Tracking the Fate of Iron-Labeled Cells: Differentiation between Live and Dead Cells

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Introduction:

The use of iron oxides to label cells for their detection by MRI is quite widely used today in MR research. In experimental cellular MRI, cells are typically loaded with magnetic particles prior to their injection or implantation. The presence of a magnetic label in cells causes a distortion in the magnetic field that appears as signal loss (negative contrast) in MR images. The use of these agents for cell-specific imaging has now been demonstrated in a number of different disease models. Even single cells can be visualized in vivo by MRI.

A question that is often posed about the areas of signal loss is whether they represent living cells; or whether it is possible to determine if cells have died, based on changes in the appearance of the areas of signal loss. For the most part we, the MR community, have assumed that if an iron-labeled cell dies it will be 'cleared' by the immune system and the area of signal loss will disappear. This assumption is the basis for investigations of the fate and/or survival of transplanted cells, for example.

In our lab we have used iron based cell labels to study many different preclinical models over the past several years. Here we present data from these experiments which suggests that whether or not the signal loss remains over time, when cells die, depends on the host tissue.

Methods:

The following preclinical mouse models have been studied: (A) transplantation (Tx) of 200 iron labeled islets into mouse liver (B) transplantation of 500 iron labeled islets under the mouse kidney capsule, (C) injection of free Feridex+PLL into the liver and under kidney capsule in the same mice, (D) adoptive transfer of iron-labeled dendritic cells (DC) into syngeneic (C57Bl6) mice by sc injection into the hind-footpad (or flank) and (E) imaging of fixed and apoptotic, iron-labeled cancer cells, in mouse brain.

Imaging was performed at 1.5T (B&D) or 3T (A, C, E) on a GE MR scanner using a custom-built gradient coil and custom-built solenoid RF coils. Images were acquired in vivo using a 3-D fully refocused (steady-state free precession) gradient-echo sequence with of 100 or 200 micron isotropic spatial resolution.

Results:

Transplanted islets were detected as hypointense regions dispersed within the liver tissue (Fig. 1A) and along the kidney capsule (Fig.1B). The regions of signal loss decreased over time in the liver (after 2 weeks) but persisted without change at the kidney capsule (for up to 4 weeks). Fig 1C shows an example of a mouse that received injections of free Feridex+PLL complex into the portal vein and under the kidney capsule. After 2 weeks, signal loss in the liver is gone, signal loss along the capsule of the kidney persists.

We were able to track the migration of DC from the injection site to the draining lymph nodes. At 2 days post footpad injection there is obvious signal void in the popliteal node and node volume has increased with cellular infiltration, at 9 days the appearance is similar (not shown) at 14 days signal loss is gone and the node volume is further increased with T cell proliferation (Fig 2). The signal loss due to iron-labeled DC at the flank injection site persists for up to 2 weeks. Apoptotic iron-labeled cells in the normal mouse brain are cleared with time. Formalin-fixed iron labeled cells in the mouse brain are not and signal loss persists for up to one month (Fig 3).

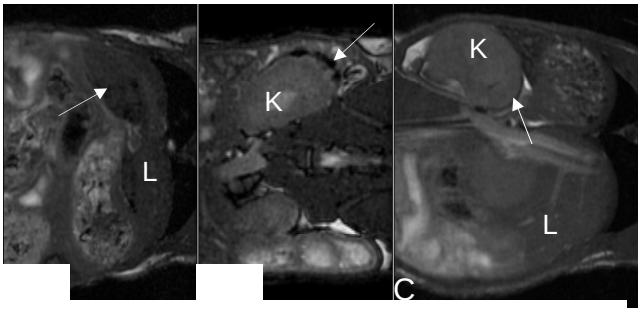

Figure 1: MR image of iron-labeled pancreatic islets transplanted into liver (**A**) and under kidney capsule (**B**) scanned 14 days after Tx. (**C**) Mouse with Feridex+PLL injected into portal vein and under kidney capsule.

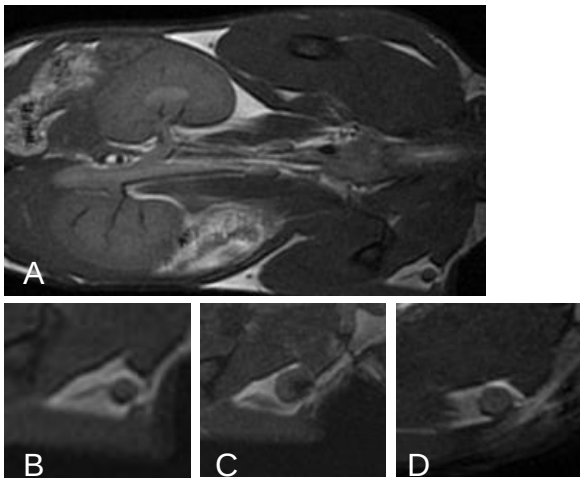

Figure 2. 3DFIESTA image of mouse body highlighting popliteal lymph node (A). Node at prescan (B), 2 days (C) and 2 weeks (D) after the injection of iron-labeled DC into footpad. Signal loss is present in node at day 2 and not at 2 weeks.

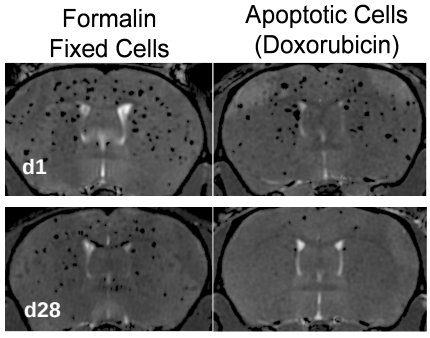

Figure 3. Mouse brain images after injection of fixed or apoptotic iron-labeled cells, at day 1 and day 28 post injection.

Discussion

The death of iron-labeled cells, and the subsequent loss of label, can be monitored by MRI. In the lymph node and in the liver when transplanted iron-labeled cells die the signal loss goes away. Free magnetic nanoparticles are similarly cleared in these tissues. This is likely because these tissues have a large number of resident immune cells. In normal tissues without a rich supply of active immune cells, (ie. here the kidney and brain), regions of signal loss attributed to the presence of dead, iron-labeled cells or free magnetic nanoparticles persist for a much longer time. This is important to consider for monitoring transplantation with MRI. Studying the rejection of islets transplanted under the kidney capsule, for example, is not be an ideal model system since the death of islets would not result in the disappearance of the area of signal loss.