Characterization of MPIO labeled primary murine bone marrow derived macrophages

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INTRODUCTION:

Early detection of the immune response is essential for identification and early treatment of diseases such as HIV, cancer, and stroke. However, the early immune response in many of these diseases has not been well-characterized. Macrophages are key players in the innate immune response and important markers of local inflammation.

Cellular MRI combines high resolution in vivo MRI with contrast agents for labeling cells. The use of micron sized iron-oxide particles (MPIOs) for cell-specific imaging by MRI has become increasingly popular for its increased iron content per labeled cell over smaller particles, and has been demonstrated in a number of different in disease models. Being phagocytotic, macrophages are highly suitable for use with MPIOs for single cell detection by MRI. Here, we evaluated the effects of MPIO labeling on macrophage functions: cytokine secretion, maintained phagocytosis, and cell migration.

MATERIALS AND METHODS:

Bone marrow cells harvested from mice were grown in culture dishes in the presence of macrophage colony-stimulating factor for 6 days. Under these conditions, the bone marrow monocyte/macrophage progenitors will proliferate and differentiate into a homogenous population of mature primary bone marrow-derived macrophages (BMM). The cell phenotype was assessed using flow cytometry analysis of Mac-1 surface antigen expression. BMMs were then labeled with MPIOs through simple incubation for 1 hour. MPIOs used are 1.63 µm diameter, polystyrene/divinyl benzene coated, and are commercially available from Bangs Laboratories. After labeling, functional assays were performed and compared to that of unlabeled BMMs. To test for cytokine secretion, BMMs were stimulated with lipopolysaccharide (LPS) for 3 hours. TNF- α and IL-12 ELISAs were performed on the supernatant. Labeled BMMs were also tested for the ability to continue phagocytosis. Green fluorescent particles were incubated with macrophages for 4 hours followed by incubation with red fluorescent particles for an additional 4 hours. Transwell migration assay was performed to evaluate migration ability. BMMs were seeded on the upper compartments of 24-well transwell plates (Costar, 5 µm pore size), and assay media with monocyte chemotactic protein-1 (MCP-1) was added to the lower compartments. After 5 hours incubation, top (non-migrated) cells were removed, and bottom (migrated) cells were stained with trypan blue and enumerated under a light microscope.

RESULTS:

BMMs were labeled with green fluorescent 1.63 µm MPIOs by simple incubation in less than 1 hour (Figure 1). TNF-α and IL-12 ELISAs performed on the supernatant of both labeled and unlabeled BMMs, without the addition of LPS stimulant, did not detect the presence of TNF- α and IL-12 (Figure 2, left). Stimulation of BMMs with 2 µg/ml LPS caused secretion of TNF- α and IL-12 for both labeled and unlabeled BMMs. For labeled BMMs, there was less TNF- α, but more IL-12 detected in the ELISAs. Transwell migration assays using MCP-1 as the chemoattractant showed both labeled and unlabeled BMMs were capable of migrating across the 5 µm pore size membrane (Results not shown). Labeling with green fluorescent particles and then subsequent labeling with red fluorescent particles demonstrates the ability of labeled BMMs to continue to phagocytosis (Figure 2, right).

DISCUSSION:

Critical to the use of magnetic particles to enable MRI-based cell tracking of immune cells is that the simple act of labeling not activate cells, nor inhibit intentional activation. It was found that labeling with MPIOs did not, on its own, stimulate the cells to produce TNF-α and IL-12, two important inflammatory cytokines. Furthermore, MPIO labeling did not inhibit macrophages to secrete these cytokines upon activation with LPS. Another important consideration is to preserve phagocytotic ability when labeled with magnetic particles. Fluorescence microscopy (Figure 2, right) shows a BMM with two different colored particle types internalized, demonstrating the ability to continue phagocytosis after labeling. Lastly, labeled cells must also maintain the ability to migrate in identical fashion to unlabeled cells. The transwell migration assays showed migration from both unlabeled and labeled BMMs, suggesting no effect on migratory ability by MPIOs.

Immune cell tracking by MRI offers the potential for early detection of a variety of diseases. Here we demonstrate a robust method of labeling monocytes/macrophages in vitro without affecting their function. Coupled with methods for magnetic labeling of other immune cell types, such as lymphocytes, MRI based cell tracking has the potential for near term clinical utility. Therefore, it is advantageous to pursue strategies for efficient, in vivo labeling not only for macrophages, but for other immune cells as well such as lymphocytes and granulocytes. Questions such as "are the cells phagocytotic?" and "how long do they live?" must be addressed in order to tailor the labeling strategy to the cell type.

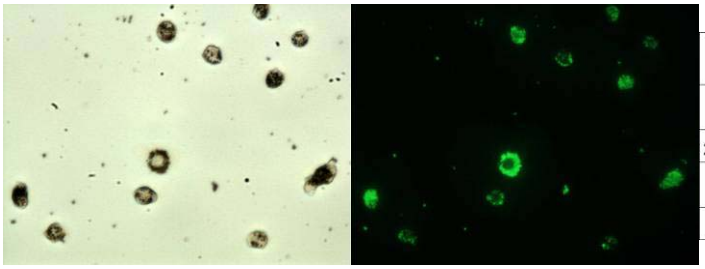

Figure 1: MPIO labeled macrophages. BMMs were cultured and labeled with 1.63 µm sized MPIOs by simple incubation for 1 hour. Optical (left) and fluorescent (right) microscopic image of BMMs labeled with MPIOs following release from the dish. Note that particles remain intracellular.

Samples	TNF-α (ng/ml)	IL-12 (ng/ml)	Transwell migration
2ug/ml LPS	2.061 +/- .034	0.1535 +/- 0.022	NA
2 ug/ml LPS + Bangs particles	1.566 +/- .136	0.275	NA
Bangs particles	-0.03 +/- 0.031	.045 +/- .048	√
Control	-.010 +/- .001	.023 +/- .012	√

Figure 2: Functional assays on BMMs. **Left)** Table showing cytokine release data for labeled and unlabeled cells, both with and without LPS treatment. Also shown are the results from transwell migration assays.
Right) Image of a BMM labeled with first green MPIOs for 1 hour and then red MPIOs for 1 hour, demonstrating continued phagocytosis. Three color fluorescence image showing blue – DAPI stained nucleus, green – first labeling MPIOs, red – second wave of MPIOs.