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Magnetic Resonance Imaging Overestimates Ferumoxide-Labeled Stem Cell Survival After Transplantation in the Heart

John Terrovitis, MD; Matthias Stuber, PhD; Amr Youssef, MD; Steve Preece, BS; Michelle Leppo, BS; Eddy Kizana, MD, PhD; Michael Schär, PhD; Gary Gerstenblith, MD; Robert G. Weiss, MD; Eduardo Marbán, MD, PhD; M. Roselle Abraham, MD

***Background*—**Stem cell labeling with iron oxide (ferumoxide) particles allows labeled cells to be detected by magnetic resonance imaging (MRI) and is commonly used to track stem cell engraftment. However, the validity of MRI for distinguishing surviving ferumoxide-labeled cells from other sources of MRI signal, for example, macrophages containing ferumoxides released from nonsurviving cells, has not been thoroughly investigated. We sought to determine the relationship between the persistence of iron-dependent MRI signals and cell survival 3 weeks after injection of syngeneic or xenogeneic ferumoxides-labeled stem cells (cardiac-derived stem cells) in rats.

***Methods and Results*—**We studied nonimmunoprivileged human and rat cardiac-derived stem cells and human mesenchymal stem cells doubly labeled with ferumoxides and β-galactosidase and injected intramyocardially into immunocompetent Wistar-Kyoto rats. Animals were imaged at 2 days and 3 weeks after stem cell injection in a clinical 3-T MRI scanner. At 2 days, injection sites of xenogeneic and syngeneic cells (cardiac-derived stem cells and mesenchymal stem cells) were identified by MRI as large intramyocardial signal voids that persisted at 3 weeks (50% to 90% of initial signal). Histology (at 3 weeks) revealed the presence of iron-containing macrophages at the injection site, identified by CD68 staining, but very few or no β-galactosidase–positive stem cells in the animals transplanted with syngeneic or xenogeneic cells, respectively.

***Conclusions*—**The persistence of significant iron-dependent MRI signal derived from ferumoxide-containing macrophages despite few or no viable stem cells 3 weeks after transplantation indicates that MRI of ferumoxide-labeled cells does not reliably report long-term stem cell engraftment in the heart. **(*Circulation*. 2008;117:1555-1562.)**

Key Words: magnetic resonance imaging ■ cells ■ transplantation

Cell transplantation is a promising new treatment modality for cardiac regeneration.[1] However, an effective, nontoxic, noninvasive method for cell tracking is required to assess cell fate after transplantation. The ideal technique would combine high sensitivity, to detect relatively small numbers of cells, and high specificity, that is, that any signal would be derived exclusively from viable, labeled cells and thus report successful engraftment.[2,3]

Clinical Perspective p 1562

Magnetic resonance imaging (MRI) is the most attractive imaging modality because it provides high-quality 3-dimensional functional and anatomic information with high soft-tissue contrast without the need for ionizing radiation, thereby allowing longitudinal follow-up for assessment of cell engraftment and migration. However, cells need to be labeled with contrast agents before injection so that they can be visualized and distinguished from resident tissues. Iron-based contrast agents of various sizes and coatings have been used to label and track stem cells by MRI.[4–6] These agents distort the magnetic field, shorten the T2 relaxation time, and generate signal voids (dark spots)[7] in T2-weighted MRIs. Ferumoxides (Feridex, Berlex, Montvale, NJ) are dextran-coated iron oxide nanoparticles (≈100-nm diameter with an iron core of 5- to 30-nm diameter) that have been approved by the Food and Drug Administration.[8] These agents usually are introduced into stem cells (ie, nonphagocytic cells) by use of transfection agents like poly-L-lysine (PLL) or protamine.[7,9] These negatively charged transfection agents form electrostatic bonds with the positively charged dextran coating of ferumoxides, and the resultant complex is then taken up by cells in membrane-bound endosomes. Significantly, no ad-

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The online Data Supplement can be found with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.732073/DC1.

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verse effects on cell viability were reported after labeling of multiple cell types,[4] and only 1 study documented interference with the potential of mesenchymal stem cells (MSCs) for differentiation into chondrocytes.[10] Hence, iron labeling of stem cells and their detection by MRI has emerged as the technique of choice for assessing stem cell engraftment in patients. The Achilles' heel of this technique is the theoretical inability to distinguish extracellular iron from intracellular iron present in stem cells or tissue macrophages. The reason is that ferumoxides persisting after stem cell death may produce a signal that is indistinguishable from that of surviving labeled cells, thus overestimating stem cell engraftment. To date, most studies assumed that the MRI signal originated from surviving ferumoxide-labeled cells, although the possibility of overestimation was acknowledged.[11,12] However, none have directly investigated whether ferumoxide-containing tissue macrophages and extracellular iron particles also contribute to the MRI signal in the heart several weeks after stem cell transplantation.

In the present study, we sought to determine the relation between signal detected by MRI and engraftment of syngeneic and xenogeneic cardiac-derived stem cells (CDCs) labeled with ferumoxides in a myocardial transplantation model. We hypothesized that if this method of labeling accurately reflects cell survival, the MRI signal should disappear in the first week after cell injection in the xenogeneic setting because of rapid rejection of the injected cells in immunocompetent animals, whereas signals would persist in the syngeneic model if large numbers of cells engrafted. Here, we report that a large MRI signal attributable to ferumoxides was detectable 2 days after cell transplantation of both syngeneic and xenogeneic cells and that much of the signal persisted for 3 weeks, despite no (xenogeneic) or very few (syngeneic) surviving cells at 21 days, indicating that persistent signal does not reflect engraftment using this imaging modality. Similar results were obtained when human MSCs (hMSCs) were injected into infarcted rat myocardium, confirming that the discordance between signal persistence and transplanted cell survival is not cell or substrate specific.

Methods

Cell Culture

Human CDCs (hCDCs) were cultured as previously described from tissue samples obtained from patients undergoing clinically indicated endomyocardial biopsies who provided consent.[13,14] Rat CDCs (rCDCs) were cultured from explanted hearts obtained from 3-month-old male Wistar-Kyoto rats (Harlan, Indianapolis, Ind). The Wistar-Kyoto rat is an inbred strain. These rats are genetically identical, making them ideal for use in cell transplantation studies in which syngeneic cells can be delivered without triggering immunological rejection. hMSCs were purchased from Cambrex (Charles City, Iowa). A detailed description of the isolation and tissue culture procedure is provided in the online Data Supplement.

Vector Production and Genetic Labeling of Cells

To enable detection of cells after transplantation, a third-generation lentiviral vector system (kindly supplied by Professor Inder Verma, Salk Institute, San Diego, Calif) was used to label hCDCs and rCDCs. Details of vector production are found in the Data Supplement.[15,16] CDCs and hMSCs were transduced at a multiplicity of infection of 20. Transduction efficiencies of >90% were achieved without impairing CDC proliferation kinetics. For titration and labeling experiments, β-galactosidase expression was assessed by X-gal (Fisher Scientific, Waltham, Mass) staining of transduced cells.

Iron Labeling Protocol

hCDCs and rCDCs were incubated with 3 doses of ferumoxides (8, 12.5, or 25 μg/mL) and 0.75 μg/mL PLL (Sigma, St Louis, Mo) for 16 hours using previously published protocols.[4,7,17,18] hMSCs were incubated with 25 μg/mL ferumoxides and 0.75 μg/mL PLL for 16 hours, a protocol with known safety and efficacy.[4,17,18]

In another series of experiments, the ability of CDCs to take up ferumoxides without facilitation by a transfection agent was assessed by incubating cells with a high dose (250 μg/mL) of ferumoxides without PLL.

Labeling efficiency was assessed by Prussian Blue staining (see Methods in the Data Supplement). To determine in vitro retention of ferumoxides by labeled cells, CDCs were labeled with ferumoxides and cultured for 3 weeks; a sample was stained with Prussian Blue on a weekly basis to test for the persistence of iron-containing cells.

Effect of Iron Labeling on Cell Viability and Proliferation

CDC viability after ferumoxide labeling was assessed by flow cytometry (7AAD and Annexin V stain; see Methods in the Data Supplement). For assessment of cell proliferation, a WST-8–based, colorimetric proliferation assay was performed on nonlabeled and ferumoxide-labeled CDCs per manufacturers' instructions (Cell Counting Kit-8, Dojindo Molecular Technologies, Gaithersburg, Md; see the Data Supplement). The absence of any detrimental effect of ferumoxide labeling on MSC viability/proliferation has been extensively validated.[4,7,17,18]

Immunogenicity of hCDCs

To assess whether CDCs are immunoprivileged, we investigated the expression of immunoregulatory molecules on the surface of hCDCs. For this purpose, CDCs from 3 patients were stained for major histocompatability complex (MHC) class I, MHC class II, β2 microglobulin, and CD80/86 (costimulatory molecules) under baseline conditions and after stimulation with 100 ng/mL interferon-γ for 30 hours. Antibodies to these antigens were directly conjugated with FITC, and labeling was assessed by flow cytometry (FACSCalibur, BD, Franklin Lakes, NJ).

To assess the functional immunogenic properties of CDCs, a 1-way mixed lymphocyte reaction was used as a measure of T-cell reactivity against allogeneic cell populations (see Methods in the Data Supplement).

In Vivo Cell Delivery and MRI

To investigate the relation of MRI signal persistence to cell survival and engraftment, 10^6 *Lacz* gene and ferumoxide-labeled CDCs were injected intramyocardially into 17 normal (immunocompetent) Wistar-Kyoto rats (Harlan). Ten animals received hCDCs (xenogeneic model) and 7 received rCDCs (syngeneic model). In another set of experiments, hMSCs were injected into 4 animals (7.5×10^5 in 2 rats, 5×10^5 in 2 rats) immediately after induction of myocardial infarction to investigate the relationship of MRI signal and cell survival using a different cell type and substrate. Finally, to investigate the kinetics of the free contrast agent in the myocardium, 2 noninfarcted rats were injected with 20 μg ferumoxides intramyocardially; this dose corresponds to the amount of intracellular iron present in stem cells using our ferumoxide-loading protocol.[4] Cells were incubated with 25 μg/mL ferumoxides and 0.75 μg PLL per 1 mL media for 16 hours 1 day before injection, rinsed thoroughly, harvested with trypsin, washed, and then suspended in 50 μL PBS before injection.

Rats underwent left thoracotomy in the fourth or fifth intercostal space under general anesthesia (isoflurane inhalation: 4% for induction and 2.5% for maintenance). The heart was exposed and the cells were injected directly into the myocardium at a single site in the anterolateral wall of the left ventricle with a 30-gauge needle.

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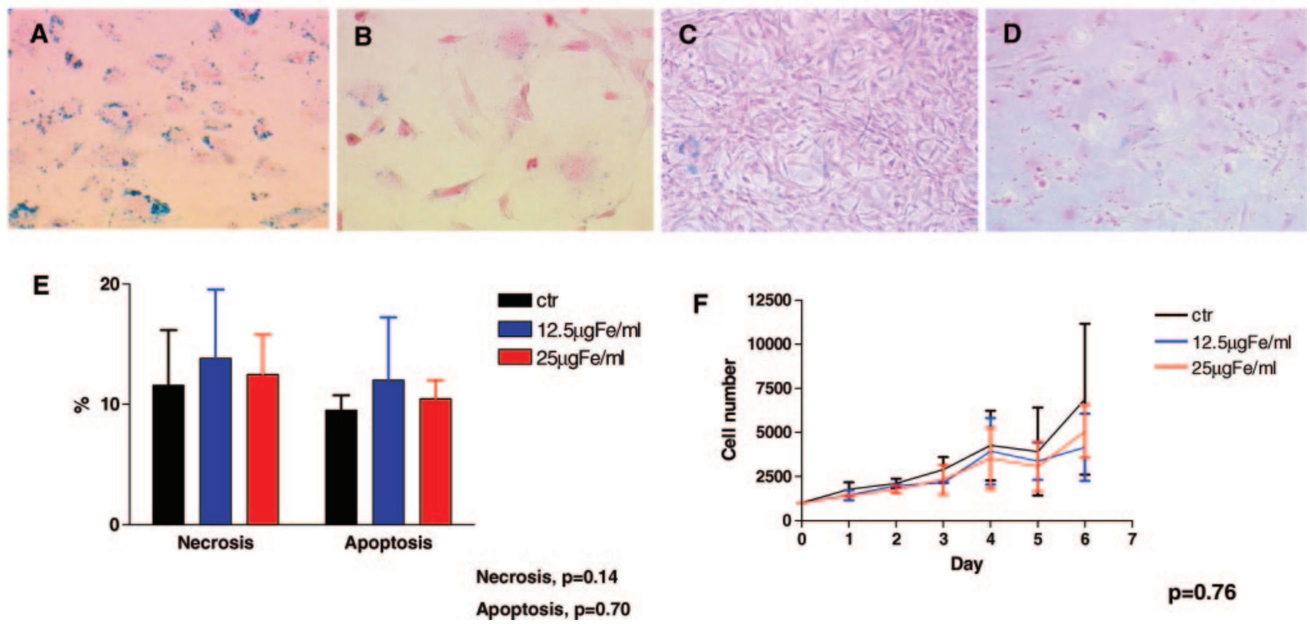

Figure 1. Prussian Blue staining of labeled hCDCs with 25 μg/mL ferumoxides and 0.75 μg/mL PLL 24 hours after labeling (labeling efficiency >90%) (A); 250 μg/mL ferumoxides without PLL 24 hours after labeling (minimal intracellular iron is seen) (B); 25 μg/mL ferumoxides and 0.75 μg/mL PLL 6 days after labeling (significant reduction in the number of Prussian Blue–positive cells) (C); and 25 μg/mL ferumoxides and 0.75 μg PLL per 1 mL media 20 days after labeling (only a single Prussian Blue–positive cell was detected) (D). E, Necrosis/apoptosis was not increased 24 hours after hCDC labeling with ferumoxides (12.5 or 25 μg/mL) and PLL (0.75 μg/mL). F, Proliferation rate of CDCs labeled with ferumoxides (12.5 μg/mL or 25 μg/mL) and 0.75 μg/mL PLL was not affected (compared with nonlabeled cells). Ctr indicates control.

Myocardial infarction was produced by permanent ligation of the left anterior descending coronary artery in 4 animals with a Prolene 7.0-mm suture immediately before cell injection; in this case, cells were injected intramyocardially into the infarct. Subsequently, the chest was closed and the animals were allowed to recover.

MRI was performed in a clinical Achieva 3T scanner (Philips Medical Systems, Best, the Netherlands) on days 2 and 21 in 15 animals (7 xenogeneic CDCs, 4 syngeneic CDCs, and 4 hMSCs) and on day 35 in 1 animal that received xenogeneic CDCs. The animals that received only ferumoxides underwent MRI at days 2, 7, 14, and 21 after injection. After completion of this follow-up period, the rats were killed and the hearts were subjected to histology. For MRI, animals were anesthetized by isoflurane inhalation (4% for induction, 2% maintenance) and then placed prone, head first in the magnet. A small-diameter (12.5×10 cm) 4-element phased-array coil was used for signal reception (Pathway MRI, Seattle, Wash). ECG-gated cine images of the heart were obtained by a spoiled gradient-echo sequence with a slice thickness of 2 mm, a flip angle of 20°, a field of view of 90 mm, a matrix of 400×400, 26 cardiac phases, a repetition time of 7.5 ms, and an echo time of 2.8 ms; using this sequence, iron particles are detected as a signal void. A "positive" signal-producing sequence[19] was not used because the sensitivity of the older negative signal-producing sequence was higher on the basis of our in vitro studies.

At least 3 consecutive short-axis slices were acquired to completely cover the area of cell injection. Images were analyzed with Image J software (NIH, Bethesda, Md). Signal intensity was measured in the myocardium (remote areas and areas of cell injection); noise was measured by creating regions of interest in the lungs. Contrast-to-noise ratios (signal intensity in the remote myocardium minus signal intensity in the areas of the cell injection divided by the SD of noise) were calculated for each slice in which the signal void was visualized. In addition, percent signal area was calculated as the area of visually determined signal void (manually defined region of interest containing area obviously darker than the surrounding myocardium) divided by the total left ventricular area in the same slice.

Histology

Histological analysis of cell engraftment was performed at 21 days in all 15 animals that were injected with doubly labeled CDCs or hMSCs and subjected to MRI. Additionally, to confirm early engraftment of the doubly labeled CDCs, 5 more animals were injected with similarly labeled cells (3 with xenogeneic hCDCs, 2 with syngeneic rCDCs) and killed 2, 5, and 7 days after cell injection (see Methods in the Data Supplement). Sections containing the largest numbers of β galactosidase–positive cells were used for quantification.

Statistical Analysis

For matched comparisons, a paired t test or repeated-measures ANOVA was used, depending on the number of groups examined. Comparisons between independent groups were performed with the standard Student t test. Repeated-measures ANOVA was used for comparison of cell proliferation rates at different time points. A value of $P<0.05$ was chosen for statistical significance. Values are reported as mean±SD.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Ferumoxide Labeling Efficiency

Incubation of CDCs with 25 μg/mL ferumoxides and 0.75 μg/mL PPL for 16 hours yielded a labeling efficiency ≥90% (Figure 1A). Reducing the ferumoxide concentration to 12.5 μg/mL did not affect the labeling efficiency; however, when the ferumoxides concentration was reduced to 8 μg/mL, only 20% of the cells demonstrated intracellular iron by Prussian Blue staining. When CDCs were exposed only to the high concentration of ferumoxides (250 μg/mL media), in the absence of the transfection reagent (PLL), a small amount of

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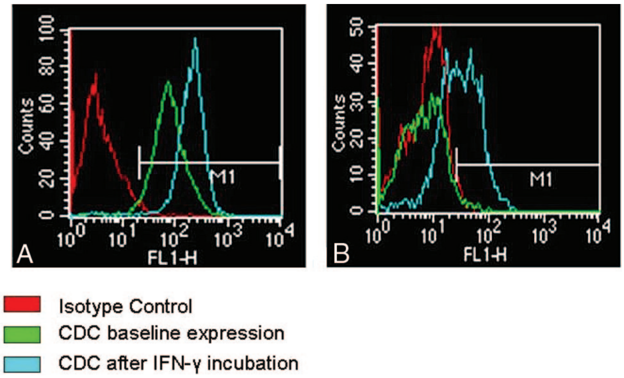

Figure 2. A, hCDCs express MHC class I molecules at baseline conditions. B, hCDCs express MHC class II molecules after interferon (IFN)-γ stimulation.

intracellular iron was detected by Prussian Blue staining in only a small fraction of cells (Figure 1B).

Retention of Iron Particles by Cells

When labeled CDCs (starting from ≈100% Prussian Blue–positive cells) were expanded in culture for 3 weeks, there was a rapid reduction in the number of labeled cells on days 7 and 14, and eventually, on day 21, very few cells stained positive for iron (Figure 1C and 1D), probably because of dilutional loss of the label with cell division. Hence, cells were doubly labeled with ferumoxides and a lentiviral vector expressing nuclear-localized β-galactosidase for the in vivo study. The vector provirus DNA is incorporated into the CDC genome and transmitted to all daughter cells during mitosis. Thus, cell tracking is possible by histology (X-gal staining for β-galactosidase detection) if cell division occurred after injection despite dilution of the iron label.

Effect of Ferumoxide Labeling on Viability and Proliferation

Iron labeling of CDCs with 12.5 and 25 μg/mL ferumoxides with PLL (0.75 μg/mL) did not increase cell necrosis or apoptosis ($P=0.14$ and $P=0.70$, respectively, by repeated-measures ANOVA) as assessed by flow cytometry (Figure 1E). This labeling protocol also did not significantly affect cell proliferation (assessed with the WST-8 assay; $P=0.76$ by repeated-measures ANOVA), suggesting that the ferumoxide-PLL combination is not toxic to CDCs (Figure 1F). Hence, 25 μg/mL ferumoxides with PLL (0.75 μg/mL) was used for subsequent in vivo experiments.

Immunogenicity of CDCs

Flow cytometry experiments revealed that hCDCs express MHC class I surface antigens at baseline and MHC class II only after stimulation by interferon-γ (Figure 2A and 2B). In the mixed lymphocyte reaction experiments, hCDCs activated allogeneic T-cell proliferation at baseline (stimulator index, 36.1), and the reaction was augmented after interferon-γ prestimulation (stimulator index, 60.2). These results indicate that hCDCs, unlike MSCs, are not immunoprivileged and that cell rejection should be expected when CDCs are transplanted into allogeneic or xenogeneic recipients.

Relation of MRI Signal to Labeled-Cell Engraftment

Two days after CDC injection into intact rat myocardium, a large area of signal void was detected in all animals at the injection site (Figure 3A and 3B). The mean signal void area was 24.3±9.5% and 22.5±6.0% ($P=0.72$ by Student's t test) of the total area of the 3 most apical left ventricular slices in animals that received syngeneic and xenogeneic cells, respectively. At 3 weeks, the signal void area was similar to that at 2 days after cell injection in animals transplanted with syngeneic cells (20.2±9.3%; $P=0.33$ versus 2 days by paired t test; Figure 3C). However, in the animals that received xenogeneic cells, at 3 weeks, when no human cells are expected to have survived rejection, an area of signal void could still be clearly identified. Although the area was smaller after xenogeneic transplantation (12.9±4.3%, $P=0.02$ versus 2 days by paired t test; Figure 3D and video A of the Data Supplement), it still represented ≈50% of the area identified 2 days after transplantation.

The contrast-to-noise ratio was similar for syngeneic and xenogeneic transplantation both at 2 days (12.4±3.8 and 10.1±1.8; $P=0.21$) and at 3 weeks (10.4±0.4 versus 10.7±4.3; $P=0.88$ by Student's t test). In addition, the contrast-to-noise ratio at 3 weeks was similar to that 2 days after transplantation ($P=0.4$ for the syngeneic cells, $P=0.78$ for the xenogeneic cells by paired t test).

In animals injected with hMSCs into the infarct area, a pattern of signal persistence similar to that of xenogeneic

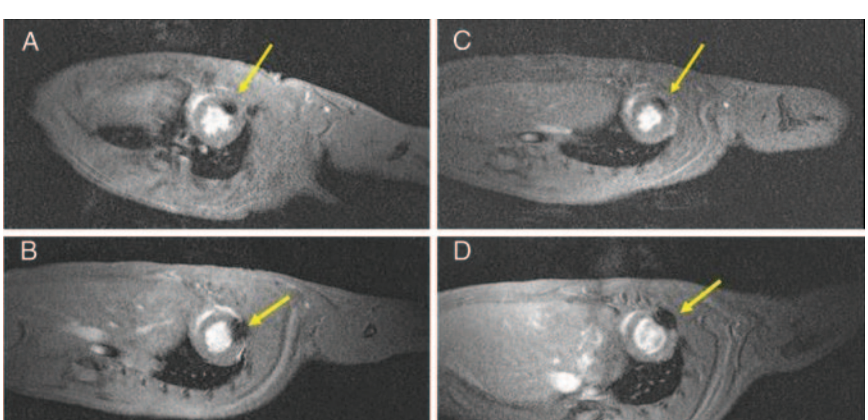

Figure 3. Representative in vivo rat heart MRI in 2 animals that received CDCs. Short-axis image at 2 days after cell injection reveals a large signal void (arrow) at the injection site in an animal that received syngeneic CDCs (A) and in an animal that received xenogeneic CDCs (B). A large signal void (arrow) persisted in the myocardium at the injection site 21 days after cell injection in the animal that received syngeneic CDCs (C) and in the animal that received xenogeneic CDCs (D).

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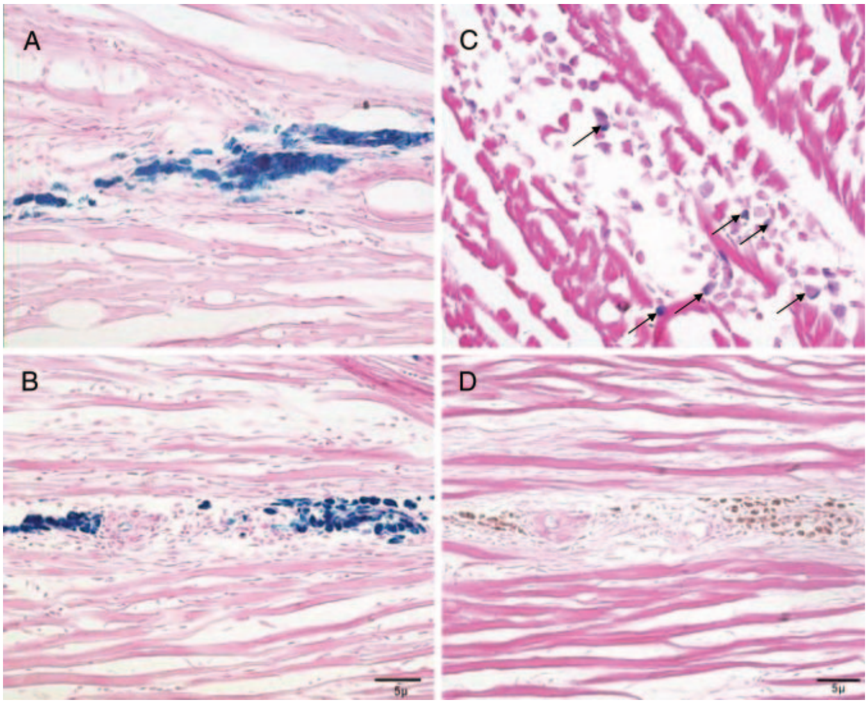

Figure 4. Representative histology of rat hearts harvested 21 days after syngeneic and xenogeneic cell injection. Prussian Blue staining demonstrates a large number of iron-containing cells in an animal injected with syngeneic cells (A) and in an animal injected with xenogeneic cells (B). X-gal stains, in sections adjacent to the Prussian Blue ones, showed few β-galactosidase–positive cells in the animal injected with syngeneic cells (arrows) (C) and no β-galactosidase–positive cells in the animal injected with xenogeneic cells (D).

cells in the noninfarct model was observed at 3 weeks (Results section, Figure IA through ID, and video B of the Data Supplement). In the 2 animals injected with contrast agent alone, a discrete, although gradually diminishing, signal void was detected in the weekly MRI studies up to weeks 3 after injection (Results section and Figure IIA through IID of the Data Supplement).

Histological Assessment of Cell Engraftment

In all animals (early and late death), Prussian Blue–positive cells were detected at the injection site in tissue sections (Figure 4A and 4B). Sections corresponding to the Prussian Blue–positive areas were tested for the presence of β-galactosidase–positive cells.

In the syngeneic model, X-gal stain revealed many positive cells (50 to 90 CDCs per high-power field) on day 2 and few cells (1 to 3 CDCs per high-power field) on days 7 and 21 (Figure 4C). In the xenogeneic model, X-gal stain revealed many positive cells (45 to 70 CDCs per high-power field) in the sections derived from the animal killed on day 2 and few cells (1 to 3 CDCs per high-power field) in the animal killed on day 5. In contrast, no β-galactosidase–positive cells were identified at later time points (7 and 21 days [Figure 4D] and 35 days) despite the presence of many Prussian Blue–positive cells, indicating that the iron-containing cells were not the injected human CDCs. To increase sensitivity for β-galactosidase detection, adjacent sections were subjected to immunocytochemistry with an anti–β-galactosidase antibody. Consistent with the X-gal staining, very few positive cells (1 to 5 CDCs per high-power field) were seen in samples on day 5 (Figure 5), and no positive cells were seen at later time points, indicating that no human cells survived >5 days in the rat myocardium, as expected in this xenogeneic study system.

To identify the iron-containing cells, sections adjacent to those displaying Prussian Blue–positive cells from both models killed at 21 days were tested for a macrophage-specific antigen (macrosialin or CD68) by immunocytochemistry. This revealed many CD68-positive cells, with a staining pattern similar to that seen with Prussian Blue–positive cells (Figure 6A and 6B). Thus, despite the loss of 95% to 100% of the stem cells between days 2 and 21, ≈50% (xenogeneic) to 80% (syngeneic) of the MRI signal persisted, demonstrating a dramatic discordance between MRI signal persistence and labeled cell viability.

We subsequently examined histological sections from the animals that received human MSCs. Despite the presence of abundant Prussian Blue–positive cells, no surviving hMSCs were found, and the iron-containing cells were again identi-

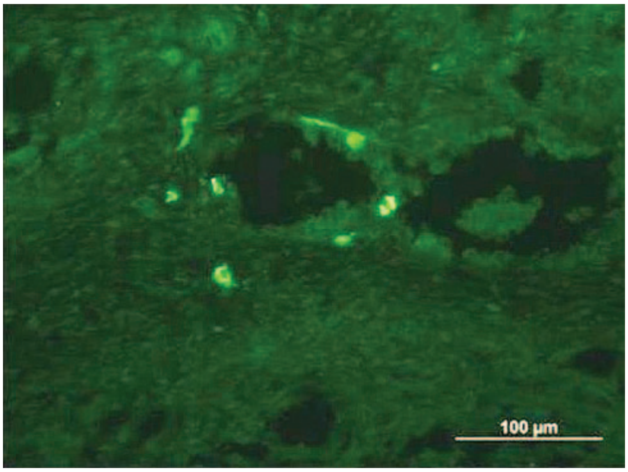

Figure 5. β-Galactosidase immunostaining of a rat heart harvested at day 5 after xenogeneic cell injection. Few β-galactosidase–positive cells were found. This is the latest time point after xenogeneic cell injection at which β-galactosidase–positive cells were identified.

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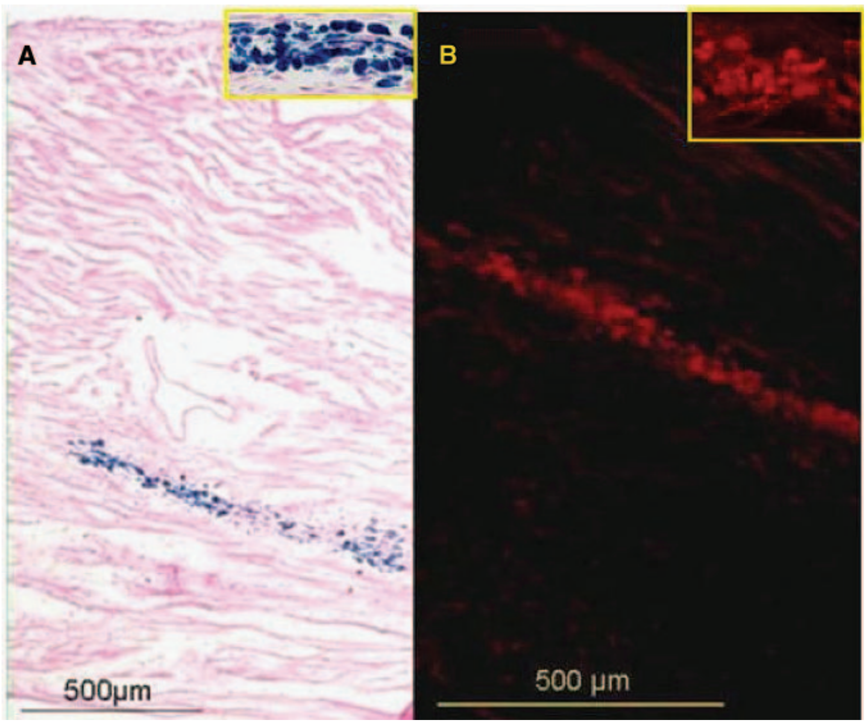

Figure 6. Representative histology of a rat heart harvested 21 days after xenogeneic cell injection. A, Prussian Blue staining demonstrating a large number of iron-containing cells. B, CD-68 immunostaining showing a large number of positive cells (macrophages) with a pattern similar to that seen with Prussian Blue–positive cells.

fied as macrophages (the Results section and Figure IIIA and IIIB of the Data Supplement).

Discussion

The major finding of this study is that MRI of ferumoxide-labeled stem cells is not a reliable technique for quantifying engraftment in the heart because of the considerable residual signals generated by the persistence of iron-laden tissue macrophages after labeled cell death.

The validity of information concerning stem cell survival and engraftment derived from the use of ferumoxides should be established in each specific application of cell transplantation by demonstrating that any signal detected is generated exclusively by viable, labeled cells. To clarify this issue, we selected a xenogeneic model of human CDCs transplanted into an immunocompetent rat. In a previous study, no xenogeneic cells (human bone marrow–derived MSCs) could be detected by pathology (assessed by in situ hybridization) in immunocompetent recipients (Sprague-Dawley rats) 5 to 7 days after transplantation, even though relatively immunoprivileged MSCs were used.[20] CDCs, on the contrary, express MHC class I and II molecules and were shown to activate allogeneic T cells in vitro. Therefore, these cells would be rejected by an immunocompetent host after allogeneic or xenogeneic transplantation.

Not surprisingly, there were no surviving human cells in rat myocardium at 7 and 21 days after injection in histological sections despite the use of a robust genetic labeling technique and 2 independent methods for detecting transgene expression. Significantly, in all animals, strong MRI signal voids persisted at 21 days after transplantation and even at 35 days in 1 animal that was followed up for this period. The contrast-to-noise ratio was similar at 2 days (when viable cells were present) and at 21 days (when all cells were dead and iron was contained in macrophages), confirming that MRI signal characteristics are unable to distinguish between iron in stem cells and that present in macrophages. Interestingly, the area occupied by the iron-containing cells decreased over 3 weeks, indicating that the iron particles were being cleared, albeit with a significant time delay in relation to cell death. Finally, immunohistochemical analysis at 21 days after cell injection identified the iron-containing cells as tissue macrophages, which were participating in the clearance of the cellular debris after death of the xenogeneic cells. The uptake of ferumoxides by these infiltrating cells led to the persistence of iron at the injection site and generation of signals on MRI.

Because in previous studies this method was used to label MSCs transplanted into infarcted myocardium,[11] we conducted similar experiments using infarcted animals and hMSCs. Despite the fact that MSCs are relatively immunoprivileged, no hMSCs survived at 3 weeks after transplantation, a result that is in accordance with previous studies.[20] However, a significant MRI signal void area persisted at this time point. These findings are not surprising because macrophages are known to readily uptake free ferumoxides without any need for transfection agents. However, clearance of these particles from the myocardium is particularly slow. Within this context, we observed the persistence of ferumoxides in the myocardium for at least 3 weeks after intramyocardial injection of pure ferumoxides (50 μL of diluted Feridex at a concentration of 0.4 mg iron per 1 mL).

In the syngeneic model, the potential for misinterpretation of the MRI results is more significant. In this setting, a certain proportion of cells is expected to survive; therefore, any signal detected may be intuitively perceived as representing genuine engraftment unless a second labeling technique is used to identify the injected cells. In the present study,

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genetic labeling with β-galactosidase revealed the presence of very few surviving CDCs at 3 weeks. This finding is not unexpected because other groups also have reported very low engraftment rates 21 days after stem cell transplantation.[21–23] Significantly, iron-derived MRI signals (size and contrast-to-noise ratio) were similar at 2 and 21 days after transplantation, falsely suggesting high engraftment rates. In contrast, histology revealed that most of the iron at the injection sites was inside macrophages with very few surviving ferumoxide-containing CDCs. We hypothesize that apoptosis, which provokes minimal inflammation,[24] is probably an important cause of cell death late after transplantation in the syngeneic setting. Slower clearance, resulting from less inflammation combined with signal from surviving labeled cells, could result in the larger signal observed in the syngeneic setting. In contrast, in the xenogeneic setting, early cell rejection and the ensuing cell necrosis are highly proinflammatory, probably resulting in faster clearance of tissue iron and a resultant smaller signal void at 21 days after transplantation.

Interestingly, to the best of our knowledge, only 2 studies in the literature directly address the issue of false-positive MRI signals in cell transplantation, and both investigated pancreatic islet transplantation.[25,26] These studies reported discrepant results as far as the nature of the cells containing the ferumoxides is concerned: mostly endocrine cells but also some macrophages in the 1 study[25] and exclusively macrophages in the other.[26] However, they both demonstrated rapid loss of the iron-related MR signals after islet rejection. A possible explanation is the difference in the iron-handling properties of the recipient organs, ie, the heart in our study and the liver in the pancreatic islet transplantation studies. The liver contains abundant Kupffer cells, which are proficient in iron handling and probably recycle the iron released from the ferumoxides rapidly.

Several studies attempted to assess engraftment after intramyocardial injection of iron-labeled stem cells.[11,12,27] Two of these acknowledged the possibility of false-positive signals generated by iron particles persisting in the myocardium despite injected stem cell death but did not specifically address this issue.[11,12] In the studies of allogeneic MSC transplantation, the MRI signal was assumed to originate from surviving iron-labeled stem cells, although the contribution of iron-containing macrophages or extracellular iron was not investigated.

Interestingly, in a previously published study, the propensity of macrophages to uptake iron oxide nanoparticles during the clearance of dead cells after cardiac transplantation was suggested as a method for noninvasively monitoring rejection after heart transplantation.[28] In this report, iron oxides were administered intravenously and accumulated in the myocardium (generating signals detectable by MRI) as a result of their uptake by the infiltrating macrophages participating in the rejection process. This finding confirms the inherent limitation of using ferumoxides as reporters of stem cell engraftment in the myocardium.

Study Limitations

An important determinant of the size of the MRI signal void is the dose of ferumoxides used to label cells. We selected a dose previously shown to be safe and effective for labeling rapidly growing adherent cells.[7,17] If lower doses of ferumoxides (or a smaller number of cells or only a fraction of labeled cells among the injected cell preparation) had been used, clearance of the iron nanoparticles by macrophages could have been faster, and the time course of the decrease in the size of the signal void might have been shorter. In our hMSC subgroup, we injected 50% fewer cells in 2 animals and found that although the size of the signal void was indeed smaller, there was still identifiable signal at 3 weeks after injection despite transplanted cell death. Therefore, our main conclusion that MRI is unable to distinguish live from dead stem cells and hence unable to quantify engraftment remains valid.

In the present study, we investigated cell numbers and iron amounts that would be meaningful for application in future clinical studies. A smaller amount of ferumoxides in the labeling mixture would have compromised immediate labeling efficiency and long-term effectiveness of the technique (because of rapid dilution of the label in the proliferating cells), as shown by our in vitro experiments. Furthermore, because we investigated ferumoxide labeling as a method to quantify engraftment, labeling only a fraction of the injected cells was not an option but could be useful for identifying cell injection sites.

Conclusions

Despite the numerous advantages of MRI and ferumoxide cell labeling, the persistence of ferumoxides in the myocardium, resulting from reuptake by tissue macrophages, for a significant time after unequivocal ferumoxide-labeled stem cell death undermines the value of ferumoxides as reporters of long-term stem cell viability and engraftment in the heart. This method appears to be useful for tracking the anatomic location of the cell injections after direct intramyocardial stem cell transplantation but does not provide reliable information on long-term cell viability.

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Disclosures

Dr Schär is employee of Philips Healthcare, Cleveland, Ohio. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Iron labeling of stem cells has been touted as a reliable method to assess engraftment and migration after cell transplantation by magnetic resonance imaging (MRI). Cardiac-derived stem cells or mesenchymal stem cells labeled with iron oxide were injected intramyocardially into rats to investigate the relationship between iron-dependent MRI signals and cell survival. Comparing in vivo images with histological results in the same hearts, we found that intense MRI signals, generated by iron in tissue macrophages, persisted for 3 to 5 weeks after rapid loss of viable transplanted cells, as in the case of xenogenic transplantation. The iron-derived MRI signals were similar whether they arose from macrophages or viable stem cells. Importantly, the results were not cell (cardiac-derived or mesenchymal stem cells) or substrate (normal versus infarcted myocardium) specific. Iron oxide labeling and MRI may be appropriate for localization of cell injection sites, but these methods are not reliable for in vivo tracking of viable cells in the heart.

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