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Transplantation of Placenta-Derived Mesenchymal Stem Cells in the EAE Mouse Model of MS

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Abstract Stem cell-based regenerative medicine raises great hope for the treatment of multiple sclerosis (MS). Bone marrow-derived mesenchymal stem cells (BM-MSCs) are being tested in clinical trials. Bone marrow is the traditional source of human MSCs, but human term placenta appears to be an excellent alternative because of its availability, without ethical issues. In this study, the therapeutic effect of human placental MSCs (PL-MSCs) was evaluated in experimental autoimmune encephalomyelitis (EAE), the mice model of MS. EAE mice were transplanted intracerebrally with PL-MSCs or with the vehicle saline 5 or 10 days after first MOG injection. The mice were monitored for a month after therapy. A daily EAE score revealed a decrease in disease severity in the transplanted animals when compared to saline. Survival was significantly higher in the transplanted animals. In vitro experiments demonstrated that conditioned media from LPS-activated astrocytes stimulated PL-MSCs to express the gene TNF-α-stimulated gene/protein 6 (TSG-6). The same mRNA expression was obtained when PL-MSCs were exposed to TNF-α or IL1-β. These results demonstrate that PL-MSCs have a therapeutic effect in the EAE mice model. We assume that this effect is caused by reduction of the anti-inflammatory protein, TSG-6, of the inflammatory damage.

Keywords Multiple sclerosis (MS) · Experimental autoimmune encephalomyelitis (EAE) · Human placenta-derived MSCs (PL-MSCs) · TNF-α-stimulated gene/protein 6 (TSG-6)

Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease of the central nervous system. It is a chronic, relapsing remitting disease characterized by patchy perivenular inflammatory infiltrates in areas of demyelination and axonal loss (Noseworthy et al. 2000).

MS is one of the most common causes of neurological disability affecting young adults. The common treatment for MS is based on immunomodulation, mostly interferon-β or glatiramer acetate (Hemmer et al. 2006). Although partially effective in symptomatic alleviation, the current immunomodulating drugs and any other available treatments do not halt the ongoing progression of neurodegeneration.

Experimental autoimmune encephalomyelitis (EAE) is the most common animal model for MS, via T cell-mediated inflammation-induced demyelination and axonal damage. The disease is generally induced by immunization with myelin antigens such as myelin oligodendrocyte glycoprotein (MOG) or myelin basic protein (MBP) and adjuvant, resulting in a CD4+ T helper-1 cell response that attacks the myelinated areas of the CNS (Whitha et al. 1991).

Previous reports have shown the potential of cell-based therapy for EAE through transplantation of oligodendrocytes

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or neural stem cells (Pluchino et al. 2003; Einstein et al. 2007; Pluchino et al. 2005; Archer et al. 1997). These in vivo experiments demonstrated an improvement in disease symptoms following intravenous (i.v.), intraperitoneal, or intracerebral administration of these cells in the EAE mice (Kassis et al. 2008; Zhang et al. 2005; Lianhua et al. 2009; Gordon et al. 2008; Barhum et al. 2010).

Most of the studies focused on mesenchymal stem cells which represent a relatively rare cell population that resides primarily in the bone marrow but can be isolated also from other adult and fetal tissues including adipose tissue, umbilical cord blood, fetal lung, and placenta (Pittenger et al. 1999; Deans and Moseley 2000; Gronthos et al. 2001; In 't Anker et al. 2004; Erices et al. 2000; Lu et al. 2006; Zheng et al. 2009). These cells can self-renew and differentiate into multilineage cells and can secrete several cytokines, growth factors, and extracellular matrix molecules (Lu et al. 2006; Chen et al. 2010; Wang et al. 2010; Liao et al. 2009; Zhao et al. 2009; Wu et al. 2009).

Human placenta has been considered as one ideal source for MSCs due to its accessibility, lack of ethical conflicts, painless procedures endured by donors, and abundant maternal and fetal mesenchymal stem cells (MSCs) it contains (Brooke et al. 2009). In accordance to all the aforementioned, we tested the therapeutic efficiency of human placental MSC (PL-MSC) transplantation to the cerebral ventricles of EAE mice and we investigated for the possible mechanism by which PL-MSCs influence disease severity.

Materials and Methods

Isolation and Culture of Human Placental MSCs

Placental MSCs were extracted from the placenta as previously described (Yust-Katz et al. 2012). The placentas of five healthy women after Caesarean section were collected and processed following their written informed consent. Several pieces of tissue closest to the umbilical cord were dissected and placed in a Petri dish. The minced tissue was transferred to a 50-ml centrifuge tube containing Hank's balanced salt solution (HBSS) (Biological Industries, Beit Haemek, Israel)+0.1 % bovine serum albumin (BSA) (Sigma-Aldrich, St. Louis, MO, USA) and digested for 45 min at 37°C with a combination of 0.1 % collagenase type IV (Sigma-Aldrich, St. Louis, MO, USA), 200 μg/ml DNAse I (Worthington, Lakewood, NJ, USA), and 0.1 % dispase (Invitrogen Carlsbad, CA, USA). The tissue was triturated every 10 min using 10-ml plastic serological pipettes. The slurry was successively filtered through a 100-μm Nytex mesh (BD Falcon, MD, USA) placed in a standard laboratory funnel, and the volume was adjusted to 35 ml HBSS (Biological Industries, Beit Haemek, Israel). Thereafter, 15 ml of Histopaque (Sigma-Aldrich, St. Louis, MO, USA) was added to the centrifuge tube and layered with the mixture using a 9-in. glass Pasteur pipette. The tube was centrifuged for 20 min at 800*g*. The cell layer was recovered and diluted with HBSS (Biological Industries, Beit Haemek, Israel) containing 0.1 % BSA (Sigma-Aldrich, St. Louis, MO, USA) to a final volume of 50 ml, and recentrifuged at 200×*g*. The cell pellet was then resuspended twice. The cell suspension was plated on dishes coated with human placental fibronectin (100 ng/ml; Sigma-Aldrich, St. Louis, MO, USA) at a density of 1×10^6 cells/80 cm^2 (i.e., 1×10-cm dish) in Knockout DMEM (Invitrogen, Carlsbad, CA, USA) containing 10 % fetal bovine serum (FBS) (Biological Industries, Beit Haemek, Israel), 1:100 nonessential amino acids (Biological Industries, Beit Haemek, Israel), 55 μM beta-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA), 10 μg/ml ciprofloxacin (Bayer, Leverkusen, Germany), and 10 μg/ml amphotericin B (Invitrogen, Carlsbad, CA, USA).

Animals

Six- to eight-week-old C3H.SW or C57bl female mice weighing 20 g were housed in standard conditions: constant temperature (22±1°C), humidity (relative, 40 %), and a 12-h light/dark cycle, and were allowed free access to food and water. The animals and protocol procedures were approved and supervised by the Animal Care Committee at the Tel Aviv University.

Induction of Chronic EAE and PL-MSC Transplantation

Chronic EAE was induced according to previously described procedures (Hunter et al. 1985). In brief, female C3H.SW or C57bl mice (6–8 weeks old) were immunized twice, at day 1 and day 8, by subcutaneous injection with an emulsion-containing MOG (pMOG35–55 3.7 mg/kg or 15 mg/kg respectively) in complete Freund's adjuvant (CFA) containing 200 μg heat-activated *Mycobacterium tuberculosis* in a total volume of 0.2 ml. The C57bl female mice were also injected with pertusis toxin (15 μg/kg) at day 0 and day 2. Five or 14 days post-EAE induction, mice received bilateral intracerebroventricular (ICV) injections of 2×10^5 MSCs or phosphate-buffered saline (PBS) in a volume of 2 μl using a Hamilton 10-μl syringe with a 26-gauge needle. The coordinates of the injections were as follows: AP +0.6 mm, ML ±0.7 mm, and V −3 mm, from bregma based on the mouse stereotaxic atlas (Paxinos and Watson).

Mice were examined daily for clinical disease severity daily by using a score scale: 0, no disease signs; 1, loss of tail tonicity; 2, mild hindlimb weakness; 3, complete hindlimb paralysis; 4, paralysis of four limbs; 5, moribund; and

6, death. Mild disease severity was defined as a clinical score of 2; moderate disease severity was defined as a clinical score of 3; and severe disease was defined as a score of 4.

Three controls and three MOG-induced EAE mice were sacrificed on day 10 and their spleens were removed in order to prepare splenocyte primary cultures. Brain and spinal cords were excised on day 30 and were stored in paraformaldeyhde for 24 h followed by 30 % sucrose for 24 h and then frozen in isomethylbutane and were kept in −70°C for histology.

Splenocyte Viability and Proliferation Assay

The proliferation response of spleen cells was tested 10 days after the first MOG injection and 5 days post-cell transplantation or sham operation. Three animals from each group were sacrificed by cervical dislocation; the spleens were removed and mechanically dissociated. Splenocytes were washed twice with PBS and placed for 1 h in RPMI 1640 medium supplemented with 2 mM glutamine, 50 μM 2-mercaptoethanol, antibiotics (100 U/ml penicillin G, 100 μg/ml streptomycin), and 10 % heat-inactivated fetal calf serum (all from Biological Industries) in 37°C. Splenocytes were then counted and plated in a concentration of 3×10^5 cells/well in the PL-MSCs' conditioned medium (DMEM incubated for 24 h with the placenta cells) or in fresh DMEM. Subsequently, MOG peptide (2 μg/well) was added in quadruplicate wells. The cells were incubated for 72 h at 37°C in humidified air containing 5 % CO_2. ^{3}H thymidine (1 μCi/well) was added for the last 16 h of incubation, and the cultures were then harvested and counted using a Matrix 96 Direct beta counter (Packard Instr., Meriden, CT, USA). The proliferative response was measured using ^{3}H thymidine incorporation expressed as mean counts per minute recorded in quadruplicate wells.

Astrocyte Cell Culture

Astrocyte primary culture was extracted from the cortex of newborn mice as previously described (Pehar et al. 2006) with small changes. Briefly, the cortex tissue was dissected from newborn mice brains (p1–p3) and was transferred to a Petri dish. All the meninges were pulled off. Pure tissue was transferred to a tube containing cold PBS. The brain mechanically dissociated and digested by incubation with 1/5 V/V trypsin for 10 min at 37°C. The reaction was stopped by the addition of complete medium (DMEM, 10 % serum, SPN, and glutamine) and 50 μl DNAse. The tissue was then mechanically digested by pipetation few more times until full dissociation into single cells was accomplished. The cells were washed with complete medium once and centrifuged at 1,100 rpm for 7 min. Supernatant was removed and the pellet was resuspended in a complete medium. Cells (1×10^6) were plated in T75 flask in 10-ml complete medium. The flasks were incubated at 37°C in 5 % CO_2. The medium was first changed after 24 h and then every 3 days until confluence is achieved (after approximately 8–10 days). Once the primary culture was confluent, it was placed on a shaker platform horizontally with medium covering the cells, sharked at 250 rpm for 18 h, and the medium that contained microglia was removed. After a few hours, the cells were trypsinized and replated to two new flasks. The astrocyte cells, from passage one, were exposed to 1 μg/ml LPS in serum-free medium (DMEM+SPN+glutamine) for 48 h; this conditioned medium was collected and were used to treat PL-MSCs.

RNA Isolation and Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from the placenta MSCs that were exposed to 1,000 pg/ml TNF-α or 100 pg/ml IL-1β or conditioned media of astrocytes, which were exposed previously to 1 μg/ml LPS (48 h), using the TRI reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions. RNA was quantified by a spectrophotometer. RT-PCR was carried out on 0.5 μg RNA samples using the 10 U enzyme RT-superscript II (Invitrogen, Carlsbad, CA, USA) in a mixture containing 1.3 μM oligo-dT12-18 (Sigma-Aldrich, St. Louis, MO, USA), 1× buffer supplied by the manufacturer, 10 mM dithiothreitol, 20 μM deoxynucleotide triphosphates, and RNase inhibitor (RNAout, Invitrogen, Carlsbad, CA USA). Reverse transcription was performed at 42°C for 2 h. RT-PCR was performed on cDNA using the ABI Prism 7700 sequence detector (Applied Biosystems, Carlsbad, CA, USA) with SYBR Green) Invitrogen, Carlsbad, CA, USA). The following primers were used: IL-10—interlukin 10: 5′-GAGAAGCATGGCCCAGAAATC-3′ (forward) and 5′-CGCATCCTGAGGGTCTTCA-3′ (reverse); IL-17—interlukin 17: 5′-TCATCTGTGTCTCTGATGCTGTTG-3′ (forward) and 5′-TCGCTGCCTTCACTGT-3′ (reverse); TSG-6—tumor necrosis factor-stimulated gene 5′-GGTGTGTACCACAGAGAAGCA-3′ (forward) and 5′-GGGTTGTAGCAATAGGCATCC-3′ (reverse); TNF-α—tumor necrosis factor alpha: 5′-GACCCTCACACTCAGATCATCTTCT-3′ (forward) and 5′-CCACTTGGTGGTTTGCTACGA-3′ (reverse); GAPDH—glyceraldehyde-3-phosphate dehydrogenase: 5′-CGACAGTCAGCCGCATCTT-3′ (forward) and 5′-CCAATACGACCAAATCCGTTG-3′ (reverse).

Data was calculated as the ratio of mean threshold-targeted gene expression to GAPDH. For each gene, the specificity of the PCR product was assessed by verifying a single peak on melting curve analysis. Findings were compared to MSC growing medium without insult.

Statistical Analysis

Results are presented as means±standard errors of the mean (SEM); p values were calculated using two-tailed unpaired Student's t test and ANOVA. Survival curves were analyzed using Kaplan–Meier analysis. In all tests, significance was assigned when $p<0.05$.

Results

Placental MSCs were isolated from the placenta tissue closest to the umbilical cord. This culture probably contains fetal and maternal cells. The seeded cells created a carpet of fibroblastlike cells. After three passages, most of the cells stained positive for the mesenchymal surface membrane markers: CD29, CD73, CD105 (>95 %), and CD90 (55 %). The cells were negatively stained for CD14, CD34, and CD45 (<1 %) to hematopoietic markers. Adherent cells also differentiated into adipocytes and osteoblasts, indicating their mesenchymal identity (Yust-Katz, 2012).

To test the possible protective effect of these placental-derived MSC in MS, we examined their effect in a MOG-induced chronic EAE model. We induced EAE in two mice strains, C3H.SW and C57bl. In both models, we transplanted PL-MSC ICV 5 days after the first MOG injection. We found that PL-MSC transplantation significantly reduced the disease severity score and prolonged survival without delay in symptoms onset. Both mice strains developed clinical symptoms on day 12. However, the maximal score in EAE mice transplanted with PL-MSCs reached 3 in C57bl and 4 in C3H.SW as compared to score 5 in saline-injected mice (Fig. 1a; Fig. 2a). To further examine whether cell transplantation after the appearance of symptoms reduces the disease severity, we transplanted the PL-MSCs in MOG-induced EAE in C57bl on day 14. We found that late transplantation resulted in similar suppression of the disease severity as when PL-MSCs were transplanted on day 5 (presymptoms) and on day 14 (at the beginning of the disease) (Fig. 2a). Moreover, we observed that cell injection significantly prolonged mice survival (Fig. 1b). While saline-injected mice showed a 30 % survival rate on day 30 after MOG injection in the cell-treated group, 70 %–80 % of the mice survived at that time point. The extended survival was evident even when transplantation was on day 14 after symptom onset (Figs. 1b, 2b).

In the histology study on day 30, we monitored the transplanted PL-MSCs, stained with PKH-26 marker prior to transplantation. We found significant amounts of transplanted cells in the brain lateral ventricles, the injection location (Fig. 3a). Moreover, few PKH-26-positive cells were also found in the spinal cord sections (Fig. 3b).

To test the possibility that PL-MSCs induce an immunosuppressive function mediated by released soluble factors, we

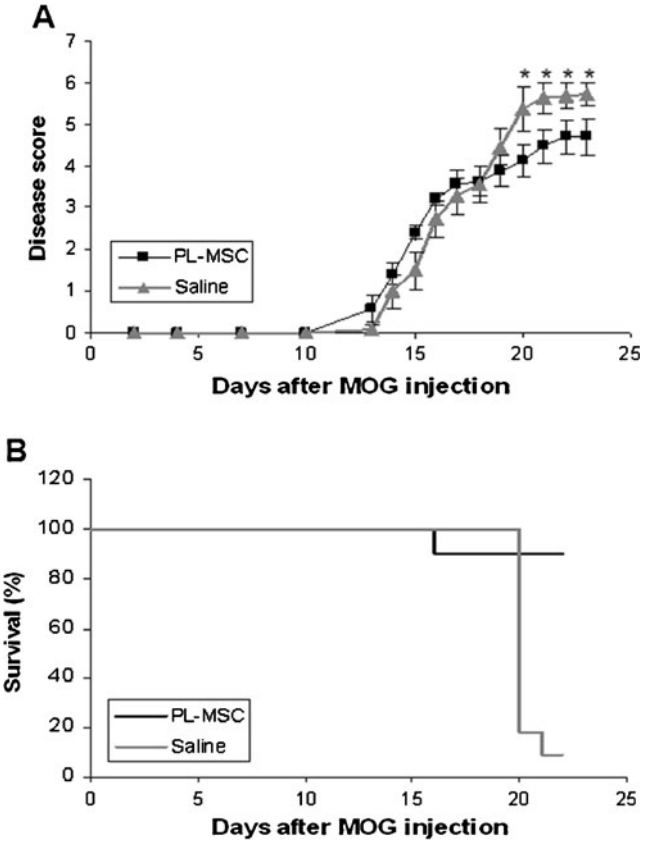

Fig. 1 PL-MSC ICV transplantation into C3H.SW-induced EAE animal model. **a** Clinical score of MOG-induced EAE mice following unilateral ICV transplantation of PL-MSCs ($n=10$) and saline ($n=11$). **b** Survival curve of MOG-induced EAE mice following unilateral ICV transplantation of PL-MSCs ($n=10$) and saline($n=11$). Saline-injected mice developed severe clinical symptoms, while PL-MSC-treated mice showed a significant reduction in the severity of clinical signs ($p<0.05$) (**a**). A similar pattern was observed evaluating the effect of cells' injection on survival 23 days after MOG injection (saline 9 % survival; PL-MSCs 80 % survival, $p<0.01$)

applied PL-MSC-conditioned medium on EAE-derived splenocytes. When MOG was added to the splenocytes, we demonstrated an increase in ^{3}H-thymidine incorporation of up to 150 %. Proliferation in the presence of PL-MSC-conditioned medium was slightly, not significantly, lower (Fig. 4a).

Next, after isolating spleen cells from mice that were transplanted ICV with PL-MSCs or saline 5 days after the first MOG injection, we measured cell proliferation. We found that the transplantation of PL-MSCs into the brain did not decrease splenocyte proliferation in response to MOG (Fig. 4b).

Furthermore, RT-PCR analysis for interleukins 10 and 17 on mRNA isolated from splenocytes of EAE-induced mice 5 days post-transplantation demonstrated comparable levels in transplanted and control mice group (Fig. 4c, d). These unchanged mRNA expression level of inflammatory cytokines show lack of peripheral immune response to the transplanted PL-MSCs.

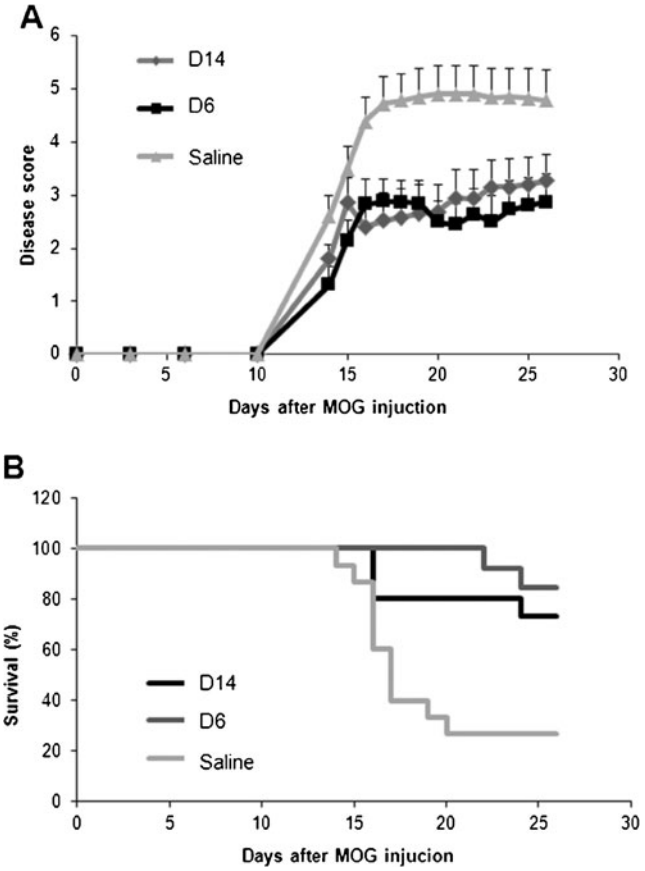

Fig. 2 ICV transplantation of PL-MSCs into C57bl-induced EAE animal model. **a** Clinical score of MOG-induced EAE mice following unilateral ICV transplantation of PL-MSCs on day 5 ($n=10$), on day 14 ($n=10$) and saline ($n=1$) ($p<0.05$). **b** Survival curve of MOG-induced EAE mice following unilateral ICV transplantation of PL-MSCs ($n=10$) and saline ($n=11$) (Kaplan–Meier analysis $p<0.05$). p values are presented in relation to saline-injected group)

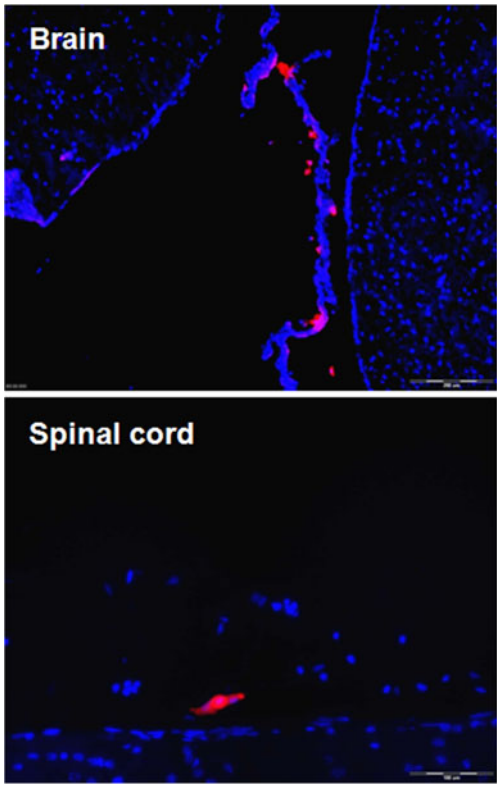

Fig. 3 Histology of transplanted brain and spinal cord. Five days postinjection, PKH26-stained PL-MSCs were found in the lateral ventricle (the injection site) in the brain. Few positive cells were found attached to the spinal cord white matter in transplanted EAE-induced mice. (*Scale bars* are brain 25 μm, spinal cord 50 μm)

To further study the protective effect of PL-MSC, we tested their possible antiinflammatory effect. To challenge these cells, we isolated astrocytes and exposed them to LPS for 48 h. TNF-α mRNA expression was induced tenfold by exposure to LPS (data not shown). We then added the LPS-activated astrocyte-conditioned medium to cultured PL-MSC. We found, using real-time PCR analysis, that in response to this conditioned medium, PL-MSCs expressed the antiinflammatory protein TSG-6 mRNA sevenfold more than saline. Additionally, in response to the inflammatory cytokines TNF-α or IL1-β, PL-MSCs highly expressed TNG-6 mRNA fivefold or 35-fold, respectively, as compared to saline (Fig. 5).

Discussion

In this study, we describe the therapeutic advantage of intracerebral ventricle transplantation of PL-MSCs in a chronic EAE mouse model for MS. Intraventricular transplantation of PL-MSCs 5 days after MOG injection resulted in significant amelioration of disease symptoms in C3H.SW and C57bl mice. Moreover, mice developed less severe disease symptoms when PL-MSCs were transplanted 14 days after the first MOG administration. PL-MSC transplantation inhibited disease progression and significantly increased mice survival, as compared to controls. The latter treatment mimics the complexity of the clinical situation and might indicate the potential of such transplantation to reverse the disease signs.

To establish cell-based therapy as a new strategy for MS, it is necessary to identify the best cell source in terms of ethical considerations and risks-to-benefit ratio. Although bone marrow remains the main source of MSC for clinical trials, the human placenta is considered an ideal source due to accessibility, it presents a painless procedure for donors, without ethical conflicts (Brooke et al. 2009; Tran et al. 2011; Fukuchi et al. 2004; Hwang et al. 2009; Parolini et al. 2008; Dzierzak and Robin 2010). Moreover, placental tissue contains abundant maternal and fetal MSCs. A huge amount of cells can be isolated from each placenta. Furthermore, the placenta cells have immunosuppressive features

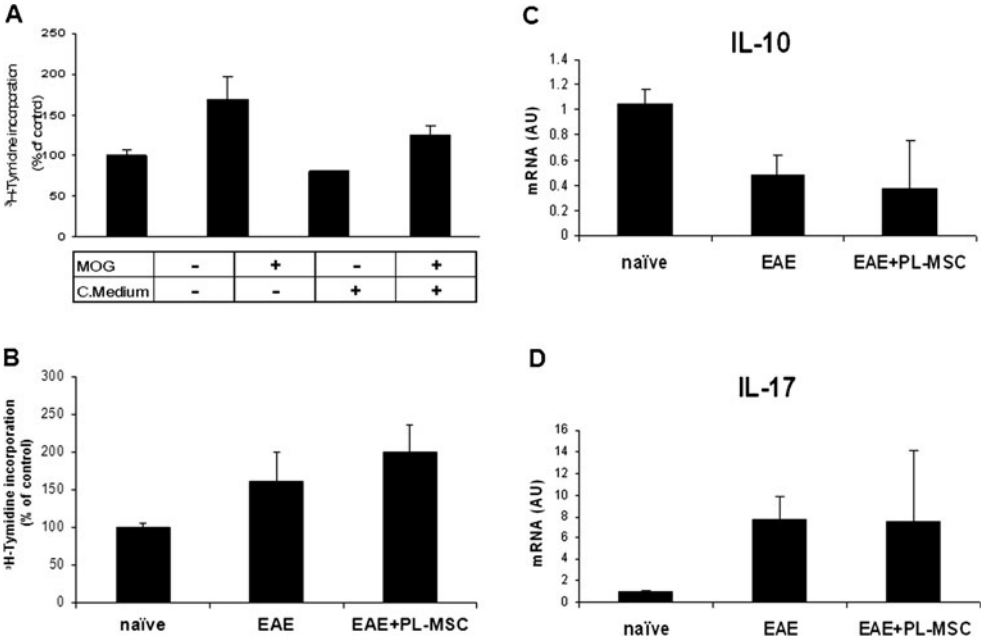

Fig. 4 **A** ^{3}H tymidine incorporation in MOG-induced splenocytes. PL-MSC conditioned medium slightly reduced the ^{3}H tymidine incorporation in MOG-induced splenocytes. **B** Splenocytes ex vivo, post-PL-MSC transplantation, proliferate the same after MOG and express the same interleukins level; ^{3}H tymidine incorporation in MOG induction of splenocytes that were collected from mice 5 days post-cell transplantation. **C** IL-10 real-time RT-PCR of splenocytes that were collected from mice 5 days post-cell transplantation. **D** IL-17 real-time RT-PCR of splenocytes that were collected from mice 5 days post-cell transplantation

and regulatory effects (e.g., T-cell suppression) on various immune cells, and PL-MSCs have the potential to directly or indirectly inhibit disease-associated Th1, Th2, and Th17 cells as well as cytotoxic T lymphocytes. PL-MSC cells express major histocompatibility complex (MHC) class I but not MHC class II antigens (In 't Anker et al. 2004), giving them an advantage for transplantation as they can, at least partially, bypass rejection responses.

A histology study indicated that the prelabeled transplanted PL-MSCs survived in the EAE mice brain at the transplantation location. Moreover, a few of the transplanted PL-MSCs were also found in the host spinal cord. The capacity to migrate towards injured tissue of MSC, even for a long distance, has already been reported by our laboratory and other groups (Sadan et al. 2008, 2009; Bahat-Stroomza et al. 2009; Hellmann et al. 2006). Our current observation demonstrates that PL-MSCs were spread in the CNS of EAE-induced mice.

Several mechanisms might explain the therapeutic effect of the PL-MSC transplantation in the EAE model, the obvious ones being neuroprotection and immunomodulation. An earlier study in our laboratory showed that ICV injection of bone marrow MSCs improved the clinical course of EAE. The bone marrow derived MSCs, undifferentiated or differentiated to secrete neurotrophic factors (NTFs), efficiently protected a neuronal cell line from oxidative stress, demonstrating a marked neuroprotective effect. We have also demonstrated that the bone marrow-derived mesenchymal stem cells (BM-MSCs) are capable of suppressing immune cell reaction to MOG (Barhum et al. 2010).

Over recent years, emerging evidence has revealed that NTFs play an important role in MS and its animal model, EAE. We have previously shown that PL-MSCs express IGF-1 in vitro as well as a small amount of GDNF and BDNF (Yust-Katz et al. 2012). Thus, we assume that transplanted PL-MSCs provide protection for EAE mice by exposing them to NTF.

Another suggested mechanism by which MSCs affect EAE is immunomodulation. Several studies have reported that i.v. infusion of BM-MSC improved the clinical course of EAE (Kassis et al. 2008; Gerdoni et al. 2007; Zappia et al. 2005). Inducing peripheral T cell tolerance to myelin proteins reduced the migration of pathogenic T cells to the CNS, on one hand, while on the other hand, homing to the CNS where they preserved axons and reduced demyelination.

Several studies showed that BM-MSCs can modulate the functions of both T and B lymphocytes. MSCs can inhibit the production of TNF-α and IFN-γ by CD4+ (helper T cells) and CD8+ cytotoxic T cells, while they can upregulate the expression of IL-10 and restore the secretion of IL-4 by CD4+ and CD8+ T cells [review (Abumaree et al. 2011)].

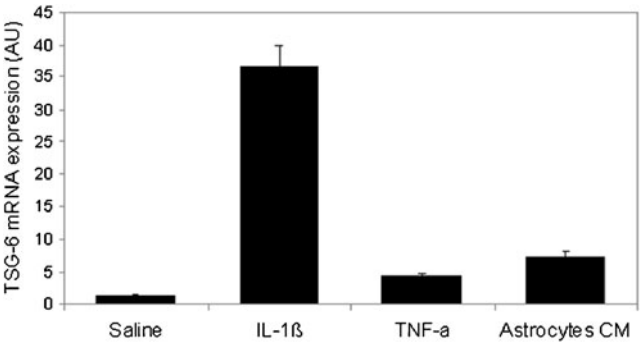

Fig. 5 PL-MSCs overexpress GST-6 mRNA in response to inflammatory cytokines, TNF-α and IL1-β or conditioned media of astrocytes that were exposed to LPS

In accordance with these findings, we measured PL-MSCs' immunomodulatory ability and found that although PL-MSC conditioned medium slightly reduces MOG-induced immune cells' proliferation, the PL-MSCs that were transplanted ICV 5 days after MOG injection had no effect on the spleen cell proliferation ex vivo.

Moreover, RT-PCR expression analysis of IL-10 and IL-17 in splenocytes of EAE-induced mice 5 days post-cell or saline transplantation revealed that the PL-MSC transplantation has no peripheral influence on T cell response to MOG. These results demonstrate that PL-MSC transplantation into the brain has only a restricted effect on the peripheral immune system.

We further studied the mechanisms that might explain the marked MSC-based therapeutic effects in EAE. We focused on the local immunomodulation in the CNS mediated by the transplanted PL-MSC. MSCs modulate their antiinflammatory effects in multiple ways that appear to be responsive to the different microenvironments created by different tissue injuries (Prockop and Oh 2012). It is known that microglia and astrocyte cells in the CNS of EAE express and release inflammatory cytokines such as TNF-α and IL1-β. We isolated astrocyte cells and found that exposure to LPS was followed by high expression of TNF-α mRNA. We measured the antiinflammatory response of PL-MSCs caused by exposure to condition media of LPS-activated astrocytes.

Prockop et al. have shown that BM-MSCs secrete the multifunctional TSG-6 in vivo in response to inflammatory conditions including lung injury, MI, and in the cornea following chemical and mechanical injury (Danchuk et al. 2011; Oh et al. 2010; Roddy et al. 2011; Lee et al. 2009). The same mechanism might be involved in EAE. We found that placental MSCs highly express TSG-6 mRNA in response to TNF-α, IL1-β, and conditioned medium collected from astrocytes that were exposed to LPS. Real-time PCR analysis of TSG-6 mRNA levels showed high response of PL-MSCs to TNF-α, IL1-β, and more importantly, to conditioned media from activated astrocytes.

TSG-6 is a 35 kDa secreted protein composed mainly of contiguous hyaluronan-binding link and CUB modules (Kohda et al. 1996; Nentwich et al. 2002). The protein is not expressed in normal cells or tissues, but is expressed in many cells after exposure to TNF-α and other proinflammatory cytokines. The antiinflammatory activity of TSG-6 was largely attributed to its ability to bind to the fragments of hyaluronan to inhibit components in the inflammatory network of proteases and to suppress neutrophil migration into the site of inflammation (Milner et al. 2006; Getting et al. 2002). The negative feedback loop created by PL-MSCs and TSG-6 can attenuate the inflammatory cascade that was initiated by resident microglia and activated astrocytes.

Our results indicate the therapeutic potential of PL-MSCs in the EAE mice model. A combination between neuroprotection and immunomodulation in EAE brain and spinal cord after PL-MSC transplantation might account for the therapeutic effects of these cells. The antiinflammatory effect could be explained by the possible effect of TSG-6 produced by brain-transplanted PL-MSCs on the CNS inflammatory state. We believe that PL-MSCs can reduce inflammation at the site of injury and may have important implications for treating MS, a disease characterized by inflammation.

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