

MR and iron magnetic nanoparticles. Imaging opportunities in preclinical and translational research

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ABSTRACT

Ultrasmall superparamagnetic iron oxide nanoparticles and magnetic resonance imaging provide a non-invasive method to detect and label tumor cells. These nanoparticles exhibit unique properties of superparamagnetism and can be utilized as excellent probes for magnetic resonance imaging. Most work has been performed using a magnetic resonance scanner with high field strength up to 7 T. Ultrasmall superparamagnetic iron oxide nanoparticles may represent a suitable tool for labeling molecular probes that target specific tumor-associated markers for *in vitro* and *in vivo* detection by magnetic resonance imaging.

In our study, we demonstrated that magnetic resonance imaging at 1.5 T allows the detection of ultrasmall superparamagnetic iron oxide nanoparticle conjugated antibody specifically bound to human tumor cells *in vitro* and *in vivo*, and that the magnetic resonance signal intensity correlates with the concentration of ultrasmall superparamagnetic iron oxide nanoparticle antibody used and with the antigen density at the cell surface. The experiments were performed using two different means of targeting: direct and indirect magnetic tumor targeting. The imaging of tumor antigens using immunospecific contrast agents is a rapidly evolving field, which can potentially aid in early disease detection, monitoring of treatment efficacy, and drug development. Cell labeling by iron oxide nanoparticles has emerged as a potentially powerful tool to monitor trafficking of a large number of cells in the cell therapy field. We also studied the labeling of natural killer cells with iron nanoparticles to a level that would allow the detection of their signal intensity with a clinical magnetic resonance scanner at 1.5 T.

Magnetic resonance imaging and iron magnetic nanoparticles are able to increase the accuracy and the specificity of imaging and represent new imaging opportunities in preclinical and translational research.

Introduction

Magnetic resonance imaging (MRI) offers a non-invasive technique to obtain anatomic and metabolic/functional information with high spatial and temporal resolution. It has two particular advantages over techniques that involve the use of radionuclides or optical probes: higher spatial resolution, and physiological, molecular and anatomical information can be extracted simultaneously. For specific detection of macromolecules using MRI, i.e., molecular imaging, ligands need to be conjugated to MR contrast agents in order to induce a different signal intensity (SI) from the non-targeted tissue. Similarly, for MRI detection of the cell populations of interest, i.e., cellular imaging, cells need to be labeled with MR contrast agents in order to make them stand out from the surrounding tissues. For both applications, gadolinium chelates may be used, but they have low relaxivity values, which further decrease upon cellu-

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lar internalization, are not biocompatible, and very little is known about their potential toxicity following cellular dechelation over time.

Superparamagnetic iron oxide particles (SPIO), which were introduced as contrast agents shortly after the use of gadolinium chelates¹⁻³, currently appear to be the preferred material. In particular, ultrasmall superparamagnetic iron oxide particles (USPIO) may represent a suitable tool for labeling molecular probes that target specific tumor-associated markers for *in vitro* and *in vivo* detection by MRI. An important property of USPIO is the strong T₂ relaxivity that produces a decrease in SI on T₂-weighted images^{4,5} and also a high T₁ relaxivity with an increase in SI on T₁-weighted images^{6,7}. The association of MRI with specific tumor iron oxide nanoparticles is able to increase the accuracy and the specificity of imaging⁸.

Monoclonal antibodies (mAbs) represent an important emerging tool in cancer therapy, and the study of specific surface markers on tumor cells has determined an important development of mAbs for use as targeted therapeutic agents. Therapeutic mAbs can act by blocking growth factors, directly signaling arrest and apoptosis, or inducing elimination of mAb-decorated target cells via activation of host defense mechanisms⁹.

The preparation and use of magnetically labeled mAbs seemed to be an extension of the earlier work in nuclear medicine carried out using radiolabeled antibodies. In this way, the detailed anatomic information on the MR images can be specifically marked in order to detect a disease in its earliest stages, particularly tumors. Immunoglobulins can be covalently linked to the dextran polysaccharide coat of the iron oxide using an established method^{10,11}. Iron oxide nanoparticles have been conjugated to polyclonal immunoglobulin G for the detection of induced inflammation¹², to mAb fragments for the specific visualization of myocardial infarction¹³, to intact mAbs for immunospecific detection of intracranial small cell lung carcinoma¹⁴, intracellular adhesion molecule-1 gene expression on transfected cell lines¹⁵ and oligodendrocyte progenitors¹⁶, and to synaptotagmin I for detection of apoptotic tumor cells¹⁷. Alternative ways of attaching mAbs to magnetic nanoparticles include glutaraldehyde cross-linking¹⁸, complexing through ultrasonication^{19,20}, using the biotin-streptavidin system^{21,22} and amine-sulfhydryl group linkage^{23,24}. The aforementioned studies provide examples of cases where molecular MRI using iron oxides has proven to be successful. Moreover, most work has been performed using an MR scanner with high field strength up to 7 T.

However, cellular imaging aims to visualize cells. It is a non-invasive method to study cellular processes which entails proper labeling of cells with appropriate MR contrast agents²⁵. Cell labeling by SPIO has emerged as a potentially powerful tool to monitor trafficking of transplanted cells by magnetic resonance, e.g. in studies

for tissue repair, but it has also become increasingly important in the development of oncologic cell therapies.

We report our experience in tumor and cell labeling. For tumor targeting, we used two different approaches: direct magnetic tumor targeting by a USPIO conjugated antibody specifically bound to human tumor cells *in vitro* and *in vivo*, and indirect magnetic tumor targeting based on a bridge of tumor-specific biotin-conjugated antibodies *in vivo*. We demonstrated that the MRI SI correlates with the concentration of USPIO antibody used and with the antigen density at the cell surface. We also studied the possible use of these conjugated monoclonal antibodies as contrast agents for *in vivo* labeling of human tumor cells.

For cell labeling, we investigated the possibility of labeling human natural killer (NK) T cells with iron nanoparticles and different types of transfection agents. We tested these types of targeting at a level that would allow their SI with a clinical MR scanner at 1.5 T.

Iron oxide nanoparticles

The *in vitro* magnetic properties of USPIO are reported to be a T₁ relaxivity (r₁) of 21.6 (mmol/liter-sec)⁻¹ and a T₂ relaxivity (r₂) of 44.1 (mmol/liter-sec)⁻¹ at 37 °C and 0.47 tesla (T), where mmol/liter is the concentration of iron oxide. Conventional, water-soluble, paramagnetic contrast agents are generally metal chelates with unpaired electrons, and they work by shortening both T₁ and T₂ relaxation times of surrounding water protons to produce a signal-enhancing effect²⁶. They distribute in the extracellular fluid and do not cross the intact blood-brain barrier²⁷. Another approach is the development of water-insoluble SPIO nanoparticles that comprise iron oxides such as magnetite (Fe₃O₄), maghemite (γFe₂O₃), or other ferrites^{28,29}. Ferromagnetic crystals are composed of magnetized domains the size of a micron. Superparamagnetism occurs when the size of the crystals is smaller than the ferromagnetic domain (~30 nm). SPIO agents typically consist of an iron oxide core and a hydrophilic coating³⁰. They have very high relaxivities (R₁ and R₂), and the significant capacity of these particles to increase the susceptibility effect of the measured spin-spin relaxation time (T₂^{*}) is especially useful in MRI. This large T₂^{*} effect is the result of the non-homogeneous distribution of these superparamagnetic particles, which accelerates the loss of phase coherence of the spins contributing to the MRI signal. Clinically, SPIO are predominantly used for their negative enhancement effect on T₂- and T₂^{*}-weighted sequences. This class of MRI agents includes large oral SPIO (300-3500 nm) agents, standard SPIO (60-150 nm) agents, USPIO (10-40 nm) agents, monocrystalline iron oxide (10-30 nm) nanoparticle agents, and cross-linked iron oxide agents (a form of monocrystalline iron oxide nanoparticles with a cross-linked dextran coating)³¹. Both the size and

the surface properties of SPIO particles affect their pharmacokinetics, organ distribution, and intracellular uptake³². Biologically, SPIO particles are usually taken up by the reticuloendothelial system and phagocytic cells. USPIO particles are less prone to liver uptake and are small enough to migrate across the capillary wall of tumors³³.

Tumor targeting

Direct tumor magnetic labeling

We used commercially available USPIO bound to an anti-CD20 monoclonal antibody (immunoglobulin G1-murine) and stabilized with sodium citrate (Miltenyi Biotec, Bergisch Gladbach, Germany). USPIO-anti-CD20 mAb is a molecular imaging agent developed for MRI of CD20 antigen-positive B cell lymphomas. The particles were composed of a biodegradable, non-toxic, ferromagnetic matrix (dextran). The overall mean particle diameter was ~30-50 nm. There were typically 10-200 antibody molecules/particle (30 nm in diameter). The *in vitro* R_1 and R_2 relaxivities measured at 37 °C and 1.5 T were 30 and 60 liter·s⁻¹·mmol⁻¹, respectively³⁴. The CD20 antigen is a 35-kDa, cell-surface non-glycosylated, hydrophobic phosphoprotein expressed on normal and malignant B cells, and it does not shed, modulate, or internalize^{35,36}. It is present on ~9% of the peripheral blood mononuclear cell (PBMC) fraction and >90% of B cells from blood and lymphoid organs. Lymphoma cells from >90% of patients with B-cell non-Hodgkin lymphoma express this antigen. Despite the presence of CD20 on normal B cells, it is a good tumor target for molecular targeting with antibodies for the management of non-Hodgkin lymphoma.

As cell lines, D430B and Raji cells (anaplastic large B-cell lymphoma) were used. The expression of surface antigens by D430B and Raji cell lines was analyzed by immunofluorescence. The presence of USPIO antibody bound to the cells was verified by staining with a phycoerythrin-labeled anti-mouse immunoglobulin antibody (Southern Biotec Inc., Birmingham, AL, USA). All samples were analyzed by cytofluorimetric analysis on a FACScan (BD Biosciences, Franklin Lakes, NJ, USA).

In vitro study. We incubated five million human D430B cells³⁷ and Raji Burkitt lymphoma cells (ICLC, Interlab Cell Line Collection) with different amounts of anti-CD20 monoclonal antibody USPIO conjugates. After incubation with the USPIO-mAb conjugates, unbound conjugates were removed and cells were included in a matrigel sponge for MRI imaging with a 1.5-T MR system (Philips Gyroscan NT-Intera). Immunofluorescence analysis showed that USPIO-anti-CD20 mAb bound to the cell surface and the D430B cells expressed five times more CD20 molecules than the Raji Burkitt cells. USPIO anti-CD20 mAb on D430B cells showed a

decrease in SI on T2*-weighted images and SI enhancement on T1-weighted images (Figure 1). In comparison, USPIO anti-CD20 mAb on Raji Burkitt cells only showed a slight hypointensity on T2-weighted images and a non-homogeneous hyperintensity on T1-weighted images (Figure 1). Quantitative analysis showed that the changes in T1 SI ($\Delta SI = SI_{\text{non-labeled}} - SI_{\text{USPIO anti-CD20 mAb}} / \text{noise}$) values from the three-dimensional fast-field echo sequences at 1.5 T for the USPIO anti-CD20 mAb on D430B cells were -36.6, -12.4, and -6.2 for 0.03 $\mu\text{mol Fe/liter}$, 0.01 $\mu\text{mol Fe/liter}$, and 0.005 $\mu\text{mol Fe/liter}$ USPIO anti-CD20 mAb, respectively. The T2 ΔSI values were -73, -24, and -12 for 0.03 $\mu\text{mol Fe/liter}$, 0.01 $\mu\text{mol Fe/liter}$, and 0.005 $\mu\text{mol Fe/liter}$ USPIO anti-CD20 mAb, respectively. In comparison, the T1 ΔSI values of the USPIO anti-CD20 mAb on Raji Burkitt cells were -25, -9, and -6 for 0.03 $\mu\text{mol Fe/liter}$, 0.01 $\mu\text{mol Fe/liter}$, and 0.005 $\mu\text{mol Fe/liter}$ USPIO anti-CD20 mAb, respectively. The T2 ΔSI values were -43, -17.7, and -12 for 0.03 $\mu\text{mol Fe/liter}$, 0.01 $\mu\text{mol Fe/liter}$, and 0.005 $\mu\text{mol Fe/liter}$ USPIO anti-CD20 mAb, respectively.

In vivo study. Experiments were approved by the Institutional Review Committee of the National Cancer Institute and were performed in accordance with the National Regulations on Animal Research Resources. MRI

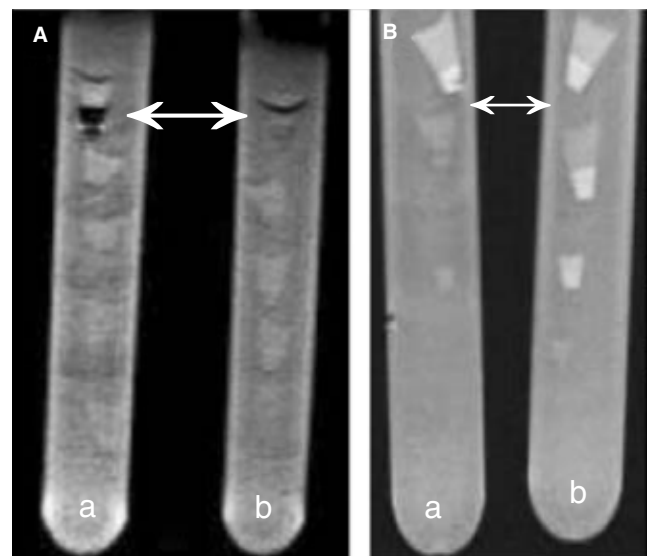


Figure 1 - Representative MR images of cell pellets in test-tubes. Each pellet contains 5×10^6 D430B lymphoma cells and 5×10^6 Raji lymphoma cells on T2*-weighted images (A) and on T1-weighted images (B), with of USPIO-anti-CD20, respectively. A) D430B cells-USPIO-anti-CD20 showed an important decrease of SI on T2*-weighted images that is more evident in the pellet treated with 0.03 $\mu\text{mol Fe/l}$ of USPIO-anti-CD20, whereas Raji cells-USPIO-anti-CD20 showed a slight hypointensity in the same sequences (double arrow). B) D430B cells-USPIO-anti-CD20 showed a SI enhancement on T1-weighted images and Raji cells-USPIO-anti-CD20 an inhomogeneous hyperintensity on T1-weighted three-dimensional fast field echo images (double arrow).

studies were performed in NOD-SCID mice bearing s.c. D430B or the Raji Burkitt tumors (0.5-1 cm²). Each mouse received an i.v. dose of 8 μ mol Fe/kg 24 h before imaging with a 1.5 T-MR system³⁴.

The D430B tumors showed a non-homogeneous SI decrease on T2*-weighted images (Figure 2) and a slight SI enhancement on T1-weighted images. In comparison, the Raji Burkitt tumors showed slight non-homogeneous hypointensity on T2*-weighted images and slight nonhomogeneous hypertensity on T1-weighted images. Quantitative analysis studies were conducted with the region-of-interest technique to obtain the signal/noise (SNR) ratios. The T2*-weighted Δ SI value (SNR_{before}/SNR_{after}) of the D403B tumor ($n = 5$) was $35 \pm 7\%$ ($82 \pm 9\%/57 \pm 11\%$), whereas the T2*-weighted Δ SI value of the Raji Burkitt tumor was $15 \pm 8\%$ ($47 \pm 10\%/40 \pm 13\%$). In comparison, tumors in mice injected with a non-specific standard SPIO agent ferumoxide (17 μ mol Fe/kg) showed a T2*-weighted Δ SI value of $5 \pm 6\%$ ($1,392 \pm 86\%/1,322 \pm 80\%$).

Indirect tumor magnetic labeling

We used commercially available USPIO bound to an anti-biotin monoclonal antibody (immunoglobulin G1-murine) and stabilized with sodium citrate (Miltenyi Biotec). The r_1 and r_2 relaxivities were 30 L \cdot sec⁻¹ \cdot mmol⁻¹ and 60 L \cdot sec⁻¹ \cdot mmol⁻¹, respectively³⁴. Anti-CD70 murine mAb, clone LD6, was purified from culture medium by affinity chromatography on Sepharose-Protein A (GE Healthcare, Chalfont, St Giles, UK) and subsequently conjugated with EZ-Link Sulfo-NHS-LC-biotin (Pierce®, Rockford, IL) according to the manufacturer's instructions^{38,39}. Surface expression of the CD70 antigen by the D430B line was analyzed by immunofluorescence and flow cytometry. To detect the binding of USPIO anti-biotin to the biotin-conjugated antibodies, D430 B cells were incubated with biotin-LD6 and biotin-CH-Leo. After washes, cells were challenged with 10 ml of the USPIO anti-biotin suspension, and the presence of USPIO-anti-biotin bound to the cells carry-

ing biotin-conjugated anti-CD70 mAb was verified by staining with a FITC-labeled anti-mouse immunoglobulin antibody (Caltag) followed by flow cytometric analysis. Mice were injected s.c. with 2×10^7 human D430B cells (palpable masses of 0.5 to 1 cm²). In a second set of experiments, 2×10^7 D430B cells per mouse were injected into the tail vein to induce pseudo-metastases, in particular localized in kidney and retroperitoneum. To target tumor-expressed human CD70 antigen, 30 μ g per mouse of biotin-labeled LD6 mAb was administered via the tail vein and MRI was performed with an MR scanner at 1.5 T. Four hours after administration of the biotin-labeled antibody, 16 μ mol Fe/kg per mouse of USPIO anti-biotin was injected in the tail vein. MRI was performed again 24 h later. As negative control, a group of mice was similarly inoculated with biotin-conjugated CH-Leo mAb. We performed the histological analysis according to standard techniques. Iron oxide-labeled cells were stained with Perl's Prussian blue stain. The D430B s.c. tumors showed a nonhomogeneous SI decrease on T2- and T2*-weighted images 24 h after USPIO anti-biotin administration (Figure 3); the D430B pseudo-metastases showed an important decrease in SI on T2* (Figure 4) and on balanced fast-field, echo-weighted images. Histopathological analysis using the Prussian blue stain demonstrated a significant iron localization in the D430B pseudo-metastases in comparison to s.c. D430B tumor.

Cell labeling

NK cells were generated from PBMC obtained from healthy donors. The PBMC were enriched for NK cell populations by negative depletion using immunomagnetic beads (NK cell isolation kit II; Miltenyi Biotec). PBMC were incubated with a biotin-conjugated antibody mix containing anti-CD3, anti-CD4, anti-CD14, anti-CD15, anti-CD19, anti-CD36, anti-CD123 and anti-235a (glycophorin A). Cells were washed and cen-

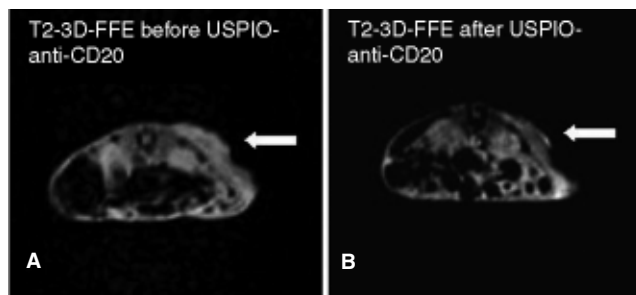


Figure 2 - Direct magnetic tumor targeting *in vivo*. On T2*-weighted images before (A) and after (B) USPIO-anti-CD20 antibody administration. The CD20 high D430B tumor showed a non-homogeneous decrease of signal.

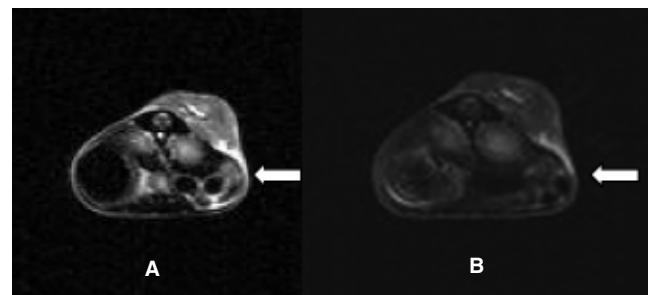


Figure 3 - Indirect tumor targeting in D430B subcutaneous lymphoma. On T2-weighted images, before (A) and after (B) USPIO-anti-biotin administration. There was a nonhomogeneous decrease in signal.

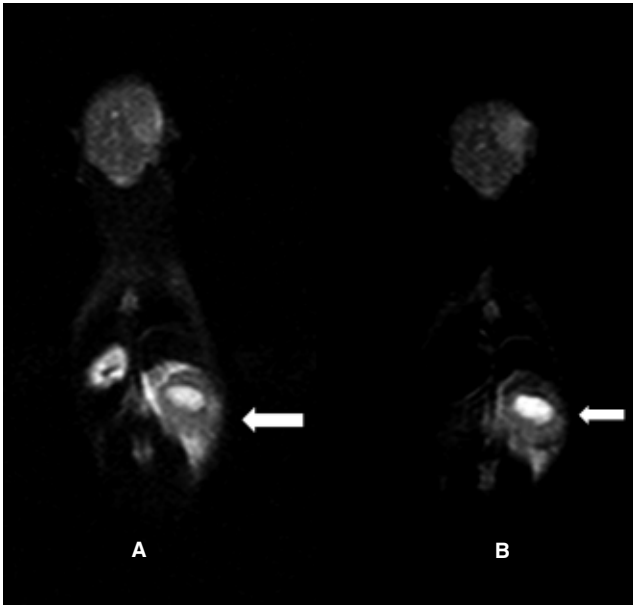


Figure 4 - Indirect tumor targeting in D430B pseudo-metastases lymphoma. On T2-weighted images, before (A) and after (B) USPIO-anti-biotin administration. There was a decrease in signal intensity in D430B.

trifuged. The supernatant was discarded and the cell pellet was resuspended in 0.1% phosphate-buffered saline human AB serum and incubated at 4 °C for 10 min after the addition of depletion anti-biotin microbeads. The cell suspension was loaded on a column in the magnetic field of a magnetic-activated cell sorter. NK cell purity was determined by flow cytometry. Enriched NK cells were expanded as bulk NK cell populations in RPMI containing 10% heat-inactivated fetal calf serum and 500 IU/ml of interleukin-2.

As a targeted contrast agent, we used a commercially available ferumoxide suspension (Endorem, Guerbet, Paris), which has a total iron content of 11.2 and 28 mg/ml, respectively. As a transfection agent, protamine sulfate (Pro, 6 mg/ml) or polylysine (PLL, 1.5 mg/ml) was added. The final FE-Pro or FE-PLL suspensions were added directly to the cells and incubated overnight. For the assessment of labeling efficiency and to study the SI on an MR scanner at 1.5 T, four groups were compared: unlabelled cells; NK-ferumoxides alone; NK-ferumoxides plus protamine; NK-ferumoxides plus PLL (Figure 5). Cell viability was measured in single cell suspensions by trypan blue exclusion. Perl's Prussian blue stain was performed to show iron localization in NK cells. On T2-weighted images, we observed a decrease in SI in all three groups due to iron in NK cells (Figure 5), but in the quantitative analysis, we calculated a significant decrease in SI in human ferumoxides-PLL-NK-labeled cells (60%) compared with human ferumoxide-Pro-NK-labeled cells (30%). Perl's Prussian blue stain demonstrated that the hypointensi-

ty on MRI was due to ferumoxides in NK cells. Moreover, there were no toxic effects after labeling cells, and with our doses no apoptosis or necrosis was observed.

Discussion

SPIO particles appear to be the preferred material for the targeting of tumor and cells. The popularity of SPIO particle labeling is mainly because: 1) they provide a strong change in signal (hypointensity) per unit of metal (on T2*-weighted images), 2) they are composed of biodegradable iron (biocompatible and can be recycled by cells using biochemical pathways for iron metabolism), 3) their surface coating (dextran) allows chemical linkage of functional groups and ligands, 4) they can be easily detected by light and electron microscopy, and 5) they can be manipulated and change their magnetic properties according to size. Iron nanoparticles can be conjugated to specific mAbs in order to label tumor and cells. These "molecular probes" are able to induce a different SI in tumor tissue compared to normal tissue. Many promising probes are being, or will be, applied to the diagnosis of cancer and may also facilitate cancer therapy. Furthermore, many antibodies directed to tumor-associated antigens are available for clinical use. A wide spectrum of commercially available SPIO antibodies used for cell-separation techniques is available, and some of them are also available as clinical-grade reagents⁸. They may be a promising tool for several *in vitro* and *in vivo* applications.

In our studies, we demonstrated the successful use of a commercially available USPIO antibody conjugate for MRI *in vitro* and in a murine model. In medicine, many

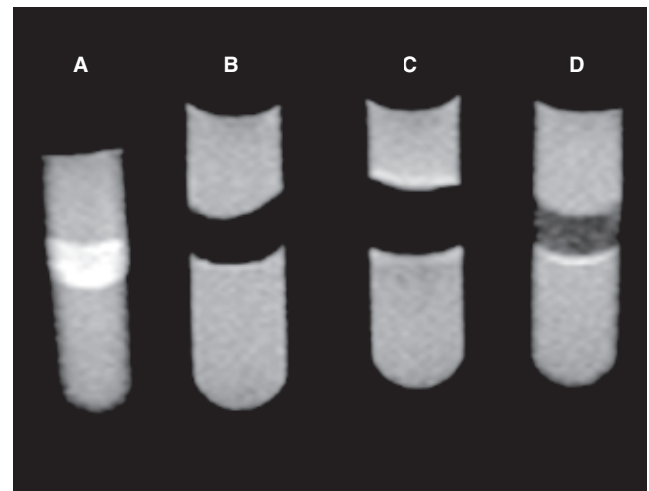


Figure 5 - T2-weighted images of USPIO natural killer labeled cells and natural killer cells. A) Unlabelled natural killer cells. B) Ferumoxides Pro-NK-labeled cells. C) Ferumoxides PLL-NK-labeled cells. D) NK-ferumoxides

approaches have been investigated for diagnosis and therapy and have offered a large variety of applications: magnetic cell separation, MRI, magnetic targeted drug delivery, and magnetically induced hyperthermia. Such particles do not have any toxic effect or incompatibility with biological organisms, therefore they have a potential use as contrast agents for MRI and as a carrier system for drugs⁴⁰.

Our *in vitro* study was designed to assess the feasibility of the use of an MR scanner at 1.5 T to detect USPIO antibodies bound to tumor cells at potentially suitable doses for small animal models in a pre-clinical setting. We demonstrated that commercially available USPIO antibody conjugates specific for a B-cell lymphoma-associated antigen binding to the human D430B cells or to the Raji Burkitt lymphoma cell line can be visualized on MRI at 1.5 T. We also demonstrated through *in vitro* assays that there is a dose-response relationship between the different amounts of USPIO anti-CD20 bound to the cells and the DSI on T2*-weighted images. This effect is more evident on cells expressing high levels of the target antigen (D430B) than on cells with a lower expression (Raji), indicating that the magnitude of DSI observed depends on the dose of the immunospecific contrast agent and on the target antigen expression of the cells. Indeed, *in vivo* experiments indicated that the intravenous administration of a single dose of USPIO anti-CD20 antibody conjugate 24 h before performing MRI at 1.5 T is sufficient to induce detectable changes of SI in a reproducible fashion (35% ± 7). Our results are consistent with those obtained in previous studies using similar USPIO contrast agents to visualize inflammatory cells infiltrating the brain in a murine model of autoimmune encephalomyelitis⁴¹. However, these previous studies used a 3.5-7 T MR scanner, whereas in the present investigation the high antigen expression by a homogeneous population of tumor cells allowed the detection of cell-bound USPIO by a 1.5 T standard scanner. With indirect magnetic tumor targeting, we assessed the feasibility of targeting superparamagnetic particles to tumors by a bridge of tumor-specific biotin-conjugated antibodies. We demonstrated that the combination of specific biotin-labeled antibodies with the USPIO anti-biotin as the common secondary reagent was able to target two types of murine models: the xenotransplant (s.c.) and the pseudo-metastases lymphoma tumors. In particular, the D430B pseudo-metastasis was localized in the kidney and retroperitoneum. These tumor sites are more vascularized than s.c. tumors. In fact, the decrease in SI at MRI visualization was more evident in D430B pseudo-metastases, and Perl's Prussian blue staining demonstrated a significant iron concentration in this type of tumor model.

A potential limitation of these molecular probes may be related to the relatively low iron concentration compared with the clinically available non-targeted MRI contrast agents, which are non-specifically internalized

by the reticular-endothelial cells. Thus the development of similar USPIO-targeted antibodies with high iron oxide content may enhance the sensitivity of detection, in order to allow MRI of tumors with small diameters or with lower target antigen density. We believe that this indirect method opens the possibility to target any cell surface antigen, thus becoming a useful tool in MRI.

Another possible application of iron nanoparticles and MRI is to label cells. In our cell labeling experiments, we illustrated the possibility to label NK cells with ferumoxides and different kinds of transfection agents. NK cells are a subpopulation of lytic effector lymphocytes that represents the first line of defense against tumor progression and metastasis. More studies were performed with stem cells, which have a significant phagocytic activity. This characteristic is less evident in NK cells. For this reason, we used different kinds of transfection agents. The complex ferumoxides-PLL-NK cells showed a substantial decrease in SI on T2*-weighted images, and Perl's Prussian blue staining confirmed the presence of ferumoxides in NK cells, thus proving that PLL is more efficient than Pro as a transfection agent for the internalization of ferumoxides by NK cells.

We have shown that significant changes in T2-weighted MRI SI can be achieved in human NK lymphocytes with a short incubation of iron oxide nanoparticles at concentrations that do not have deleterious effects on cellular viability or function. The detected contrast change lasted for at least 24 h and raises the possibility that iron oxide nanoparticle labeling of lymphocytes could be used to track these cells in tumors and other tissues using high spatial resolution MRI. Iron nanoparticles might be a type of targeting to study *in vivo* NK cell migration at a level that would allow the detection of their SI with a clinical MR scanner at 1.5 T. We believe that by optimizing molecular-targeted contrast agents, MRI technology and the generation of new USPIO or SPIO antibodies, or other USPIO or SPIO ligands, may provide useful immunospecific contrast agents for the diagnosis of tumors and for targeting cells for cell therapy. In this way, it promises to provide new methods for the early detection of cancer and support for personalized cancer therapy. Nevertheless, there has been sufficient experience with specifically targeted contrast agents and high-resolution techniques for MRI and other modalities that we must begin moving these new technologies from the laboratory to the clinic.

Conclusions

MRI combined with iron oxide contrast agents is able to increase the accuracy and specificity of imaging and represents a new imaging opportunity in preclinical and translational research.

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