Non-invasive Direct in-vivo Imaging of Single Immune Cells in Acute Allograft Rejection after Heterotopic Heart and Lung Transplantation in Rats

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INTRODUCTION:

The current gold standard for detecting organ rejection, biopsy, is not only invasive but also prone to sampling errors. We explore the possibility of using micrometer-sized iron oxide particle, Bangs particles, to non-invasively track individual immune cells, mainly macrophages, at the rejection site after heart and lung transplantation. Bangs particles, 0.9 or 1.6 μm in size, are styrene/divinyl benzene polymer microspheres that contain magnetite core as well as a fluorescent dye.

METHODS:

1. Animal model: We use an abdominal heterotopic working heart and lung transplantation model in rats (DA to BN pair).
2. MRI methods: EKG and respiration gated T_2^*-weighted cine imaging on Bruker 4.7T ADVANCE system is used for in-vivo imaging with the in-plane resolution of 156 μm.
3. Iron oxide particle labeling: (1) ***In-vivo*** labeling: immune cells, mostly macrophages, are labeled with *in-vivo* labeling, by direct intravenous injection 1 day prior to MRI scans (2) ***Ex-vivo*** labeling: isolated macrophages are labeled with Bangs particles, and then the labeled cells are infused into the animal.

RESULTS:

Macrophages are labeled with Bangs particles by either *in-vivo* or *ex-vivo* methods. In the *ex-vivo* labeling method, macrophages are isolated and labeled in culture, then the labeled cells are infused to the animal. Alternatively, with the *in-vivo* labeling method, Bangs particles were introduced to the animal by direct intravenous infusion, and macrophages then uptake Bangs particles inside the animal.

Distinct hypointensity can be observed 10 hours after infusion of *ex-vivo* labeled macrophages in the transplanted allograft heart and lung (Fig.1). Areas with concentrated macrophage accumulation can be readily seen with intense signal reduction. Interestingly, in addition to the regions with concentrated hypointensity, there are discrete circular dark "spots" seen in the allograft heart and lung, particularly near RV and septal wall.

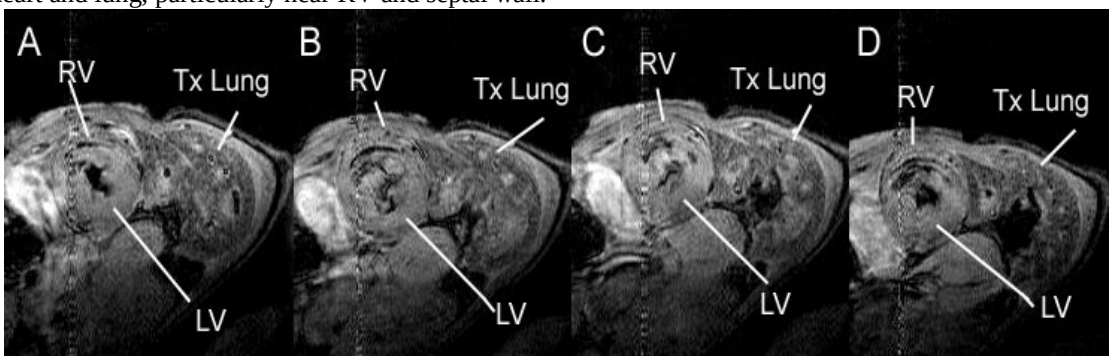

Figure1 Visualizing macrophages at the rejected heart and lung with *ex-vivo* labeling. Macrophages are labeled with Bangs (1.6μm) particles in culture, then are infused through vein to an allograft at post-operational day (POD) 6. T_2^* images shown are taken 10 hours after cell infusion. A-D are consecutive imaging slices.

With *in-vivo* labeling, similar distinct punctuate circular high contrast spots can be seen in the transplanted allograft heart (Fig. 2 A & B) and lung (Fig. 2 C), one day after particle administration. This dotted pattern can be seen as early as 2 hours after Bangs particle administration. Each "dot" of the high contrast could possibly represent a single cell, or even a single particle, for the background gradient generated by iron oxide particles can propagate as large as 50 times of the radius. The punctate pattern with Bangs particle labeling is not due to random interstitial deposition of iron oxide particles, but uptake of the particles into cells. Electron micrograph reveals the uptake iron is within membrane bound vesicles.

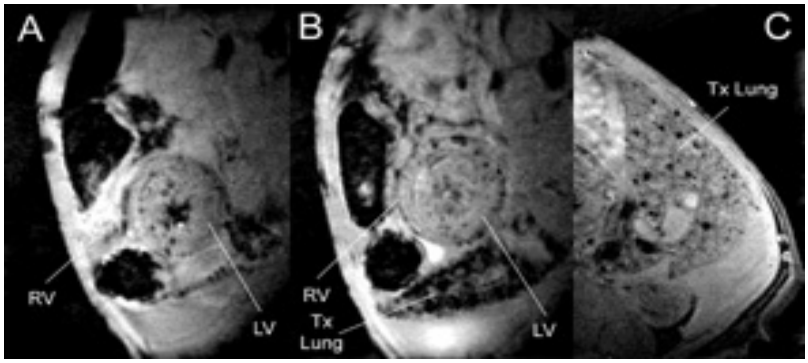

Figure 2 Visualizing macrophages at the rejected heart and lung with *in-vivo* labeling. Bangs (0.9μm) particles are administered through direct intravenous injection at POD 5, and the T_2^* images shown are taken 1 day after particle infusion at POD6.

CONCLUSION:

Our preliminary results indicate that a more sensitive contrast agent, such as Bangs particles, provides potential for monitoring single cells in vivo. With better cell labeling strategies, much better methodologies can be developed that are not only important and useful for detecting organ rejection, but also for many other biological processes.