Antibody mediated cell labeling of peripheral T cells with micron sized iron oxide particles (MPIOs) allows single cell detection by MRI

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Introduction

MRI is a useful tool for tracking lymphocyte migration in intact animals. Indeed, the earliest cellular MRI experiments involved MRI-based lymphocyte tracking [1]. Currently, successful labeling of lymphocytes for MRI tracking makes use of TAT peptide functionalized USPIOs [2] or SPIO-transfection agent complexes [3], achieving labeling ranges from ~ 1-2 pg iron per cell. Receptor mediated endocytosis of SPIOs has been demonstrated for lymphocytes [4] as well as dendritic cells [5]. Recently, in vivo single cell detection by MRI has been demonstrated, however cells were labeled with ~ 100 pg iron per cell [6]. Micron sized iron oxide particles, MPIOs, efficiently pack 0.1 pg to 10 pg of iron in an individual particle, allowing single cells labeled with only one or a few particles to be detected by MRI [7]. While adherent cells can be labeled by direct overnight incubation with MPIOs [8], cells that grow in suspension have been difficult to label with MPIOs. Herein is described an antibody-mediated procedure for efficiently labeling peripheral T cells with MPIOs with enough iron oxide to allow detection of single lymphocytes by MRI.

Methods

Peripheral blood was harvested from adult Sprague-Dawley rats. Fresh, whole blood (5 x 10^6 T cells/ml) was incubated with various amounts of biotinylated rat anti-CD5 (Cedarlane Laboratories, Ontario, Canada) for one hour, both on ice and at room temperature, and with and without shaking. Antibody concentrations were 0X, .04X, .40X, 2X, 8X, 40X and 200X saturation of all CD5 receptors on each T cell, assuming 30,000 receptors/cell. After one hour, green fluorescent, 1.63 micron streptavidin coated MPIOs (1.0 pg iron/particle, Bangs Laboratories, Fishers, IN) were added to the same whole blood and incubated one hour, again both on ice and at room temperature, and with and without shaking. MPIO concentrations were 0, 1, 5, and 50 beads/cell. Lymphocytes were harvested from whole blood by standard Ficoll centrifugation, followed by red blood cell lysis. Flow cytometry and manual counting under both stereo and confocal fluorescence microscopy were used to determine percent labeling and particle number per cell. Viability was assessed by Trypan blue exclusion tests. Labeled lymphocytes were suspended in 1% agarose containing 1 mM Gd-DTPA and imaged using 3D gradient echo at 11.7 T using the following parameters: 100 ms TR, 10 ms TE, 100 microns isotropic, 30 minutes.

Results

Cell labeling increased with increasing antibody concentration, reaching a maximum at 2X saturation, for each MPIO concentration. Results from flow cytometry showed that at 2X saturation, % cell labeling was 16 ± 5%, 53 ± 7%, and 83 +/- 15% for 1, 5 and 50 MPIOs/cell added, respectively. Manual counting of % cell labeling was 18%, 42% and 76% for the same MPIO/cell ratios. Interestingly, there was wide heterogeneity in number of incorporated MPIOs per T cell. Flow cytometry revealed two distinct populations of labeled lymphocytes, both equal in cell size, but one much more granular than the other. The more granular cells had many more incorporated MPIOs (Range 16-56 beads/cell, Mean 35.0 +/- 11.4) than the less granular population (Range 1-5 beads/cell, Mean 2.5 +/- 1.3). These two cellular subsets were equally populated for the highest labeling conditions. Neither the temperature of the labeling reaction nor shaking the samples during labeling had any statistically significant effect on labeling efficiency. Viability was >95% for the highest labeled cell populations.

MRI showed multiple punctate, hypointense spots from the labeled cells. The heterogeneity of the sizes of the spots is reflective both of the heterogeneity in the number of particles incorporated on each cell, as well as partial volume effects from the large voxel sizes, with respect to the size of the cells. Signal attenuation from single cells at 100 microns isotropic was between 84 and 91% for the largest dark spots, with dimensions of the enhancement on the order of 300 x 300 microns.

Discussion

T cells have been implicated in a number of diseases, in maladies such as multiple sclerosis (MS), and therapeutically in immunotherapy of cancer. The ability to detect the presence of single T cells would, in the case of MS assist in an early detection of MS lesions, or in the case of immunotherapy allow an analysis of homing kinetics and efficiency of T cells to gliomas. Measuring these processes at the single cell level facilitates quantification of these parameters, as well as studying rare or potentially novel populations of cells. The large iron load delivered by MPIOs to T cells, as much as 25 times larger than that currently achievable with (U)SPIOs, is advantageous for detecting single cells using MRI parameters well suited for animal studies. Additionally, as a result of large iron loads, gradient echo times can be shortened, reducing spurious dark spots generated in areas of susceptibility differences. Lastly, antibody-mediated magnetic cell labeling with MPIOs should be particularly useful for labeling cells where low receptor copies are expressed.

References

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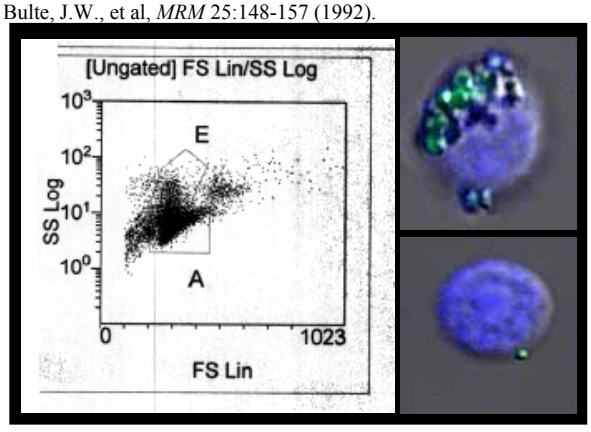

Figure 1: (A) FACS analysis of labeled cells. Gate A is the less granular T cells, gate E is the more granular T cells. (B) Representative labeled cell from gate E, MPIOs are green, nucleus is stained blue with DAPI. (C) Representative cell from gate A. Each T cell measures approximately 10 microns in diameter.

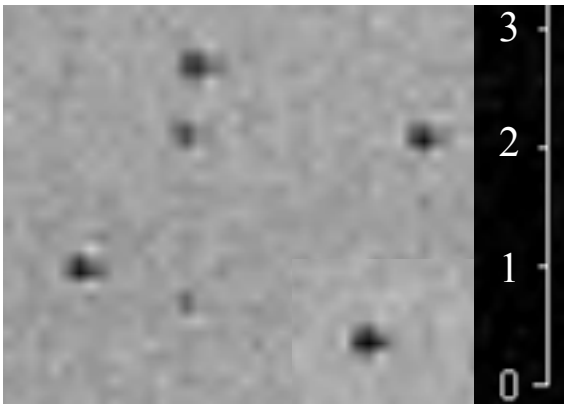

Figure 2: MRI of lymphocytes. Individual well labeled lymphocytes create a hypointense contrast region 300 microns across, even though they themselves are only 10 microns in diameter. Signal reduction in the center pixel was 84-91% for the largest dark spots. The scale bar is mm.