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Human bone marrow stromal cell treatment improves neurological functional recovery in EAE mice

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Abstract

We investigated the treatment of remitting–relapsing experimental autoimmune encephalomyelitis (EAE) in mice with human bone marrow stromal cells (hBMSCs). hBMSCs were injected intravenously into EAE mice upon onset of paresis. Neurological functional tests were scored daily by grading clinical signs (score 0–5). Immunohistochemistry was performed to measure the transplanted hBMSCs, cell proliferation (bromodeoxyuridine, BrdU), oligodendrocyte progenitor cells (NG2), oligodendrocytes (RIP), and brain-derived neurotrophic factor (BDNF). The maximum clinical score and the average clinical scores were significantly decreased in the hBMSC-transplanted mice compared to the phosphate-buffered-saline-treated EAE controls, indicating a significant improvement in function. Demyelination significantly decreased, and $BrdU^+$ and $BDNF^+$ cells significantly increased in the hBMSC-treated mice compared to controls. Some $BrdU^+$ cells were colocalized with $NG2^+$ and RIP^+ immunostaining. hBMSCs also significantly reduced the numbers of vessels containing inflammatory cell infiltration. These data indicate that hBMSC treatment improved functional recovery after EAE in mice, possibly, via reducing inflammatory infiltrates and demyelination areas, stimulating oligodendrogenesis, and by elevating BDNF expression.

Keywords: Experimental autoimmune encephalomyelitis; Human bone marrow stromal cells; Oligodendrocyte progenitor cell; Brain-derived neurotrophic factor; Mice

Introduction

Multiple sclerosis (MS) is believed to be an immune-mediated response that recognizes myelin peptide determinants and initiates attacks directly against myelin constituents, and causes myelin destruction and axonal loss (Hemmer et al., 2002; Lucchinetti et al., 2000). MS is characterized by patchy perivenular inflammatory infiltrates in areas of demyelination and axonal loss (Kieseier et al., 1999). It is a chronic, remitting–relapsing disease of the central nervous system (CNS), and the most common cause of neurological disability affecting young adults. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS (Pluchino et al., 2003).

To date, experimental cell therapy for EAE has been based mainly on the transplantation of oligodendrocytes or neural stem cells to enhance CNS regeneration and neural cell replacement (Archer et al., 1997; Pluchino et al., 2003). However, there are still many problems to be solved before we can reliably use cell therapy to repair mature brain and spinal cord (Franklin and Blakemore, 1997; Jefferson et al., 1997). Our previous studies have demonstrated that rat bone marrow stromal cells (Chen et al., 2001) and human bone marrow stromal cells (hBMSCs, Li et al., 2002) intra-

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venously injected after stroke migrate selectively and target damaged brain, enhance neurogenesis, promote neural stem cell proliferation, and improve functional recovery (Chopp and Li, 2002). Intravenous administration of BMSCs also increases the expression of brain-derived neurotrophic factor (BDNF) after traumatic brain injury, which possibly contributes to the improvement in functional outcome (Mahmood et al., 2004). In this study, we investigated whether intravenously injected hBMSCs reach multiple demyelinating areas of the CNS, and whether hBMSCs provide therapeutic benefit in EAE mice.

Materials and methods

Animals

Female SJL/J mice (8–10 weeks old, Jackson Laboratory, Bar Harbor, ME) were used in our experiments. All experimental procedures have been approved by the Institutional Animal Care and Use Committee of Henry Ford Health Systems.

hBMSC preparation

hBMSCs were generously provided by the Cognate Therapeutics Inc. (Baltimore, MD). Briefly, bone marrow was obtained from adult human donors and the nucleated cell fraction was aspirated and placed into culture with media consisting of Dulbecco's Modified Eagle's Medium-low glucose and 10% selected fetal bovine serum. The adherent cells were fed every 3–4 days during the primary culture period, which generally lasted for about 2 weeks. When dense colonies of spindle-shaped cells covered greater than 80% of the surface area of the culture dish, the cells were harvested by trypsinization and passaged into the secondary culture that consisted of feeding the cultures and passaging. The cells were then harvested and cryopreserved in appropriate dose-related aliquots in Plasma-Lyte containing human serum albumin and dimethyl sulfoxide. The cells are tested for purity at the end of each passage by flow cytometry. BMSCs cells were washed by flow wash buffer [1 × DPBS (Hyclone, Logan, UT), 0.5% BSA (Sigma). and 0.1% sodium azide (Sigma)], then suspended in blocking buffer [wash buffer+25 μg/ml mouse Ig (Sigma) at 1×10^7 cells/ml. Propidium iodide (PI) analysis of cell viability was performed. The cell suspensions were incubated on ice and protected from light for 30 min, washed in wash buffer and fixed in 1% paraformaldehyde. The BMSC populations were analyzed for the following surface antigens for phenotypic characterization: CD13, CD14, CD29 (Caltag, Silverstone Towcester, UK), CD34, CD44 (Cell Sciences Inc., Canton, MA), CD45, CD54, CD63, CD73, CD90, CD105 (Caltag), CD166, HLA-A, B, C, and HLA-DR. All antibodies were purchased from BD-Pharmingen (San Diego, CA), unless otherwise noted. Final analysis of expression was based on percent (+) events and median fluorescence values with respect to the appropriate isotype control: mouse IgG1 FITC, mouse IgG1 PE, mouse IgG1 APC, mouse IgG2a APC, mouse IgG2b APC, and IgG2a PE. Our findings are consistent with those of other laboratories in showing that the cells are positive for MHC class I, CD29, CD90, CD105, CD13, CD44, CD63, CD73, and CD166. They are negative for MHC class II, CD45, CD14, and CD34.

EAE induction and animal groups

Myelin proteolipid protein (PLP) (p139–151, HSL-GKWLGHPDKF, SynPep Corporation, Dublin, CA) was used for immunization. The purity of the peptide was greater than 95% as measured by High Performance Liquid Chromatography. EAE was induced in female SJL/J mice by subcutaneous injection with 25 μg PLP dissolved in 50 μl complete Freund's adjuvant (CFA, Difco Laboratories, Livonia, MI). On the day of immunization and 48 h later, pertussis toxin (PT, List Biological Laboratories, Inc., Campbell, CA) 200 ng in 0.2 ml phosphate buffered saline (PBS) was injected into the mouse tail vein (Youssef et al., 2002). Mice were randomly divided into: Normal group: mice without immunization; hBMSC treatment group: hBMSCs (0.5, 2, or 3×10^6 per mouse) were administered intravenously in 1 ml total fluid volume PBS on the day of clinical symptom onset (score $\geq$ 1); PBS treatment group: PBS (1 ml) was injected into the tail vein of the EAE mice on the day of clinical symptom onset as EAE controls. hBMSC doses were selected based on our experience in the treatment of stroke (Chen et al., 2001; Li et al., 2002) and traumatic brain injury (Mahmood et al., 2001) with hBMSCs.

Bromodeoxyuridine (BrdU, Sigma Chemical), the thymidine analog that is incorporated into the DNA of dividing cells during S-phase, was used for mitotic labeling. BrdU (100 mg/kg bw) was intraperitoneally injected once a day for 14 consecutive days into EAE mice starting on the day of clinical symptom onset.

Neurological functional measurement

Mice in the hBMSC treatment group (0.5, 2, and 3×10^6 groups, $n = 10$ per group) and PBS treatment group ($n = 10$) were scored daily for clinical symptoms of EAE, as follows: 0, healthy; 1, loss of tail tone; 2, ataxia and/or paresis of hindlimbs; 3, paralysis of hindlimbs and/or paresis of forelimbs; 4, tetraparalysis; 5, moribund or dead (Pluchino et al., 2003). We tested the neurological functions of EAE mice treated with hBMSCs or PBS daily until 90 days after clinical symptom onset. For histopathological and immunohistochemical analysis, we selected brains and spinal cords from mice treated with the dose of hBMSCs yielding the best functional test score.