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ORIGINAL RESEARCH REPORT

Mesenchymal Stem Cells Enhance the Engraftment and Myelinating Ability of Allogeneic Oligodendrocyte Progenitors in Dysmyelinated Mice

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Multiple sclerosis is an autoimmune disease characterized by demyelination and axonal loss throughout the central nervous system. No regenerative treatment exists for patients who fail to respond to conventional immunosuppressive and immunomodulating drugs. In this scenario, stem cell therapy poses as a rational approach for neurological regeneration. Transplantation of embryonic-derived oligodendrocyte progenitor cells (OPCs) has been shown to promote remyelination and ameliorate animal models of neurodegenerative diseases. However, its therapeutic application is limited due to potential transplant rejection. In multiple sclerosis, an added concern is that transplant rejection would be most pronounced at sites of previous lesions, exacerbating a hyperactive immune response which could prevent remyelination and precipitate additional demyelination. Routine systemic immunosuppression may not be sufficient to prevent transplant rejection-associated immune reactions in the cerebral microenvironment. Mesenchymal stem cells (MSCs), due to their homing properties and inherent immunosuppressive nature, are a promising tool for clinical application targeted toward immunosuppression at sites of injury. In this study, we used a co-transplantation strategy to investigate the effect of syngeneic MSCs on the survival and remyelination abilities of allogeneic OPCs in adult nonimmunosuppressed shiverer mice. At all time points examined, cotransplantation with MSCs increased OPC engraftment, migration, and maturation in myelinating oligodendrocytes, which produced widespread myelination in the host corpus callosum. In addition, MSCs reduced microglia activation and astrocytosis in the brain of transplanted animals as well as T-cell proliferation in vitro. These data suggest that combining the immunomodulatory and trophic properties of MSCs with the myelinating ability of OPCs might be a suitable strategy for promoting neurological regeneration in demyelinating diseases.

Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) characterized by loss of myelin and neurodegeneration. It is the most common cause of disability in young adults after trauma [1]. The pathological features of MS include immune cell infiltration, oligodendrocyte death, demyelination, and axonal damage [2–4]. Failure of the CNS to remyelinate MS lesions [5] and the resulting axonal damage [6,7] leads to irreversible functional decline in patients with MS [8,9]. Available treatments focus on modulating or suppressing the autoimmune response. These treatments are partially effective in delaying the progression of the disease, but with the accrual of disease burden and the associated clinical disability, they offer little benefit [10]. Thus, there is a compelling need for the development of therapies for MS aimed at preventing or improving disability, and stopping disease progression by promoting remyelination and preventing axonal loss [11,12].

Transplantation of oligodendrocyte progenitor cells (OPCs) and the neural stem cells from which they are derived has been shown to significantly ameliorate experimental models of MS and other neurodegenerative diseases [13,14]. Human embryonic stem cells (ESCs) may potentially serve as an unlimited experimental and therapeutic source of transplantable cells. However, their clinical application in neurological disease is limited in part because of tissue rejection and inflammation [15,16]. Further, in a disease such as MS that is pathologically characterized by inflammatory demyelination [2–4], the tissue rejection induced immune hyperactivity may enhance autoimmune demyelination [17]. In this scenario, it is crucial to develop alternative strategies to the use of conventional immunosuppressants for promoting graft cell survival. Autologous bone marrow-derived

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mesenchymal stem cells (MSCs) are a possible solution to this problem. These multipotent progenitor cells have immunosuppressive properties and prevent tissue damage [18]. These functions may be of therapeutic value in allogeneic transplantation, because they allow a tolerant milieu, thereby decreasing graft rejection [19].

In this study, we investigated the effect of syngeneic MSCs on the survival and remyelinating abilities of allogeneic OPCs co-transplanted in the brain of adult shiverer mice, a model of congenital and irreversible dysmyelination that carries a spontaneous mutation of myelin basic protein (MBP) resulting in a lack of central myelin [20,21]. Although the myelinating potential of OPCs from different sources has been examined in this experimental model [22–25], this is the first study to report an alternative strategy to the use of conventional immunosuppressants to enhance graft survival and myelinogenic capacity of allogeneic ESC-derived OPCs transplanted in adult immunocompetent animals. Our findings demonstrate that by creating a tolerant immune environment for graft cell survival, and by providing trophic support for cell engraftment and maturation, MSC transplantation represents a powerful alternative (or complement) to immunosuppressant drugs in allogeneic transplant paradigms.

Materials and Methods

Mouse embryonic stem cells and oligodendroglial differentiation

An ES cell line constitutively expressing enhanced green fluorescent protein (GFP) was derived from transgenic C57BL/6J mice (The Jackson Laboratories) expressing enhanced GFP under the chicken β-actin promoter [26,27]. Oligodendroglial differentiation was carried out according to published protocol [28]. Briefly, ES cells were cultured on feeder cells (mouse embryonic fibroblasts) with serum-media supplemented with leukemia inhibitory factor to expand undifferentiated colonies (stage 1). For neuroepithelial differentiation, a single cell suspension of ES cells was cultured under nonadherent conditions in serum-containing media without leukemia inhibitory factor to induce formation of embryoid bodies (EBs/stage 2). After 5 days, EBs were plated on polyornithine-coated flasks in ITSF (insulin, transferrin, sodium selenite, and fibronectin) serum-free media (stage 3). After 7 days, cells were cultured in neural expansion media in the presence of 20 ng/mL fibroblast growth factor (FGF) for several passages (stage 4). To obtain terminally differentiated neurons, FGF was withdrawn for 2 weeks. For oligodendroglial differentiation, 5 days into stage 4 (common stage) cells were cultured in neural expansion media supplemented with 20 ng/mL EGF and 10 ng/mL FGF for 2 weeks, followed by several passages in N2 media supplemented with10 ng/mL platelet-derived growth factor (PDGF)/FGF to obtain highly pure, transplantable OPCs. To obtain terminally differentiated oligodendrocytes, growth factors were removed from cultured OPCs for 10 days.

Bone marrow derived MSCs

Mouse MSCs were derived from the bone marrow of 3 week old C3HeB/FeJ mice, the parental strain of the shiverer mouse used in this study. Femurs and tibias were dissected from each mouse, and bone marrow was flushed with a 22-G or 25-G needle, respectively, containing D-PBS + 2% FBS. Washed cells were plated in a 10 cm dish in Mouse MesenCult (StemCell Technologies, Vancouver, Canada) media supplemented with 20% FBS. Nonadherent cells were eliminated at day 3 by replacing the entire media. Media was changed every 3–4 days until cells reached 70% confluency. MSCs were passaged 2 to 4 times before injection. On the day of injection, MSCs were labeled with 800 nM CellTracker™CM-DiI (Invitrogen), washed twice with D-PBS, and resuspended in D-PBS for injection.

FACS analysis

Mouse MSCs were labeled with FITC-conjugated anti-CD9, anti-CD44, anti-Sca1, and anti-CD105, or the appropriate isotype controls (BD Biosciences) and analysis was performed with a BD FACS Aria.

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction was performed on the Applied Biosystems 7900 HT Fast Real-Time PCR System using a customized TaqMan low density array and Taqman Gene Expression Master Mix (Applied Biosystems). Fold change in RNA levels was calculated using the ΔΔ Ct method (relative quantitation), and 18S rRNA expression was used as an internal control.

Stem cell transplantation and sample collection

Homozygous C3Fe.SWV-MBP$^{shi/shi}$ shiverer mice were purchased from The Jackson Laboratory. Animals were divided into 5 groups based on the type of cell transplant they received: (1) 10 μL ($\sim 5\times10^5$) ESC-derived oligodendrocyte progenitor cells constitutively expressing GFP (GFP-OPCs) alone, (2) 10 μL ($\sim 5\times10^5$) DiI labeled MSCs alone, (3) 5 μL (5×10^5) GFP-OPCs + 5 μL (5×10^5) MSCs, (4) saline (10 μL), or 10 μL ($\sim 5\times10^5$) GFP-OPCs alone in cyclosporine-A treated animals (CSA control). In the CSA control group, animals were immunosuppressed daily by subcutaneous administration of cyclosporine A (10 mg/kg; Sandimmune, Novartis) beginning 3 days before OPC grafting and continuing until sacrifice. All animals were 8 weeks old at the time of cell transplantation. Injections were administered intracranially into the corpus callosum with the following stereotactic coordinates in reference to the bregma: +0.17 mm (anteroposterior axis), 0 mm (lateromedial axis), +2.1 mm (vertical axis). Mice were sacrificed at 3 time points: 2, 4, and 8 weeks. Each time point was repeated thrice (3 mice per group) using a single batch of cells (MSCs or OPCs). Animals were anesthetized with ketamine and intracardially perfused first with saline and then with 4% paraformaldehyde in 0.1M PBS, pH 7.4. Brains and spinal cords were fixed in formaldehyde for at least 24 h, then removed, cut coronally into 3 pieces, and submitted to paraffin processing. Tissue was then embedded in a paraffin cassette, sliced at 5μm with a microtome, and mounted on Unifrost Plus Microscope Slides (VWR). Slides were dried overnight before staining. All animal experiments were approved by IACUC committee of the St Luke's Roosevelt Hospital Center and conformed to NIH guidelines.

Immunofluorescent staining

Microscope slides were deparaffinized with a xylene-alcohol scale, and antigen retrieval was performed using antigen unmasking solution from Vector Laboratories. Slides were then washed and blocked in 10% normal goat serum with 0.2% Triton X-100 for 1 h. After blocking, tissue was incubated with primary antibodies or the appropriate isotype control (negative control) overnight at 4°C. Antibodies used included mouse anti-MBP (1:1,000), mouse anti-Neurofilament SMI312 (1:1,000) (Covance), chicken anti-GFP (1:1,000) (Abcam), rabbit anti-glial fibrillary acid protein (GFAP) (1:500) (Dakocytomation), rabbit anti-Iba1 (1:200) (Wako Chemicals), mouse anti-O4 (1:100), mouse anti-Nestin (1:100), rabbit anti-Olig2 (1:500), rabbit anti-NG2 (1:200), rabbit anti-Oct4 (1:200), rabbit anti-Sox2 (1:1,000), mouse anti-SSEA1 (1:200), rat anti-SSEA3 (1:200), mouse anti-Olig1 (1:500), and mouse anti-A2B5 (1:100) (Millipore). The next day, slides were washed in PBS and then incubated for 2 h at room temperature in the dark with AlexaFluor conjugated secondary antibodies diluted in blocking solution. Secondary antibodies used included goat anti-mouse 488/555/647 IgM, goat anti-mouse 488/555/647 IgG1, goat anti-mouse 488/555/647 IgG2b, goat anti-mouse 488/555/647 IgG2a, rabbit anti-mouse 488/555/647 IgG, goat anti-chicken 488 IgY, and rat anti-mouse 488/555 IgG (all from Invitrogen). Slides were then washed for 5 min in 4′,6-diamidino-2-phenylindole diluted in PBS (1:5,000), followed by PBS washes. Tissue was sealed under a cover slip using Fluoromount (Sigma-Aldrich). Samples were analyzed using a Zeiss LSM 510 Meta Confocal microscope running Zen imaging software at 10×, 20×, 40×, and 63×magnification. For immunocytochemistry, cells were cultured on 8-well chamber slides, fixed with 4% paraformaldehyde, and stained as just described. Semi-automated cell counts were performed using Image J software (Image Processing and Analysis in Java).

Luxol Fast Blue staining

Microscope slides were baked for 30 min at 37°C to dry and deparaffinized with a xylene-alcohol scale. Slides were immersed in 2 changes of 95% ethanol for 1 min each and then placed in Luxol Fast Blue solution (0.1% Solvent Blue 38 (Sigma) in 95% Ethanol, +5 mL 10% glacial acetic acid, filtered) at 58°C for at least 3 h. After staining, slides were immersed in 95% alcohol and then distilled water to wash off excess stain. Differentiation was accomplished by submerging the slides in 0.05% lithium carbonate for 10–20 s, followed by 2 changes (1 min each) in 70% EtOh. These steps were repeated if further differentiation was required. Slides were washed in distilled water and then stained for 5 min in Cresyl Violet Solution (0.25% Cresyl violet acetate (Sigma) +5 drop 10% glacial acetic acid per 300 mL solution, filtered). The slides were then washed in 2 changes of 95% EtOH (1 min each) and 100% EtOH (2 min each) and then cleared in 3 changes of xylene. Slides were dried and mounted using cytoseal (Thermo Scientific).

Myelinated axon counts

Myelinated axons were counted as described [25]. Briefly, uniform coronal sections of the corpus callosum were selected for neurofilament and MBP staining and analyzed with regard to the percentage of myelinated host axons. A 500 μm stack of 25 superimposed optical slices taken at 20 μm intervals (Zeiss Axio observer 2) was made for each mouse, beginning rostrally and progressing caudally from the injection site. Three parallel, equidistant lines were laid over the images perpendicular to the axons. Axons were scored at intersections with the lines as either myelinated (closely apposed to MBP on both sides) or unmyelinated. To assess the extent of corpus callosum remyelination, double-positive MBP-NF sections from the above stack were summed to determine the total length of remyelinated brain tissue.

Microglia/macrophage and reactive astrocytes quantification

The number of Iba1+ microglia/macrophages and GFAP+ reactive astrocytes in the corpus callosum were quantified using Image J software. A 500 μm stack of 25 superimposed optical slices taken at 20 μm intervals (Zeiss Axio observer 2) was made for each mouse, beginning rostrally and progressing caudally from the injection site. A uniform region from each optical slice was analyzed by counting the percentage of Iba1+ or GFAP+ cells over the total number of 4′,6-diamidino-2-phenylindole + cells.

ELISA and western blot assays

OPCs were seeded at a concentration of 35,000 cells×cm^2 alone in MSC conditioned media (CM) or with MSCs (6,000 cells×cm^2). Supernatant was collected every 3 days and filtered, and aliquots were stored at −80°C until use. ELISAs for transforming growth factor beta (TGF-β), PDGF-AA, and insulin-like growth factor 1 (IGF-1) all from R&D systems were performed according to the manufacturer's instructions and analyzed on a Synergy HT Multi-Mode Microplate Reader (Biotek). Proteins for western blot analysis were harvested from OPCs cultured in conditioned and non-conditioned media using RIPA Buffer (Cell Signaling) with Halt Protease and Phosphatase Inhibitor Single-Use Cocktail (Thermo Scientific) following the manufacturers' instructions. Five micrograms of protein sample were loaded per lane on a NuPage 4%–12% Bis-Tris Gel 1.5 mm×10-well (Invitrogen) and stained with MBP (SMI-99; Covance) and anti-NG2 Chondroitin Sulfate Proteoglycan (Millipore) antibody at 1:1,000 and 1:500 dilution, respectively. Secondary detection was performed using 1:500 Goat anti-mouse IgG-HRP (Santa Cruz Biotechnology) and 1:1,000 Goat anti-Rabbit IgG (Fc) (AbD Serotec). Subsequently, the membranes were stripped using Re-Blot Plus-Mild (Millipore), restained with 1:4,000 Mouse anti B-actin (Sigma-Aldrich), and visualized using Immobilon Western Chemiluminescent HRP Substrate (Millipore).

Antibody neutralization assay

OPCs were cultured with nonconditioned media, CM, or CM supplemented with 15 μg/mL of mouse IGF-1 neutralizing antibody from R&D Systems. The antibody concentration used was one hundred times the neutralization dose of the maximum IGF-1 concentration detected by ELISA (1.4 ng/mL). Media was changed every third day, and cells were cultured in triplicate treatment groups for 10 days.

Protein extraction and western blots to detect MBP were performed as previously described.

T-cell proliferation assay

Mouse MSCs were plated at a concentration of 10,000 cells/cm^2 1 day before T cell isolation. CD4$^+$/CD25$^-$ T cells were isolated from C3HeB/FeJ mouse splenocytes using the untouched CD4$^+$ T cell isolation kit (Miltenyi) followed by the CD25 microbead kit (Miltenyi) to deplete activated T cells. CD4$^+$/CD25$^-$ cells were labeled with 2.5 μM CFSE and plated at a 10:1 ratio with MSCs in RPMI media with 10% FBS. Cells were either left unstimulated or stimulated with anti-CD3/CD28 Dynabeads (Invitrogen). All conditions were tested in duplicate. After 72 h, T cells were collected, stained with anti-mouse CD4-Pacific Blue (BD), and analyzed for CFSE staining by flow cytometry with a BD FACS Aria.

Statistical analysis

Microsoft Excel and SPSS were used for statistical analysis. Data were presented as mean ± standard error of the mean (SEM) and student *t*-test, or one-way ANOVA with *post-hoc* analysis (*Tukey HSD Test*) was used to assess the significance of the data. *P*-values <0.05 were considered statistically significant.

Results

Generation and characterization of transplantable cells

Oligodendroglial precursor cells (OPCs) allogeneic for the shiverer recipient were generated from a mouse ES cell line constitutively expressing enhanced green fluorescent protein (mESC-GFPs) as described in the Materials and Methods section. Gene expression changes were tracked along differentiation stages by quantitative real-time polymerase chain reaction (Fig. 1) and immunocytochemistry (Fig. 2) to characterize transplantable OPCs. Pluripotency of mESC-GFP cells was confirmed by expression of SSEA1 (Fig. 2A), absence of SSEA3, and expression of oct-4 mRNA (Fig. 1A). Differentiation of mESC-GFPs (stage 1) into neural precursor cells (stage 4) was associated with downregulation of oct-4 and upregulation of the neural stem cell-associated genes NCAM and nestin (Fig. 1A), as well as by protein expression of Nestin and Sox2 (Fig. 2B). Neural precursor cells (common stage) were further differentiated into OPCs whereupon OPC markers NG2, NKX2.2, and Olig1/2 were expressed at peak levels (Fig. 1B). At the protein level, OPCs lacked Nestin (Fig. 2C) and acquired expression of oligodendroglial lineage markers such as A2B5 (Fig. 2D), Olig1/2 (Fig. 2E), and NG2 (Fig. 2F). Terminal differentiation of OPCs proceeded through a pro-oligodendrocyte stage expressing O4

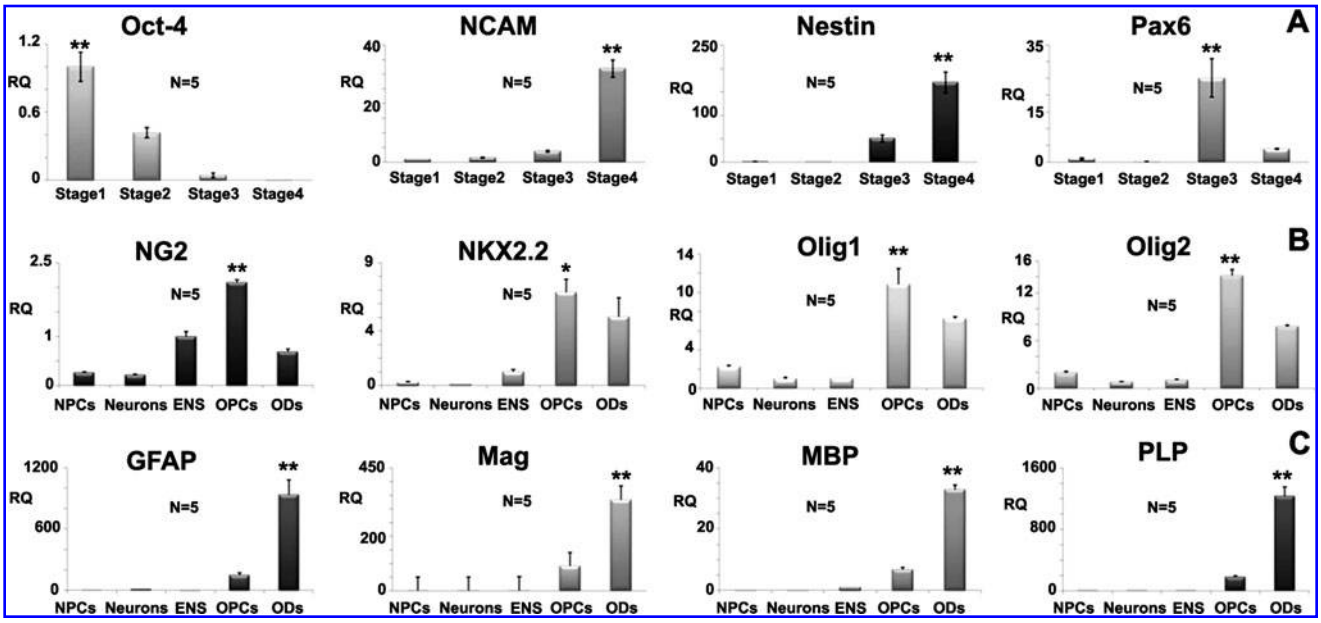

FIG. 1. Differentiation of mESC-GFPs into oligodendroglial cells: RNA level comparisons. **(A)** Gene expression changes of pluripotent (oct4) or neural stem cell (NSC)-associated genes (ncam, nestin, and pax6) along the differentiation of mESC-GFP (stage1) into NSC (stage 4); **(B)** expression changes of OPC genes (NG2, NKx2.2, olig1/2) from an early NSC stage (ENS) into either a neuronal (NPCs and Neurons)) or an oligodendroglial (OPCs and ODs) differentiation pathway; **(C)** expression changes of astrocytes (GFAP) and mature oligodendrocytes (MAG, MBP, and PLP) genes, into either a neuronal (NPCs and Neurons) or an oligodendroglial (OPCs and ODs) differentiation pathway. Stage 1 and ENS were taken as calibrator in **A,** and **B, C**, respectively. $^{**}P<0.01$ and $^{*}P<0.05$, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* **(A**: $P<0.01$ for Oct-4 stage1 vs. stage 2, 3, or 4; $P<0.01$ for NCAM and Nestin stage 4 vs. stage 1, 2 or 3; $P<0.01$ for Pax6 stage 3 vs. stage 1, 2 or 4. **B**: $P<0.01$ for NG2, Olig1, and Olig2 OPCs vs. NPCs, neurons, ENS, or ODs; $P<0.01$for NKX2.2 OPCs vs. NPCs, neurons, or ENS, and $P<0.05$ for NKX2.2 OPCs vs. ODs. **C**: $P<0.01$ for GFAP, MAG, MBP, and PLP ODs vs. NPCs, neurons, ENS, or OPCs**)**]. Error bars represent SEM. N = number of samples examined. mESC, mouse embryonic stem cell; GFP, green fluorescent protein; MBP, myelin basic protein; GFAP, glial fibrillary acid protein; NPCs, neuronal precursor cells; OPCs, oligodendrocyte progenitor cells; ODs, oligodendrocytes.

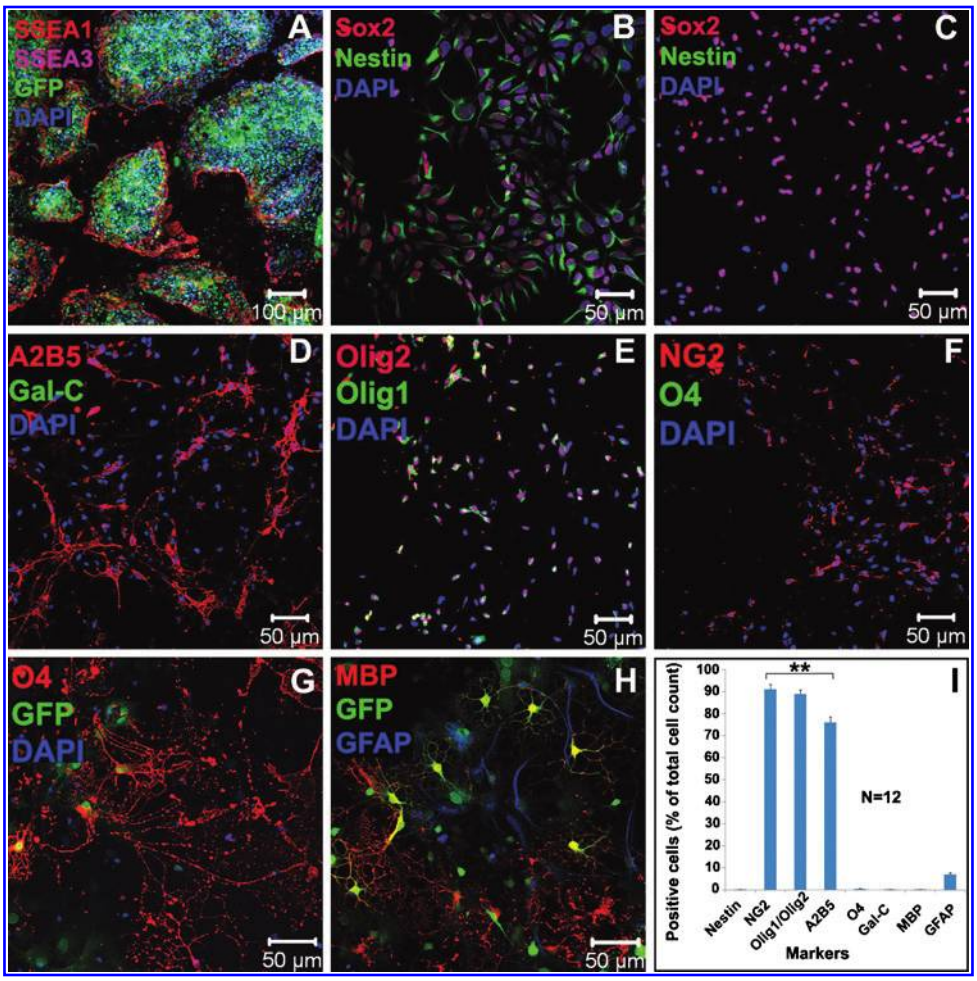

FIG. 2. Differentiation of mESC-GFPs into mature oligodendrocytes: protein expression. mESC-GFPs were characterized by immunocytochemistry during their differentiation into mature oligodendrocytes. **(A)** undifferentiated ES cells expressing SSEA1 but not SSEA3; **(B)** neural stem cells expressing nestin and sox2; **(C–F)** transplantable OPC population that lacks nestin expression **(C)**, and acquires the expression of oligodendroglial lineage markers A2B5 **(D)**, Olig1/2 **(E)**, and NG2 **(F)**, but not yet O4 **(F)** or Gal-C **(D)**; **(G)** prooligodrendrocytes expressing O4; **(H)** mature glia cells -oligodendrocytes (MBP) and astrocytes (GFAP); **(I)** Quantification of different markers during the OPC stage. $^{**}P < 0.01$, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* ($P < 0.01$ for NG2,olig1/olig2, or A2B5 vs. nestin, O4, Gal-C, MBP, or GFAP. No significant differences between NG2, olig1/olig2 and A2B5)]. Error bars represent SEM. N = number of samples examined.

(Fig. 2G) and into mature oligodendrocytes (MAG, MBP, and PLP) and astrocytes (GFAP) (Figs. 2H and 1C).

A single batch of highly pure OPCs, which are known to possess greater mitotic, migratory, and reparative properties compared with mature oligodendrocytes on transplantation into demyelinated [29,30] or hypomyelinated [31] hosts, was used for all the transplantation studies. Immunohistochemistry on the day of transplantation followed by semi-automated cell counts (Fig. 2I) revealed that the transplanted OPC population was primarily of oligodendroglial lineage and contained 91%±2.38% NG2, 89%±1.8% Olig1/Olig2, and 76%±2.5% A2B5positive cells. There were 7%±0.81% GFAP positive astrocytes and very few nestin, O4, Gal-C, or MBP positive cells, indicating that the cells were neither under-differentiated nor too mature for transplantation.

MSCs were derived from the bone marrow of syngeneic mice and expanded in vitro for several passages to eliminate nonmesenchymal cells. MSCs were characterized by immunocytochemistry (Fig. 3A) and FACS analysis (Fig. 3B) and were found to be positive for the MSC markers CD9, CD44, Sca1, and CD105 and virtually negative for CD11b or CD45. To facilitate their localization in vivo, MSCs were labeled with DiI before transplantation. A single batch of MSCs was used for all the transplantation experiments. Bright field pictures of cell types described in Figure 2 and 3 are shown in Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/scd.

MSCs enhance the survival and myelinating abilities of allogeneic oligodendrocytes in the brain of adult immunocompetent shiverer mice

To examine the effect of syngeneic MSCs on the survival and myelinating properties of allogeneic OPCs, a combination of these cell populations was injected into the corpus callosum of adult shiverer mice. The corpus callosum, the largest white matter tract in the brain, was chosen to facilitate the localization of transplanted cells and to easily visualize and quantify myelination in the dysmyelinated shiverer CNS. Except for the CSA control group, animals did not receive any immunosuppressive treatment before or after transplantation. Two weeks post-transplantation (Fig. 4A–H), allogeneic GFP^+ OPCs transplanted alone (Fig. 4C, G) or in combination with syngeneic MSCs (Fig. 4D, H) integrated into the host parenchyma and migrated along the corpus callosum, where they produced extensive myelination of the host axons, as visualized by MBP staining (Fig. 4) and luxol fast blue (Supplementary Fig. S2C). MBP signal was not found in animals that received MSCs alone (data not shown) or saline (Fig. 4B, F and Supplementary Fig. S2B). The number of myelinated axons, visualized by MBP/neurofilament

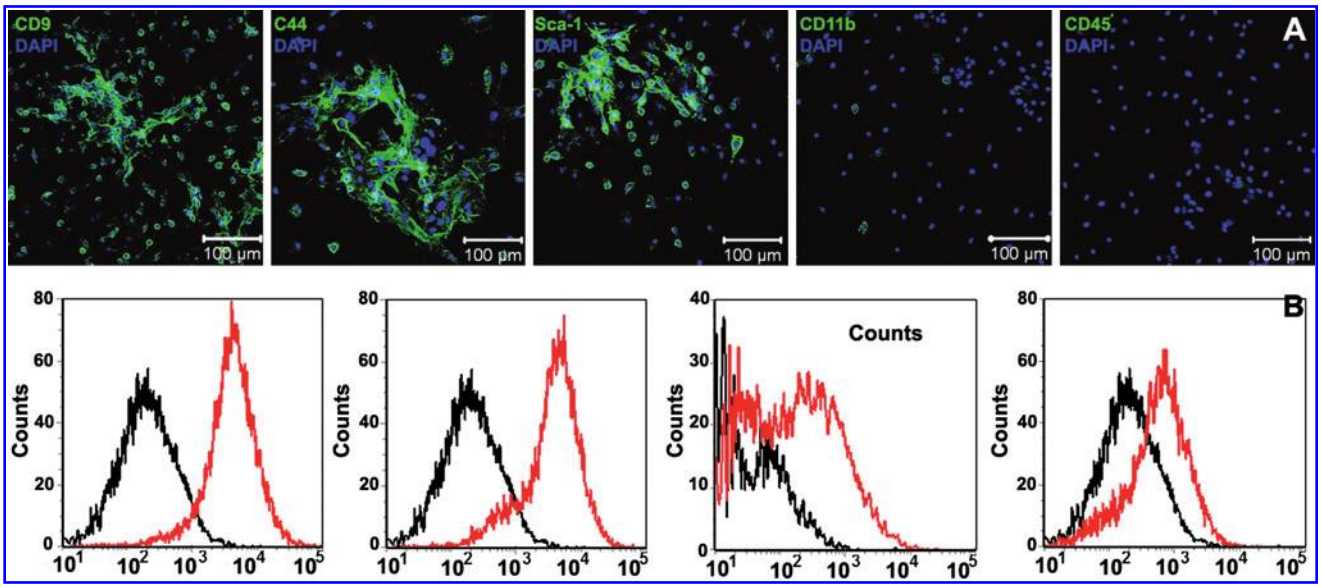

FIG. 3. In vitro characterization of bone marrow derived MSCs: MSCs were characterized by immunocytochemistry **(A)** and flow cytometry **(B)** before transplantation. *Black histograms* represent isotype controls, and *red histograms* represent antibody-specific shift in fluorescence. MSC, mesenchymal stem cell.

(NF) double staining, promoted by the allogeneic OPCs (Fig. 4C, G) was increased in the presence of syngeneic MSCs (Fig. 4D, H) by ~35% at 2 weeks, ~47.5% at 4 weeks, and, ~51% at 8 weeks post-transplantation (Fig. 4I). Similar results were found when the extent of corpus callosum myelination (rostral-caudal) was examined (Fig. 4J). In addition, the number of GFP+ cells was increased in the presence of MSCs at all time points examined, and the distribution of these cells in the corpus callosum resembled the MBP pattern (data not shown).

MSCs reduce inflammation and astrocytosis induced by allogeneic OPCs transplantation

It has been well established that MSCs are immunoprivileged and have immunosuppressive properties [18]. These functions may be of therapeutic value in allogeneic transplantation, because they could allow for a tolerant milieu and thereby decrease graft rejection [19]. To investigate whether MSC-mediated immune suppression was involved in the survival of allogeneic OPCs, we looked for evidence of inflammation-driven graft rejection in the brain of transplanted shiverer mice. We examined microglia activation by staining transplanted brains with Iba1 antibody at 2 weeks post-transplantation (Fig. 5). In the OPCs alone group, we found a massive infiltration of microglia cells (Iba1) throughout the corpus callosum (Fig. 5C). Microglia infiltration was decreased considerably in the OPCs+MSCs group (Fig. 5D), where the number of Iba1$^+$ cells was similar to animals that were immunosuppressed with cyclosporine-A and received OPCs alone (Fig. 5E).

These data indicate that the presence of allogeneic OPCs is required to induce a massive infiltration of microglia cells in the recipient's CNS and that such infiltration is strongly reduced by co-transplanted MSCs. To investigate whether the OPC-induced microglia infiltration was associated with astrogliosis, we stained transplanted brains with GFAP antibody (Fig. 5) and quantified the number of reactive astrocytes at 2 weeks post-transplantation (Fig. 5F). Similar to the effect seen in microglia data, the presence of MSCs strongly reduced astrocytosis in the brains of transplanted animals. Taken together, these data demonstrate that mouse MSCs possess anti-inflammatory properties in vivo which mitigate the host immune response to allogeneic engraftment of OPCs.

MSCs produce the anti-inflammatory cytokine TGF-β and reduce T-cell proliferation in vitro

To further characterize the immunosuppressive properties of our MSC preparation, we first performed an ELISA to measure TGF-β levels in vitro and found that cultured MSCs produced this anti-inflammatory cytokine (Fig. 6A). Further, we co-cultured splenocyte-derived T-cells from C3HeB/FeJ mice with syngeneic MSCs and found that the MSCs reduced activated T-cell proliferation in vitro (Fig. 6B).

MSCs promote oligodendrocyte maturation in vitro

Since the number of GFP+ cells and their induced myelination were significantly higher in the OPC+ MSC group compared with CSA control (data not shown), which displayed significantly higher values than untreated OPCs alone, we investigated whether trophic support, in addition to immunosuppression, was a mechanism by which MSCs modulate OPC properties. We cultured OPCs in the presence of MSCs or MSC-CM and measured the release of PDGF-AA, which is a mitogenic factor involved in maintenance of undifferentiated OPCs, and IGF-1, which promotes OPC maturation into myelinating oligodendrocytes. In addition, we quantified the amount of cellular NG2 and MBP as markers of OPC and mature oligodendrocytes, respectively. These data show that MSCs, either co-cultured with OPCs (Fig. 7A, C) or supplied as CM (Fig. 7B, D), produced IGF-1 (Fig. 7C,

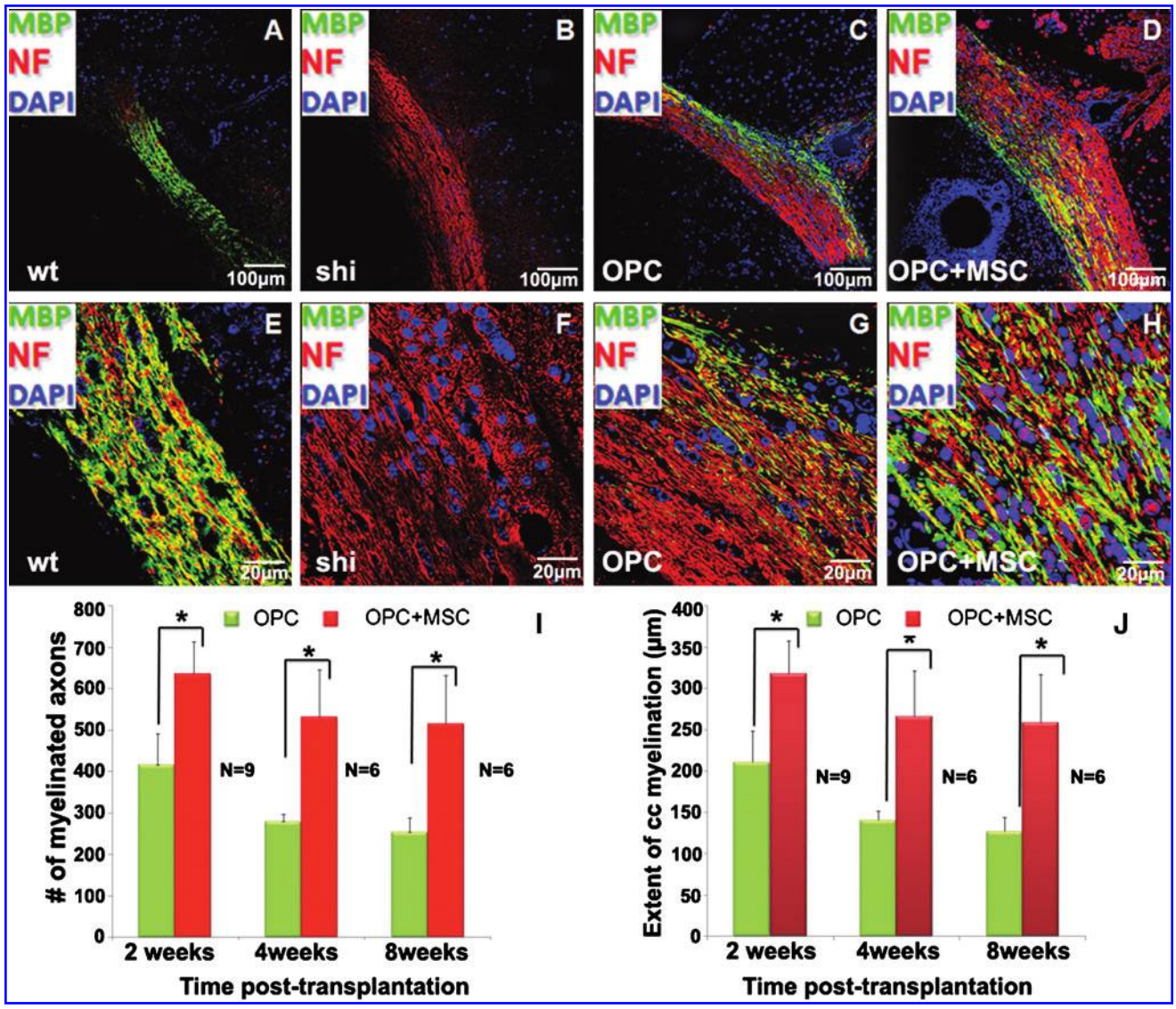

FIG. 4. MSCs increase OPC-induced myelination in the brain of adult shiverer mice: Axonal myelination visualized by double staining for MBP and neurofilament (NF). **(A, E)** wild-type control (wt); **(B, F)** shiverer mouse (shi) injected with saline; **(C, G)** shi injected with OPCs alone; **(D, H)** shi injected with OPCs + MSCs. Comparison of the number of myelinated axons **(I)** and the extent of corpus callosum myelination **(J)** between the OPC and OPC + MSC groups at different time points post-transplantation. At each time point examined, both the number of myelinated axons **(I)** and the extent of corpus callosum myelination **(J)** were increased when OPCs were co-injected with MSCs $^*P < 0.05$, student's *t*-test. Error bars represent SEM. N = number of animals examined.

D) and correlated with reduced OPC production of PDGF-AA (Fig. 7A, B). These findings correlated with a reduction of OPCs expressing NG2 (Fig. 7E) and an increase in mature oligodendrocytes expressing MBP protein (Fig. 7F) in response to CM from MSCs. OPC maturation into MBP expressing oligodendrocytes was inhibited by the application of function blocking antibody against IGF-1 (Supplementary Fig. S3). These results indicate that MSCs promote maturation of OPCs into myelinating oligodendrocytes through the release of trophic factors.

Discussion

Neural regeneration and repair strategies are an intense area of research, because almost all neurological disorders at present are associated with irreversible damage. For conditions such as traumatic brain and spinal cord injury, Alzheimer's disease, cerebral palsy, amyotrophic lateral sclerosis, Parkinson's disease, and progressive MS, there are currently limited or no regenerative therapeutic options. A number of research avenues are under investigation, including the use of growth factors, [32,33], excitatory neural stimulation [34,35], and hyperbaric oxygen therapy [36,37], but the application of stem cell biology in its broadest sense represents the most promising approach. The versatility and the prolific proprieties of ES cells make them an attractive candidate for a starting point in repair strategies. However, therapeutic use of ES cells is limited by ethical issues and concerns over tumor formation and tissue rejection in recipients. By comparison, syngeneic bone marrow derived

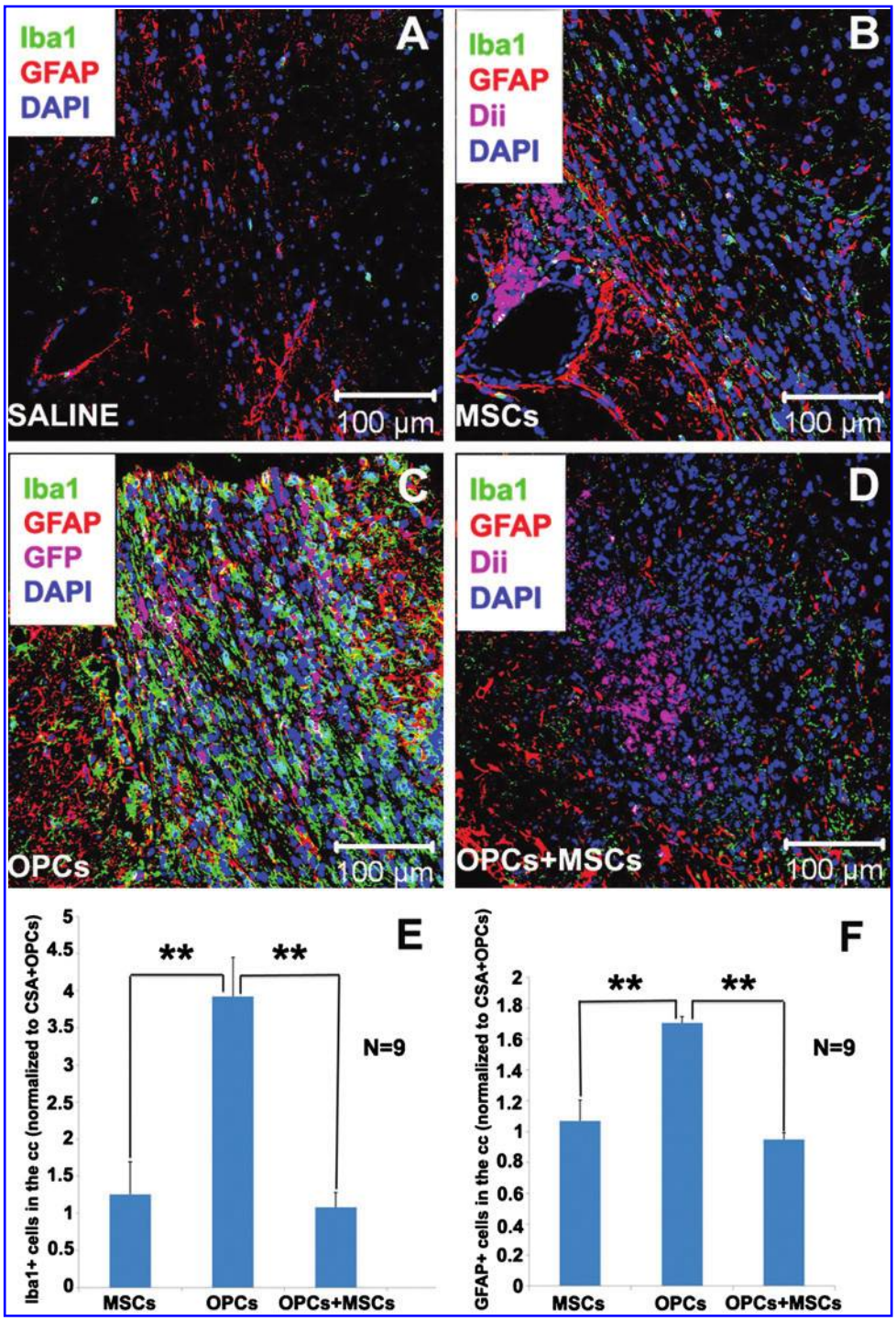

FIG. 5. MSCs reduce inflammation and astrocytosis induced by allogeneic OPC transplantation: Immunostaining for microglia ($Iba1^+$) and reactive astrocytes ($GFAP^+$), at 2 weeks post-transplantation in **(A)** saline control, **(B)** MSCs alone, **(C)** OPCs alone; and **(D)** OPCs + MSCs group. GFP and DiI positive cells represent OPCs and MSCs, respectively. **(E, F)** Quantification of microglia/macrophage **(E)** and reactive astrocytes **(F)** after stem cell treatments compared with OPC + cyclosporine-A (CSA) control. $^{**}P < 0.01$, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* ($P < 0.01$ for MSCs vs. OPCs; MSCs vs. OPCs + MSCs nonsignificant; $P < 0.01$ for OPCs vs. OPCs + MSCs)]. Error bars represent the SEM. N = number of animals examined.

MSCs are less likely to result in tumor formation. Although tissue rejection is not an issue with MSCs, their restricted differentiation potential limits their use as source of transplantable cells for neurological disease. It has been established that MSCs have immunosuppressive qualities and, in addition, these cells are home to areas of tissue damage and inflammation [18,19,38]. The goal of our study was to investigate the feasibility of using ES cells for their reparative potential and moderating the risk of rejection by concomitant use of MSCs.

We studied the efficacy of combinatory stem cell delivery to promote myelination in dysmyelinated shiverer mice. We transplanted highly pure embryonic-derived OPCs—allogeneic for the recipient—with or without syngeneic MSCs into the corpus callosum of adult immunocompetent animals. Using this strategy, we were able to show that MSCs increase the survival, migration, and myelinating ability of ESC-derived OPCs in a dysmyelinated CNS environment. Our data suggest that adjunctive treatment with MSCs allows for prolonged allogeneic OPC engraftment and enhanced myelination by a bystander mechanism: (1) MSCs mitigate the host's immune response to allogeneic cells (microglia and astrocytosis reduction) by releasing anti-

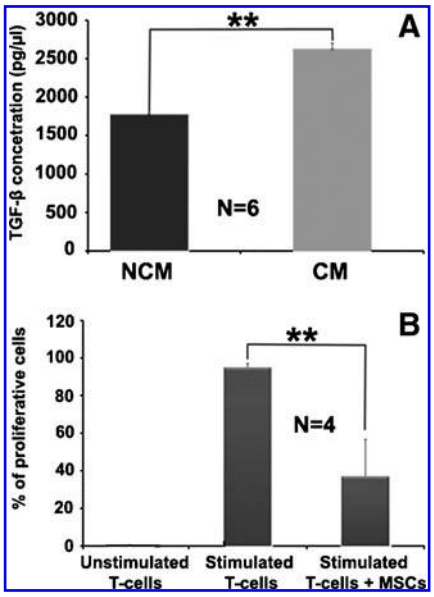

FIG. 6. MSCs produce the anti-inflammatory cytokine TGF-β and reduce T-cells proliferation in vitro. **(A)** ELISA to measure TGF-β levels in MSCs conditioned media (CM) and nonconditioned media (NCM). TGF-β levels are increased in CM; **(B)** T-cell proliferation assay with or without MSCs. T-cell proliferation is diminished by co-cultured MSCs. $^{**}P < 0.01$, student's *t*-test. Error bars represent SEM. N = number of samples examined. TGF-β, transforming growth factor beta.

inflammatory cytokines such as TGF-β; (2) MSCs provide trophic support by releasing growth factors such as IGF-1, which, in turn, promote the maturation of OPCs into myelinating oligodendrocytes.

Several groups have reported the capacity of ESC-derived OPCs to promote myelination in the dysmyelinated shiverer CNS using allogeneic [39] or xenograft [40] transplantation paradigms. However, these studies relied on the use of neonatal mice and/or immunosuppressants (like cyclosporine-A) to prevent graft rejection. The use of conventional immunosuppressants is problematic due to their inherent toxicity and because they may not be highly effective in the CNS due to lack of blood brain barrier penetration [41]. In this study, we have shown for the first time that prolonged allograft survival and enhanced CNS myelination can be achieved in adult animals without the use of immunosuppressant drugs, by combining the myelinating abilities of ESC-OPCs with the immunomodulatory and trophic support properties of adult stem cells such as MSCs. Thus, at sites of demyelination, the MSCs may create a local immune-tolerant and growth-factor-enriched environment enabling ESC-OPC engraftment and maturation and allowing for reparative myelination without the need for systemic immunosuppression.

Our data are in accordance with previous studies which have shown that functional recovery exerted by MSCs in animal models of CNS diseases are likely mediated through trophic [42] and immunosuppressive [18] mechanisms, whereby MSCs secrete bioactive factors that influence repair

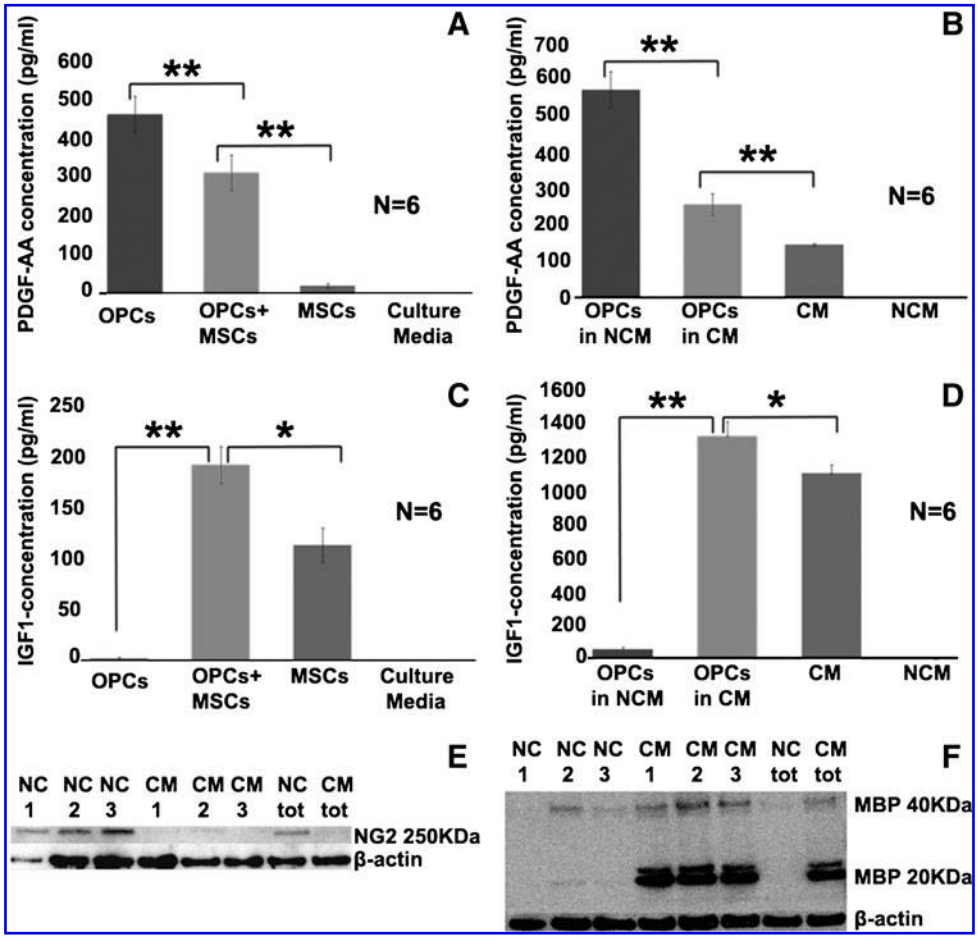

FIG. 7. MSCs promote oligodendrocyte maturation in vitro. **(A–D)** ELISA to measure PDGF-AA **(A, B)** or IGF-1 **(C, D)** levels in co-cultured **(A, C)** or condition media **(B, D)** experiments. **(E, F)** Western blot on OPCs cultured with MSC conditioned (CM) or nonconditioned (NC) media to measure NG2 **(E)** and MBP **(F)** levels. $^{*}P < 0.05$, $^{**}P < 0.01$, one-way ANOVA [post-hoc analysis: *Tukey HSD Test* **(A**: $P < .001$ for: OPCs vs. OPCs + MSCs; OPCs + MSCs vs. MSCs; OPCs vs. MSCs. **B**: $P < 0.01$ for: OPCs in NCM vs. OPCs in CM; OPCs in CM vs. CM; OPCs in NCM vs. CM. **C**: $P < 0.01$ for OPCs vs. OPCs + MSCs; OPCs vs. MSCs. $P < 0.05$ for OPCs + MSCs vs. MSCs. **D**: $P < 0.01$ for: OPCs in NCM vs. OPCs in CM; OPCs in NCM vs. CM. $P < 0.05$ for OPCs in CM vs. CM**)**]. Error bars represent the SEM. N = number of samples examined. PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor 1.

and regeneration in surrounding tissue. The immune-regulatory effects of MSCs have been shown to be mediated through a number of different cytokines including TGF-β, hepatocyte growth factor, indoleamine 2,3-ioxygenase, and interleukin 6 [18]. Further, MSCs produce trophic factors such as brain-derived neurotrophic factor [43], ciliary neurotrophic factor [44], and IGF-1 [45] and were recently found to induce neurogenic and oligodendrogenic fate and differentiation [46–48]. Moreover, several studies have shown that oligodendrocytes also secrete a variety of growth factors including brain-derived neurotrophic factor (BDNF), midkine, hepatocyte growth factor, TGF-β, and glial cell line-derived neurotrophic factor [49–51]. Collectively, these factors might modulate inflammation and promote allograft cell survival, as well as migration and differentiation within the shiverer CNS, ultimately resulting in enhanced myelination of dysmyelinated tissue. In addition, this synergistic effect may stimulate resident neural stem/progenitors cells to become first OPCs and then mature oligodendrocytes, which could support the transplanted cells in overcoming the obstacles to remyelination in MS where although neural stem/progenitors cells and OPCs are present around MS plaques, migration of OPCs and differentiation/maturation into myelinating cells are impaired [52,53].

Conclusions

The potential of exogenous stem cell or progenitor cell transplantation as novel therapeutic strategies to address unmet medical needs is a vast and important area of investigation. In this study, we reported the first combined use of embryonic-derived and adult stem cells to promote myelination in a dysmyelinated CNS. We showed that the survival and myelinating abilities of allogeneic ESC-OPCs transplanted in the brain of adult shiverer mice can be increased by adjunctive treatment with autologous MSCs instead of conventional immunosuppressants. Our findings represent a first step toward the development of novel therapeutics for the treatment of patients with disability associated with demyelinating disease. Use of this combinatorial stem cell strategy should next be studied in a disease model that has widespread inflammatory demyelination akin to MS, such as experimental allergic encephalomyelitis. It would be of interest to note whether the OPC-MSC combination has a dual effect on suppressing inflammation and ameliorating disease as well as on remyelination. In addition, multiple routes of cell administration (intrathecal, intravenous, and intracerebral) should be explored in experimental allergic encephalomyelitis to establish the best approach to administer stem cell therapy in demyelinating diseases. This is particularly important in MS where a systemic (eg, intravenous, intrathecal) transplantation of stem cells might be therapeutically efficacious owing to the ability of transplanted cells to follow, via the blood stream or cerebrospinal fluid circulation, a gradient of chemoattractants (eg, proinflammatory cytokines and chemokines) occurring at the site of inflammatory lesions [54,55]. We hope that by utilizing innovative strategies such as those we have described, the differentiation potential of ESCs seen experimentally may be exploited with safety in clinical disease.

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Author Disclosure Statement

The authors have nothing to disclose.

References

1. Noseworthy JH, C Lucchinetti, M Rodriguez and BG Weinshenker. (2000). Multiple sclerosis. N Engl J Med 343:938–952.
2. Gironi M, A Bergami, E Brambilla, F Ruffini, R Furlan, G Comi and G Martino. (2000). Immunological markers in multiple sclerosis. Neurol Sci 21:S871–S875.
3. Hemmer B, S Cepok, S Nessler and N Sommer. (2002). Pathogenesis of multiple sclerosis: an update on immunology. Curr Opin Neurol 15:227–231.
4. Lassmann H. (2002). Mechanisms of demyelination and tissue destruction in multiple sclerosis. Clin Neurol Neurosurg 104:168–171.
5. Franklin RJ. (2002). Why does remyelination fail in multiple sclerosis? Nat Rev Neurosci 3:705–714.
6. Kornek B, MK Storch, R Weissert, E Wallstroem, A Stefferl, T Olsson, C Linington, M Schmidbauer and H Lassmann. (2000). Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal injury in active, inactive, and remyelinated lesions. Am J Pathol 157:267–276.
7. Trapp BD, J Peterson, RM Ransohoff, R Rudick, S Mork and L Bo. (1998). Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338:278–285.
8. Ferguson B, MK Matyszak, MM Esiri and VH Perry. (1997). Axonal damage in acute multiple sclerosis lesions. Brain 120 (Pt 3):393–399.
9. Lovas G, N Szilagyi, K Majtenyi, M Palkovits and S Komoly. (2000). Axonal changes in chronic demyelinated cervical spinal cord plaques. Brain 123 (Pt 2):308–317.
10. Aktas O, B Kieseier and HP Hartung. (2010). Neuroprotection, regeneration and immunomodulation: broadening the therapeutic repertoire in multiple sclerosis. Trends Neurosci 33:140–152.
11. Pluchino S and G Martino. (2008). The therapeutic plasticity of neural stem/precursor cells in multiple sclerosis. J Neurol Sci 265:105–110.
12. Pluchino S, L Zanotti, E Brini, S Ferrari and G Martino. (2009). Regeneration and repair in multiple sclerosis: the role of cell transplantation. Neurosci Lett 456:101–106.
13. Goldman S. (2005). Stem and progenitor cell-based therapy of the human central nervous system. Nat Biotechnol 23:862–871.
14. Keyoung HM and SA Goldman. (2007). Glial progenitor-based repair of demyelinating neurological diseases. Neurosurg Clin N Am 18:93–104, x.
15. Tambuyzer BR, I Bergwerf, N De Vocht, K Reekmans, J Daans, PG Jorens, H Goossens, DK Ysebaert, S Chatterjee, E Van Marck, ZN Berneman and P Ponsaerts. (2009). Allogeneic stromal cell implantation in brain tissue leads to robust microglial activation. Immunol Cell Biol 87:267–273.
16. Wu DC, AS Boyd and KJ Wood. (2008). Embryonic stem cells and their differentiated derivatives have a fragile immune privilege but still represent novel targets of immune attack. Stem Cells 26:1939–1950.
17. Tepavcevic V and WF Blakemore. (2005). Glial grafting for demyelinating disease. Philos Trans R Soc Lond B Biol Sci 360:1775–1795.
18. Nauta AJ and WE Fibbe. (2007). Immunomodulatory properties of mesenchymal stromal cells. Blood 110:3499–3506.

19. English K, A French and KJ Wood. (2010). Mesenchymal stromal cells: facilitators of successful transplantation? Cell Stem Cell 7:431–442.
20. Privat A, C Jacque, JM Bourre, P Dupouey and N Baumann. (1979). Absence of the major dense line in myelin of the mutant mouse "shiverer". Neurosci Lett 12:107–112.
21. Roach A, K Boylan, S Horvath, SB Prusiner and LE Hood. (1983). Characterization of cloned cDNA representing rat myelin basic protein: absence of expression in brain of shiverer mutant mice. Cell 34:799–806.
22. Gumpel M, N Baumann, M Raoul and C Jacque. (1983). Survival and differentiation of oligodendrocytes from neural tissue transplanted into new-born mouse brain. Neurosci Lett 37:307–311.
23. Gumpel M, O Gout, C Lubetzki, A Gansmuller and N Baumann. (1989). Myelination and remyelination in the central nervous system by transplanted oligodendrocytes using the shiverer model. Discussion on the remyelinating cell population in adult mammals. Dev Neurosci 11:132–139.
24. Windrem MS, MC Nunes, WK Rashbaum, TH Schwartz, RA Goodman, G McKhann, 2nd, NS Roy and SA Goldman. (2004). Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. Nat Med 10:93–97.
25. Windrem MS, SJ Schanz, M Guo, GF Tian, V Washco, N Stanwood, M Rasband, NS Roy, M Nedergaard, LA Havton, S Wang and SA Goldman. (2008). Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. Cell Stem Cell 2:553–565.
26. Okabe M, M Ikawa, K Kominami, T Nakanishi and Y Nishimune. (1997). "Green mice" as a source of ubiquitous green cells. FEBS Lett 407:313–319.
27. Abbondanzo SJ, I Gadi and CL Stewart. (1993). Derivation of embryonic stem cell lines. Methods Enzymol 225:803–823.
28. Brustle O, KN Jones, RD Learish, K Karram, K Choudhary, OD Wiestler, ID Duncan and RD McKay. (1999). Embryonic stem cell-derived glial precursors: a source of myelinating transplants. Science 285:754–756.
29. Groves AK, SC Barnett, RJ Franklin, AJ Crang, M Mayer, WF Blakemore and M Noble. (1993). Repair of demyelinated lesions by transplantation of purified O-2A progenitor cells. Nature 362:453–455.
30. Blakemore WF, JM Gilson and AJ Crang. (2000). Transplanted glial cells migrate over a greater distance and remyelinate demyelinated lesions more rapidly than endogenous remyelinating cells. J Neurosci Res 61:288–294.
31. Warrington AE, E Barbarese and SE Pfeiffer. (1993). Differential myelinogenic capacity of specific developmental stages of the oligodendrocyte lineage upon transplantation into hypomyelinating hosts. J Neurosci Res 34:1–13.
32. Schulte-Herbruggen O, A Braun, S Rochlitzer, MC Jockers-Scherubl and R Hellweg. (2007). Neurotrophic factors—a tool for therapeutic strategies in neurological, neuropsychiatric and neuroimmunological diseases? Curr Med Chem 14:2318–2329.
33. Zuccato C and E Cattaneo. (2009). Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 5:311–322.
34. Iwata NK and Y Ugawa. (2005). The effects of cerebellar stimulation on the motor cortical excitability in neurological disorders: a review. Cerebellum 4:218–223.
35. Thickbroom GW. (2007). Transcranial magnetic stimulation and synaptic plasticity: experimental framework and human models. Exp Brain Res 180:583–593.
36. Steele J, D Zutshi and WG Bradley. (2007). Negative results of a phase II study of hyperbaric oxygen therapy for amyotrophic lateral sclerosis. Amyotroph Lateral Scler 8:274–275.
37. Dave KR, R Prado, R Busto, AP Raval, WG Bradley, D Torbati and MA Perez-Pinzon. (2003). Hyperbaric oxygen therapy protects against mitochondrial dysfunction and delays onset of motor neuron disease in Wobbler mice. Neuroscience 120:113–120.
38. Chamberlain G, J Fox, B Ashton and J Middleton. (2007). Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 25:2739–2749.
39. Liu S, Y Qu, TJ Stewart, MJ Howard, S Chakrabortty, TF Holekamp and JW McDonald. (2000). Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. Proc Natl Acad Sci USA 97:6126–6131.
40. Nistor GI, MO Totoiu, N Haque, MK Carpenter and HS Keirstead. (2005). Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 49:385–396.
41. Uchino H, E Elmer, K Uchino, PA Li, QP He, ML Smith and BK Siesjo. (1998). Amelioration by cyclosporin A of brain damage in transient forebrain ischemia in the rat. Brain Res 812:216–226.
42. Caplan AI and JE Dennis. (2006). Mesenchymal stem cells as trophic mediators. J Cell Biochem 98:1076–1084.
43. Neuhuber B, B Timothy Himes, JS Shumsky, G Gallo and I Fischer. (2005). Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. Brain Res 1035: 73–85.
44. Wislet-Gendebien S, F Bruyere, G Hans, P Leprince, G Moonen and B Rogister. (2004). Nestin-positive mesenchymal stem cells favour the astroglial lineage in neural progenitors and stem cells by releasing active BMP4. BMC Neurosci 5:33.
45. Abboud SL, CR Bethel and DC Aron. (1991). Secretion of insulin like growth factor I and insulin like growth factor-binding proteins by murine bone marrow stromal cells. J Clin Invest 88:470–475.
46. Bai L, A Caplan, D Lennon and RH Miller. (2007). Human mesenchymal stem cells signals regulate neural stem cell fate. Neurochem Res 32:353–362.
47. Rivera FJ, S Couillard-Despres, X Pedre, S Ploetz, M Caioni, C Lois, U Bogdahn and L Aigner. (2006). Mesenchymal stem cells instruct oligodendrogenic fate decision on adult neural stem cells. Stem Cells 24:2209–2219.
48. Rivera FJ, FA Siebzehnrubl, M Kandasamy, S Couillard-Despres, M Caioni, AM Poehler, B Berninger, B Sandner, U Bogdahn, M Goetz, I Bluemcke, N Weidner and L Aigner. (2009). Mesenchymal stem cells promote oligodendroglial differentiation in hippocampal slice cultures. Cell Physiol Biochem 24:317–324.
49. Du Y and CF Dreyfus. (2002). Oligodendrocytes as providers of growth factors. J Neurosci Res 68:647–654.
50. Wilkins A, H Majed, R Layfield, A Compston and S Chandran. (2003). Oligodendrocytes promote neuronal survival and axonal length by distinct intracellular mechanisms: a novel role for oligodendrocyte-derived glial cell line-derived neurotrophic factor. J Neurosci 23:4967–4974.
51. Zhang YW, J Denham and RS Thies. (2006). Oligodendrocyte progenitor cells derived from human embryonic

stem cells express neurotrophic factors. Stem Cells Dev 15:943–952.
52. Williams A, G Piaton, MS Aigrot, A Belhadi, M Theaudin, F Petermann, JL Thomas, B Zalc and C Lubetzki. (2007). Semaphorin 3A and 3F: key players in myelin repair in multiple sclerosis? Brain 130:2554–2565.
53. Rivera FJ, C Steffenhagen, D Kremer, M Kandasamy, B Sandner, S Couillard-Despres, N Weidner, P Kury and L Aigner. (2010). Deciphering the oligodendrogenic program of neural progenitors: cell intrinsic and extrinsic regulators. Stem Cells Dev 19:595–606.
54. Herrmann MM, S Gaertner, C Stadelmann, J van den Brandt, R Boscke, W Budach, HM Reichardt and R Weissert. (2005). Tolerance induction by bone marrow transplantation in a multiple sclerosis model. Blood 106:1875–1883.
55. Martino G and S Pluchino. (2006). The therapeutic potential of neural stem cells. Nat Rev Neurosci 7:395–406.

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