Differential Play of Macrophages and T-lymphocytes in Acute Allograft Cardiac Rejection: in vivo Cellular MRI Detection with a New Iron-oxide Particle

Yijen Lin Wu[1], Li Liu[1], Qing Ye[1], Brent D Barbe[1], T. Kevin Hitchens[1], and Chien Ho[1,2]

[1]*Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States,* [2]*Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, United States*

INTRODUCTION

Cellular MRI with iron-oxide based contrast agents offers the potential to track many cell types *in vivo*.at high resolution. T-lymphocytes are key players in cell-mediated immunity and the ability to monitor T-cells *in vivo* is useful in developing therapeutic strategies and understanding biological processes, including organ rejection. However, T-cells generally exhibit low iron-oxide particle incorporation by simple co-incubation, which makes tracking them with MRI *in vivo* challenging. To overcome this low incorporation electroporation, transfection agents, or conjugating with HIV-tat peptides can be employed to increase cell loading,. However, the effects of these manipulations on the processes of interest or T-cells themselves are not well studied. It is advantageous if various classes of T-lymphocytes can be labeled with simple co-incubation with no need for external aids. We have developed a new class of iron-oxide nano-particles, ITRI-IOPC-NH_2 particles with increased positive charge and terminal amino groups, which can label T-cells by simple co-incubation. We explored the feasibility of tracking T-cells in a rodent heterotopic heart and lung transplantation model, and compared to effects with macrophages, another gateway cells for immunity.

METHODS

Iron-oxide nano-particles and cell labeling: Cationic ITRI-IOPC-NH_2 particles were synthesized from IOPC particles, coated with polyethylene glycol (PEG), and contain a terminal carboxyl group on the surface, which allows versatile chemical manipulation (1). Various fluorescent probes, including FITC, DyLight 649, Cy3, Cy5, can be incorporated with the particles. T-lymphocytes or macrophages are isolated from spleens and are labeled in culture by co-incubation with IOPC-NH_2 particles.

Animal model: A rodent heterotopic heart and lung transplantation mode in abdomen is implemented for this study. The natural configuration of pulmonary and coronary circulation is preserved; the graft heart receives sufficient volume and pressure loading and exhibits wall motion close to native hearts. The acute allograft rejection progresses as time goes on. The allograft hearts and lungs exhibited moderate to severe acute allograft rejection over time from post-operational day (POD) 4 to 7.

MRI: IOPC-NH_2-labeled T-cells or macrophages are monitored with T_2^* MRI both *in vivo* and *ex vivo*.

RESULTS

After single administration of ITRI-IOPC-NH_2-labeled T-cells (Fig. 1 A-F) or IOPC-NH_2-labeled macrophages (Fig.1 G-L) on POD 4, patches hypointensity can be seen in the transplanted allograft hearts and lungs with T_2^* MRI on POD 5 (Fig. 1, A, B, D, E, G, H, J, K) and POD 6 (Fig. 1 C, F, I, L). The areas with hypointensity are immune cell infiltration foci due to rejection. In this transplant model, allograft heart and lung developed moderate acute rejection on POD 5 to 6, and more severe rejection on POD 6 to 7. Interestingly, as acute rejection progresses over time, the signal intensity continues to decrease with macrophage administration (Fig. 2 B), but the signal intensity recovers on POD 6 with T-cell administration, despite of increasing in rejection severity. The same outcome is also observed if the labeled immune cells are administered one day earlier on POD 3. This preliminary observation indicates that macrophages and T-cells might play different roles with different timing during the rejection processes. *Ex vivo* MRI and pathology shows that hypointensity detected are ITRI-IOPC-NH_2-containing CD3-positive T-cells or ED1-positive macrophages.

CONCLUSION

Our preliminary results show that the IOPC-NH_2 particles can label T-cells by co-incubation without additional manipulation, and the IOPC-NH_2-labeled T-cells can be detected in rejecting allograft transplanted hearts and lungs. Moreover, the IOPC-NH_2-labeled T-cells and macrophages show different temporal and spatial distribution as the rejection progresses. This indicates that the T-cells and macrophages play different roles in rejection, and our IOPC-NH_2 particles conjugated with various fluorescent probes can be valuable in mechanistic investigation.

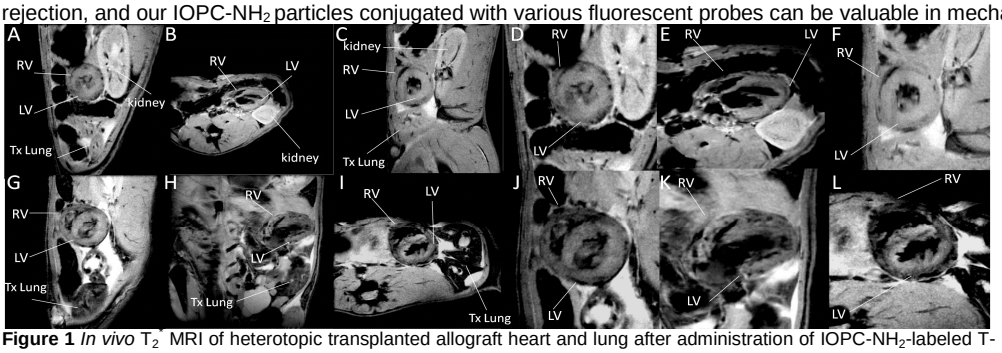

Figure 1 *In vivo* T_2^* MRI of heterotopic transplanted allograft heart and lung after administration of IOPC-NH_2-labeled T-cells (top panels, A-F) or IOPC-NH_2-labeled macrophage (lower panels, G-L) on POD 4. (A, D, G, J) short-axis view on POD 5; (B, E, H, K) long-axis view on POD 5; (C, F, I, L) short-axis view on POD 6. The right panels (D-F; J-L) are enlarged partial filed-of-view from the same images on the left (A-C, G-I). LV: left ventricle of transplanted heart; RV: right ventricle of transplanted heart; TxLung: transplanted lung. The *In vivo* T_2^* MRI is acquired with 156-micrometer in-plane resolution at 7-Tesla.

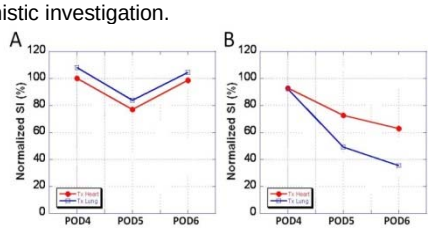

Figure 2 Temporal signal progression of allograft heart (red filled circles) and lung (blue open squares) after NH_2-labeled T-cells (A) IOPC-NH_2-labeled macrophage (B) administration on POD 4. Signal intensity is taken from *In vivo* T_2^* MRI and normalized to muscle in the same imaging planes. POD: post-operation day

REFERANCE:

Liu L, Ye Q, Wu Y, Hsieh WY, Chen CL, Shen HH, Wang SJ, Zhang H, Hitchens TK, Ho C. "Tracking T-cells in vivo with a new nano-sized MRI contrast agent." (2012) *Nanomedicine,* 8(8):1345-54