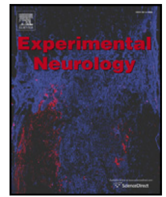
Experimental Neurology 275 (2016) 154–161

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr



Research paper

Co-transplantation of syngeneic mesenchymal stem cells improves survival of allogeneic glial-restricted precursors in mouse brain

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ARTICLE INFO

Article history:
Received 24 May 2015
Received in revised form 16 October 2015
Accepted 24 October 2015
Available online 26 October 2015

Keywords:
Mesenchymal stem cell
Co-transplantation
Glial-restricted precursor
Bioluminescence imaging
Cell survival
Immunomodulation

ABSTRACT

Loss of functional cells from immunorejection during the early post-transplantation period is an important factor that reduces the efficacy of stem cell-based therapies. Recent studies have shown that transplanted mesenchymal stem cells (MSCs) can exert therapeutic effects by secreting anti-inflammatory and pro-survival trophic factors. We investigated whether co-transplantation of MSCs could improve the survival of other transplanted therapeutic cells. Allogeneic glial-restricted precursors (GRPs) were isolated from the brain of a firefly luciferase transgenic FVB mouse (at E13.5 stage) and intracerebrally transplanted, either alone, or together with syngeneic MSCs in immunocompetent BALB/c mice ($n = 20$) or immunodeficient Rag2$^{-/-}$ mice as survival control ($n = 8$). No immunosuppressive drug was given to any animal. Using bioluminescence imaging (BLI) as a non-invasive readout of cell survival, we found that co-transplantation of MSCs significantly improved ($p < 0.05$) engrafted GRP survival. No significant change in signal intensities was observed in immunodeficient Rag2$^{-/-}$ mice, with transplanted cells surviving in both the GRP only and the GRP + MSC group. In contrast, on day 21 post-transplantation, we observed a 94.2% decrease in BLI signal intensity in immunocompetent mice transplanted with GRPs alone versus 68.1% in immunocompetent mice co-transplanted with MSCs and GRPs ($p < 0.05$). Immunohistochemical analysis demonstrated a lower number of infiltrating CD45, CD11b^{+} and CD8^{+} cells, reduced astrogliosis, and a higher number of FoxP3^{+} cells at the site of transplantation for the immunocompetent mice receiving MSCs. The present study demonstrates that co-transplantation of MSCs can be used to create a microenvironment that is more conducive to the survival of allogeneic GRPs.

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1. Introduction

Stem cell-based therapies have emerged as a promising mode of treatment in neurodegenerative diseases for which there is no effective cure. One of the major obstacles to the success of stem cell-based therapies is the loss of transplanted cells after engraftment (Lawrence et al., 1994; Li and Duncan, 1998; Tambuyzer et al., 2009). Pathogenic immune signaling that results from the disease process itself (Chen and Palmer, 2008), immunorejection (Zhao et al., 2011), or non-immunologic factors, such as the lack of trophic factors in the host microenvironment, greatly reduce transplanted cell survival and functional integration. One way to improve transplanted cell survival and differentiation is to attenuate the host immune response (Chen et al., 2011). Classic immunosuppressive drugs can be used to inhibit activity of the immune system and minimize transplanted cell rejection (Gorelik et al., 2012). However, treatment with immunosuppressive drugs is often associated with low specificity that may lead to toxicity detrimental to both host and grafted cells (Chan et al., 1995; Mohebbi et al., 2009; Oliveira et al., 2004).

Transplanted cell survival can also be improved by supplementing trophic factors (Apostolides et al., 1998) or co-transplantation of "helper cells" engineered to overexpress growth factors such as basic fibroblast growth factor (bFGF) (Liang et al., 2013; Smith and Snyder, 2013).

Mesenchymal stem cells (MSCs) are multipotent stromal cells that play a role in shaping the bone marrow microenvironment where hematopoiesis occurs. The use of MSCs for the improvement of engrafted cell survival is very appealing because in addition to their regenerative abilities, MSCs also exhibit a multitude of positive effects, including the release of pro-survival trophic and anti-inflammatory/immunomodulatory factors (Abboud et al., 1991; Aggarwal and Pittenger, 2005; Jitschin et al., 2013; Wilkins et al., 2009). These cells

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http://dx.doi.org/10.1016/j.expneurol.2015.10.008
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also have a strong capacity for exosome secretion in response to cellular injuries (Baglio et al., 2012; Li et al., 2013; Liang et al., 2014). Therapeutic MSCs have been used in several neurological conditions with inflammation, such as multiple sclerosis, amyotrophic lateral sclerosis (Karussis et al., 2010; Vercelli et al., 2008; Zhao et al., 2011), Huntington's disease (Bantubungi et al., 2008), and spinal cord injury (Torres-Espin et al., 2013), and to enhance engraftment of therapeutic cells such as hematopoietic stem cells (Le Blanc et al., 2007), human embryonic stem cells (Puymirat et al., 2009), oligodendrocyte progenitors (Cristofanilli et al., 2011) and islet cells (Kerby et al., 2013).

Among the therapeutic cell types considered for transplant therapy, glial-restricted precursors (GRPs) have been found to have positive effects in different neurological disorders. GRPs show high A2B5 immunoreactivity and can be differentiated into oligodendrocytes and astrocytes under appropriate differentiation signals (Rao and Mayer-Proschel, 1997; Rao et al., 1998). GRPs, when transplanted into the central nervous system (CNS), can generate extensive remyelination in a rodent models of dysmyelination, preserve electrophysiological function in rats with focal inflammatory spinal cord demyelination (Walczak et al., 2011), and support regeneration in an animal model of spinal cord injury (Haas and Fischer, 2013). However, the long-term survival of these cells post-transplantation remains a challenge. In a previous study it was reported that, in immunocompetent animals, GRP graft survival was compromised despite the use of immunosuppressive drugs (Gorelik et al., 2012). In this study, we aimed to develop a co-transplantation strategy in which the pro-survival and immunomodulatory properties of MSCs can be used to improve the survival of allografted GRPs in mouse brain.

2. Materials and methods

2.1. Isolation and characterization of MSCs

Syngeneic MSCs were isolated from an aspirate of bone marrow harvested from the tibia and femoral marrow compartments of 4-week-old BALB/c mice (Jacksons Laboratory), the immunocompetent cell recipients in this study. Bone marrow was cultured in a medium with Dulbecco's modified Eagle's medium (DMEM) and 20% fetal bovine serum (FBS) for three days at 37 °C in a 5% CO_2 incubator. After three days, non-adherent cells were removed and fresh medium was replaced. When primary cultures became 80–90% confluent, cells were treated with TrypLE™ Express (Gibco) for five minutes at 37 °C, harvested, washed with 10 mM phosphate buffered saline (PBS), pH = 7.4, and replated. An adherent population of MSCs was obtained three weeks after the initiation of culture. For characterization, MSCs (1×10^6 cells) were incubated in 2% FBS in PBS at 4 °C for 30 min with 1 μl of monoclonal antibody specific for CD34, CD45, CD90, and CD105 (Novus Biological), or left unstained, and were analyzed by FACSCalibur with CellQuest software (Becton Dickinson).

2.2. Isolation and characterization of GRPs

Allogeneic GRPs were isolated from the cortical region of the brain of a homozygous firefly luciferase transgenic mouse (FVB background, Jackson Laboratories, strain 008,450) at the E13.5 stage, as described elsewhere (Mujtaba et al., 1999). Cells were maintained in serum-free DMEM-F12 medium (Invitrogen) supplemented with N2, B27, bovine serum albumin, and bFGF (Invitrogen) as previously described (Lepore et al., 2006). GRPs were characterized using an immunocytochemical assay. In brief, cells were fixed in 4% paraformaldehyde (PFA) for 30 min. Nonspecific binding was blocked by incubating a solution of 10% donkey serum and 0.1% Triton X-100-PBS for two hours at room temperature. Cells were incubated with the appropriate dilutions of primary antibodies in blocking solution overnight at 4 °C, then rinsed with PBS and incubated with corresponding secondary antibodies (Alexa Fluor-488 and Alexa Fluro-546, Invitrogen) in blocking buffer for one hour at room temperature. The culture was rinsed three times with PBS and images were obtained with a Zeiss AX10 fluorescence microscope. Primary antibodies used for these experiments were: anti-A2B5 (1:1000, Santa Cruz); anti-Olig1 (1:1000, EMD Millipore); and anti-firefly luciferase (1:3000, GeneTex).

2.3. Bioluminescent imaging (BLI) of GRPs in vitro

Transgenic luciferase expressing GRPs were plated into poly L-lysine/laminin-coated 96-well plates at 10,000, 1000, 100, and 10 cells per well. For bioluminescence measurements, the medium was removed and 0.15 mg/ml D-luciferin in PBS was added. The luminescence signal was measured using an IVIS Spectrum/CT instrument (Perkin Elmer). Images were acquired at one-minute intervals for 10 min until peak signal was observed. BLI was quantified by drawing of regions of interest (ROIs) over the wells, with data were expressed as photon flux (p/s).

2.4. Cell transplantation

Twenty immunocompetent male BALB/c (4 weeks old, Jacksons Laboratory) and eight male immunodeficient Rag2$^{-/-}$ mice (4–6 weeks old, Taconic) were used in this study. Animals were divided into experimental and control groups. The experimental group was subdivided into: (i) immunocompetent mice transplanted with MSC + GRP (n = 10); (ii) immunocompetent mice transplanted with GRP alone (n = 10); and a non-immunorejection control group subdivided into (i) immunodeficient mice transplanted with MSC + GRP (n = 4); and (ii) immunodeficient mice transplanted with GRP only (n = 4).

All animal procedures were conducted according to an approved protocol from our Institutional Animal Care and Use Committee. Animals were anesthetized with 2% isoflurane in oxygen, shaved and stabilized in a Cunningham adaptor mounted on a stereotactic frame (Stoelting). MSCs and GRPs were harvested, washed, and suspended in PBS at a density of 1×10^5 cells/μl. A 2 μl cell suspension containing 1 μl MSCs and 1 μl GRPs or 1 μl GRPs and 1 μl PBS was injected into the brain (AP = 0 mm; ML = 2.2 mm; DV = 2.0 mm) at a rate of 0.5 μl/min using a Hamilton 31G microinjection needle (Hamilton) and a nano-injector (Stoelting). The needle was kept in place for two min after injection to minimize backflow and then withdrawn slowly. Rectal temperature was monitored and maintained between 36.5 and 37.5 °C using a self-regulating heated blanket (Harvard Apparatus) for the duration of surgery. No immunosuppressive drugs were given to any animal before or after surgery. Animals were kept in a normal day-night cycle (12/12 h) with ad libitum access to food and water.

2.5. BLI of transplanted GRPs in vivo

Bioluminescent images were acquired using an IVIS Spectrum/CT instrument (Perkin Elmer). Animals were anesthetized with 2% isoflurane gas in oxygen, and 150 mg/kg D-luciferin (Gold Biotechnology) was injected intraperitoneally (i.p.). Images were acquired 10 min after injection at the peak of the bioluminescence signal. To generate 2D bioluminescent images, no emission filter was used during imaging. Images were quantified by drawing ROIs over the brain region, with data expressed as photon flux (p/s). To generate 3D bioluminescent images, four spectrally resolved images were acquired using emission filters at 600, 620, 640, and 660 nm, with a bandwidth of 20 nm each. Imaging parameters were an exposure time of 180 s, an aperture of f/1, a FOV = 13 cm, and 2048 × 2048 pixel resolution. Pixel binning was set to an 8 × 8 bin width for an effective image resolution of 256 × 256 pixels. Imaging parameters were identical for both 2D and 3D imaging.

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