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**Immunologic regulatory effects of human umbilical cord blood-derived mesenchymal stem cells
in a murine ovalbumin asthma model**

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Abstract

Background: Mesenchymal stem cells (MSCs) have multiple immunomodulatory properties and hold therapeutic potential for inflammatory diseases. However, the therapeutic and immunologic effects of human umbilical cord blood-derived MSCs (huMSCs) remain largely unexamined for asthma.

Objective: This study was to investigate the immunomodulatory properties of huMSCs in an ovalbumin (OVA)-induced murine asthma model.

Methods: Mice were injected intraperitoneally with OVA and an aluminum hydroxide adjuvant. huMSCs were administered via the tail-vein (5×10^5 cells/100uL) to female BALB/c mice prior to the initial OVA challenge. The effects of huMSCs were assessed by investigating airway hyperresponsiveness, histological changes, inflammatory cell numbers, serum allergen-specific antibodies, cytokine production in spleen, lung tissue, and BAL fluid as well as expansion of regulatory T cells.

Results: Administration of huMSCs significantly reduced methacholine bronchial hyperresponsiveness and eosinophil counts in BAL cells. Similarly, there was a significant decrease in serum OVA-specific IgE and IgG1 levels along with Th2 cytokine production (IL-4, IL-5, and IL-13) in the lung and spleen tissues, whereas increased percentage of regulatory T cells was observed after treatment with huMSCs.

Conclusions: Our results suggest that huMSC treatment reduces OVA-induced allergic inflammation, which could be mediated by regulatory T cells.

Introduction

Asthma is a chronic respiratory disease characterized by persistent airway inflammation and remodeling [1]. Traditional management strategies for asthma focus on two therapies: controllers to treat the underlying inflammation and relievers that relax the airway muscles. Despite these strategies, asthma remains uncontrolled in numerous patients [2, 3]. Thus, alternative treatment options are required. Recent study suggests that transplantation of mesenchymal stem cells (MSCs) could inhibit airway inflammation and remodeling for treatment of heterogeneous airway diseases [4]. Human mesenchymal stem cells (hMSCs) are non-hematopoietic multipotent stromal cells that can differentiate into multiple connective tissue lineages, including adipocytes, chondrocytes, osteoblasts, myocytes, and neuronal cells [5, 6]. hMSCs can also repair damaged and inflamed tissue and regulate innate and adaptive immune responses [7-9]. hMSCs are well tolerated following allo- and xeno-transplantation and are isolated from bone marrow, adipose tissue, skeletal muscle, synovium, spleen, thymus, lung, amniotic fluid, and the umbilical cord [10]. Human umbilical cord blood-derived mesenchymal stem cells (huMSCs) are readily isolated and can be expanded following a painless and noninvasive isolation procedure [11]. Several reports of allogeneic and xenogeneic MSCs have revealed the immunomodulatory properties of MSCs; however, none has investigated the immunomodulatory outcomes of huMSCs in a murine asthma model [12-20]. Thus, the immunomodulatory effects of huMSCs were investigated using an ovalbumin (OVA)-induced murine allergic asthma model.

Methods

Mouse model

Six-week-old female BALB/c mice (16–18 g) were purchased from SLC Inc. (Hamamatsu, Kotoh-cho, Japan), and maintained under specific pathogen-free conditions. All experiments were approved by the Institutional Animal Care and Use Committee at the Institute of Laboratory Animal Resources at Seoul National University (SNU-130829-3-2). A total of 18 BALB/c mice were divided into 3

groups and used in this study: Group A (control group, n=6), Group B (ovalbumin-induced asthma + PBS, n=6), and Group C (ovalbumin-induced asthma + huMSCs, n=6).

Preparation of human umbilical cord blood-derived mesenchymal stem cells

Isolation and multiplication of huMSCs were performed as previously described [21, 22]. huMSCs were collected from the umbilical vein following neonatal delivery with maternal informed consent, and prepared according to the proper manufacturing practices at MEDIPOST Co., Ltd (Seoul, Korea). huMSCs were collected using a 16-gauge needle that was joined to a huMSCs collection bag containing 23 ml of CPDA-1 anticoagulant (Greencross Co., Yongin, Kyunggi-do, Korea). huMSCs were harvested within 24 hr of collection, and cell viability was always greater than 90%. Mononuclear cells were isolated by centrifugation in a Ficoll-Hypaque gradient (density 1.077 g/cm³, Sigma, St Louis, MO, USA). The separated mononuclear cells were washed, suspended in α -minimum essential medium (α -MEM, GibcoBRL, Carlsbad, CA, USA), supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide with a twice-weekly medium change. One to three weeks later, when the monolayer of adherent cell colonies reached confluence, the cells were trypsinized (0.25% trypsin, HyClone), re-suspended in culture medium (α -MEM supplemented with 10% FBS and 50ug/ml gentamicin) and subcultured at a concentration of 5×10^5 cells/cm². We used the sixth passage cells for experiments. Flow cytometric analysis was performed to verify the cultured cells at passage 6, which were stained with antibodies against human CD13, CD14, CD34, CD45, HLA-DR (FITC, Becton Dickinson, San Jose, CA, USA), CD31, CD90, CD51/61, CD64 (FITC, Pharmingen, Los Angeles, CA, USA) and CD29, CD44, CD106 (PE, Pharmingen, Los Angeles, CA, USA). Corresponding mouse isotype antibodies were used as controls. The labeled cells were analyzed by FACSCalibur flow cytometer using Cell Quest Pro software (Becton Dickinson, San Jose, CA, USA).

OVA sensitization and airway challenge

BALB/c mice in Groups B and C were treated intraperitoneally on days 1 and 14 with 75 µg of ovalbumin (Grade V, Sigma-Aldrich, St. Louis, MO, USA) and 2 mg of aluminum hydroxide (Alum, Pierce, Rockford, IL, USA) in 200 µL of phosphate-buffered saline (PBS). Mice in Group A were treated with saline with aluminum hydroxide only. Following sensitization, huMSCs were administered via the tail-vein (5×10^5 cells/100µL) to Group C mice prior to the initial OVA challenge. As the control, PBS was administered to mice in Groups A and B prior to the initial OVA challenge. All mice were challenged intranasally with 50 µg OVA in 20 µL of PBS on days 29, 30, and 31 (Fig. 1).

Airway physiology

One day after the final OVA challenge, airway hyperresponsiveness (AHR) after methacholine exposure was measured using a barometric plethysmographic chamber (OCP 3000, Allmedicus, Anyang, Korea). The 3-min enhanced pause (Penh) value was also measured.

Analysis of bronchoalveolar lavage

Twenty-four hours after assessing AHR, mice were sacrificed, and bronchoalveolar lavage (BAL) fluid were collected. BAL was performed by inserting a cannula into the bronchi and infusing 2ml of PBS. BAL fluid was recovered by aspirating with a syringe. Cells were separated from the lavage fluid, and microscope slides were prepared using Cytospin 3 (Thermo Scientific, Pittsburgh, PA, USA) and stained using the Diff-Quik staining kit (Sysmex Co., Kobe, Japan). A total of 300 cells per slide were assessed by light microscopy using specific morphological criteria to quantify for each inflammatory cell type.

Histopathological analysis

Lung tissues were obtained from the middle zone of the left lung to evaluate pathological changes of lung parenchyma. Samples were fixed in 4% neutral buffered formalin, processed, paraffin embedded, sectioned at 3-mm intervals. Two different staining methods were used for light microscopic evaluation. Hematoxylin and eosin (H&E) stain was used for recognizing peribronchial and perivascular inflammation and thicknesses of epithelium as well as subepithelial smooth muscle layers. Mucous secreting goblet cells were detected in Periodic acid-Schiff (PAS) staining. All sample slides for comparison were observed under same magnification. The degree of peribronchial and perivascular inflammation was evaluated on a subjective scale of 0-3, as described previously [23, 24]. A value of 0 represents no detectable inflammation, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 for most bronchi or vessels surrounded by a thin layer (1-5 cells thick) of inflammatory cells, and a value of 3 for most bronchi or vessels surrounded by a thick layer (> 5 cells thick) of inflammatory cells. To grade the extent of goblet cell hyperplasia, subjective five-point scoring system (PAS score) was used as described elsewhere [25, 26]. The scoring criteria were as follows: 0, < 0.5% PAS positive cells; 1, < 25%; 2, 25–50%; 3, 50–75% and 4, > 75%. Eight random fields were counted for each slide and mean score was calculated from six mice. Analyses were performed by a pathologist in a blinded fashion, and slides were presented in random order for each examination.

Analysis of cytokine expression in the lung, spleen tissue and BAL fluid

To measure cytokine responses in lung tissue, lung lysates were prepared and assayed from all mice used in this study. Total RNA (2µg) was reverse transcribed into cDNA using a single-strand cDNA synthesis kit (Promega, Madison, WA, USA). Gene expression was measured using an ABI 7500 real-time polymerase chain reaction system (Applied Biosystems, Foster, CA, USA) with cDNA, gene-specific primers, and SYBR Green master mix. The expression of each gene was normalized against β -actin and presented as fold changes compared with untreated cells. Mouse primer sequences used for PCR analysis were as follows;

Interleukin-4:

(Forward primer) 5'-ACTTGAGAGAGATCATCGGC-3'

(Reverse primer) 5'-ACTTGAGAGAGATCATCGGC-3'

Interleukin-5:

(Forward primer) 5'-ACCACAGTGGTGAAAGAGAC-3'

(Reverse primer) 5'-CATCGTCTCATTGCTTGCTCA-3'

Interleukin-13:

(Forward primer) 5'-CCTGGCTCTTGCTTGCCT T-3'

(Reverse primer) 5'-GGTCTTGTGTGATGTTGCTCA-3'

The protein levels of IL-4, IL-5 and IL-13 in spleen as well as IL-4, IL-10, IL-13 and TGF- β in BAL fluid were determined by a sandwich ELISA (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's protocol. Erythrocyte-depleted splenocytes were obtained to measure cytokine concentrations from supernatant after re-stimulation with OVA (100 μ g/ mL) for 72 hours.

Analysis of serum OVA-specific antibody

Forty-eight hours following the final OVA challenge, blood samples were obtained by cardiac puncture, and OVA-specific antibody concentrations (IgG1, IgG2a, and IgE) were measured. OVA-specific antibody concentrations were measured by sandwich ELISA (R&D Systems Inc., Minneapolis, MN, USA) using 5- μ g/ml grade V OVA (Sigma-Aldrich, St. Louis, MO, USA) for coating and anti-mouse isotype-specific antibodies (Southern Biotechnology Associates, Birmingham, AL, USA) for detection.

Flow cytometric analysis of lung-draining lymph nodes and spleen

To evaluate whether injected huMSCs affected regulatory T (Treg) cell expansion, single-cell suspensions of lung-draining lymph nodes and spleen were prepared from non-treated and huMSCs-treated samples. A phenotypic analysis of Treg cells was performed using three-color flow cytometry,

where cells were stained with anti-mouse CD4-FITC, anti-mouseCD25-PE, and anti-mouse Foxp3–PE–cyanine 5. For the Foxp3 antibody, an isotype-matched control was used to determine the background. Treg cells were identified as CD4⁺CD25⁺Foxp3⁺ triple-positive and expressed as the percentage of total CD4⁺ T cells. Data were collected using an LSRII flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using FlowJo 10.0 software (Tree Star, Ashland, OR, USA).

Statistics

Data were analyzed using Stata 14.1 software (StataCorp LP, College Station, TX, USA). All values were expressed as the means $\pm$ standard error (SEM). All experiments were repeated at least three times. Comparisons among all groups were made using the Kruskal–Wallis test, whereas the Mann–Whitney U-test was used to compare two groups. A p-value < 0.05 was considered statistically significant.

Results

Methacholine AHR

Methacholine significantly increased AHR in the OVA groups compared with the control group. However, BALB/c mice receiving huMSCs showed a significant decrease in the development of AHR following methacholine treatment (Fig. 2).

BAL and histologic findings

To examine if huMSCs could modify allergen-driven airway inflammation in mice, lung histologies and cellularity were assessed in tissue and bronchoalveolar lavage samples from BALB/c mice. As shown in Figure 3A, non-OVA challenged mice exhibited minimal cellularity in BAL fluid, whereas

OVA-challenged mice exhibited significant cell increases in macrophages, neutrophils, lymphocytes, and eosinophils. Even though there were significant decreases in inflammatory cells of the huMSC-treated mice, the lung inflammation, especially the eosinophilic infiltration, was more significantly reduced by more than 50% compared with OVA challenged mice. Microscopically, OVA exposure significantly increased peribronchial and perivascular infiltration of inflammatory cells with eosinophil, lymphocytes, and goblet cells compared with PBS-treated mice. The basement membrane, epithelium, and subepithelial smooth muscle layers were thickened in those cells. Treatment with huMSCs alleviated the thickness of the basement membrane, epithelium, and subepithelial smooth muscle layers in the OVA-treated group (Fig. 3B). PAS staining of the lung tissue showed that the mucus production was also markedly decreased after huMSC treatment (Fig. 3B). Microscopic findings as described above were further confirmed by subjective scoring of the inflammation and PAS positive cells showing significant reduction in the airway inflammation and goblet cell hyperplasia upon treating with huMSCs, as compared with those of OVA-treated group alone (Fig. 3C).

ELISA analysis of ovalbumin-specific immunoglobulin expression

To evaluate whether huMSCs affected the production of serum OVA-specific antibodies, the concentrations of OVA-specific IgG1, G2a, and IgE were determined by ELISA. As shown in Figure 4, IgG1 and IgE levels were elevated in the OVA groups compared with controls. Notably, IgG1 and IgE levels in the OVA-treated groups were significantly lower in mice treated with huMSCs. No significant differences in IgG2a levels were observed under any conditions (data not shown).

Levels of cytokines measured by RT-qPCR and ELISA

The physiologic and histologic findings described above could be explained by huMSCs-mediated inhibition of airway hyperreactivity and Th2-mediated eosinophilic lung inflammation. Therefore, the effect of huMSCs on T cell-mediated immunity was examined by quantitative real-time RT-PCR and ELISA. huMSCs treatment significantly reduced IL-4, IL-5, and IL-13 mRNA expression in lung

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tissue in the OVA-treated group (Fig. 5). Cytokine levels of IL-10 and TGF- β in BAL fluid determined by ELISA were also increased in huMSCs-treated OVA group as compared with non-treated OVA group, whereas concentrations of IL-4 and IL-13 were decreased with huMSCs-treated OVA group (Fig. S1). However, protein levels changed by treatment in BAL fluid were minimal when converted into protein concentration. The levels of IL-4 and IL-5 levels in the supernatants of OVA-treated splenocytes were significantly higher in the OVA-treated group compared with control and were significantly reduced upon treatment with huMSCs (Fig. 6), while IL-13 levels detected in spleen was too low to compare differences between groups (data not shown).

Comparison of regulatory T cells in lymph nodes and spleen by flow cytometry

The expansion of the Treg population was assessed in the lung-draining lymph nodes and spleen of huMSCs-treated and untreated cells challenged with OVA. The percentage of CD4⁺CD25⁺Foxp3⁺ Treg cells in the OVA-treated group was significantly higher following treatment with huMSCs compared with group challenged with OVA alone in lung lymph nodes. The frequency of Treg cells from the spleen was also observed an increasing trend in corresponding huMSCs treatment (Fig. 7).

Discussion

MSCs are present in multiple tissue types, including bone marrow, adipose tissue, amniotic fluid, and the umbilical cord [10]. Unlike hematopoietic stem cells, MSCs differentiate into almost any cell type, which makes them appealing for regenerative medicine applications [4]. Moreover, due to the hypo-immunogenic properties of MSCs, they survive and differentiate in MHC-mismatched allogeneic or xenogeneic transplant recipients [27]. Bone marrow-derived MSCs (BM-MSCs) are the major source of human MSC and the most widely studied [28]. BM-MSCs elicit immunomodulatory effects and downregulate the Th2 response in airways in allergic inflammatory diseases [13-19]. BM-MSCs, however, are difficult to obtain in sufficient quantities from total bone marrow cells. Moreover, BM-

MSCs decrease in concentration and proliferative/differentiation capacity with age. In contrast, umbilical cord cells are easily collected without pain, and the procedure avoids other ethical and technical issues [11]. huMSCs harvested from umbilical cords exhibit multipotency and self-renewal capacities. They accumulate at the site of damage or inflammation and promote tissue repair and modulate the immune response [29-33]. Thus, huMSCs are being used in multiple clinical trials for the treatment of patients with neurodegenerative disease, autoimmune disease, liver disease, and severe acute graft-versus-host disease (www.clinicaltrials.gov) [34-37]. Therefore, umbilical cord cells exhibit significant clinical benefits, including their ease of accessibility and regenerative capacities.

In the present study, we demonstrated that all the data collectively support a role for huMSCs in regulation of Th2-induced inflammation in a murine asthma model. To determine whether injected huMSCs migrated into the lung, we performed *in vivo* bioluminescent imaging as described previously [14]. We observed that huMSC are deposited in the lungs whereas virtually no cells were seen in other organs within 48 hours after tail vein injection of huMSCs (data not shown). huMSCs significantly attenuated airway hyperresponsiveness and eosinophilic infiltration into lung tissue and BAL fluid. huMSCs also conferred multiple histopathological changes. huMSCs downregulated IL-4, IL-5, and IL-13 and decreased the production of IgE and IgG1, which are indicative of a Th2 response.

These findings are comparable with those of previous studies using allogeneic bone marrow or adipose-derived MSCs in an asthmatic mouse model [12-20]. The mechanisms of action both innate and adaptive responses have been reported in MSCs immunomodulation, which encompass the induction of soluble factors, anergy, apoptosis, tolerogenic dendritic cells, and regulatory T (Treg) cells [38]. Treg cells defined by expression of transcription factor Foxp3 have an important role in the regulation of immune responses and in the prevention of allergic disease. Recent studies have shown the ability of MSCs to generate Treg cells, and MSC expansion or induction of Treg cells has been associated with protection allergic airway disease [13, 14]. To evaluate the mechanism underlying the immunomodulatory effects of huMSCs in a murine asthma model, Treg cell populations derived from lung lymph nodes and spleen as well as regulatory cytokines IL-10 and TGF- β from BAL fluid were explored as reported by Cho *et al* [20]. We showed that huMSCs increased the population of FoxP3⁺

Treg cells in lung lymph node together with increasing trends from the spleen. Previous studies have demonstrated that regulation of an active immune response by Treg cells occurs in the draining lymph node as well as at the site of inflammation [39, 40]. We also found that the expansion of FoxP3+ Treg cells was accompanied by upregulation of IL-10 and TGF- β 1 production. These findings are in agreement with those of a previous report in a murine model of allergic asthma demonstrating that Treg cells from adipose tissue by activated MSCs might secrete anti-inflammatory cytokines such as IL-10 and TGF- β , which ultimately lead to decrease of lung eosinophilic inflammation, as well as allergy-specific Th2 cytokines and IgE antibody production [20]. However, this is a limitation that the differences were low when converted into protein concentration determined by ELISA. In our studies, albeit with limitation, not only did huMSCs decrease the pathology and inflammation in a murine asthma model, but also there was a significant decrease in serum OVA-specific antibodies (IgE and IgG1) and Th2 cytokine levels in lung (IL-4, IL-5, and IL-13 mRNA) as well as spleen (IL-4 and IL-5 protein) consistent with decreased allergic inflammation as seen by systemic effects. Therefore, we reasoned that huMSCs could provide immunomodulatory effects in asthma and speculated that their immunomodulatory effects might be related to Treg cells. We fully acknowledge that this study could not rigorously prove mechanistic link between huMSCs and Treg cells function, and must be interpreted with caution, but therapeutic outcomes of huMSCs on alleviation of allergic inflammation were obvious. As far as we know, this is the first investigation detailing the effects of xenogenic huMSCs on allergic inflammation in an acute asthma model. Despite our remarkable findings, further studies should be accumulated to clarify whether huMSCs efficiently regulate T cells and protect against allergen-driven pathology in a murine asthma model.

In conclusion, our study demonstrated that xenogeneic huMSCs attenuate allergic airway inflammation in a murine asthma model. The therapeutic effects of huMSCs are similar to those of bone marrow- or adipose-derived MSCs and do not have any serious immunologic consequences. These findings support further investigations into the mechanisms and development of huMSCs for the prevention or treatment of asthma.

References

1. Busse WW, Lemanske RF, Jr. Asthma. *N Engl J Med*. 2001; **344**: 350-62.
2. Agbetile J, Green R. New therapies and management strategies in the treatment of asthma: patient-focused developments. *J Asthma Allergy*. 2011; **4**: 1-12.
3. Chung KF, Wenzel SE, Brozek JL *et al*. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*. 2014; **43**: 343-73.
4. Weiss DJ. Concise review: current status of stem cells and regenerative medicine in lung biology and diseases. *Stem Cells*. 2014; **32**: 16-25.
5. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol*. 2000; **28**: 875-84.
6. Pittenger MF, Mackay AM, Beck SC *et al*. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; **284**: 143-7.
7. Bassi EJ, de Almeida DC, Moraes-Vieira PM, Camara NO. Exploring the role of soluble factors associated with immune regulatory properties of mesenchymal stem cells. *Stem Cell Rev*. 2012; **8**: 329-42.
8. Liu TM, Martina M, Hutmacher DW *et al*. Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. *Stem Cells*. 2007; **25**: 750-60.
9. Shi Y, Hu G, Su J *et al*. Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. *Cell Res*. 2010; **20**: 510-8.
10. Iyer SS, Rojas M. Anti-inflammatory effects of mesenchymal stem cells: novel concept for future therapies. *Expert Opin Biol Ther*. 2008; **8**: 569-81.
11. Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. *World J Stem Cells*. 2014; **6**: 195-202.
12. Park HK, Cho KS, Park HY *et al*. Adipose-derived stromal cells inhibit allergic airway inflammation in mice. *Stem Cells Dev*. 2010; **19**: 1811-8.
13. Kavanagh H, Mahon BP. Allogeneic mesenchymal stem cells prevent allergic airway

- inflammation by inducing murine regulatory T cells. *Allergy*. 2011; **66**: 523-31.
14. Nemeth K, Keane-Myers A, Brown JM *et al*. Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. *Proc Natl Acad Sci U S A*. 2010; **107**: 5652-7.
 15. Goodwin M, Sueblinvong V, Eisenhauer P *et al*. Bone marrow-derived mesenchymal stromal cells inhibit Th2-mediated allergic airways inflammation in mice. *Stem Cells*. 2011; **29**: 1137-48.
 16. Bonfield TL, Koloze M, Lennon DP *et al*. Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. *Am J Physiol Lung Cell Mol Physiol*. 2010; **299**: L760-70.
 17. Lee SH, Jang AS, Kwon JH *et al*. Mesenchymal stem cell transfer suppresses airway remodeling in a toluene diisocyanate-induced murine asthma model. *Allergy Asthma Immunol Res*. 2011; **3**: 205-11.
 18. Firinci F, Karaman M, Baran Y *et al*. Mesenchymal stem cells ameliorate the histopathological changes in a murine model of chronic asthma. *Int Immunopharmacol*. 2011; **11**: 1120-6.
 19. Ou-Yang HF, Huang Y, Hu XB, Wu CG. Suppression of allergic airway inflammation in a mouse model of asthma by exogenous mesenchymal stem cells. *Exp Biol Med (Maywood)*. 2011; **236**: 1461-7.
 20. Cho KS, Park MK, Kang SA *et al*. Adipose-derived stem cells ameliorate allergic airway inflammation by inducing regulatory T cells in a mouse model of asthma. *Mediators Inflamm*. 2014; **2014**: 436476.
 21. Jin HJ, Bae YK, Kim M *et al*. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. *Int J Mol Sci*. 2013; **14**: 17986-8001.
 22. Yang SE, Ha CW, Jung M *et al*. Mesenchymal stem/progenitor cells developed in cultures from UC blood. *Cytotherapy*. 2004; **6**: 476-86.
 23. Kwak YG, Song CH, Yi HK *et al*. Involvement of PTEN in airway hyperresponsiveness and

- inflammation in bronchial asthma. *J Clin Invest.* 2003; **111**: 1083-92.
24. Tournoy KG, Kips JC, Schou C, Pauwels RA. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy.* 2000; **30**: 79-85.
25. Myou S, Leff AR, Myo S *et al.* Blockade of inflammation and airway hyperresponsiveness in immune-sensitized mice by dominant-negative phosphoinositide 3-kinase-TAT. *J Exp Med.* 2003; **198**: 1573-82.
26. Yoshioka M, Sagara H, Takahashi F *et al.* Role of multidrug resistance-associated protein 1 in the pathogenesis of allergic airway inflammation. *Am J Physiol Lung Cell Mol Physiol.* 2009; **296**: L30-6.
27. Atoui R, Chiu RC. Concise review: immunomodulatory properties of mesenchymal stem cells in cellular transplantation: update, controversies, and unknowns. *Stem Cells Transl Med.* 2012; **1**: 200-5.
28. Galderisi U, Giordano A. The gap between the physiological and therapeutic roles of mesenchymal stem cells. *Medicinal research reviews.* 2014; **34**: 1100-26.
29. Zhang Y, Xia Y, Ni S *et al.* Transplantation of umbilical cord mesenchymal stem cells alleviates pneumonitis of MRL/lpr mice. *J Thorac Dis.* 2014; **6**: 109-17.
30. Liu Y, Mu R, Wang S *et al.* Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. *Arthritis Res Ther.* 2010; **12**: R210.
31. Lim JY, Jeong CH, Jun JA *et al.* Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells after intrathecal administration by lumbar puncture in a rat model of cerebral ischemia. *Stem Cell Res Ther.* 2011; **2**: 38.
32. Guo J, Yang J, Cao G *et al.* Xenogeneic immunosuppression of human umbilical cord mesenchymal stem cells in a major histocompatibility complex-mismatched allogeneic acute graft-versus-host disease murine model. *Eur J Haematol.* 2011; **87**: 235-43.
33. Chang JW, Hung SP, Wu HH *et al.* Therapeutic effects of umbilical cord blood-derived mesenchymal stem cell transplantation in experimental lupus nephritis. *Cell Transplant.* 2011; **20**: 245-57.
34. Wang L, Li J, Liu H *et al.* Pilot study of umbilical cord-derived mesenchymal stem cell

- transfusion in patients with primary biliary cirrhosis. *J Gastroenterol Hepatol*. 2013; **28** Suppl 1: 85-92.
35. Shi M, Zhang Z, Xu R *et al*. Human mesenchymal stem cell transfusion is safe and improves liver function in acute-on-chronic liver failure patients. *Stem Cells Transl Med*. 2012; **1**: 725-31.
 36. Kim HJ, Seo SW, Chang JW *et al*. Stereotactic brain injection of human umbilical cord blood mesenchymal stem cells in patients with Alzheimer's disease dementia: A phase 1 clinical trial. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*. 2015; **1**: 95-102.
 37. Dongmei H, Jing L, Mei X *et al*. Clinical analysis of the treatment of spinocerebellar ataxia and multiple system atrophy-cerebellar type with umbilical cord mesenchymal stromal cells. *Cytotherapy*. 2011; **13**: 913-7.
 38. English K. Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol Cell Biol*. 2013; **91**: 19-26.
 39. Carson Wft, Guernsey LA, Singh A *et al*. Accumulation of regulatory T cells in local draining lymph nodes of the lung correlates with spontaneous resolution of chronic asthma in a murine model. *Int Arch Allergy Immunol*. 2008; **145**: 231-43.
 40. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity*. 2009; **30**: 636-45.









