Neural precursor migration following intracerebroventricular delivery during the chronic phase of experimental allergic encephalomyelitis is reduced as compared to the acute phase

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Introduction

In multiple sclerosis (MS), oligodendrocytes cannot sustain the repair of ongoing white matter damage caused by infiltrating autoreactive immune cells. To attenuate the disease burden in MS, therapies are necessary to preserve endogenous oligodendrocytes, improve migration of native neural stem cells towards demyelinated CNS lesions, or replace lost oligodendrocytes using exogenous cell sources. During the acute inflammatory phase of experimental allergic encephalomyelitis (EAE) intracerebroventricular (ICV)-transplanted neural precursor cells (NPCs) migrate in response to signals present within the inflamed CNS[1] and contribute to the attenuation of inflammatory markers and EAE symptoms[2]. However, little is known about the migratory disposition of neural stem cells following ICV transplantation in the diseased CNS. Also, it is not clear whether therapeutic benefits obtained following NPC transplantation in the acutely inflamed CNS can also be found upon NPC delivery within the chronically demyelinated tissue environment that is characteristic of CNS lesions during remission in MS. We have used serial MR imaging of Feridex- labeled NPCs to detect potential differences in the migratory response of ICV transplanted NPCs to the acute inflammatory and chronic demyelinated host environment. *In vivo* and ex vivo MR data of NPC biodistribution in EAE were confirmed by detecting markers for labeled NPCs and correlated with histological and immunohistochemical markers of inflammation and myelination.

Materials and Methods

Induction of EAE: Female C57Bl/6 mice were immunized with 200 µg of MOG_{35-55} peptide diluted in saline and emulsified with complete Freund's adjuvant containing 5 mg mycobacterium tuberculosis (H37RA). Pertussis toxin (300 µg i.p.; Sigma) diluted in 0.3 ml saline was injected immediately after delivery of MOG emulsion and 48 hrs later. Disease progression and severity was scored as follows: 0 = normal; 1 = flaccid tail; 2 = mild hind limb weakness; 3 = hind limb paralysis; 4 = marked weakness all limbs; 5 = moribund.

Cellular labeling: Feridex (25 µg Fe/ml) and 375 ng/ml poly-L-lysine hydrobromide were combined in 1 mL of culture medium and mixed gently for 1 hour at room temperature to create transfection complexes. Feridex complexes (1 mL) were added to medium containing neurospheres for 24 hours at 37°C. At the same time, BrdU (20 µM) was added to the culture medium for histological detection of transplanted cells in host tissue.

ICV transplantation of neurospheres**:** Feridex/BrdU labeled neurospheres were sterotaxically injected into the right lateral ventricle during either the acute phase of EAE (n = 10; day 14 post-induction) or the chronic phase of EAE (n = 10; day 28 post-induction). Control animals without disease (n=5) were included.

Serial *in vivo* MRI: The biodistribution of Feridex-labeled neurospheres within the CNS of control, acute EAE, and chronic EAE mice was monitored at 9.4T using T2-weighted 3D RARE on days 1, 3, and 7 after ICV transplantation. The head was centered within a 30 mm receiving coil and a region of the CNS spanning from the anterior commisure to the cerebellum was imaged using the parameters: FOV 1.9 X 1.9 X 0.8 cm, 148 X 148 X 250 µm, TE 11.4 msec, TR=1500 msec, RARE Factor = 2, AVG = 1.

Statistical difference mapping: *In vivo* 3D RARE image slices were aligned to a template using 20 manually placed landmarks for affine image transformation. The image intensity for each aligned subject was normalized to the template dataset based on intensity histogram and matching image slices from each animal within each experimental group (control, n =4; acute EAE, n = 10; and chronic EAE, n = 9) were summed to obtain average pixel intensity values. Average pixel values from acute EAE and chronic EAE were subtracted from control values in matching slices to create a difference map and the probability that a hypointense signal is detected was computed for each pixel.

Ex vivo MRI and iron oxide detection with DAB-enhanced Prussian Blue staining. One week following ICV transplantation mice were transcardially perfused with PBS followed by 4% paraformaldehyde. 3D multigradient (MGE) echo (65 µm isotropic) T2* MR images were obtained using a 11.7 T NMR spectrometer and a 15 mm coil. Hypointensities detected by ex vivo 3D MGE MRI were correlated with Prussian Blue histochemistry in 10 µm coronal cryosections. Cryosections were incubated with freshly prepared Perls' reagent for 1 hr followed by DAB enhancement.

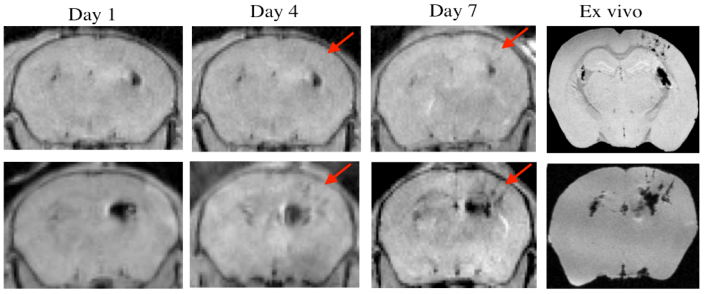

Fig. 1

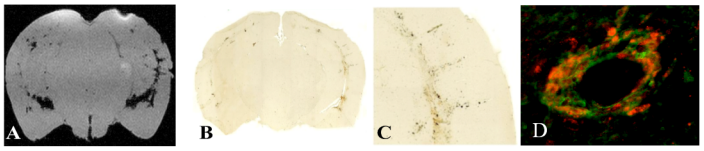

Fig. 2

Results and Conclusions: *In vivo* and *ex vivo* MR imaging revealed that NPCs migrated extensively along the corpus callosum in acute EAE whereas, in chronic EAE, cell migration was less extensive and appeared limited to regions of the corpus callosum ipsilateral to the site of cell injection. Only in the acute EAE brain, NPCs were found to exit from caudal regions of the corpus callosum and enter the brain parenchyma (Fig. 1, red arrows); a pronounced and surprising tangential migration into regions of the somatosensory cortex was observed in 5 of 10 acute EAE brains. The distribution of hypointensities (Fig. 2) detected by *ex vivo* MR imaging (A) correlated highly with the distribution of iron oxide labeled cells as detected histologically using DAB-enhanced Prussian Bue staining (B). Higher magnification revealed a characteristic radial spreading of labeled cells into the parenchyma of the somatosensory region (C) that, to the best of our knowledge, has been hitherto unreported. Immunohistochemistry (D) for the vascular endothelial marker Reca-1 (green) demonstrated that BrdU+ neural precursors (red) are present in close proximity to cortical blood vessels. We conclude that cell movements are determined by the phase of EAE as mediated by inflammatory factors within the microenvironment of the CNS. These findings have important ramifications for clinical translation of NPC therapy in that the cellular distribution and potentially disease attenuation will depend on the clinical presentation and disease status of MS. Funded by NMSS RG3630, the TEDCO Maryland Stem Cell Fund ESC 06-29-01, and The Israel Science Foundation 140/50.

References: [1]T. Ben-Hur et al., Magn. Reson. Med. 57, 164-171 (2007), [2]Einstein et al., Exp. Neurol. 198, 275-294 (2006).