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Intravenous Administration of Bone Marrow Mesenchymal Stem Cells Benefits Experimental Autoimmune Myasthenia Gravis Mice Through an Immunomodulatory Action

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Abstract

Mesenchymal stem cells (MSC) are potent in immunomodulation. It has been proven that MSC functioned to correct immune disorder in several immune diseases. Here, we tested the hypothesis that MSC from human bone marrow (hMSC) can provide a potential therapy for experimental autoimmune myasthenia gravis (EAMG). EAMG mice model was established by subcutaneous injection of synthetic analogue of acetylcholine receptor (AchR), then, hMSC were intravenously delivered into these mice repeatedly. The results showed that hMSC could specifically home to spleen tissue and hMSC treatment significantly improved the functional deficits of EAMG mice. In addition, AchR antibody level was dramatically decreased in cell-treated group when compared with untreated control on 10 days after the second cell injection. Moreover, both *in vivo* and *in vitro* mixed lymphocyte proliferation assays revealed that hMSC could definitely inhibit the proliferation of AchR-specific lymphocyte. In conclusion, our study demonstrated that hMSC treatment was therapeutically useful in autoimmune myasthenia gravis mice, and the underlying mechanism may relate with their immunomodulatory potential.

Introduction

Myasthenia gravis (MG) is a T-cell dependent, acetylcholine receptor (AchR) antibody-mediated complex autoimmune neuromuscular disease. The pathogenesis of MG involves the hyperactivity of various immune cells, activation of complement system, deficiency of immunomodulation and impairment of immune homeostasis [1, 2]. It has been suggested that a dysfunction of stromal progenitors, endothelial progenitors and the bone marrow microenvironment usually occurs for autoimmune disease [3]. Mesenchymal stem cells (MSC) are resident of the microenvironment; and also, they are able to give rise to other cellular constituents of the microenvironment including reticular cells, adipocytes and osteoblasts [4]. MSC could express various molecules such as intercellular adhesion molecule (ICAM)-1, ICAM-2, lymphocyte function-associated antigen (LFA) 3, fibronectin, lamin and collagen, which are involved in the process of immune reaction [5–11]. It has been reported that MSC are able to suppress T lymphocytes activation and proliferation in mixed lymphocyte experiment [12]. The underlying mechanisms relate with their ability to secrete bioactive cytokines in addition to their direct physical contact with T lymphocytes. Furthermore, MSC are capable of modulating the functions of other immune cells such as natural killer cells, dendritic cells and B cells [13–15]. Therefore, MSC can be potentially used for immunomodulation and correction of impaired immune homeostasis in diseases like MG.

Recently, researchers in our laboratory have reported MSC from foetal bone marrow are able to suppress the proliferation of human peripheral blood mononuclear cells (PBMC) and CD4+ T cells, which is consistent with the observation of other groups [16]. Meanwhile, accumulating evidence has revealed that human bone marrow-derived MSC transplantation could improve autoimmune diseases in animal models such as experimental allergic encephalomyelitis (EAE) [17]. MG is characterized by the presence of Th cells and autoantibodies (IgG type) specific for the AchR. Therefore, it is reasonable to speculate that MSC will be therapeutically effective when

SCANDINAVIAN JOURNAL OF Immunology AN INTERNATIONAL MONTHLY JOURNAL

introduced to MG. In this study, we successfully induced EAMG mouse model by subcutaneous injection of synthesis peptide, which has been demonstrated to be an ideal alternative to traditional MG model induced by AchR [18, 19]. Previous studies have shown that murine bone marrow MSC could ameliorate the symptoms of MG animals [18, 19]. While there is no report about human MSC' (hMSC) effect on EAMG so far. It is necessary, regarding the development of MSC-based therapy in humans, to test if hMSC are also able to function effectively after transplantation into MG animal models. This study was designed to determine the potential of hMSC in improving the functional deficits of EAMG mice and the related mechanism.

Methods

Isolation and culture of human bone marrow MSC. Human foetal bone marrow for research purpose was approved by the Medical Ethics Review Board of Chinese Academy of Medical Sciences and Peking Union Medical College. Pregnant women gave written consent for the clinical procedure and for the use of tissue for research purposes. Foetal bone marrow was obtained after termination of pregnancy, under conditions where it was clear that tissues would otherwise be discarded. Foetal gestational age was about 13–15 weeks, and bone marrow was collected from foetal long bones. The mononuclear cells (MNC) of bone marrow were isolated by using Ficoll gradient centrifugation and cultivated in uncoated plastic flasks. The growth medium was composed of DF12 (Gibco Life Technologies, Paisley, UK) culture medium containing 40% MCDB-201 (Sigma, St. Louis, MO, USA), 2% foetal calf serum (FCS; Hyclone, Logan, UT, USA), 1 × insulin-transferrin-selenium (ITS; Gibco Life Technologies), 10^{-8} M dexamethasone (Sigma), 50 U/ml penicillin/streptomycin, 10 ng/ml human basic fibroblast growth factor (bFGF; Peprotech, London, UK), 10 ng/ml human epidermal growth factor (EGF; Peprotech) and 2 mM L-glutamine (Gibco Life Technologies). After 3 days, the non-adherent cells were discarded, and BM-MSC were adherent on plastic flasks. The media was changed every 3 days. After 7–14 days of plating, cells were detached by treatment with 0.125% trypsin and 0.1% EDTA (Sigma) and replated at a 1:3 dilution using the same growth media.

Immunophenotypic analysis. For immunophenotypic analysis of the expanded clonal cells, hMSC of passage 3 were analysed for the following markers: CD90, HLA-ABC, HLA-DR, CD19, CD34, CD45, CD29, CD14, CD31, CD166, CD73, CD117 (all from BD PharMingen, San Jose, CA, USA) and CD105 (Ancell Corporation, Bayport, MN, USA). Then, 1×10^5 cells were incubated for 30 min at 4 °C in the dark with fluorescence-conjugated monoclonal antibodies and control mouse isotypes. After two washing steps with phosphate-buffered saline (PBS; Gibco), cells were acquired using a FACSCalibur (BD, San Diego, CA, USA) flow cytometer using Cell Quest software.

Multipotent differentiation. To evaluate adipogenic and osteogenic differentiation, cells of passages 3–5 were seeded at 1000 cells/cm^2 and incubated as above for 24 h before changing to the induction media. For adipogenic differentiation, 1 μM dexamethasone, 5 μg/ml insulin, 0.5 mM isobutyl methylxanthine (Sigma) and 60 μM indomethacin (Sigma) were added to the culture medium. For osteogenic differentiation, 0.1 μM dexamethasone (Sigma), 0.05 mM ascorbic acid-2-phosphate (Sigma) and 10 mM β-glycero-phosphate (Sigma) were added to the growth medium. Cells were then maintained for 21 days with a media change every 3–4 days. After induction, the cells were fixed with 4% paraformaldehyde and stained with Oil Red O (Sigma) and Alizarin red (Sigma) to view neutral lipid vacuoles in adipocytes and calcium deposition in osteocytes, respectively. To assess chondrogenic differentiation, pellets of passage 5 cells were cultured for 3 weeks in chondrogenic medium. Chondrogenic medium consisted of DMEM (high-glucose), 1 × ITS (Gibco), 1 × linoleic acid–bovine serum albumin, ascorbate 2-phosphate (50 μg/ml), dexamethasone (100 nM) and 10 ng/ml hTGF-β1 (R&D Systems, Abingdon, Oxon, UK). This medium was replaced every 3–4 days for 21 days. Development of chondrogenic differentiation was determined by staining the pellet with Alcian blue. In addition, the expression of adipocyte-specific marker PPAR-γ, osteoblast-specific marker osteocalcin and cartilage-specific marker collagen II (all from Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) at the protein level was assessed by flow cytometry. The undifferentiated cells were maintained in the growth medium.

Mice and Ag preparation. Six-week-old female C57BL6/J mice were purchased from Vital River Laboratory Animal Technology Co. Ltd in Beijing. The mice were maintained in the animal laboratory of the institute of Hematology on a 12-h light/12-h dark cycle under pathogen-free conditions and had a standard diet and water. All animal experiments in this study were performed according to the institutional guidelines for animal care and approved by the institutional review board of the Chinese Academy of Medical Science and Peking Union Medical College. Analogue of Torpedo acetylcholine (AchR)α146-162 peptide (KKGKSYCEIIVTHFPFDQQN CTIKLG) was synthesized by Sangon Ltd (Shanghai, China). Peptides were synthesized using automated peptide synthesizer and purified by reversed phase HPLC, and their synthesis was confirmed by mass spectroscopy.

Induction and clinical evaluation of EAMG. To induce EAMG, mice were anesthetized and immunized once in both hind footpads by subcutaneous (s.c) injection of Ag

(100 μg/mice in 200 μl) emulsified in CFA (Sigma) supplemented with additional non-viable Mycobacterium tuberculosis H37RA (1 mg/mouse; Sigma) every 30 days. Control mice received an equal volume of PBS in CFA group. Clinical signs of EAMG were monitored on alternative days for 8 weeks after disease induction, and clinical scoring, conducted by two individuals blinded to the experimental groups, was assessed according to the presence of tremor, hunched posture, muscle strength and fatigability as described by Mario [20]. In briefly, 0 indicates no obvious abnormalities; 1, no abnormalities before testing, but reduced strength at the end; 2, clinical signs present before testing, i.e. tremor, head down, hunched posture, weak grip; 3, severe clinical signs present before testing, no grip; 4, moribund, dead.

Transplantation procedure. On the fourth day after first immunization, EAMG (n = 20)-induced group were randomly divided into groups and injected intravenously with either 1×10^6 MSC in 200 μl PBS per animal or 200 μl PBS alone. Equal volume of PBS was administrated in the control group (n = 10). The injection was repeated once on the 4 days after second immunization.

Mixed lymphocyte assays. We examine the effect of hMSC on proliferation of spleen mononuclear cells (MNC) both *in vivo* and *in vitro. In vivo*, MNC were obtained from either hMSC administrated or non-treated mice on day 56 after first immunization. Cell suspensions were prepared using 70-μm filters and separated by Ficoll gradient. Subsequently, MNC were washed twice in PBS and suspended in complete RPMI 1640 containing 2 mM glutamine (Sigma-Aldrich, Louis, MO, USA), 100 IU/ml penicillin/streptomycin, 10% FCS (Invitrogen, Life Technologies, Carlsbad, CA, USA) and 2×10^{-5} M_2-mercaptoethanol. Triplicate aliquots (200 μl) of MNC suspensions, each containing 2×10^5 cells, were plated into 96-well round-bottom microtiter plates and pulsed for 16 h with 5-bromo-2′-deoxyuridine (BrdU). The BrdU uptake was quantified by cell proliferation enzyme-linked immunosorbent assay through the use of a BrdU kit (Roche Diagnostics, Penzberg, Germany). The optical density (OD) values were determined in triplicate against a reagent blank at a test wavelength of 492 nm to reflect the growth characteristics of MNC.

To examine the effect of hMSC on MNC proliferation *in vitro*, different numbers of hMSC were cocultured with the MNC from non-treated EAMG group on 9 days after the first immunization [12]. Briefly, concentrations of hMSC added to each well were irradiated at 40 Gy, then 2×10^5 MNC were seeded at a final cell density of 1×10^6/ml, in the presence of serial concentrations of AchR (10 μg/ml), or 5 μg/ml concanavalin A (Sigma). The cocultures were incubated for 48 h in a humidified atmosphere of 5% carbon dioxide at 37 °C and then pulsed for 16 h with Brdu as described *in vivo* assays to test the OD value.

ELISA for anti-mouse AchR antibody isotypes. Mice were bled on day 10 after the second treatment (n = 3). AchR (0.5 ug/ml) was used to coat 96-well microtiter plates (Corning Costar 96-well plate, eBioscience) with 0.1 M carbonate bicarbonate buffer (pH 9.6) overnight at 4 °C. Serum samples diluted 1:100 to 1:3200 in blocking buffer were added and incubated at 37 °C for 120 min. After five washes, horseradish peroxidase-conjugated (HRPO) goat anti-mouse IgG diluted in 1:2000 in blocking buffer was added and incubated at 37 °C for 120 min. Subsequently, o-phenylenediamine substrate solution (eBioscience) was added, and colour was allowed to develop at room temperature in the dark for 15 min. The reaction was stopped by adding 2 M H_2SO_4. The OD values were determined in triplicate against a reagent blank at a test wavelength of 492 nm.

hMSC labelling and immunofluorescence. For cell tracking studies, 2×10^7 cells were labelled with 3 μg/ml CM-DiI (Invitrogen) at 37 °C for 5 min and then at 4 °C for another 15 min. The cells were washed with PBS for three times, and the labelling efficiency was detected under fluorescence microscope (Olympus). The recovery rate of this procedure was 80–85% viable cells. One week after hMSC injection, the mice abdominal cavity was exposed quickly after anaesthesia with isoflurane, then placed in a ventral recumbent position in the IVIS imaging system chamber and imaged for 2 s with the whole body cooled CCD (IVIS 200 Series Imaging System; Xenogen, Alameda, CA, USA)camera system at the highest sensitivity. When imaging, first a black and white image of the animal is taken in low light conditions, then the photons emitted from the animal are collected by a sensitive cooled charge-coupled device camera. Photons emitted from the liver region were quantified using Living Image software (Xenogen).

Four weeks after the last cell therapy, mice were killed, and spleens were removed carefully and immersed in 4% paraformaldehyde followed by acetone fixation. A series of adjacent 6-μm-thick frozen sections of spleen were incubated for 30 min at room temperature with 5% BSA in PBS. Monoclonal antibodies against human cell surface glycoproteins CD54 and CD44 (form BD) were used, respectively, to examine the existence of hMSC. 4′,6-Diamidino-2-phenylindole (DAPI; Sigma-Aldrich) was used to identify nuclear cells. Control sections were incubated with antibody against mouse IgG1 (BD).

Statistical analysis. Data are presented as mean ± SD. One-way analysis of variance was used to make comparisons of parameters among groups. If the omnibus tests among groups were significantly different, post hoc tests between groups using unpaired t tests were used. $P < 0.05$ was considered significant. The SPSS (SPSS, Chicago, IL, USA) software package was used for the statistical analysis.

Table 1 Immunophenotype of hMSC.

Surface markers	The positive percentage (%) $n = 3$
CD14	0.80 ± 0.05
CD19	0.42 ± 0.07
CD29	98.75 ± 1.36
CD31	1.07 ± 0.23
CD34	0.29 ± 0.09
CD45	0.34 ± 0.11
CD73	99.91 ± 1.88
CD90	99.17 ± 1.45
CD105	99.29 ± 2.01
CD117	1.80 ± 0.29
CD166	99.96 ± 1.94
HLA-ABC	88.93 ± 1.25
HLA-DR	1.09 ± 0.31

Results

Immunophenotype and multilineage differentiation of hMSC

hMSC were successfully isolated and *in vitro* expanded from foetal bone marrow. Flow cytometric results illustrated that hMSC were positive for CD44, CD105, CD166, CD90 and HLA-ABC and negative for CD45, CD34, CD31, CD19, CD14, CD117 and HLA-DR (Table 1). These data clearly demonstrate that the cell possesses the immunophenotype of MSC [21]. Furthermore, hMSC were able to differentiate into adipogenic, osteogenic and chondrogenic cells *in vitro* (Fig. 1). Together, our results suggest that hMSC fit the recommended definition of MSC.

hMSC inhibit the proliferation of MNC

The proliferation of MNC in coculture group was affected at some degree by irradiated hMSC compared with the MNC alone group. As shown in Fig. 2A, when the number of hMSC was abundant, the proliferation was inhibited (e.g. the number is 4×10^4 and 2×10^4). Especially, on the ratio of hMSC and MNC was 1:5, MNC display the lowest proliferation level. While the number of hMSC was deficient, the proliferation was promoted (e.g. the number is 0.5×10^4). Furthermore, the suppression of hMSC on MNC appeared variant in different stimulate groups. It was more obvious in Con A group than that in AchR group and no-antigen group.

After cell transplantation, we also examined the proliferation of MNC *in vivo*. We found that the proliferation of MNC in hMSC treatment group was markedly diminished compared to the no-treatment group (Fig. 2B).

Additionally, we tested the effect of the hMSC supernatants (third passage hMSC and 2 days culture) on the proliferation of MNC from EAMG. In AchR group and non-treated group, we did not observe a significant inhibition, while in the Con A group the proliferation of MNC was gently decreased (Fig. 2A). Interestingly, we found that MNC were attracted by and consequently attached to the hMSC (Fig. 2C). Therefore, both solution molecule and cell–cell contact may be involved in the inhibiting process.

Tracing of hMSC in EAMG mice

To study the distribution of hMSC after transplantation, we prelabelled hMSC with fluorescent dye CM-DiI and analysed the EAMG mice in imaging system 1 week after cell administration. We found that a part of hMSC migrated to lymphoid organs in abdominal part (Fig. 3A). In the sections of spleen, we also found the hMSC as shown by CD54 (red) and CD44 (red) staining

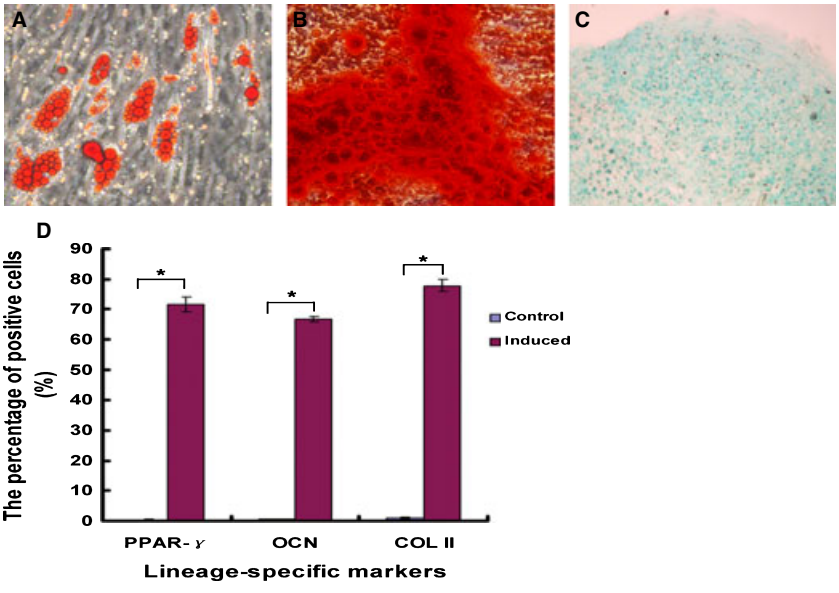

Figure 1 Multilineage differentiation of hMSC. (A) Oil Red O staining of induced adipocytes from hMSC. (B) Differentiation of hMSC into osteoblasts as evaluated by Alizarin red staining. (C) Chondrogenic potential of hMSC as shown by Alcian blue staining. (D) The expression of lineage-specific markers for adipogenesis (PPAR-γ), osteogenesis (OCN) and chondrogenesis (COL II) as determined by flow cytometry analysis ($n = 3$), $^*P < 0.05$. Error bars represent SD. *P* values were calculated with Student's *t*-test. PPAR-γ indicates peroxisome proliferation–activated receptor-γ; OCN, osteocalcin; and COL II, collagen II. Original magnification: (A–C) ×200.

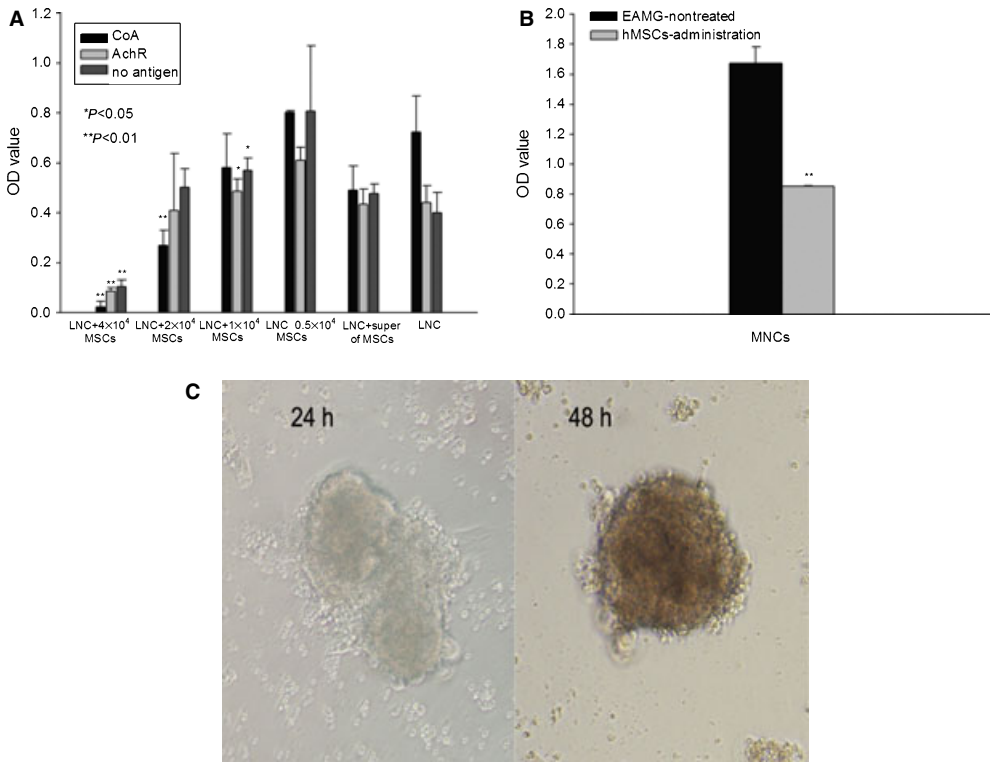

Figure 2 hMSC inhibit the proliferation of mononuclear cells (MNC). (A) hMSC could inhibit the proliferation of MNC *in vitro*. The proliferation of MNC in coculture group was affected at some degree by irradiated hMSC compared with the MNC alone group. Especially, on the radio of hMSC and MNC was 1:5, MNC display the lowest proliferation level. The hMSC supernatants (third passage hMSC and 2 days culture) were able to decrease the proliferation of MNC from experimental autoimmune myasthenia gravis (EAMG) in Con A group. (B) hMSC inhibit the proliferation of MNC *in vivo*. The proliferation of MNC in hMSC-administrated group was markedly diminished compared with the no-treatment group. (C) The attraction between hMSC and MNC in different times point. MNC were attracted by and consequently attached to the hMSC.

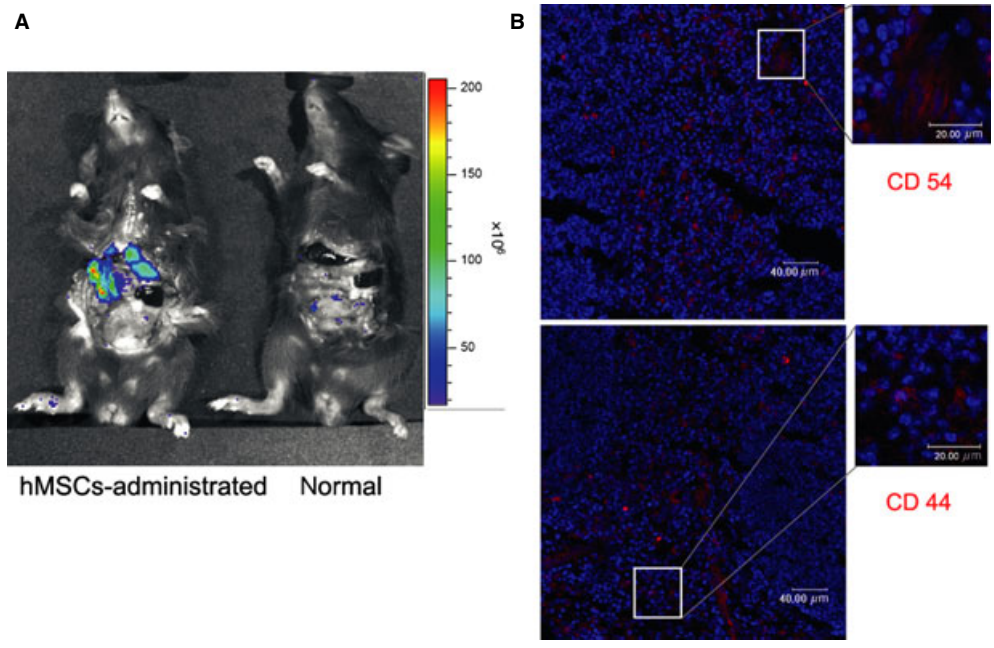

Figure 3 hMSC labelling and immunofluorescence. (A) *In vivo* imaging of hMSC of distribution in experimental autoimmune myasthenia gravis (EAMG) mice. Percentage of hMSC migrated to lymphoid organs in abdominal part. (B) Representative fluorescence microscopic spleen slice. hMSC were showed by CD 54 (red) and CD44 (red). DAPI (blue) staining was used to identify nuclear cells. hMSC were found in the sections of spleen.

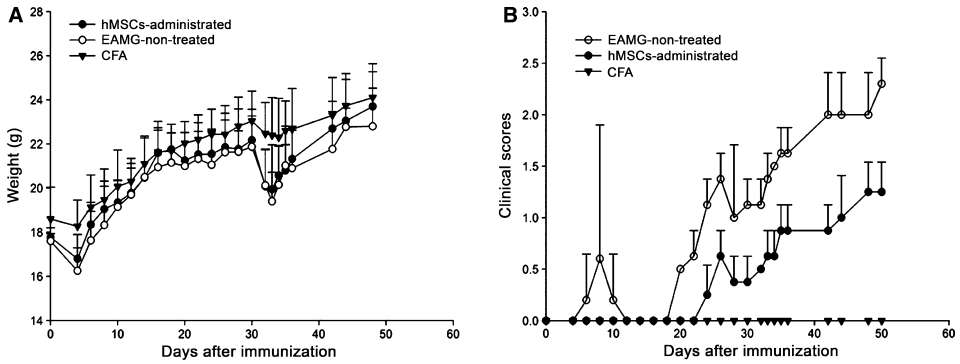

Figure 4 Administration of hMSC ameliorates experimental autoimmune myasthenia gravis (EAMG). Body weight and clinical score in hMSC-administrated group, non-treated and CFA group. We did not observe significant difference of the body weight between hMSC-administrated group, non-treated group and CFA group (Fig. 4A). The mean clinical scores of the hMSC administration group were significantly improved compared with that of non-treated group. (P clinical score <0.05).

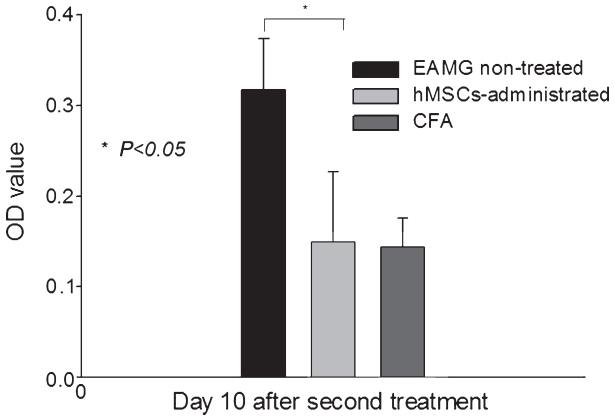

Figure 5 The antibody of acetylcholine receptor (AchR) level in hMSC-administrated group, non-treated group and CFA group ($^*P < 0.05$).

(Fig. 3B). These observations indicate that hMSC are able to circulate to the immune organs *in vivo*, which may relate to their immunomodulatory function.

Administration of hMSC ameliorates EAMG

For non-treatment group, 90% mice had clinically demonstrable fatigable muscle weakness. As we can see in Fig. 4B, the mean clinical scores of the hMSC administration group were significantly improved, and the AchR levels were reduced when compared with that of no-treatment group (Fig. 5), which means hMSC administration ameliorated the disease situation of EAMG. However, the death rate between the different groups was apparent (data not show), suggesting that hMSC might be less effective for the serious EAMG. In addition, we did not observe significant difference of the body weight between hMSC-administrated group and non-treated group (Fig. 4A).

Discussion

In this study, we examined a stem cell-based therapeutic strategy for EAMG using human MSC. In EAMG mice, human MSC were able to reduce the proliferation of AchR-special T cells and decrease the autoantibodies levels, which may explain the ameliorated EAMG mice symptoms by hMSC injection. Our findings suggest that, the beneficial effect of MSC in EAMG animals was not restricted to allogeneic transplantation [19], xenogeneic MSC (hMSC) were as efficient as murine MSC in mitigating the clinical signs of mice EAMG. The immunoprivileged feature of MSC [21] indicates their promising future as a candidate for cell therapy.

There are several potential mechanisms for the effect of hMSC on the EAMG mice. Clinically, the acute phase of patients with MG relates with an over-activation of immune system, although the exact mechanism is still unclear. In the over-activated immune system, proliferation of AchR-special T cell plays critical roles for the development of disease. In our study, we observed that active MNC were inhibited by hMSC both *in vivo* and *in vitro* mixed lymphocyte cell experiments. Especially, when the ratio of MNC and hMSC was 5:1, the proliferation of MNC was completely suppressed in Con A group (Fig. 2A). The supernatant of hMSC also decreased the proliferation of MNC to some extent in Con A group, which suggested that soluble molecules secreted by hMSC played certain roles in the inhibitory process. This speculation is supported by several previous studies, in which hepatocyte growth factor (HGF), transforming growth factor (TGF-β), indoleamine 2,3-dioxygenase (IDO) and interleukin-10 (IL-10) [10] were proved to be involved in the process. Moreover, cell–cell contact may be another factor contributing to the inhibition. As seen in our study, much more MNC were attracted to the hMSC bodies (Fig. 2C), indicating direct physical interaction between them may be a necessary step for the

inhibition consequence. Indeed, a recent study reported the similar observation [22]. They found that the immunosuppressive function of MSC was elicited by IFN-gamma and the concomitant presence of any of three other proinflammatory cytokines including TNF-alpha, IL-1alpha or IL-1beta. These cytokine combinations provoked the expression of high levels of several chemokines and inducible nitric oxide synthase (iNOS) by MSC. Finally, chemokines-derived T cell migrates into the proximity with MSC, where T-cell responsiveness was suppressed by nitric oxide (NO).

hMSC could affect the Th1/Th2 balance, which may be another mechanism in the immunomodulatory process. In patients with MG, the suppression of disease was often accompanied by decreased Th1 cell proliferation [23–25], and Th1-type chemokine signallings were altered in the patients with MG. In our former study, we found that hMSC decreased IFN-gamma-producing Th1 cells [16], another study also showed that, in the EAE animal model, inflammatory T cells including IFN-gamma producing Th1 cells and their associated cytokines were reduced along with concomitant increases in IL-4 producing Th2 cells and anti-inflammatory cytokines after hMSC administration [26]. More importantly, two other studies found that the benefits of MSC in EAMG were mediated by their ability to alter the balance of Th1/Th2/Treg cell subsets through the secretion of TGF-$\beta$and IDO [18, 19].

That hMSC diminished the AchR antibody may also be one of the explanations to the therapeutical effect of cell treatment. The decrease in autoantibodies in the serum of patients with MG is often coupled with the recovering of the patients. In our study, we found a reduced AchR antibody levels in serum of hMSC-administrated group. Indeed, previous studies reported that MSC can effectively inhibit the proliferation, differentiation to plasma cells and antibody production of B cells [15], [27]. More importantly, the concentration of MSC is very important to the MSC' inhibitory effect on B cells [28]. In our study, we used 10^6 hMSC in the EAMG mice, which might be an appropriate cell dose for inhibiting the production of the antibody. In parallel with our study, in the EAE animal model, administration of MSC led to the inhibition of pathogenic antibodies [29]. This effect was recently assumed to be a result of metalloproteinase processing of CCL2 produced by MSC, which leads to the suppression of STAT3 [30].

hMSC could home to spleen and lymph nodes and contact with the lymphocytes. After systemic administration, we found human-derived cells in mice spleen. hMSC expressed homing receptors and was able to home to site of inflammation and lymphoid tissue. However, the selective homing hMSC was limited and disappeared after several weeks. Perhaps, a therapeutic benefit could also be obtained by local paracrine production of growth factors and a provision of temporary antiproliferative and immunomodulatory properties [21, 31–33]. Furthermore, because the immune privilege of hMSC may not be guaranteed in an immune competent host after systemic administration in large amount, it is of interest to test if local delivery of cells could raise the cell numbers in target tissue and bring about more significant results.

In conclusion, we demonstrated that systemic administration of hMSC was beneficial to EAMG mice, and the underlying mechanisms may correlate with immunomodulatory potential of hMSC. Further studies are needed to reveal the signal pathways involved in the effects of hMSC, in order for facilitating the MSC-based therapy in autoimmune diseases like MG.

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Disclosures

The authors have no financial conflicts of interest.

References

1 Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis Prevalence, clinical correlates, and diagnostic value. *Neurology* 1976;26:1054–9.
2 Rosen A, Casciola-Rosen L. Autoantigens in systemic autoimmunity: critical partner in pathogenesis. *J Intern Med* 2009;265:625–31.
3 Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007;110:3499–506.
4 Liu M, Guang Yang S, Shi L, *et al.* Mesenchymal stem cells from bone marrow show a stronger stimulating effect on megakaryocyte progenitor expansion than those from non-hematopoietic tissues. *Platelets* 2010;21:199–210.
5 Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284:143–7.
6 Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. *J Cell Physiol* 1999;181:67–73.
7 Chichester CO, Fernandez M, Minguell JJ. Extracellular matrix gene expression by human bone marrow stroma and by marrow fibroblasts. *Cell Adhes Commun* 1993;1:93–9.
8 Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats–similarities to astrocyte grafts. *Proc Natl Acad Sci USA* 1998;95:3908–13.
9 Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000;28:875–84.
10 Majumdar MK, Thiede MA, Mosca JD, Moorman M, Gerson SL. Phenotypic and functional comparison of cultures of marrow-derived

mesenchymal stem cells (MSCs) and stromal cells. *J Cell Physiol* 1998;176:57–66.
11 De Ugarte DA, Alfonso Z, Zuk PA, *et al.* Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. *Immunol Lett* 2003;89:267–70.
12 Zappia E, Casazza S, Pedemonte E, *et al.* Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 2005;106:1755–61.
13 Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. *Blood* 2006;107:1484–90.
14 Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. *Transplantation* 2007;83:71–6.
15 Corcione A, Benvenuto F, Ferretti E, *et al.* Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006;107:367–72.
16 Guo Z, Zheng C, Chen Z, *et al.* Fetal BM-derived mesenchymal stem cells promote the expansion of human Th17 cells, but inhibit the production of Th1 cells. *Eur J Immunol* 2009;39:2840–9.
17 Bai L, Lennon DP, Eaton V, *et al.* Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia* 2009;57:1192–203.
18 Kong QF, Sun B, Bai SS, *et al.* Administration of bone marrow stromal cells ameliorates experimental autoimmune myasthenia gravis by altering the balance of Th1/Th2/Th17/Treg cell subsets through the secretion of TGF-beta. *J Neuroimmunol* 2009;207:83–91.
19 Kong QF, Sun B, Wang GY, *et al.* BM stromal cells ameliorate experimental autoimmune myasthenia gravis by altering the balance of Th cells through the secretion of IDO. *Eur J Immunol* 2009;39:800–9.
20 Losen M, Stassen MH, Martinez-Martinez P, *et al.* Increased expression of rapsyn in muscles prevents acetylcholine receptor loss in experimental autoimmune myasthenia gravis. *Brain* 2005;128:2327–37.
21 Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum* 2009;60:1006–19.
22 Ren G, Zhang L, Zhao X, *et al.* Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;2:141–50.
23 Fuchs S, Feferman T, Meidler R, Brenner T, Laub O, Souroujon MC. The disease-specific arm of the therapeutic effect of intravenous immunoglobulin in autoimmune diseases: experimental autoimmune myasthenia gravis as a model. *Isr Med Assoc J* 2008;10:58–60.
24 Fuchs S, Feferman T, Meidler R, *et al.* Immunosuppression of EAMG by IVIG is mediated by a disease-specific anti-immunoglobulin fraction. *Ann N Y Acad Sci* 2008;1132:244–8.
25 Suzuki Y, Onodera H, Tago H, *et al.* Altered expression of Th1-type chemokine receptor CXCR3 on CD4+ T cells in myasthenia gravis patients. *J Neuroimmunol* 2006;172:166–74.
26 Bai Y, Liu R, Huang D, *et al.* CCL2 recruitment of IL-6-producing CD11b+ monocytes to the draining lymph nodes during the initiation of Th17-dependent B cell-mediated autoimmunity. *Eur J Immunol* 2008;38:1877–88.
27 Comoli P, Ginevri F, Maccario R, *et al.* Human mesenchymal stem cells inhibit antibody production induced in vitro by allostimulation. *Nephrol Dial Transplant* 2008;23:1196–202.
28 Crop M, Baan C, Weimar W, Hoogduijn M. Potential of mesenchymal stem cells as immune therapy in solid-organ transplantation. *Transpl Int* 2009;22:365–76.
29 Gerdoni E, Gallo B, Casazza S, *et al.* Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol* 2007;61:219–27.
30 Rafei M, Hsieh J, Fortier S, *et al.* Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production via STAT3 inactivation and PAX5 induction. *Blood* 2008;112:4991–8.
31 Prockop DJ. "Stemness" does not explain the repair of many tissues by mesenchymal stem/multipotent stromal cells (MSCs). *Clin Pharmacol Ther* 2007;82:241–3.
32 Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008;8:726–36.
33 Tyndall A, Uccelli A. Multipotent mesenchymal stromal cells for autoimmune diseases: teaching new dogs old tricks. *Bone Marrow Transplant* 2009;43:821–8.