Does Prussian Blue Staining Correlate with Dextran Staining of Ferumoxides Labeled Cells?

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Introduction: Ferumoxides, as dextran coated superparamagnetic nanoparticles (SPION), complexed with protamine sulfate (FePro) are being used for magnetic labeling of cells to non-invasively monitor cell trafficking and tissue distribution by in vivo MRI (1). The MRI findings are usually correlated to histology by Prussian Blue (PB) stain for iron, or by the detection of dextran coating with anti-dextran antibodies and standard immunofluorescence or immunohistochemistry protocols. Since it is known that the dextran molecules may detach from SPION (2), the objective of this study was to investigate the reliability of dextran staining compared to PB staining of FePro labeled rapidly proliferating and non-dividing cells over time.

Methods: Primary human monocytes from volunteers were plated in RPMI media supplemented with 50ng/mL MCSF and cultured for 4 days to allow for differentiation into macrophages (PM). THP-1 cells, derived from acute monocytic leukemia, were grown to 90% confluence and differentiated into mature macrophages (MM) via treatment with 25ng/mL PMA for 36 hours. The PM, MM, and rapidly dividing LadMAC mouse macrophage cells were labeled with FePro using standard protocol (1). Cells were plated in triplicate and each experiment was repeated at least twice. Throughout the experiment, media was changed twice weekly, and cells were collected at days 1, 7, and 14. At each time point, viability of FePro labeled and unlabeled cells was measured by Trypan Blue and cells were analyzed by flow cytometry (FACS) using a FITC conjugated anti-dextran antibody, as well as by staining for iron with Pearl's staining technique. Quantitation of percentage of PB +cells on cytospins with average of >5 high powered fields was performed using Image J. Iron content of each sample was measured with T2 relaxometry (1)π.

Results: FePro labeling of all cell type resulted in 100 % labeling efficiency. Two weeks post-labeling only 20% of FePro labeled or unlabeled human PM or LadMAC cells remained viable while 80% of differentiated labeled or unlabeled THP-1 cells were alive. FACS revealed 51% of PM to be dextran positive at day 7, decreasing to 30% by day 14. However, the corresponding PB staining showed that 70% of cells at day 7 and 62% at day 14 were positive for iron (Figure 1a). Forty six percent of differentiated THP-1 cells were dextran-positive at day 7 after labeling and 18% after two weeks, compared with PB staining that showed 63% of cells as iron positive at day 7 and 32% at day 14 (Figure 1b). Furthermore, FACS analysis of FePro labeled and rapidly dividing LadMAC cells showed only 20% of cells to be dextran positive at day 7 and 3% on day 14 after cell labeling. Seventy six percent of FePro labeled LadMAC cells were PB positive on day 7 and 57% on day 14 post-labeling (Figure 1c). The iron content in all cell types correlated with PB staining (Figure 1d).

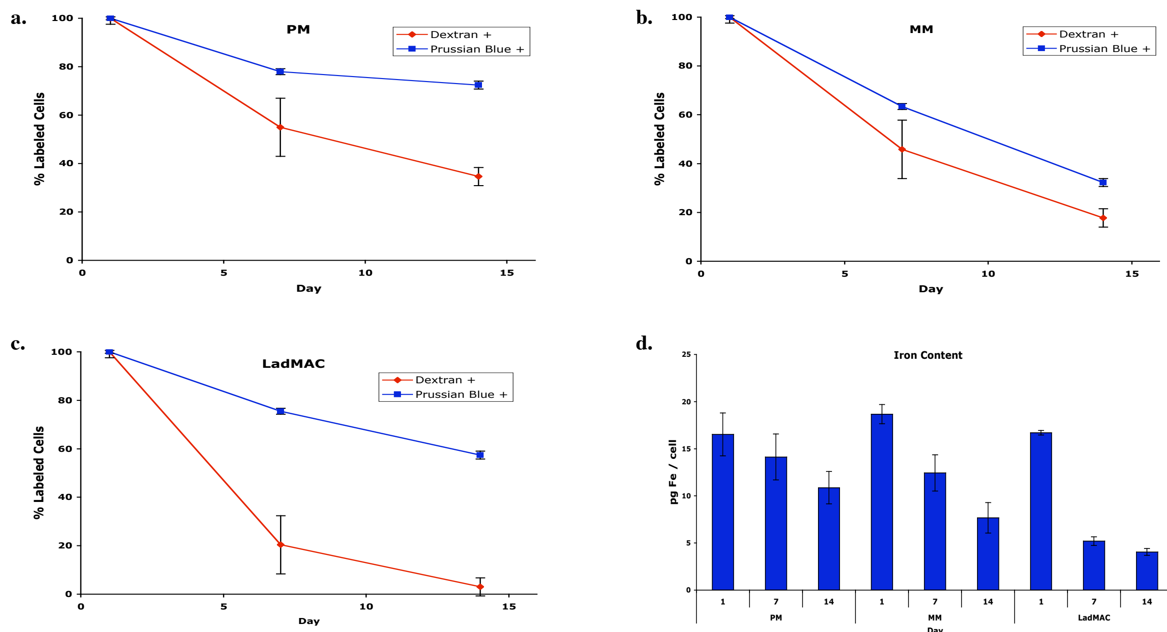

Figure 1. Graphs of dextran levels compared to iron staining by Prussian Blue in primary macrophages (a), in mature macrophages (b), and in LadMAC (c). Iron content (pg/cell) in all cell types over time (d).

Conclusions: In all three types of cells studied, dextran was detected at lower levels as compared to PB staining. The fastest decline in dextran detection occurred in the most rapidly proliferating cells, due to the dilution effect of the label. Decrease in PM and MM viability over 14 days is consistent with lifespan of cells in vivo. These results indicate that tracking cells one or two weeks post-labeling using dextran staining may not the most reliable and sensitive method of detecting SPION label.

References: 1. Arbab AS et al. Blood 2004; 104:1217. 2. Bourrinet P et al. Invest Radiol. Mar 2006; 41(3):313-324.