In vivo MR tracking of magnetically labeled neural spheres transplanted in chronic EAE mice: relation between cell migration and inflammation

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Introduction

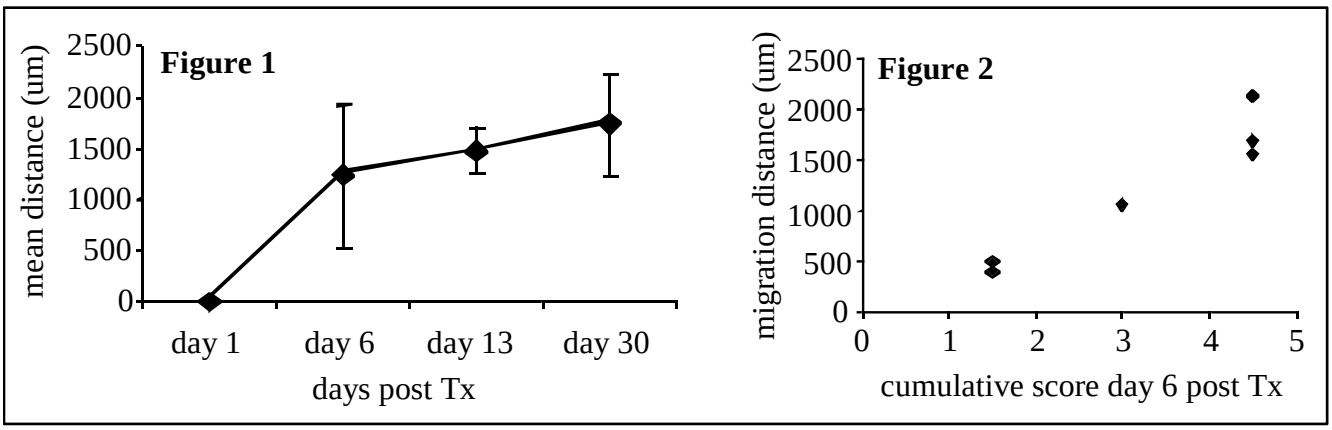
Central to the future success of cell transplantation in multiple sclerosis is the ability of transplanted cells to migrate from the site of transplantation to relevant foci of disease and to survive for prolonged periods of time, while exhibiting remyelinating and trophic properties. We have previously shown that the inflammatory process during EAE induces the targeted migration of transplanted rat neural precursor cells from the ventricular and subarachnoid spaces into the inflamed white matter tracts (1), and, using magnetically labeled cells, migration into white matter structures could be observed by *ex-vivo* MR microscopy (2). *In vivo* serial MR imaging, however, allows one to assess when and at what speed cells are migrating, and for how long they persist in the target organ. We report here on the transplantation of magnetically labeled mouse and human neural spheres in a mouse chronic EAE model, and correlated the distance of migation (as assessed by MR imaging) to the clinical disease status.

Materials and Methods

Mouse neural spheres were derived from the brains of newborn C57Bl/6 mice, and differentiation of GFP-transfected human ES cells into neural precursors was accomplished as previously described (3). Chronic EAE was induced in C57Bl/6 mice by immunization with MOG35-55 peptide, complete Freund's adjuvant, and Pertussis toxin. Immunized mice were observed daily and assigned a score for neurological symptoms according to a standard protocol. Cells were magnetically labeled with a mixture of 25µg/ml Feridex and 0.375µg/ml poly-L-lysine (PLL) (4). Transplantation was performed on day 6 following EAE induction. 1,500 mouse neural spheres (~100 cells per sphere) or 250 human ES-derived neural spheres (~3,000 cells/sphere) were transplanted into both lateral ventricles of 8 and 8 EAE mice, respectively (total n=16). In addition, as controls, 4 EAE mice received injections of PLL-Feridex-labeled mouse (n=2) and human (n=2) dead cells. MRI was performed *in vivo* using a 4.7 Tesla horizontal bore NMR system (Bruker), using a 3D RARE sequence with 50-78 µm resolution. Ex vivo imaging was performed at 9. 4 Tesla, using a 9.4 T GE Omega NMR spectrometer and 50 µm resolution. From the 3D MRI dataset, the distance of migration was calculated according to the number of MR slices counted from the most proximal site of hypointense MR signal at the ventricular edge to the most distant site of hypointense signal.

Results

MRI was performed at days 1-2, 6-7, 13-14 and 30 after transplantation, thus representing different stages of disease: the first MRI was obtained before onset of clinical disease, the second MRI was obtained very early in the course of disease, the third procedure was at peak of disease and the fourth was at the chronic stage of disease. *In vivo* MRI showed that at days 1-2 after transplantation, the grafts were located mainly in the lateral ventricles. As early as 6 days after transplantation, hypointense MRI signals were observed in white matter tracts. These were further observed on images taken at days 13-14 and 30 post transplantation. Migration was most commonly observed in the corpus callosum and internal capsule towards the cerebral peduncles and in the stria-medullaris and fornix. Measurement of the distance of transplanted cell migration in the brain, as measured from the MR images, indicated that most of the migration occurred within the first 2 weeks, during the acute phase of disease (Figure 1). Comparison of human and mouse cell migration revealed that the human cells migrated slower and to a shorter distance than the mouse cells in this system. The variance in clinical behavior and in MR-based measurements of distances of migration between mice that were transplanted with mouse neural spheres demonstrated a good correlation between disease severity and extent of migration (Figure 2).



Conclusion

The observation that the greatest degree of migration occurred very early in the course of disease highlights the narrow time window in which transplantation of remyelinating cells may be effective for obtaining clinical results. The results in our syngeneic model show, for the first time, that inflammatory signals associated with the clinical score modulate migration in a positive manner.

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References

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