bone marrow progenitors or blood precursors and loaded with selected antigens for injection into patients (2,3). Recent studies have demonstrated the clinical benefit of DC-based vaccines to induce protective antitumor immunity in patients with solid tumors (4,5). DC vaccines are generated from hematopoietic progenitors or circulating monocytes cultured in vitro and loaded with selected antigens for

injection into patients. Because they may be fully characterized, ex vivo generated DCs offer an opportunity to establish or define many parameters of DC vaccination including the definition of the DC subset, the antigen formulation, and the activation pathway. In this context, we and others have demonstrated that vaccination with ex vivo generated antigen-loaded DCs can induce immune response in a limited number of patients with cancer (5,6). These studies indicate that the quality of CD8⁺T cell immunity represents a key factor to reach therapeutic success. However, the clinical response obtained in patients who received DC vaccines remains variable and infrequent (6). As a result, identifying the factors that limit current DC vaccine efficacy is critical to improve clinical responses in patients. Factors influencing efficacy are related to both DC vaccine quality (as its intrinsic ability to engage an effector response) and the ability of the ex vivo generated DCs to migrate to lymphoid organs. Cytokines and toll-like receptor ligands used in DC cultures greatly influence the DCs ability to prime tumor-specific effector T cells in vivo (5). However, how these culture conditions affect DC migration to lymphoid organs has yet to be examined. One of the issues hindering the evaluation of DC migration is related to the ability to accurately track the migration of DC vaccines in vivo. Although prior clinical studies have used radioactive tracers to follow in vivo DC migration, the low spatial resolution and short radioactive half-lives of these tracers, which do not typically exceed 2 to 3 days, limit the use of radio-labeled DCs for monitoring in vivo DC vaccine therapy (7).

Magnetic resonance imaging (MRI) is currently an important diagnostic modality for in vivo cell tracking (8-16). In vivo monitoring by MR requires that the DCs are labeled with MR active materials, such as iron oxide particles. Iron labeled cells produce significant MR signal loss (T2/T2* effects) due the diffusion of water protons through the inhomogeneous local magnetic fields generated by the cells (17). Although a linear correlation between the dipolar R2 values and Feridex concentration is observed in aqueous solution, compartmentalization of the individual iron particles within intracellular endosomes and lysosomes may induce nonlinearity. In vitro studies using Feridex labeled lymphocytes and gliosarcoma cells show that the R2 values of labeled cells are two to three times lower than values obtained for equivalent Feridex concentrations in agarose gel (16). Additionally, studies have shown that the R2 values obtained for iron labeled cells are strongly dependent on the cell type, cell size, and concentration of intracellular iron (15,16). For large iron particles (such as Feridex) and at high magnetic field strengths (often used for preclinical imaging), R2 may be described using the Ayant spectral density shown in the equation below (18).

$$R_2 = \left(\frac{32\pi}{405} \right) \gamma^2 \mu^2 \left(\frac{N}{RD} \right) \cdot \left[4.5 J_A \left(\sqrt{2} w_0 \tau_D \right) + 6 J_A(0) \right]$$

where γ is the gyrometric ratio of a water proton, μ is the magnetic moment at a given field, N is the number of iron particles per volume unit, R is the particle radius, D is the diffusion coefficient, J_A is the Ayant spectral density function, w_0 is the proton Larmor frequency, and τ_D is the modulation of the relative translational diffusion time.

In agar gel or aqueous solution where the diffusion of water protons is unrestricted and the iron particles are distributed homogeneously within a unit volume, R2 will correlate linearly with N (or Feridex concentration). However, when compartmentalized within the lysosome or endosome of a cell the following factors may influence the R2 value observed: (1) water diffusion may be restricted (at a given echo time) causing modulation of the transverse magnetization (19). (2) Aggregated or flocculation of the iron particles within the vesicles of the cell may increase the effective radius thereby causing the production large susceptibility effects between the cells and other tissue, and (3) the number of particles per unit volume

may become variable as the particles are sequestered within a limited volume. Studies have shown that R_2 values first increase during agglomeration, reach a maximum value, and then decrease as the agglomeration process continues (20). As a result, R_2 may not always scale linearly with concentration when the iron particles are sequestered within a cell.

Studies have, however, shown that a simple linear relationship exists between iron concentration (for Feridex labeled cells) and R_2^* (15,21). The linear relationship is based upon the fact that $R_2^* \propto cM^n$, where c is the iron oxide concentration, M is the magnetization associated with the iron oxide core (field dependent), and n (1 or >1) is dependent upon the local magnetic field distribution generated by the iron particles. For most iron oxides, n is approximately 1 so that the R_2^* values are roughly equal to Feridex concentration at any given applied field strength (where M is constant) (21). In this case, strong local magnetic fields are generated by intracellular compartmentalization of the iron within a fraction of the total cell volume. The net magnetization created by the cell containing Feridex is a sum of all the individual magnetic moments of each particle within the cell and is therefore proportional to the net magnetization at any given field. The result is often described as the generation of one giant magnetic particle with the diameter and shape of the cell that contains the compartmentalized iron.

There are currently three types of iron oxide particles that may be used for cell labeling: polymer encapsulated micron sized iron oxide particles (MPIOs), 90–100 nm sized superparamagnetic iron oxide particles, and 12–35 nm ultrasmall superparamagnetic iron oxide particles. Because of their size, MPIOs are able to deliver a high payload of iron into cells. As a result, they are nearly 50% more effective (based on the dipolar R_2 values) than ultrasmall superparamagnetic iron oxide particles with equivalent iron content (22,23). The high sensitivity of these particles enables the in vivo detection of a single MPIO labeled cell within murine neural tracks (22,23). However, the biologically inert coating associated with the MPIO particles limits the ability of cells to metabolize and excrete the MPIO particles. As a result, clinical translation of an MPIO platform may potentially be limited due to issues associated with bioretention. Feridex is a superparamagnetic iron oxide particle (superparamagnetic iron oxide particles) that has been used extensively in stem cell labeling and in vivo tracking by MRI (13,15,24,25). Feridex is composed of 100 nm dextran coated iron oxide particles that are easily taken up by phagocytic cells (such as macrophages and DCs) without the need for transfection agents or electroporation.

Although superparamagnetic iron oxide particles are less effective than MPIOs with respect to delivery of iron into the cell, studies have shown that these particles exhibit high magnetization values and may be metabolized by a variety of cells (13,14,26,27). Although Feridex has been approved by the Food and Drug Administration for MR liver indications, the use of Feridex for cell labeling is still considered *off-label* in the United States. However, clinical studies performed outside of the United States have shown the diagnostic potential and utility of this material for the in vivo detection and tracking of cells (28). Ultrasmall superparamagnetic iron oxide particles, such as monocrystalline *iron oxide* nano-compound (MION) and NC100150 injection, exhibit relatively low magnetization values and require extensive cell uptake to deliver iron loads that are equivalent to MPIOs or superparamagnetic iron oxide particles. As a result, ultrasmall superparamagnetic iron oxide particles are not typically used as cell labels. As the focus of the current study is eventual clinical translation, Feridex was chosen as the cell label.

Additionally, a recent study in patients showed increased monocyte-derived DC uptake in draining lymph nodes of patients after intranodal administration, relative to intradermal administration (7). Both injection routes, however, resulted in a large percentage of the injected cells remaining at the injection site. Although preclinical studies using murine-

derived DCs have been performed to evaluate lymphoid organ homing, it is difficult to extrapolate these data to a clinical setting due to variations in murine DC responsiveness and activation, relative to human DCs (17). As a result, new in vivo preclinical models are required to determine the most effect injection route for human DC homing and immune response activation. Here we sought to establish the ability of MRI to determine the migration of Feridex labeled human DCs in mice as a function of injection route. The MR methodology was validated by correlating the in vivo R2* values to the ex vivo Feridex tissue content.

MATERIALS AND METHODS

Animals

Five- to 8-week-old wild type C57BL/6 mice and B6.Cg-Foxn1nu/J nude mice were purchased from the Jackson Laboratory (Jackson Laboratory, Bar Harbor, USA). All animal protocols were approved by the Mount Sinai Medical Center Institutional Animal Care and Use Committee.

Isolation of DC Cells

Human monocytes were obtained from peripheral blood mononuclear cells using ficoll-hypaque gradient separation of buffy coats obtained from the New York Blood Center. Monocytes were purified by positive selection using CD14 pure magnetic beads (Miltenyi Biotec, Bergisch-Gladbach, Germany). To obtain immature DCs, monocytes were cultured for 7 days in the following medium: 50% Roswell Park Memorial Institute Medium (Mediatech, Manassas, VA), 50% serum-free hematopoietic cell medium (X-VIVO, Biowhittaker, MD) in the presence of 50 ng/mL of granulocyte-macrophage colony-stimulating factor and 34 ng/mL of Interleukin-4 (Peprotech, NJ). The purity of the culture was then determined as percent lineage negative, major histocompatibility complex (MHC) II positive, and CD1a positive cells using flow cytometry. DCs were matured with 0.2 mg/mL prostaglandin E2, 10 ng/mL tumor necrosis factor, 10 ng/mL recombinant IL-1, and 1000 U/mL recombinant IL-6 (all Peprotech, Rocky Hill, NJ) for 48 h.

Murine DCs were generated from bone marrow cells cultured in Roswell Park Memorial Institute-1640 medium supplemented with 10% fetal calf serum in the presence of 50 ng/mL granulocyte-macrophage colony-stimulating factor and 50 ng/mL interleukin-4 (Pepro-Tech) for 7 days. On day 7, the culture was analyzed by flow cytometry for the expression of CD11c, CD11b, and MHC class II.

Feridex Uptake

Titration experiments were performed to determine the optimal Feridex uptake conditions for human DCs. The CD14 positive cells were adjusted at 1.106 per milliliter of medium and cultured at 0.2×10^6 cells/cm² (= 2 mL per well on a 6 well plate). Feridex (Berlex, NJ) was added to immature and mature DCs at 2 days (150 µg Fe/mL), 2 and 3 days (for a total of 300 µg Fe/mL added over a 48 h time period), 3 days (150 µg Fe/mL), and 4 days (150 µg Fe/mL) post DC culture. Feridex (150 mg Fe/mL) was added to murine DCs 4 days after the onset of DC cultures. At day 5, cells were washed extensively, the medium was renewed, and the cells were cultured for an additional 2 days. All cells were then washed, counted, and sent to inductively coupled plasma mass spectrometry (ICP-MS, Cantest, Burnaby, BC) for determination of total iron content. The percent Feridex uptake was then determined based upon the amount of iron detected in the cells by ICP-MS versus the total concentration of Feridex added during incubation.

Cytospins were prepared by diluting 2×10^4 cells in 300 μ L PBS. The cells were then spun down on microscope slides using a Cytospin 3 centrifuge (Shandon, UK). Perl's staining was performed by fixing the samples with 4% paraformaldehyde for 10 min followed by incubation with 2% potassium ferrocyanide in 2% hydrochloric acid. The slides were washed with distilled water and counterstained with nuclear fast red and dehydrated in ethyl alcohol (90, 95, and 100%) before cover-slipping. Images were acquired with a Nikon microscope using specialized software (SOFT, Diagnostic Instruments, MI). The location of the Feridex within the cell was then evaluated using Transmission Electron Microscopy (model CX-100; JEOL, Tokyo, Japan), according to established protocols (29).

Cell Viability and Phenotype

Cell viability was determined using Trypan Blue staining. Phenotypic characterization was performed with respect to histocompatibility complex class II (HLA-DR) molecules, costimulatory molecules (CD80, CD86), dendritic cell maturation (CD83), and mobility (chemokine receptor CCR7) using a fluorescence activated cell sorter (Becton Dickinson LSRII). The following fluorochrome-conjugated monoclonal antibodies were used: anti-CD1a, anti-CD14, anti-HLA DR/DP (Q5/13), anti-CD80, anti-CD83, anti-CD86 (all Becton Dickinson, CA), and anti-Chemokine Receptor 7 (R & D SYSTEMS, MN).

Cell Function

The capacity of Feridex labeled DCs to stimulate T-cells was analyzed in a mixed lymphocyte reaction. All alloreactive T-cells were isolated from human blood mononuclear cells using Ficoll-Hypaque centrifugation. Immature and mature DCs were irradiated at 3000 absorbed ionizing radiation (RAD) and cocultured with alloreactive T cells for 4 days at different DC/T cell ratio. After 3 days of culture, 1 mCi/well of tritiated thymidine was added for 16 h, and incorporation of tritiated thymidine was measured in a beta-counter.

Cell Metabolism

Feridex was added to human DCs at 2 and 3 days (total of 300 μ g Fe/mL) post DC culture. A fraction of the cells were removed, washed, counted, and sent to ICP-MS for determination of iron content. The remaining cells were washed extensively, and the cells cultured for an additional 4 days in a renewed medium. Cells were then washed and counted before being sent to ICP-MS for determination of total iron content. The percent cell metabolism was determined based upon the relative change in iron content 3 days post culture and 7 days post culture.

Ex Vivo DC Phantoms

The sensitivity of the MR method was tested using ex vivo DC phantoms. Phantoms were prepared by adding known numbers of Feridex labeled human DCs to 0.2-mL warm 2% agarose gel in 0.5-mL plastic tubes (diameter of 1 cm at top). The samples were mixed and snap frozen in dry ice to allow for a homogenous distribution of cells within the gel. MR imaging was performed immediately after the gel was set using a 9.47 Tesla (Bruker, Billerica, MA) dedicated mouse scanner. The phantoms were placed in the same 30 mm whole body mouse coil used for all in vivo imaging. Multiple echo gradient echo (GRE) pulse sequences with the following pulse sequence parameters were used to obtain $R2^*$ -maps: TR 29.1 msec, TE = 5.1 msec to 10 msec ($n = 5$), 30 slices, flip angle = 30° , slice thickness = 0.5 mm, number of signal averages (NEX) = 6, in-plane resolution = 0.098 mm^2 , and 100% z -rephasing gradient. In addition to the conventional GRE sequences, the white marker imaging sequence GRASP (GRAdient echo Acquisition for Superparamagnetic particles with Positive contrast) was also used. For the GRASP sequence, all sequence parameters were equivalent to those used for the GRE sequence except that the z -rephasing

gradient was reduced to 50%. Regions-of-interest were drawn to encompass the entire diameter of each phantom tube (for any given slice). $R2^*$ fitting was performed on a pixel-by-pixel basis using a custom made program in Matlab (The Mathworks, R2007b). The signal intensity of each pixel was normalized to the standard deviation of adjacent noise before linear fitting of the signal-to-noise ratio versus echo time. Linear fits of cell number (determined using a bright line counting chamber, Hausser Scientific, USA) and iron content (determined by ICP-MS) versus $R2^*$ (obtained by MRI) were then constructed and the resulting correlation coefficients reported.

In Vivo Detection by MRI

Eight- to 12-week-old nude male mice were administered 500,000 Feridex labeled human DCs via intravenous (i.v., retro-orbital, $n = 6$) or subcutaneous (footpad, $n = 6$) injection. Additionally, age matched wild-type (WT) = mice received 500,000 Feridex labeled murine DCs via retro-orbital ($n = 6$) or footpad ($n = 6$) injection. MR images of the liver, spleen, and draining lymph nodes were obtained immediately before injection and 24 h post injection. All in vivo MR imaging was performed at 9.4 Tesla using the pulse sequence parameters described for the ex vivo DC phantom. A respiratory gating system (SA Instruments, Inc., Stony Brook, NY) was used to gate the sequences and monitor the animals during imaging. $R2^*$ -mapping was performed on a pixel-by-pixel basis using a Matlab program, as described earlier. Regions of interest were drawn to encompass as much of the tissue as possible within a given slice. The $R2^*$ values were obtained for at least three slices (maximum of five slices) and the average values calculated for each tissue at each imaging time point post injection. Immediately after the last MR scan, the mice were sacrificed and the liver, spleen, and draining lymph nodes isolated. A section of tissue was isolated and fixed in 4% paraformaldehyde for 10 min followed by incubation with 2% potassium ferrocyanide in 2% hydrochloric acid for Perl's staining. The slides were washed with distilled water and counterstained with nuclear fast red and dehydrated in ethyl alcohol (90, 95, and 100%) before cover-slipping. Images were acquired with a Nikon microscope using specialized software (SOFT, Diagnostic Instruments, MI). The remaining tissue was weighed and either sent to ICP-MS (lymph node) or homogenized (liver/spleen) for determination of the iron content by relaxometry (27).

In Vivo Longitudinal Tracking

The MR signal of the liver and spleen was evaluated over a 2 week time interval after i.v. injection of 500,000 Feridex labeled human DCs ($n = 8$ total). Mice were sacrificed, saline perfused, and the liver and spleens excised at days 7 ($n = 1$), 10 ($n = 1$) and 14 ($n = 6$) post injection. Tissue sections were fixed for Perl's staining and the remaining tissue was weighed and homogenated for the evaluation of the total iron content by relaxometry (27).

Iron Content in Liver and Spleen

Because of the high concentration of endogenous iron in the liver, spleen, and blood, relaxometry methods were used to determine tissue Feridex concentrations. Dose-response curves were generated by spiking ex vivo liver homogenate and spleen homogenate with known concentrations of Feridex (0–1 mM Fe, $n = 6$). Because of the relatively small size of the spleen, all spleen homogenates were diluted 1:1 with PBS. The transverse relaxation times ($T2$) were determined at 60 MHz (40°C) using a Bruker Minispec spectrometer (Bruker Medical GmbH, Ettlingen, Germany). $T2$ values were calculated based upon a monoexponential fit of echo amplitude versus time. The following relationship between the transverse relaxation rates (denoted as y) and Feridex concentration (denoted as x) was observed for the spiked samples: for the liver homogenate $y = 247x + 18$ ($R^2 = 0.994$) and for diluted spleen homogenate $y = 193x + 0.18$ ($R^2 = 0.991$). The limits of quantification were determined as 0.039 mM Fe and 0.068 mM Fe for the liver and diluted spleen,

respectively. The dose-response curves were then used to approximate the Feridex concentration in liver homogenate and diluted spleen homogenate obtained from the treated mice. Correlations between in vivo $R2^*$ values and Feridex concentration were then determined.

Statistics

The number of mice per group was determined based upon pilot data that suggested an effect size of 6 was required to evaluate significant changes in the $R2^*$ values of the spleen after i.v. DC injection. The critical value was 4.8 and actual power was 0.8552 as determined using GPOWER. One-way ANOVA with Bonferroni post hoc tests was used to evaluate the significance associated with change in $R2^*$ values as a function of time post injection.

RESULTS

Feridex Labeling Does Not Alter Cell Viability, Maturation, and Function

As shown in Fig. 1b, Feridex labeling of human DCs (>99%) did not significantly affect cell viability, relative to control cells, under any of the incubation conditions tested. Perl's Prussian blue staining (Fig. 1c) and transmission electron microscopy (Fig. 1d) showed significant Feridex uptake into intracellular endosomes/lysosomes. No significant metabolism of Feridex was observed over a 7 day time period in cell culture (as confirmed by ICP-MS).

Immature Feridex labeled human DCs expressed lower levels of MHC II costimulatory molecules (HLA-DR) and chemokine receptor CCR7 compared with unlabeled human cells (Fig. 2a). The reduction in HLA-DR and CCR7 for the immature Feridex labeled cells are indicative of: (1) a potential reduction in an effector response (T-cell activation, HLA-DR) and (2) a reduction in cell mobility (CCR7). No significant differences were observed, however, between unlabeled control DCs and activated (or mature) Feridex labeled DCs (Fig. 2b). This finding is consistent with results reported by other groups for Feridex labeled murine DCs (17). No significant differences ($P > 0.05$) in the upregulation of HLA-DR and costimulatory molecules (CD80, CD86) were observed between activated DC controls and activated Feridex labeled DCs. Additionally, Feridex labeling did not influence cell maturation (CD83) or cell mobility (CCR7). As suggested by the HLA-DR data, Feridex labeling of activated human DCs did not alter their ability to prime allo-reactive T-lymphocytes. No functional effect on the induction of T-cell proliferation at cell populations greater than 10,000 was observed.

Ex Vivo DC Phantoms

A linear relationship between $R2^*$ and Feridex concentration was observed in the agar gel phantoms ($y = 206x + 23$, $R^2 = 0.9785$). These results correlate well with previous studies that showed a linear relationship between $R2^*$ and Feridex concentration (21,27). For the ex vivo dendritic cell phantom, signal loss was observed when using conventional GRE sequences were applied (Fig. 3a). For the GRASP sequence, cells that contain iron appeared white due to matching of the z-rephasing gradient to local field inhomogeneities. Figure 3a shows good correlation between the signal loss observed using GRE sequences and signal enhancement by GRASP. A linear relationship was also observed between Feridex labeled DC number and $R2^*$ (Fig. 3b). The results clearly show that it is possible to detect 500 Feridex labeled DCs suspended in 0.2 mL agar gel. If one assumes that $1 \text{ cm}^2 = 1 \text{ mL}$ and the cells are distributed homogeneously, then the number of cells in a 0.5 mm imaging slice is approximately 125. As a result, the current methodology may allow us to detect as low as 125 Feridex labeled cells within a given slice. However, to label a voxel as positive for iron

we need at least 52 cells that are Feridex labeled out of a theoretical maximum of 3430 cells (based size of the voxel and the reported size of human DCs).

In Vivo Detection of Human DCs

Significant DC homing was seen in the liver, spleen, and popliteal lymph nodes (cranial lymph nodes) 24 h after i.v. injection (Fig. 4a). Good correlation between MR images and histology was observed. GRASP images showed strong signal enhancement in the popliteal and axilar lymph nodes. Analysis of the excised popliteal lymph node revealed 0.092 μg Fe (or 0.3% injected dose) was present as determined by ICP-MS. Significant increases in the $R2^*$ values of the liver and spleen were observed, relative to baseline values (Fig. 5).

No significant uptake was observed in the liver or spleen 24 h after footpad injection. Significant uptake was, however, observed in the nearest major draining inguinal lymph node (Fig. 6). Analysis of the inguinal lymph node by ICP-MS showed 3.2% of the injected dose within the node 24 h after injection. Good correlation between MR imaging and histology was observed. Because of the position of the draining lymph node to the coil, fringe effects were observed on MR images obtained using GRE sequences. Application of the GRASP sequence allowed for clear identification of the lymph node 24-h post injection.

In Vivo Longitudinal Tracking of Human DCs

The ability to longitudinally track Feridex labeled human DC was evaluated over a 14 day time interval after injection (i.v. injection, $n = 8$). Liver $R2^*$ values reached baseline values 240 h (10 days) post injection (Fig. 7a). These results were confirmed by both histology and relaxometry of homogenized liver samples. Significant iron oxide concentrations were found in the spleen for up to 240 h (10 days) post injection, as confirmed by histology (Fig. 7b). Figure 7c shows the % injected dose found in the liver and spleen as a function of time post injection.

Correlations of In Vivo $R2^*$ Values to Feridex Concentration

The in vivo $R2^*$ values of the liver and spleen of $n = 20$ mice ($n = 12$ at 24 h and $n = 8$ over 2 weeks) were correlated to the Feridex concentration obtained by relaxometry. A linear relationship was observed between the in vivo $R2^*$ values and the estimated Feridex concentrations for both liver ($R^2 = 0.7822$) and spleen ($R^2 = 0.8883$).

Comparison of the Homing of Human DCs to Murine DCs

Although immune deficient nude mice were used to longitudinally track human DCs, the homing of human cells in mice may deviate from similar murine cells. Therefore, murine DCs were isolated, Feridex labeled, and injected into wild-type mice. No significant changes in liver $R2^*$ values were observed 24 h after i.v. injection of murine DCs (Fig. 8a). This suggests that in contrast to human DCs, murine DCs exhibited limited homing to the liver after i.v. injection. No significant difference in DC homing to the spleen was observed for murine and human DCs following i.v. injection. These results suggest a limited effect of DC origin on the homing of DCs to lymphoid organs in mice.

DISCUSSION

In this study, we have established the ability of MRI to track Feridex labeled human monocyte-derived DCs in the spleen of immunodeficient mice for up to 2 weeks post i.v. injection. In vitro monocyte incubation at both days 2 and 3 of culture resulted in >99% uptake of Feridex into endosomes/lysosomes with limited cellular toxicity. Although Feridex labeling reduced the levels of MHC II and costimulatory molecules on monocyte-derived DCs at day 5 of culture, once activated, the Feridex-labeled DCs were able to

mature and prime allo-reactive T cells to a similar degree as unlabeled cells. These results strongly suggest that Feridex labeling will not alter the capacity of DC vaccines to initiate adaptive immunity in vivo.

Intravenous injection of 500,000 Feridex labeled cells resulted in significant cell homing to lymphoid tissue. Based ex vivo tissue assays, approximately 35% of the injected dose was found within the spleen 24 h post i.v. injection, whereas 0.3% of the injected dose was found within subcutaneous lymph nodes. After footpad injection, only 3% of the injected dose was found in the draining inguinal lymph node 24 h post injection with no significant homing observed to the liver and spleen. These results suggest that i.v. injection may allow for substantial lymphoid organ uptake, relative to subcutaneous footpad injection.

A similar homing profile was obtained when murine ex vivo generated DCs were injected i.v. into mice. The only significant difference between murine DCs and human DCs was related to liver uptake. Whereas significant liver uptake was observed after i.v. injection of human DCs, only limited liver uptake was observed following administration of murine DCs. The results suggest that immune-deficient mice models represent a useful model for the evaluation of Feridex labeled human DC migration to lymphoid organs. The distribution into nonlymphoid tissue (such as the liver), however, may be significantly altered. Despite variations in liver uptake, similar preclinical models could be used to determine the effect of different maturation cocktails on DC migration to lymphoid tissue in vivo.

The longitudinal studies showed that it was possible to detect Feridex-labeled DCs in the spleen of mice for up to 2 weeks post i.v. injection. As in vitro studies show limited DC metabolism of Feridex after 1 week in culture, it is unlikely that the cells are able to significantly metabolize Feridex during the first week post injection. However, as it is difficult to maintain viable DCs for more than 1 week in culture it is not possible to evaluate in vitro metabolism at later time points. As a result, it is not clear if breakdown products such as ferritin contribute to the in vivo $R2^*$ values observed at late time points post injection (27,30). Additionally, the current methodology cannot be used to predict the in vivo fate of the iron oxide particles. MR signal loss is observed regardless of whether the iron particles were present within viable DCs, apoptotic DCs, or within phagocytic cells. To determine if the human DCs were present and/or viable at late time points post injection, attempts were made to isolate and stain the human cells within the murine tissue. Unfortunately, the results from these studies did not allow us to formally conclude the presence of injected human cells within the tissue. Future studies using fluorescently labeled Feridex may allow for the use of ex vivo cell sorting methods to identify and phenotype the Feridex labeled cells present in lymphoid tissue (31).

Although the high spatial resolution associated with MRI allows for accurate evaluation of lymphoid organ morphology, the sensitivity and ability to quantify MR data is limited (relative to nuclear medicine based techniques). For MR cell tracking to be clinically useful, the detection limits of the MR method used must be defined. For example, clinical studies in patients at 3 T suggest that it is possible to detect 150,000 Feridex labeled cells injected directly into the lymph nodes of patients using $T2^*$ -weighted sequences (7). Although these results are encouraging, most therapeutic interventions require i.v. administration of DCs. As a result, the method sensitivity is likely to be affected by greater distribution volumes and lower DC density/compartimentalization within any given lymphoid organ or tissue. The results of the current study indicate that it is possible to detect 125 Feridex labeled DCs within a given 0.5 mm slice. Although a linear relationship between $R2^*$ and cell number was observed, it is not advisable to use $R2^*$ values to predict cell number in longitudinal tracking studies because this method is not sensitive to cell viability and may be affected by the presence of Feridex degradation products (15,27). Good correlation was, however,

observed between the ex vivo Feridex concentration obtained in the liver and spleen (by relaxometry) and the in vivo $R2^*$ values obtained over a 2 week time interval post i.v. injection. Based upon the linear correlation between ex vivo iron concentration and in vivo $R2^*$ values, the quantification limits of the current method were calculated as 0.038 mM Fe (7.6% injected dose) and 0.016 mM Fe (3.25% injected dose) for the liver and spleen, respectively. This data suggest that $R2^*$ values may be used to *estimate* the concentration of Feridex present within the spleen as long as the tissue concentrations are greater than 0.016 mM Fe. At concentrations less than 0.016 mM, the $R2^*$ values may still be significant (relative to baseline) but the $R2^*$ should not be used to estimate Feridex concentration. The results of the current study correlate well with other reported studies that show a good correlation between $R2^*$ values and Feridex concentration (15,16,27).

Issues associated with volume averaging and other artifacts may limit the clinical utility of MRI to detect iron labeled cells in tissues other than the brain. Specifically, the signal loss generated by the iron laden cells may be confused with signal caused by other sources (motion, perivascular effects, coil inhomogeneities, etc.). To aid in detection of iron laden cells, several white marker techniques have been developed to differentiate between the signal generated by the cells and signal loss caused by various artifacts (29,32). In the current study, the GRASP sequence was used to detect iron-laden cells. In this sequence, the z-rephasing gradient is reduced so that dipolar fields generated by the cells are rephased and positive signal is observed. The GRASP study was able to both detect and confirm the presence of the Feridex labeled DCs in the draining lymph node of the mouse 24 h after footpad injection. This was particularly important as the proximity of the lymph node to coil generated as severe coil fringe effects at long echo times. It should be noted, however, that the GRASP sequence was not able to detect the Feridex labeled cells within the liver and spleen. Reported studies have shown that if the iron particles are homogeneously distributed over a large volume (such as the liver or spleen) then the dipole effects are limited and signal enhancement is not observed. As a result, white marker sequences such as GRASP are most effective when the iron laden cells are compartmentalized within a limited volume (lymph nodes, tumors, or myocardium).

CONCLUSIONS

The results of the current study show that it is possible to detect and longitudinally track ex vivo human DC vaccines for up to 2 weeks in the spleen of mice. Greater lymphoid targeting was observed following i.v. injection, relative to subcutaneous foot-pad injection. Good correlation between in vivo $R2^*$ values and Feridex concentration were observed, with detection limits of 3.2% observed for the spleen. The white marker sequence, GRASP, allowed for accurate detection and identification of Feridex labeled DCs in superficial lymph nodes. The use of appropriate animal models and MR validated imaging strategies may allow for the optimization of human DC vaccine therapies and improved therapeutic success.

Acknowledgments

This study was partially supported by Baylor Health Care foundation (J.B.) and the National Institutes of Health (J.B.).

Grant sponsors: U19-A1057234, NIH RO1-CA078846, AR054083-01 (J.B.) and Baylor Health Care foundation (J.B.).

REFERENCES

1. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature*. 1998; 392:245–252. [PubMed: 9521319]
2. Banchereau J, Schuler-Thurner B, Palucka AK, Schuler G. Dendritic cells as vectors for therapy. *Cell*. 2001; 106:271–274. [PubMed: 11509176]
3. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature*. 2007; 449(7161):419–426. [PubMed: 17898760]
4. Schuler PB, Leblanc PA, Marzilli TS. Effect of physical activity on the production of specific antibody in response to the 1998-99 influenza virus vaccine in older adults. *J Sports Med Phys Fitness*. 2003; 43:404. [PubMed: 14625524]
5. Palucka AK, Ueno H, Fay JW, Banchereau J. Taming cancer by inducing immunity via dendritic cells. *Immunol Rev*. 2007; 220:129–150. [PubMed: 17979844]
6. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol*. 2005; 5:296–306. [PubMed: 15803149]
7. de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, Oyen WJ, Bonenkamp JJ, Boezeman JB, Adema GJ, Bulte JW, Scheenen TW, Punt CJ, Heerschap A, Figdor CG. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nat Biotechnol*. 2005; 23:1407–1413. [PubMed: 16258544]
8. Arbab AS, Janic B, Knight RA, Anderson SA, Pawelczyk E, Rad AM, Read EJ, Pandit SD, Frank JA. Detection of migration of locally implanted AC133+ stem cells by cellular magnetic resonance imaging with histological findings. *FASEB J*. 2008; 22:3234–3246. [PubMed: 18556461]
9. Arbab AS, Liu W, Frank JA. Cellular magnetic resonance imaging: current status and future prospects. *Expert Rev Med Devices*. 2006; 3:427–439. [PubMed: 16866640]
10. Bos C, Delmas Y, Desmouliere A, Solanilla A, Hauger O, Grosset C, Dubus I, Ivanovic Z, Rosenbaum J, Charbord P, Combe C, Bulte JW, Moonen CT, Ripoche J, Grenier N. In vivo MR imaging of intravascularly injected magnetically labeled mesenchymal stem cells in rat kidney and liver. *Radiology*. 2004; 233:781–789. [PubMed: 15486216]
11. Budde MD, Frank JA. Magnetic tagging of therapeutic cells for MRI. *J Nucl Med*. 2009; 50:171–174. [PubMed: 19164242]
12. Bulte JW, Duncan ID, Frank JA. In vivo magnetic resonance tracking of magnetically labeled cells after transplantation. *J Cereb Blood Flow Metab*. 2002; 22:899–907. [PubMed: 12172375]
13. Ke YQ, Hu CC, Jiang XD, Yang ZJ, Zhang HW, Ji HM, Zhou LY, Cai YQ, Qin LS, Xu RX. In vivo magnetic resonance tracking of Feridex-labeled bone marrow-derived neural stem cells after autologous transplantation in rhesus monkey. *J Neurosci Methods*. 2009; 179:45–50. [PubMed: 19428510]
14. Kim D, Chun BG, Kim YK, Lee YH, Park CS, Jeon I, Cheong C, Hwang TS, Chung H, Gwag BJ, Hong KS, Song J. In vivo tracking of human mesenchymal stem cells in experimental stroke. *Cell Transplant*. 2008; 16:1007–1012. [PubMed: 18351016]
15. Liu W, Frank JA. Detection and quantification of magnetically labeled cells by cellular MRI. *Eur J Radiol*. 2009; 70:258–264. [PubMed: 18995978]
16. Rad AM, Arbab AS, Iskander AS, Jiang Q, Soltanian-Zadeh H. Quantification of superparamagnetic iron oxide (SPIO)-labeled cells using MRI. *J Magn Reson Imaging*. 2007; 26:366–374. [PubMed: 17623892]
17. Dekaban GA, Snir J, Shrum B, de Chickera S, Willert C, Merrill M, Said EA, Sekaly RP, Foster PJ, O'Connell PJ. Semiquantitation of mouse dendritic cell migration in vivo using cellular MRI. *J Immunother*. 2009; 32:240–251.
18. Gossuin Y, Gillis P, Hocq A, Vuong QL, Roch A. Magnetic resonance relaxation properties of superparamagnetic particles. *WIREs Nanomed Nanobiotechnol*. 2009; 1:299–310. 2.
19. Athiraman H, Jiang Q, Ding GL, Zhang L, Zhang ZG, Wang L, Arbab AS, Li Q, Panda S, Ledbetter K, Rad AM, Chopp M. Investigation of relationships between transverse relaxation rate, diffusion coefficient, and labeled cell concentration in ischemic rat brain using MRI. *Magn Reson Med*. 2009; 61:587–594. [PubMed: 19107898]

20. Muller RN, Gillis P, Moyny F, Roch A. Transverse relaxivity of particulate MRI contrast media: from theories to experiments. *Magn Reson Med*. 1991; 22:178–182. Discussion 195–176. [PubMed: 1812343]
21. Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. Gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP): sequence characterization in membrane and glass superparamagnetic iron oxide phantoms at 1.5T and 3T. *Magn Reson Med*. 2006; 55:126–135. [PubMed: 16342148]
22. Shapiro EM, Sharer K, Skrtic S, Koretsky AP. In vivo detection of single cells by MRI. *Magn Reson Med*. 2006; 55:242–249. [PubMed: 16416426]
23. Shapiro EM, Skrtic S, Koretsky AP. Sizing it up: cellular MRI using micron-sized iron oxide particles. *Magn Reson Med*. 2005; 53:329–338. [PubMed: 15678543]
24. Cai J, Zhang X, Wang X, Li C, Liu G. In vivo MR imaging of magnetically labeled mesenchymal stem cells transplanted into rat liver through hepatic arterial injection. *Contrast Media Mol Imaging*. 2008; 3:61–66. [PubMed: 18381616]
25. Walczak P, Kedziorek DA, Gilad AA, Barnett BP, Bulte JW. Applicability and limitations of MR tracking of neural stem cells with asymmetric cell division and rapid turnover: the case of the shiverer dysmyelinated mouse brain. *Magn Reson Med*. 2007; 58:261–269. [PubMed: 17654572]
26. Arbab AS, Yocum GT, Rad AM, Khakoo AY, Fellowes V, Read EJ, Frank JA. Labeling of cells with ferumoxides-protamine sulfate complexes does not inhibit function or differentiation capacity of hematopoietic or mesenchymal stem cells. *NMR Biomed*. 2005; 18:553–559. [PubMed: 16229060]
27. Briley-Saebo KC, Johansson LO, Hustvedt SO, Haldorsen AG, Bjornerud A, Fayad ZA, Ahlstrom HK. Clearance of iron oxide particles in rat liver: effect of hydrated particle size and coating material on liver metabolism. *Invest Radiol*. 2006; 41:560–571. [PubMed: 16772849]
28. Bulte JW. In vivo MRI cell tracking: clinical studies. *AJR Am J Roentgenol*. 2009; 193:314–325. [PubMed: 19620426]
29. Mani V, Briley-Saebo KC, Hyafil F, Fayad ZA. Feasibility of in vivo identification of endogenous ferritin with positive contrast MRI in rabbit carotid crush injury using GRASP. *Magn Reson Med*. 2006; 56:1096–1106. [PubMed: 17036302]
30. Briley-Saebo K, Hustvedt SO, Haldorsen A, Bjornerud A. Long-term imaging effects in rat liver after a single injection of an iron oxide nanoparticle based MR contrast agent. *J Magn Reson Imaging*. 2004; 20:622–631. [PubMed: 15390223]
31. Maxwell DJ, Bonde J, Hess DA, Hohm SA, Lahey R, Zhou P, Creer MH, Piwnicka-Worms D, Nolta JA. Fluorophore-conjugated iron oxide nanoparticle labeling and analysis of engrafting human hematopoietic stem cells. *Stem Cells*. 2008; 26:517–524. [PubMed: 18055451]
32. Mani V, Adler E, Briley-Saebo KC, Bystrup A, Fuster V, Keller G, Fayad ZA. Serial in vivo positive contrast MRI of iron oxide-labeled embryonic stem cell-derived cardiac precursor cells in a mouse model of myocardial infarction. *Magn Reson Med*. 2008; 60:73–81. [PubMed: 18581415]

FIG. 1.

a: Percent uptake of Feridex by human monocyte-derived DCs. One hundred and fifty Lg Fe/mL of Feridex was added at day 2, day 2 and 3, day 3 or day 4 of culture. All cells were washed 5 days post culture, new cell culture media added and cultured for an additional 2 days. Mature DCs were obtained by culturing day 7 monocytederived DCs in the presence of an activating cocktail for two more days. **b:** Viability of immature and mature human DCs after incubation with Feridex as a function of time, **(c)** Perl's Prussian blue stain, and **(d)** TEM of human DC after incubation (days 2 and 3) with Feridex.

FIG. 2.

Phenotype and function of immature Feridex-labeled DCs (**a**), relative to control DCs. Immature Feridex labeled human DCs expressed lower levels of MHC II costimulatory molecules (HLA-DR) and chemokine receptor CCR7 compared with unlabeled human cells. **b**: Phenotype and function of activated or mature Feridex-labeled DCs, relative to control DCs. No significant differences in the upregulation of HLA-DR, costimulatory molecules (CD80, CD86), cell maturation (CD83), or cell mobility (CCR7) were observed.

FIG. 3.

Ex vivo DC phantoms. **a:** Representative MR images obtained for Feridex labeled human DCs in 0.2-mL agarose gel (diameter approximately 1 cm). GRE images were obtained using a TE of 8 msec. GRASP images were obtained for equivalent slice locations (z-rephasing gradient of 50%). **(b)** Correlation between R2* obtained for the DC phantom and cell number.

FIG. 4.

Representative in vivo MR images obtained 24 h after i.v. injection of 500,000 Feridex labeled human DCs. “L” indicates liver and “S” indicates spleen. GRASP images were only acquired for lymph node imaging. ICP-MS indicated that 0.3% of the injected dose was taken up by the popliteal lymph nodes 24 h after i.v. injection. Matching Perl’s Prussian blue staining for iron oxide deposition (blue) is shown for all tissue.

FIG. 5.

In vivo $R2^*$ values obtained in the liver and spleen before and 24 h after i.v. cell transplant.

FIG. 6.

a: Representative in vivo MR images obtained 24 h after footpad injection of 500,000 Feridex labeled human DCs. The left hind leg of the mouse is shown. The location of the draining lymph node is indicated by the letter "L." GRE (TE = 6 msec) and matching GRASP images are shown. Perl's staining of the lymph node (histology) showed the presence of iron laden cells within the boarder zones of the node, as indicated by the blue regions within the tissue. Additionally, ICP-MS confirmed that 3.2% of the injected dose was found within the draining lymph node 24 h post footpad injection. **b:** In vivo $R2^*$ values obtained in the liver, spleen, and draining lymph node before and 24 h after footpad injection.

FIG. 7.

Longitudinal tracking of Feridex labeled human DCs in immunodeficient mice after i.v. injection. Percent change in the in vivo R2* values (relative to baseline) (**a**) and percent injected dose (**c**) for the liver and spleen, as a function of time post i.v. injection. “*” indicates that the percent change is not significant ($P > 0.05$). The percent injected dose was estimated using the correlation between the in vivo R2* values and Feridex concentration, as determined by relaxometry. “#” reflects values under the quantification limit of the method. Perl’s staining of the liver and spleen at various time points post injection are also shown (**b**).

FIG. 8.

Comparison of the homing of Feridex labeled murine DCs compared with human DCs in mice. Percent change in the in vivo $R2^*$ values (relative to baseline) for the liver and spleen 24 h post i.v. injection.