Longitudinal Tracking of Recipient Macrophages in Chronic Cardiac Rejection with Non-invasive In Vivo MRI Using Micrometer-sized Paramagnetic Iron Oxide (MPIO) Particles

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INTRODUCTION:

Chronic rejection (CR) remains the major hindrance to long-term allograft survival after heart transplantation. Although the participation of cell-mediated alloimmunity in CR is well documented, the identity of the immune cells responsible for CR is uncertain. Studies, however, indicate the involvement of macrophages. For example, the absence of macrophages can inhibit CR, while early macrophage infiltration correlates with a poor prognosis after transplantation (1, 2). Thus, an increase in long-term graft survival will require better understanding of the mechanistic relationship between recipient macrophages and CR.

The purpose of this study was to utilize *in vivo* cellular MRI to longitudinally track recipient immune cells, primarily macrophages, in a heart graft experiencing CR. We employ a rat heterotopic transplantation model for chronic cardiac allograft rejection by transplanting the heart and lung from a PVG.1U ($RT1.A^uB^uD^uC^u$) rat into the abdomen of a PVG.R8 ($RT1.A^aB^uD^uC^u$) rat. In this model, without any immunologic manipulation of the recipient, chronic cardiac rejection is evident on post-operation day (POD) 20 and is extensive by POD 100 (3). *In vivo* cellular labeling of recipient immune cells is achieved by injecting micrometer-sized paramagnetic iron oxide (MPIO) particles one day prior to transplantation. The accumulation of labeled immune cells in the rejecting graft can then be followed by T2*-weighted MRI.

METHODS:

The abdominal heterotopic working heart model employed in this study is similar to our rat model of acute cardiac rejection (4) with the exception that the transplantation is between PVG.1U and PVG.R8 strains. Syngeneic transplantation between the same strains experience no rejection and serves as the experimental control. The transplanted hearts have cardiac parameters similar to that of the native heart. Recipient immune cells are labeled by intravenous injection of 0.9 μm MPIO (Bangs Laboratories, Fishers IN) at a dose of 4.5 mg Fe per Kg body weight one day prior to transplantation. EKG-respiratory gated T2* MRI was performed with a 4.7 Tesla/40 cm Bruker Avance AV scanner. Longitudinal *in vivo* MRI was performed for up to 16 weeks to follow the accumulation of labeled immune cells in the rejecting grafts. Following the *in vivo* studies, grafts were harvested for MR microscopy and histology. MR microscopy was performed using a Bruker 11.7 Tesla/89 mm system and 3D images were collected with an isotropic resolution of 40 μm. Sections of the heart grafts were stained with hematoxylin-eosin (H&E), anti-rat ED1 (for macrophages) and anti-rat $RT1.A^{a,b,l}$ (for PVG.R8) antibodies.

RESULTS AND DISCUSSION:

Following intravenous injection of the MPIO particles into the host one day before transplantation, trafficking of recipient immune cells to the rejecting graft was monitored by *in vivo* MRI every week for four weeks, then monthly for the next three months. One week after transplantation, the heart grafts exhibit a few, but very distinct, punctate circular spots of hypointensity (Figure 1A). More areas of hypointensity appear in the grafts at subsequent time points (figures 1B and 1C). As reported in our study of acute rejection in rats, each spot of hypointensity is believed to be a single macrophage infiltrated to the graft (4). These spots can be clearly observed by MRM (Figure 1D). This accumulation of labeled immune cells in the CR model can be followed for period of more than 10 weeks after a single MPIO injection. The observation that immune cells, mainly macrophages, are present in the early stages of chronic cardiac rejection was confirmed by histology (Figure 2A) and shown to be recipient immune cells by immunofluorescence staining (figures 2B and 2C). In conclusion, our approach of tracking immune cells non-invasively by *in vivo* MRI may have great potential to further our understanding of the cellular mechanisms involved in chronic rejection. Moreover, this study demonstrates the feasibility of non-invasively observing individual targeted cells over long time periods by *in vivo* MRI.

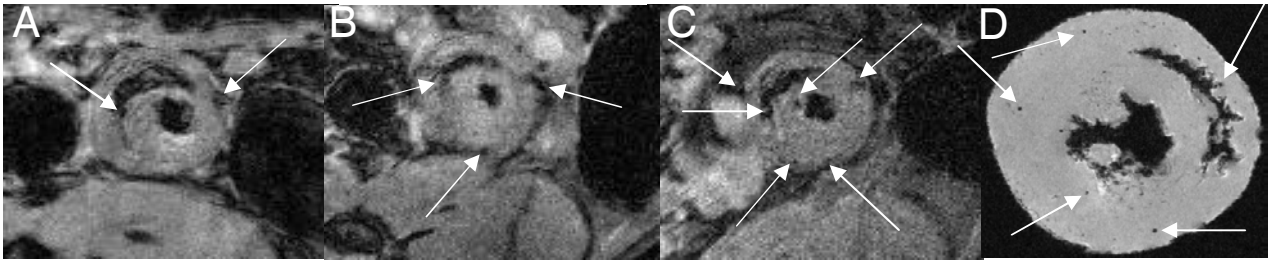

Figure 1 T2*-weighted *in vivo* MRI for an allograft on POD 7 (A), POD 14 (B) and POD 20 (C) after MPIO labeling. Few but very sparse punctate and distinct contrast spots can be seen and confirmed by MRM (D), as indicated with arrowheads, which represent MPIO labeled immune cells, mainly macrophages.

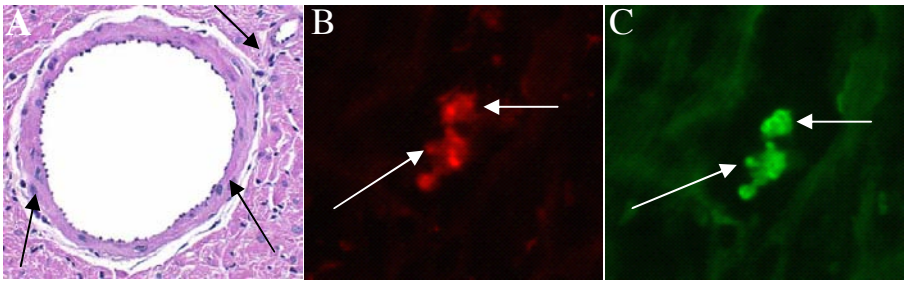

Figure 2 H&E staining of an allograft heart, POD 20 (A), shows arterial intimal thickening and perivasular inflammation (arrows). Double fluorescence staining with anti-rat ED1 (B, red, for macrophages) and anti-rat $RT1.A^{a,b,l}$ (C, green, for rat PVG.R8) on frozen tissue section from an allograft on POD 20. The images shown in B and C were taken over the same field of view indicated that recipient immune cells, mainly macrophages, are present at very early stage of chronic cardiac rejection (X400 magnification).

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