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Original Article

Transplantation of Fas-deficient or wild-type neural stem/progenitor cells (NPCs) is equally efficient in treating experimental autoimmune encephalomyelitis (EAE)

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Abstract: Studies have shown that neural stem/progenitor cell (NPC) transplantation is beneficial in experimental autoimmune encephalomyelitis (EAE), an established animal model of multiple sclerosis (MS). It is unclear whether NPCs have the ability to integrate into the host CNS to replace lost cells or if their main mechanism of action is via bystander immunomodulation. Understanding the mechanisms by which NPCs exert their beneficial effects as well as exploring methods to increase post-transplantation survival and differentiation is critical to advancing this treatment strategy. Using the EAE model and Fas-deficient (*lpr*) NPCs, we investigated the effects of altering the Fas system in NPC transplantation therapy. We show that transplantation of NPCs into EAE mice ameliorates clinical symptoms with greater efficacy than sham treatments regardless of cell type (*wt* or *lpr*). NPC transplantation via retro-orbital injections significantly decreased inflammatory infiltrates at the acute time point, with a similar trend at the chronic time point. Both *wt* and *lpr* NPCs injected into mice with EAE were able to home to sites of CNS inflammation in the periventricular brain and lumbar spinal cord. Both *wt* and *lpr* NPCs have the same capacity for inducing apoptosis of Th1 and Th17 cells, and minimal numbers of NPCs entered the CNS. These cells did not express terminal differentiation markers, suggesting that NPCs exert their effects mainly via bystander peripheral immunomodulation.

Keywords: Experimental autoimmune encephalomyelitis (EAE), neural progenitor cells (NPC), Fas-deficient (*lpr*) NPC, neuroprotection

Introduction

Multiple Sclerosis (MS) is a chronic debilitating disease characterized by multifocal lesions of the white matter and a variable clinical course. It is the leading cause of non-traumatic neurological disability in young adults. MS is also a major public health issue with a considerably high socio-economic burden, affecting over 400,000 people in the US. MS commonly presents first as a relapsing-remitting disease and later progresses to a state of chronic neurodegeneration with white matter loss and cognitive decline. Unfortunately, currently used immunosuppressive and immunomodulatory therapies such as interferon beta and glatiramer acetate are only partially effective, and they do not inhibit or reverse neurological deterioration in advanced stages of the disease.

Neural stem/progenitor cell (NPC) transplantation holds significant promise as a novel treatment strategy for MS. NPCs are a heterogeneous population of self-renewing and multipotent cells [1]. They can proliferate almost indefinitely in serum-free cultures supplemented with epidermal growth factor (EGF) and fibroblast growth factor (FGF). When growth factors are withdrawn, they will spontaneously differentiate into neurons, astrocytes, or oligodendrocytes (post-mitotic daughter cells) [2]. Multiple groups have shown that transplanting NPCs into animal models of MS--EAE animals--results in reduced inflammation, decreased demyelination and axonal damage, and consequently improved clinical outcomes [3-5]. However, the major mechanisms responsible for such improved outcomes remain unclear. Furthermore, poor post-transplantation survival, lack

of significant terminal differentiation of transplanted cells, and debate over appropriate administration route remain issues that need to be addressed before this therapy can reach its full potential.

Fas is a member of the tumor necrosis factor (TNF) death receptor superfamily, which has a well-defined role in the immune system but uncharacterized functions in the adult CNS. Our lab and others have recently started to characterize the role of Fas in the CNS by examining NPCs. We demonstrated that Fas is a major modulator of NPC biology [6-8]. Its diverse, cell-specific functions continue to unfold in the literature [9-11]. Previous studies in our lab have also shown that Fas-deficient (*lpr*) NPCs exhibit increased survival, decreased proliferation, and increased differentiation capabilities compared to wild-type (*wt*) [12]. In addition, both *wt* and *lpr* NPCs have the same capacity for inducing apoptosis of pathogenic and pro-inflammatoryTh1 and Th17 cells [8]. The beneficial anti-inflammatory effects of NPCs may provide bystander immunosuppression in EAE.

Utilizing the unique pro-survival and anti-inflammatory features of *lpr* NPCs [8, 12], we hypothesized that both *lpr* and *wt* NPC transplantation will be beneficial in EAE. Furthermore, to determine whether pro-survival or peripheral immunomodulation is the main mechanism of *lpr* NPC transplantation, we compared the clinical outcomes in EAE between *wt* and *lpr* NPCs. If the pro-survival mechanism prevails, one would expect *lpr* NPC transplantation to be more efficacious than the *wt* NPC transplantation. Otherwise, one would expect equal efficacy with either *lpr* or *wt* transplantation as both *lpr* and *wt* NPCs have equal anti-inflammatory effects by inducing pathogenic Th1 and Th17 cell apoptosis. We injected either *wt* or *lpr* NPCs intravenously into mice during the height of EAE inflammation (14 days post-immunization). Clinical scores for NPC or vehicle control injected mice were recorded over acute and chronic time points. Mice brains and spinal cords were analyzed for inflammatory infiltrate via immunohistochemical analysis for $CD45^+$ inflammatory cells. GFP labeled NPCs were visualized in periventricular and parenchymal areas of the brain as well as in the spinal cord. Our data show that *wt* and *lpr* NPCs exhibited the same efficacy in EAE, suggesting that peripheral bystander immunomodulation is likely the main mechanism of NPC transplantation.

Methods

Neuroprogenitor cell isolation

Brains were dissected from 4-day post-natal *wt* (C57BL/6) or *lpr* mouse pups. NPCs were extracted using NeuroCult® Enzymatic Dissociation Kit (Stem Cell Technologies) and plated in media containing EGF and FGF. Neurospheres were formed after 8-12 days in culture, and expression of putative stem cell marker Nestin was confirmed using confocal microscopy.

Flow cytometry

Cells were lifted from culture using 0.05% trypsin/EDTA (Gibco) at 37°C. Trypsin enzymes were stopped using 20% fetal calf serum (FCS) in Neural basal media after 7 minutes. Cells were placed in Falcon® tubes (#352058), rinsed with phosphate buffered saline (PBS) and spun at 1400 rpm for 5 minutes before proceeding with staining. All stains were added to a 100 µL volume cell suspension containing approximately one million cells.

FasR Stain: Samples were first blocked with hamster IgG (50 ug/mL). Samples were then stained with either anti-Fas or isotype control (Hamster IgG2, λ1) phycoerythrin (PE)-conjugated antibodies (1:30, BD Biosciences). All rinses and dilutions were done with 1% BSA in PBS. All incubations were performed at 4°C (15 minutes for block, 30 minutes for PE-conjugated antibodies). Flow cytometry analysis was performed with a BD LSRII flow cytometer equipped with 3 lasers (488, helium neon, and UV), and data were analyzed with FlowJo software.

Neural progenitor cell growth

Stably transduced GFP^+ NPCs (Courtesy Dr. Jeffery Spees, Director of the UVM Stem Cell Core; greater than 85% purity) were plated in a monolayer on Poly-D lysine and laminin coated T-75 flasks (Nunc) in mouse-NPC complete media consisting of Gibco® Neural basal media, B-27 supplement, penicillin/streptomycin (10 mg/mL), L-glutamine (29.20 mg/mL) EGF (10 ng/mL) and FGF (10 ng/mL) and allowed to grow to confluence for 3-5 days.

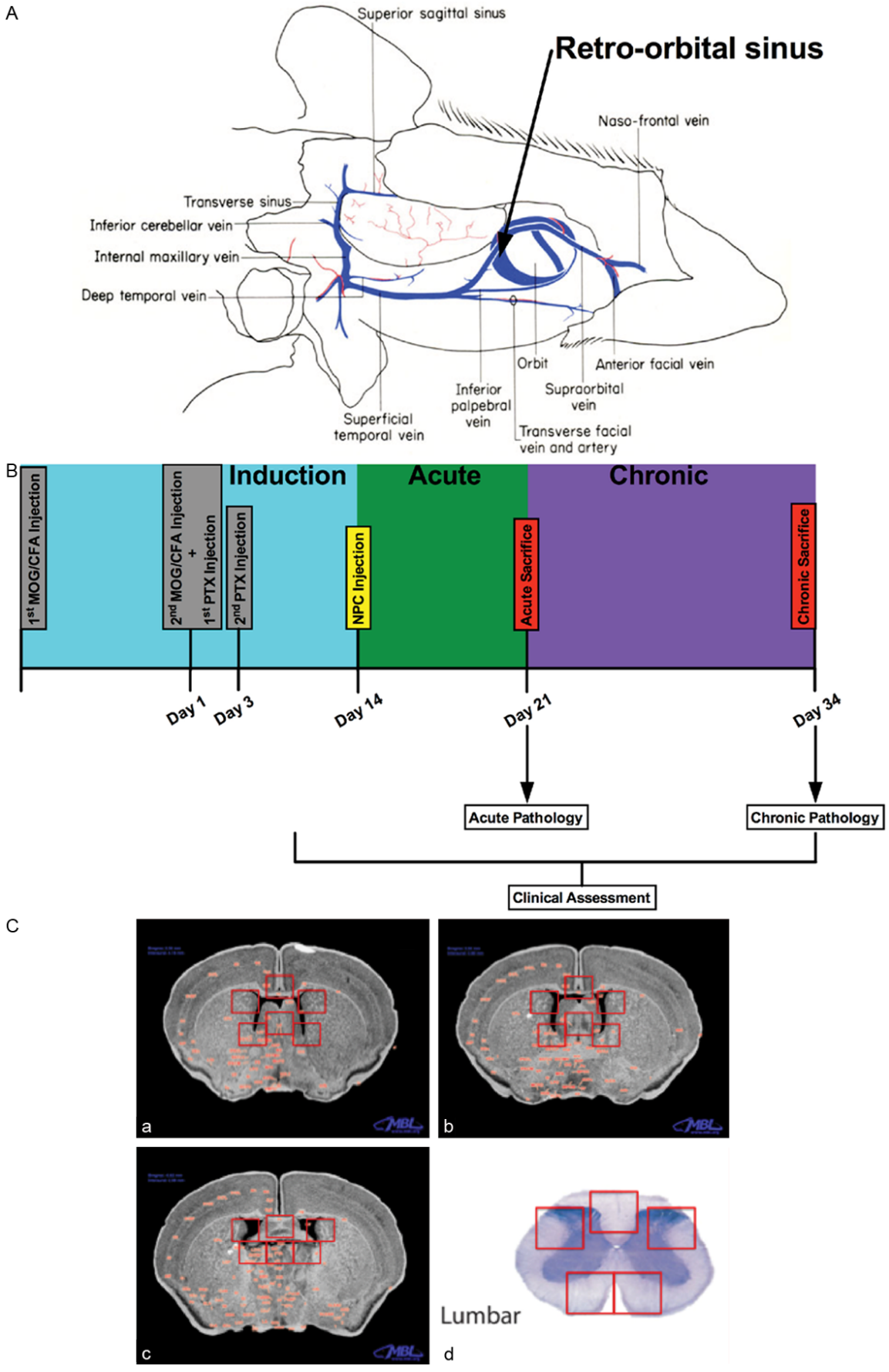
A
Superior sagittal sinus
Retro-orbital sinus
Naso-frontal vein
Transverse sinus
Inferior cerebellar vein
Internal maxillary vein
Deep temporal vein
Orbit
Anterior facial vein
Inferior palpebral vein
Supraorbital vein
Superficial temporal vein
Transverse facial vein and artery
B
Induction
Acute
Chronic
1st MOG/CFA Injection
2nd MOG/CFA Injection + 1st PTX Injection
2nd PTX Injection
NPC Injection
Acute Sacrifice
Chronic Sacrifice
Day 1
Day 3
Day 14
Day 21
Day 34
Acute Pathology
Chronic Pathology
Clinical Assessment
C
a
b
c
Lumbar
d

Figure 1. EAE induction protocol and experimental design. A: Map of blood flow in the mouse head along with injection site of the retro-orbital sinus of the adult mouse [19]. B: Timeline of experimental protocol illustrating induction procedures, treatment time-point, and clinico-pathological chronology. C: Templates used for image capture and quantification. a-c: represent the three periventricular brain depths (anterior to posterior) used, while d represents a lumbar spinal section. Red squares on the sections denote image locations.

NPCs were removed from the flasks on the day of injection, using trypsin to lift the cells from monolayer and 20% FCS in Neural basal media to stop the reaction. Cells were then counted and rinsed before injection.

Injection of treatments

Both *wt* and *lpr* cells were suspended in sterile PBS at a concentration of one million cells/50 μL. Mice were first anesthetized using isoflourane (Webster Veterinary), injected 50 μL of the cell solution into the retro-orbital sinus (**Figure 1A**) using a 27 ½ gage syringe and then observed until full motility returned. Mice receiving the vehicle control of sterile PBS were injected with the same volume of solution (50 μL) as the mice injected with cell solution. Cells were injected immediately after suspension in sterile PBS in order to minimize the amount of cell death during the injection procedure.

EAE induction and clinical time points

Mice were each immunologically primed with a 200 μg myelin oligodendrocyte glycoprotein(MOG)/complete Freund's adjuvant (CFA) subcutaneous injection with a second 200 μg MOG/CFA injection seven days later, which is Day 1 of the protocol. On Day 1, mice were also given a 200 ng pertussis toxin (PTX) intraperitoneal (IP) injection, followed by a second 200 ng PTX injection 2 days later. NPCs were injected at Day 14, 7 days after initiating clinical assessment. Mice were sacrificed at Day 21 (acute time point) or at Day 34 (chronic time point), generating two pathological time points. The induction and clinical protocol is represented visually in **Figure 1B**.

EAE clinical scoring

Clinical scoring of EAE symptoms began on Day 7 of the induction protocol and continued daily up to, and including the day of sacrifice. The scoring criterion used is a well-established 5 point scoring scale where: 0 = no clinical symptoms, 1 = limpness in tail, 2 = hind limb ataxia, 3 = hind limb paralysis, 4 = hind limb paralysis with incontinence and 5 = quadriplegia or paraplegia with clear forelimb weakness and moribund state. To eliminate bias, EAE clinical scores were recorded by a blinded investigator. Mice typically began to present clinical symptoms around Day 9.

Tissue sample harvesting

Mice were given a 50 μL lethal dose of Nembutal® (Ovation) through an IP injection and were then intracardially perfused with PBS followed by 10% formalin. Brains and spinal cords were then dissected, placed in 15 mL of 10% formalin and left to fix at room temperature for 24 hours. Samples were cryoprotected by rinsing three times with PBS using 15 mL of a 15% sucrose solution (with 0.2% azide in PBS) on a rocker at 4°C for 24 hours followed by a 30% sucrose solution (with 0.2% azide in PBS) on a rocker 4°C for 24 hours. Samples were then frozen in NEG 50™ (Richard Allen Scientific) and stored in sealed plastic tubes at -80°C until sectioning. Samples of 20 μm thickness were sliced using a cryostat and were stored and stained on microscope slides (FisherbrandÒ SuperfrostÒ Plus).

Sample staining

Sample slides were incubated at 37°C for 15 minutes before the staining process began. For samples stained for inflammation, slides were first blocked in 10% goat serum/0.4% triton in PBS for 1 hour at room temperature, and then stained with an αCD-45 1° antibody (rat, Millipore 1:750) at 4°C for 24 hours. Slides were then rinsed with PBS, and an αRT-CY3 2° antibody (Jackson 1:500) was applied for 1 hour at room temperature. Before staining was completed, slides were stained with Hoecsht (1:2000). Samples stained for putative stem cell markers were blocked in the same fashion as those stained for inflammation. The primary antibodies used to stain stem cell markers included platelet-derived growth factor receptor-alpha (PDGFR-alpha, rabbit, Santa Cruz 1:50 in 10% horse serum (HS) in PBS), beta III-tubulin (rabbit, Sigma 1:100 in 10% goat

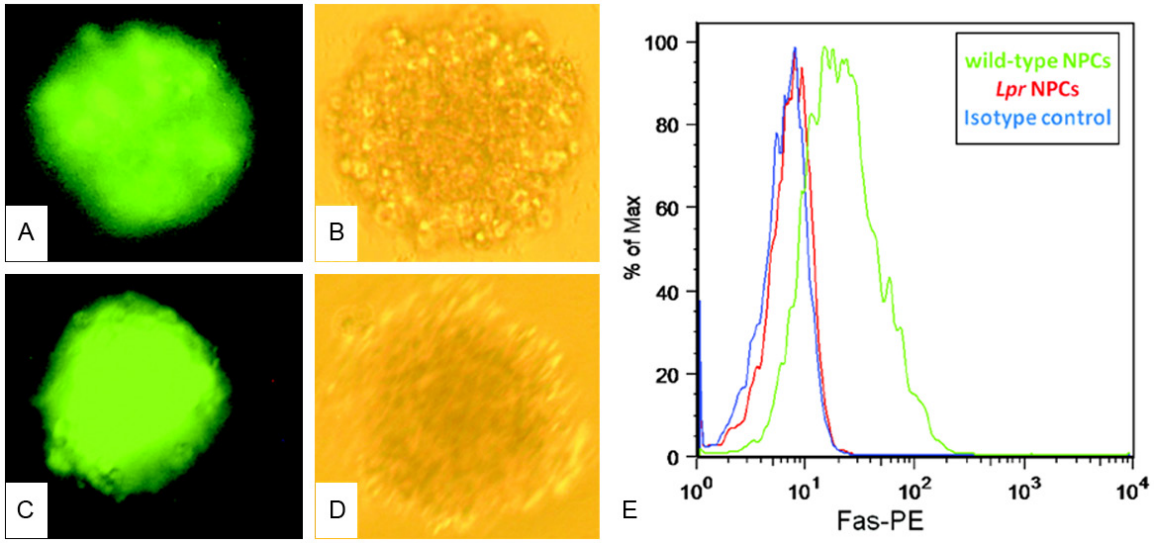

Figure 2. Characterization of NPCs in *vitro*. (A) *Lpr* GFP$^+$ NPCs form neurosphere and auto-fluoresce green under fluorescence. Images (20x) of 10-day-old GFP$^+$ neurospheres grown in culture. GFP$^+$ NPCs fluorescing green, with (B) a phase contrast image of the same sphere. (C) *Wt* GFP$^+$ NPCs fluorescing green with (D) a phase contrast image of the same sphere. (E) Confirmation that *Lpr* NPCs lack FasR. *Lpr* mice have a large insertion in the FasR gene that results in improper splicing and absence of the protein. We confirmed absence of FasR in *lpr* NPC using a phycoerythrin (PE)-conjugated anti-FasR antibody. Background staining was assessed using an isotype control IgG antibody (blue line). Unlike wild-type NPCs (green line), *lpr* NPCs (red line) do not express FasR (+ peak completely overlaps with isotype control). Y-axis represents the percent of maximum staining intensity (arbitrary units), while the X-axis represents the voltage of positive PE fluorophore staining.

serum/0.4% triton in PBS), glial fibrillary acidic protein (GFAP, rabbit, dako 1:250 in 10% goat serum/0.4% triton in PBS) and polydendrocytes/oligoprogenitor cells (NG2, rabbit, Santa Cruz 1:500 in 10% goat serum/0.4% triton in PBS). Similar to the protocol for inflammation staining, the primary antibody was rinsed off with PBS after the 24 hour incubation at 4°C, and an αRb-CY3 2° antibody (Jackson 1:500) was applied for 1 hour at room temperature and slides were stained with Hoecsht (1:2000 in PBS) before mounting.

Image capture and quantification

Images used for quantification were taken using the same Nikon Eclipse E800 microscope. For each mouse brain sample, 3 predetermined depths from the periventricular region were determined. At each depth, 6 images were taken at predetermined locations. For each mouse spinal cord sample, 3 random lumbar sections were used and 5 images were taken from each section at predetermined locations. Optical density of inflammatory cells was determined for each image using the kodalith feature of Neurolucida Version 9 software. Image depth and area selection templates are included in **Figure 1C**.

Results

Characterization of NPCs in vitro

To ensure ability to locate and identify transplanted NPCs in the CNS, stably transduced GFP$^+$ NPCs were allowed to grow in complete neural stem cell media for seven days, resulting in proliferation and formation of neurospheres. Spheres were then imaged under phase contrast as well as under fluorescent light to ensure that cells were positive for GFP (**Figure 2A-D**). Both *wt* and *lpr* NPCs used for injection were >85% GFP positive as determined by cell sorting using flow cytometry.

To confirm the phenotype of the *lpr* NPCs, both *wt* and *lpr* NPCs were stained for the Fas receptor (FasR). Cells were also stained with an isotype control from another animal as a true negative staining control. Flow cytometry showed that *lpr* NPCs matched the isotype control, demonstrating that *lpr* cells do not express the FasR whereas the *wt* NPCs do express it (**Figure 2E**).

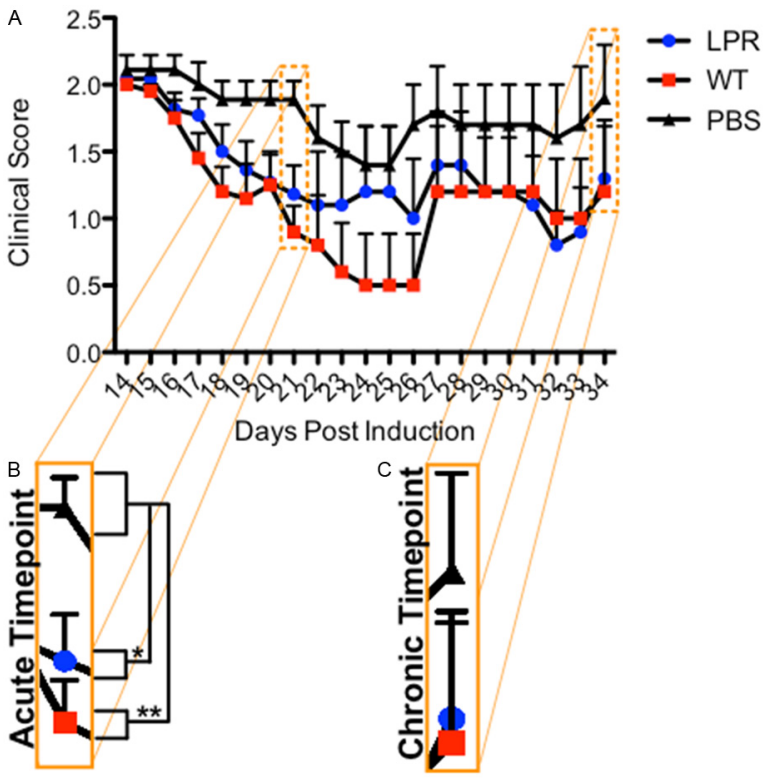

Figure 3. *Wt* and *lpr* NPC transplantation decreases EAE clinical scores. A: Mean clinical scores from each day after injection (error bars represent SEM). B: Mean clinical scores at the day of harvesting at the acute time point day 21 for *lpr* (n=11, p≤0.05), *wt* (n=10, p≤0.01) and PBS (n=9) treated mice. C: Mean clinical scores at the day of harvesting at the chronic time point day 34 for *lpr* (n=5), *wt* (n=5), and PBS (n=5) treated mice.

NPC treated mice show a decrease in clinical scores with no difference between lpr and wt treatments

After retro-orbitally injecting EAE mice with *wt* NPCs, *lpr* NPCs or sterile PBS, clinical scores of mice in each group were taken daily. At the acute time point (Day 21 of the protocol), mice in the *wt* NPC treatment group had clinical scores that were significantly lower (n=10, p≤0.01) than mice in the PBS treatment group. Similarly, mice in the *lpr* NPC treatment group also had clinical scores that were significantly lower (n=11, p≤0.05) at the acute time point than mice in the PBS treatment group (**Figure 3B**). At the chronic time point, there was a noticeable trend similar to what was observed at the acute time point with mice in the *lpr* (n=5) and *wt* (n=5) NPC treatment groups, having scores that were lower than scores of mice in the PBS (n=5) treatment group, although this trend failed to reach significance (**Figure 3C**). Mice in the *wt* NPC and *lpr* NPC treatment groups were not significantly different when compared to each other at either the acute or chronic time points.

At the acute time point, *lpr* and *wt* NPC treatment groups showed significantly greater mean improvement in clinical score than the PBS treatment group (p≤0.05 and p≤0.01 respectively). Improvement in clinical score was determined by the difference between the clinical scores at time of treatment and the time of sacrifice. As with average clinical scores at the acute and chronic time points, mean improvement in clinical score did not differ between *wt* and *lpr* NPC treated mice (**Figure 4A**). A measurement of percent of mice with recovery at the acute time point showed that mice in the *lpr* NPC treatment group had a significantly greater percent of mice with recovery (p≤0.05) compared to mice of the PBS treatment group (**Figure 4B**). A mouse was considered to show recovery if there was any improvement in clinical score at the time of treatment compared to at the time of sacrifice. It is important to note that mice in the *wt* NPC treatment group also appear to contain a greater percentage of mice with recovery when compared to mice in the PBS treatment group although large variance yields an insignificant p value (p>0.10). Further, the percent of mice showing recovery was not significantly different between the *wt* and *lpr* NPC treatment groups.

Periventricular brains and lumbar spines of mice treated with NPCs show a significant decrease in the amount of inflammation at the acute time point when compared to mice treated with PBS

When lumbar spinal sections and periventricular brain sections were stained for the inflammatory cell marker CD-45, the amount of inflammation in mice treated with *wt* (n=4, p≤0.05) and *lpr* (n=6, p≤0.05) NPCs was signifi-

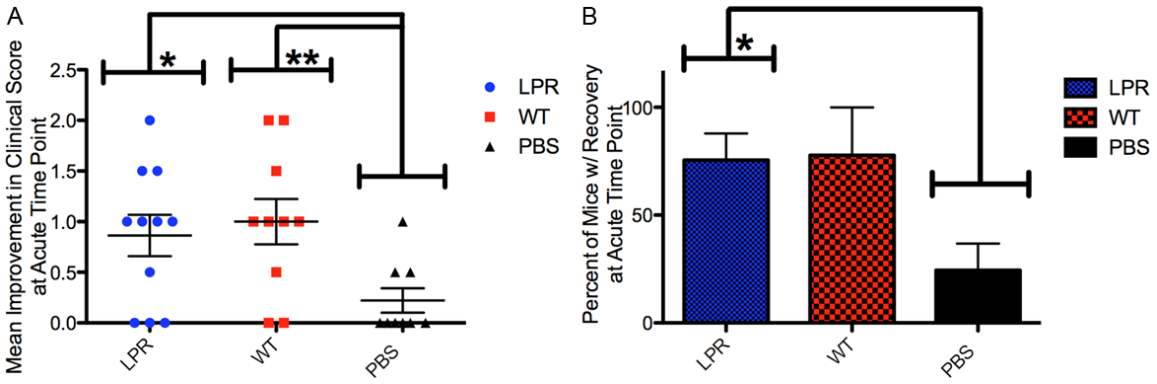

Figure 4. *Wt* and *lpr* NPCs have the same efficacy in EAE. A: Mean improvement in clinical score for *lpr* (n=11, p≤0.05) *wt* (n=10, p≤0.01) and PBS treated mice at the acute time point. Improvement in clinical score was determined by subtracting the clinical score at sacrifice from the clinical score at NPC injection (error bars represent SEM). B: Percent of mice showing recovery at acute time point for *lpr* (n=11, p≤0.05), *wt* (n=10) and PBS (n=9). A mouse was considered to be showing recovery if the clinical score at the time of sacrifice was less than the clinical score at the time of NPC injection (error bars represent SEM).

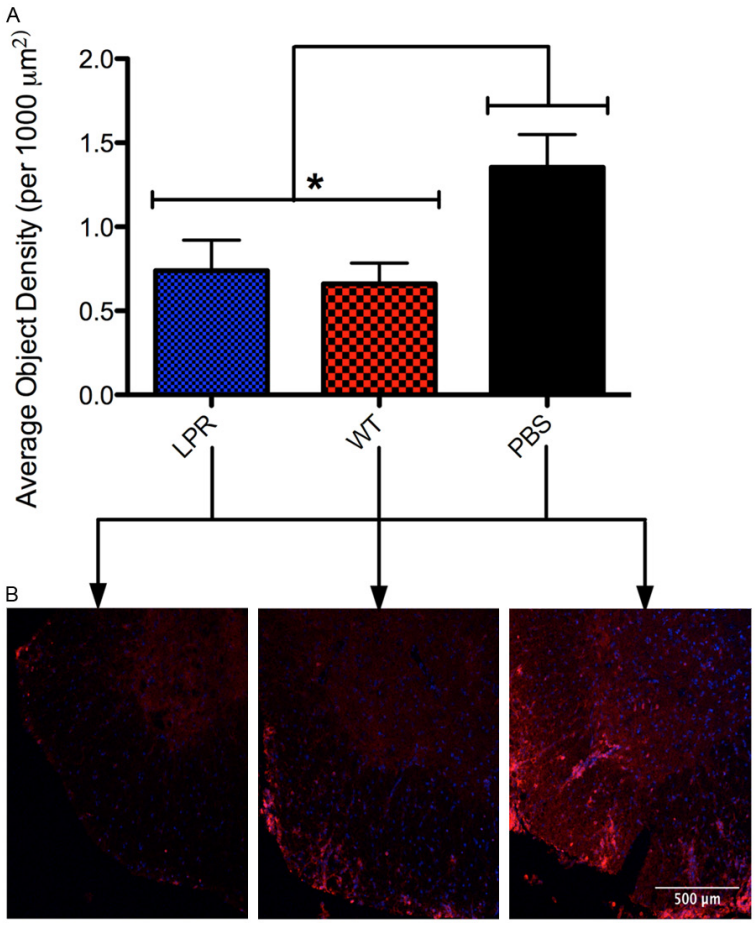

Figure 5. *Wt* and *lpr* NPCs decrease inflammation in EAE. A: Mean average object density of CD-45 positive cells in *lpr* (n=6, p≤0.05), *wt* (n=4, p≤0.05), and PBS determined using Neurolucida image processing software (error bars represent SEM). B: Representative 20x images of lumbar spinal sections of *lpr* (left), *wt* (middle), and PBS (right) stained for CD-45 positive cells (red cells).

cantly lower than in mice of the PBS treatment group (n=5) at the acute time point. When the *lpr* and *wt* treatment groups were compared, no difference in amount of inflammation was found (**Figure 5**). Sections from all different treatment groups were scored for inflammation using an inflammation scale based on the extensiveness of inflammation in each region [13]. Graphing inflammation scores against the corresponding clinical score showed a positive correlation between the two.

Retro-orbitally injected NPCs home to and reside in the CNS at the acute and chronic time points

Both *wt* and *lpr* NPCs injected into mice with EAE were able to home to sites of CNS inflammation in the periventricular brain and lumbar spinal cord (**Figure 6A-E**, sections shown are from an *lpr* treated mouse). These cells were able to survive in the post-transplantation host environment for up to 20 days with transplanted GFP$^+$ NPCs observed in both spine

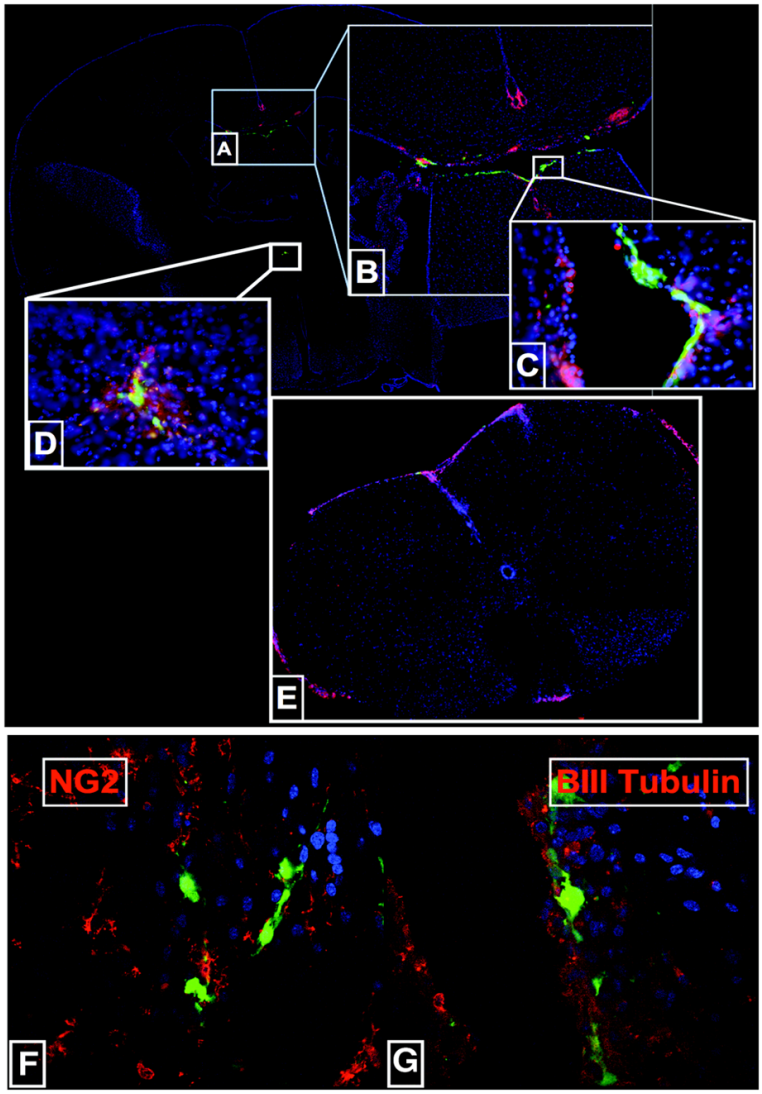

Figure 6. NPCs home to the CNS and reside there at the acute and chronic time points. A: 5x image of transplanted NPCs (green cells) and CD-45 positive cells (red cells) in the periventricular region of the brain. B-D: Close-up images of the periventricular brain at 5x, 40x, and 40x respectively. E: 5x compilation image of the lumbar spine of a mouse transplanted with NPCs. F: Transplanted NPCs express a negligible amount of oligoprogenitor and neuroprogenitor cell lineage markers at the acute and chronic time points. (left) Image of a periventricular brain section stained for NG2 (an oligoprogenitor cell marker) in red and transplanted NPCs in green. (right) 60x image of a periventricular brain section stained for BIII Tubulin (a neuron specific cell marker) in red and transplanted NPCs in green.

and brain sections at the acute and chronic time points. The amount of homing NPCs *in vivo* are similar between *wt* and *lpr* transplantation.

Transplanted NPCs express a negligible amount of oligoprogenitor and neuroprogenitor cell lineage markers at the acute and chronic time points

Transplanted NPCs were stained for multiple differentiation markers. Lack of double positive (both green and red) staining demonstrated that NPCs remained in the progenitor state in areas around the CNS lesions (**Figure 6F** and **6G**, section shown is from an *lpr* treated mouse). This finding was consistent at both the acute and chronic time points.

Discussion

To test whether *wt* and *lpr* NPCs have clinical efficacy in EAE, we transplanted GFP-labeled NPCs (either *wt* or *lpr*) into mice afflicted with EAE (see **Table 1** for summary). Both *lpr* and *wt* NPC transplantations resulted in similar reductions in EAE clinical scores and CNS inflammation. Both *lpr* and *wt* NPCs decreased EAE clinical scores to the same extent at the acute time point. Furthermore, amount of CNS infiltrating immune cells decreased equally in both *lpr* and *wt* NPC injected groups compared to controls. At the chronic time point, average clinical scores in NPC treated groups were not significantly different compared to PBS control mice. However, the cumulative disease score was much lower in both *lpr* and *wt* treated groups compared to PBS controls.

Our previously published co-culture data reveal that the immunomodulatory capacity of NPCs is not affected by their Fas-expression [8], i.e., both *wt* and *lpr* NPCs are equal in immunomodulation *in vitro*. Our previous data showed that both *wt* and *lpr* NPCs have the same capacity to induce pathogenic pro-inflammatory Th1 and Th17 cell apoptosis *in vitro*. Therefore, it is not surprising that *lpr* and *wt* NPCs have the same efficacy *in vivo* in EAE. Consistent with other publications, peripheral/"bystander" immunoregulatory functions of NPCs are most likely primarily responsible for improving disease outcomes [14, 15].

Table 1. Summary of key clinical and pathological results with *wt* and *lpr* NPC transplantation in EAE

Treatment	Number of Mice	Mean Disease Score[α,β]	Cumulative Disease Score[γ]	Inflammatory Infiltrates[δ] (per 1000 μm^2)
Acute (Sacrifice at 21 days)				
LPR	11	1.182±0.216*		0.740±0.181*
WT	10	1.000±0.224**		0.661±0.123*
PBS	9	1.889±0.139		1.353±0.194
Chronic (Sacrifice at 34 days)				
LP	5	1.300±0.436	144.5*	
WT	5	1.200±0.490	110.0**	
PBS	5	1.900±0.400	191.5	

[α]Data are mean numbers (± SEM) from a total of n=3 independent experiments. [β]The mean disease score represents the score of all mice in each group at the endpoint of each clinical period. [γ]The cumulative disease score is the summation of all scores over the entire clinical period (20 days) in each group. [δ]Inflammatory infiltrates are characterized by the mean optical density of CD45 positive cells in periventricular brain and lumbar spinal cord sections. *$p \leq 0.05$ when compared to PBS injected controls. **$p \leq 0.01$ when compared to PBS injected controls.

Even though *lpr* NPCs have significantly higher differentiation and decreased apoptosis compared to *wt* NPCs *in vitro* [12], this did not translate to promoting terminal differentiation and post-transplantation survival *in vivo*. It is possible that there are environmental factors unique to the CNS that prevent the differentiation of transplanted NPCs, regardless of their inherent *in vitro* differentiation capacities. Conceivably, inflammation itself may contribute to the inhibition of differentiation. This hypothesis is supported by several prior reports of injected NPCs remaining in a "progenitor", undifferentiated state [3, 16, 17]. Identifying the molecules responsible for inhibiting NPC differentiation *in vivo* warrants further investigation since modulating these signals could promote oligodendrocyte production and remyelination, ultimately resulting in more effective CNS repair.

Although a limited number of NPCs entered the CNS in the current study, NPCs exhibited a strong capacity for localizing to inflammatory sites. Pluchino et al. demonstrated that NPCs respond to inflammatory stimuli and traffic to the inflamed regions of the CNS [18]. Molecules such as very late antigen 4 (VLA4) and CD44 are suggested to have a role in the homing of NPCs [18]. As MS lesions are disseminated throughout the CNS, it is essential for transplanted cells to be able to reach areas of inflammation and damage. It has been suggested that the inability of immuno-therapies to prevent neurodegeneration in MS may be related to the failure of these treatments to concentrate in localized areas of CNS damage. Therefore, NPCs' potent immunomodulatory capacity, coupled with their ability to home to CNS sites of inflammation, could translate to improved therapeutic responses.

At present, no treatments are effective in preventing or reversing the neurodegeneration that occurs in MS. There is a pressing need to develop therapeutics that exhibit neuroprotective effects by acting directly on the CNS. NPC therapies represent a novel and promising avenue for developing neuroregenerative treatments [1, 19]. However, stem cell therapies currently in development are unable to completely repair lost or damaged CNS tissue [20, 21]. If endogenous or transplanted NPCs could be activated to terminally differentiate, CNS damage and consequent long-term disability could be minimized or even reversed. Therefore, determining modulators of NPC differentation and their mechanisms of action is critical to advancing therapies for neurodegenerative disease.

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Disclosure of conflict of interest

None.

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