Long-Term MR Imaging of Immunocompetent and Immunodeficient Mice Reveals Distinct Differences in Contrast Clearance in the Brain

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Introduction: Cell-based therapy for neurodegenerative diseases is actively pursued in preclinical studies and has potential for clinical translation. Non-invasive MR imaging is the preferred imaging modality for monitoring the transplantation and migration of stem cells in patients. However, there are several issues complicating interpretation of long-term tracking of grafted cells. The most critical problem is the potential for persistence of the contrast agent following cell death. The focus of this study was to evaluate the persistence or clearance of the contrast agent following the death of transplanted, labeled cells. Superparamagnetic iron oxide nanoparticles (SPIO) labeled neural stem cells were transplanted into the brain of immunocompetent, allograft rejecting mice, in which the cells will die, and into immunodeficient, allograft accepting mice, in which the cells will proliferate. Transplanted, labeled cells were monitored by MRI over a period of 93 days.

Methods: Mouse neural stem cells (C17.2) were transduced with the lentiviral vector pLenti4-CMV-fLuc2 in order to express the bioluminescent (BL) reporter gene firefly luciferase. Cells were labeled with fluorescently tagged SPIO nanoparticles (Molday ION Rhodamine B, BioPAL) and stereotaxically injected ($3x10^5$/3µL) into the right corpus callosum of immunocompetent (Balb/C) and immunodeficient (Rag2) mice at ML +2.0 mm, DV -1.5 mm relative to Bregma. BL imaging was performed weekly from 0 to 92 days after cell implantation using a Xenogen IVIS 200 optical imaging device. *In vivo* MRI was performed using a Bruker 9.4T spectrometer at days 2, 11, 30, 50, 72, and 93 after cell implantation. A T2-weighted spin echo sequence (TE=12 ms, TR=2000 ms, AV=4, FOV=2.0 x 2.0 cm, Matrix=256 x 256) and a T2*-weighted multigradient echo sequence (TE=5 ms, TR=500 ms, AV=4, FOV=1.70 x 1.70 cm, Matrix=256 x 256) were used. Tissue samples were processed for histology and fluorescence microscopy to identify transplanted cells and characterize the SPIOs. The fluorescent SPIOs were detected directly by fluorescence microscopy as well as with Prussian blue staining for detection of iron oxide. The transplanted cells were detected using anti-ß-galactosidase antibody (Cappel). Immunohistochemistry with anti-Iba1 (Wako) for microglia, anti-CD45 (AbD Serotec) for leukocytes, and anti-GFAP (Dako) for astrocytes to further characterize the SPIOs in relation to endogenous cells.

Results: The day after transplantation, SPIO-labeled, luciferase-expressing C17.2 cells generated detectable BLI signal in all mice. In immunocompetent Balb/C mice, the BLI signal was undetectable at day 15 after transplantation in all but one mouse, indicating allograft rejection. The BLI signal was gradually increasing in immunodeficient Rag2 mice for about 35 days and then reached a plateau at the level of 600-fold that of the initial signal (Figure A). While MRI signal hypointensities decreased gradually over time in all mice, some degree of signal persisted over the time course of the experiment. Unexpectedly, signal clearance in Balb/C mice that rejected the cells was significantly reduced (* $p<0.05$, ** $p<0.01$) compared to signal clearance in Rag2 mice that accepted the cells (Figure B). Iron oxide nanoparticles were detected histologically by fluorescence microscopy (Figure C) and Prussian blue in both rejecting and non-rejecting mice. Transplanted ß-gal+ cells (Figure C, green) were only present in Rag2 mice that accepted the cells. In rejecting Balb/C mice, clusters of SPIOs were detected in microglia while CD-45 positive cells (Figure D, green) surrounded the site of injection.

Conclusions: Death of magnetically labeled, transplanted cells results in the release of iron oxide to the surrounding brain tissue. Released iron nanoparticles can be retained in the brain for an extended period of time. Clearance of MR hypointensities from SPIOs is faster for surviving, proliferating cells than for cells undergoing cell death. Death of transplanted cells and release of contrast agent to surrounding tissue is a significant limitation of long-term tracking.

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