OVEREXPRESSION OF HEME OXYGENASE-1 IN MESENCHYMAL STROMAL CELLS: A NOVEL THERAPEUTIC APPROACH FOR ALLERGIC RHINITIS

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ABSTRACT

Objective: *Allergic rhinitis (AR) is an atopic disorder of the nose induced after allergen exposure through IgE-mediated inflammation. Although AR has become a significant global public health concern and led to heavy financial burden, the curative effect of the available treatments is not completely satisfactory. Mesenchymal stem cells (MSCs) therapy has been considered as a promisingly alternative approach for allergic diseases with their immunomodulatory effect. However, many issues remain to be conquered, for example, MSCs would lose their phenotypes after several passages in vitro, and their immunomodulatory effect still needs to be improved. Heme oxygenase-1 (HO-1), the rate-limiting enzyme for heme degradation, is noticed by its ability to improve the anti-allergic effect of MSCs in vitro. Nonetheless, few study has been conducted to test this effect in vivo.*

Methodology: *Here, we modified MSCs therapy strategy by transduction of SV-40 into MSCs to make them immortalized in vitro (iMSCs) and further transduced HO-1 gene into iMSCs (HO-1 iMSCs). Then we tested the immunomodulatory effect of HO-1 iMSCs in the mouse model of AR. Our results demonstrate that the modified strategy is technically feasible, and HO-1 iMSCs has stronger anti-allergic effect accompanied with higher generation of $CD25^+Foxp3^+$ regulatory T cells than their controls in vivo.*

Conclusion: *These findings suggest that HO-1 iMSCs may become a novel therapeutic strategy for future clinical use to treat AR patients.*

Keywords: *Allergic rhinitis, mesenchymal stem cells (MSCs), heme oxygenase-1 (HO-1), AR mouse model, $CD25^+Foxp3^+$ Treg.*

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Introduction

Allergic rhinitis (AR) is a chronic allergic airway disease that has become a significant global public health concern[(1)]. It is characterized by its Th2-skewed eosinophilic inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR). Epidemiologic studies indicate that AR may occur in over 500 million people around the world and the prevalence of AR is increasing globally over the past decades[(2)]. Standard drug therapy of AR is not completely satisfactory and several new treatment approaches are currently underway for allergic disease. Mesenchymal stem cells (MSCs) are pleiotropic progenitor cells that can be isolated and expanded from bone marrow and other tissues. With their powerful immunomodulatory ability[(3)], MSCs have been suggested as a potential modality for immune mediated diseases therapy, and have been used in many clinical settings such as experimental autoimmune encephalomyelitis[(4)] and collagen-induce arthritis[(5)]. The therapeutic potential of MSCs on allergic diseases has been reported by several teams[(6, 7)]. Our previous study also showed that the MSCs derived from mouse nasal mucosa have the possibility to treat AR[(8)]. However, many issues

remain to be solved, for example, the MSCs would lose their phenotypes after several passages in vitro[9]. The studies to increase the immunomodulatory effect of MSCs are still in progress.

Heme oxygenase-1 (HO-1), the rate-limiting enzyme for heme degradation, catalyzes the stepwise degradation of heme to produce equimolar quantities of iron, biliverdin and carbon monoxide[10]. HO-1 serves as a protective gene, and its overexpression has significant biological consequences such as anti-oxidative, anti-inflammatory, and anti-proliferative effects as well as modulation of the cell cycle activities. HO-1 is expressed in many cells, including MSCs[11], and several studies have investigated its therapeutic effect in MSCs for allergic diseases. Li reported HO-1 in MSCs contributed to the up-regulation of $CD4^+$ $CD25^+$ CD127low/-regulatory T cells (Treg)[12]. Mougiakakos also observed the overexpressed HO-1 in MSCs could induce Treg[13]. However, to the best of our knowledge, there are no data examining the anti-allergic effects of MSCs with overexpressed HO-1 in vivo, which may be a new strategy to improve the therapy effect of MSCs on AR. In this study, we proposed a modified MSCs therapy strategy by transduction of the SV-40 genes into the cells to make MSCs immortalized (iMSCs) in vitro[14] and further transduced HO-1 into the iMSCs (HO-1 iMSCs).

Then, we tested the immunomodulatory effect of HO-1 iMSCs in ovalbumin (OVA) induced AR mouse model. The symptom of AR, cytokines of allergic inflammation and the markers of Treg cells were tested and compared between the HO-1 iMSCs and its control groups.

Materials and methods

Animals

BALB/c mice (6-week-old) were purchased from Shanghai SLAC Laboratory Animal Company (Shanghai, China) and bred in the animal laboratory of Fudan University. Mice were maintained in a temperature controlled facility with a 12-hour light/12-hour dark cycle and were given free access to food and water. All experimental procedures in this study were followed the guidelines and protocol approved by the Animal Care and Use Committee of the Eye and ENT Hospital of Fudan University.

Culture of Bone Marrow MSCs

After sacrificing the mice, the ends of the femurs and tibias were clipped to expose the marrow. Bone Marrow was washed several times by complete medium with 10% Fetal Bovine Serum (FBS) (Gibco, Invitrogen, Paisley, UK) until the BM was white. After centrifuged for 1 min at 600 g, the cell pellets derived from the bones were resuspended in complete medium with 10% FBS, and cultured in a 25 cm2 of flask. After 24h of culturing in a humidified incubator with 5% of CO_2 at 37°C, the non-adherent cells were removed by changing the medium. Then the culture medium was changed every 3-4 days.

Immortalized MSCs

To establish iMSCs in vitro, early passages of MSCs (<3 passage) were seeded in 25 cm of flasks and infected with packaged retrovirus PHY-802 (Hanyin Biotechnology Limited Company, Shanghai, China) expressing SV40 T antigen. Stable iMSCs pools were established by selecting the infected cells with hygromycin B (at 4 mg/mL) for 1 week. Aliquots of iMSCs were kept in liquid nitrogen tanks.

HO-1 MSCs

The HO-1 sequence was amplified from the NCBI and subcloned into the retroviral expression vector PHY-022 (Hanyin Biotechnology Limited Company, Shanghai, China) via EcoRI. HO-1 iMSCs were obtained by the transduction of HO-1 gene into iMSCs at passage 4 with the PHY-022. Then, the culture was expanded in parallel until entering the senescent state.

Immunophenotypic analysis

CD29, CD44, SCA-1, CD45 and CD11b were detected by Flow cytometry. Briefly, MSCs and HO-1 iMSCs were washed twice with stain buffer, then centrifuged and resuspended in 100 μL of stain buffer. The cells were stained with 30 μg of antibodies (PE-conjugated hamster anti-mouse CD29, PerCP-Cy 5.5 conjugated rat anti-mouse CD44, Alexa Fluor 647-conjugated rat anti-mouse SCA-1, PE-Cy7-conjugated rat anti-mouse CD45, and PE-Cy7-conjugated rat anti-mouse CD11b, all from eBioscience, San Diego, CA, USA) for 30 minutes at room temperature. Then the cells were analyzed by flow cytometry (BD FACS Calibur and FlowJo software, New Jersey, USA) at passage 4.

Mouse sensitization and treatment

Twenty mice were equally divided into normal control, OVA, OVA + iMSCs and OVA +HO-1 iMSCs groups. The OVA, OVA + iMSCs and OVA + HO-1 iMSCs groups were sensitized via intraperitoneal

injection of 20 μg of ovalbumin (OVA, grade V, Sigma-Aldrich, St. Louis, MO, USA) and 1 mg of aluminum hydroxide (Sigma-Aldrich, St. Louis, MO, USA) in 200 μl of PBS on days 1, 7, and 14. After sensitization, OVA, OVA+iMSCs and OVA+HO-1 iMSCs group were injected with PBS, iMSCs and HO-1 MSCs respectively on days 18, 19 and 20 via tail vein. From days 21 to 27, OVA, OVA+iMSCs and OVA+HO-1 iMSCs groups were challenged with aerosolized 5% OVA through an air-compressing nebulizer (Yuyue403A, Jiangsu, China).

The normal control mice were sensitized, injected and challenged with PBS in the whole procedure. ALL mice were sacrificed on day 29 and the samples were collected (Figure 1). On day 28, nasal allergic symptoms were assessed by counting the number of sneezing per hour.

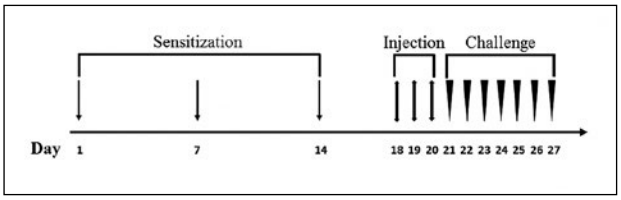

Figure 1: Schematic drawing of the mouse model of allergic rhinitis. Mice were sensitized on days 1, 7 and 14 with OVA or PBS. On days 18, 19 and 20, the mice were injected with purified iMSCs, HO-1 iMSCs or PBS before challenge of OVA or PBS from 21 to 27 days. The mice were sacrificed and the samples were collected on day 28. The mice were divided into four groups in accordance with their different treatments.

Cytokine examination

24 hours after the final challenge of OVA, blood samples were collected and tested by enzyme-linked immunosorbent assay (ELISA). ELISA kits for assessing OVA-IgE and IL-10 were purchased from eBioscience (San Diego, CA, USA). ELISA was performed according to the manufacturer. In brief, the serum was put into 96-well specific antibody-coated plate.

Microplate reader (Molecular Devices Corporation, Sunnyvale, CA) was used to record absorbance at 450 nm. Each cytokine standard and sample was run in duplicate.

Western Blot

The spleen and nasal mucosa were removed 24 hours after the last OVA challenge. Spleens were homogenized with ice-cold radioimmune precipitation assay buffer (Beyotime, Shanghai, China) containing protease inhibitors. The samples from HO-1 iMSCs and nasal mucosa were extracted with ice-cold radioimmune precipitation assay buffer. The extracted samples containing 50μg of proteins were separated by a 12% Tris-sodium dodecyl sulfate-polyacrylamide gel and transferred to a nitrocellulose membrane.

The membrane was blocked with Tris-buffered saline Tween-20 buffer containing 5% skimmed milk, and then incubated with rabbit anti-mouse HO-1 at 1:1500 dilution (Abcam, Cambridge, EN), goat anti-mouse Foxp3 at 1:1500 dilution (Abcam, Cambridge, EN) or rabbit anti-mouse CD25 at 1:200 dilution (Santa Cruz Biotechnology, Santa Cruz, CA) overnight.

This was followed by addition of appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies. β-actin was subsequently detected with mouse anti-β-actin antibody (Chemicon, Temecula, CA, USA) and HRP-labeled secondary antibody (Cappel Laboratories, Malvern, PA, USA) as an internal control. The bands of HO-1, Foxp3 and CD25 were detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL) reaction using the ECL system.

Quantitative RT-PCR

HO-1 iMSCs were harvested, and the total RNA was extracted with TRIzol reagent (Invitrogen, Burlington, Canada). Spleen tissue was removed and homogenized after the last challenge, and total RNA was isolated with TRIzol reagent according to the manufacturer's instructions.

Reverse transcription was performed with random primers and Superscript III (Invitrogen, Burlington, Canada) to obtain cDNA samples. Real-time PCR was performed using an ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Massachusetts, USA) with the following program: 95 °C for 2 mins and 40 cycles of amplification at 94 °C for 10 s, 60 °C for 10 s, and 72 °C for 40 s. Relative levels of target mRNA were compared with β-actin using the 2-ΔΔCt method. All primers were synthesized by Shanghai Shengon Biotech Company (Shanghai, China).

Sequences were as follows: GAPDH forward 5'- GGCAAATTCAACGGCACAG-3', reverse 5'-ACGACATACTCAGCACCGGC-3'; CD25 forward 5'- GAGCCACGCTTGCTGATGTT-3', reverse 5'-GGCTTTGAATGTGGCATTGG-3'; Foxp3 forward 5'- GACCCCCTTTCACCTATGCC-3', reverse 5'-GGCGAACATGCGAGTAAACC-3'; HO-1 forward 5'- CGCAACAAGCAGAACCCAG-3', reverse 5'-TGATTTCCTGCCAGTGAGGC-3'.

Statistical Analysis

Data are expressed as mean ± SD. An unpaired two-tailed t-test was used for between-group analyses. One-way ANOVA followed by Bonferroni's post hoc tests were exploited for comparison of 3 or more groups. All analyses were performed using GraphPad5.0 (Graph Pad Software Inc., San Diego, CA, USA). The difference was considered statistically significant when $p<0.05$.

Results

Characterization of HO-1 iMSCs

SV40 and HO-1 genes were transduced into MSCs by lentiviruses in a stepwise manner. The cell-surface antigen profiles of MSCs and HO-1 iMSCs were detected by flow cytometry. Our results shown that the transduction of SV40 and HO-1 didn't change the cell surface antigen profiles in HO-1 iMSCs group compared with MSCs group (Figure 2). After transduction, the expression of HO-1 mRNA and protein in HO-1 iMSCs was significantly upregulated compared with iMSCs group (Figure 3).

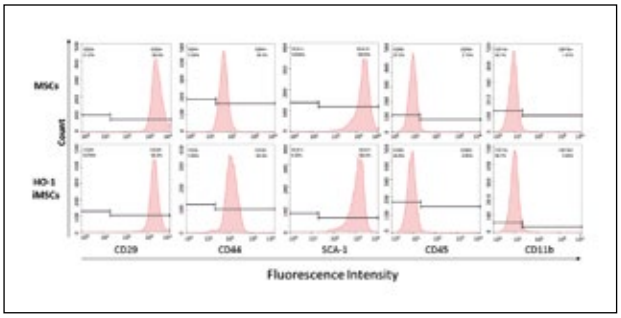

Figure 2: The molecular biological features of HO-1 iMSCs. iMSCs was characterized for the cell surface markers of CD29, CD44, SCA-1, CD11b and CD45 by flow cytometry.

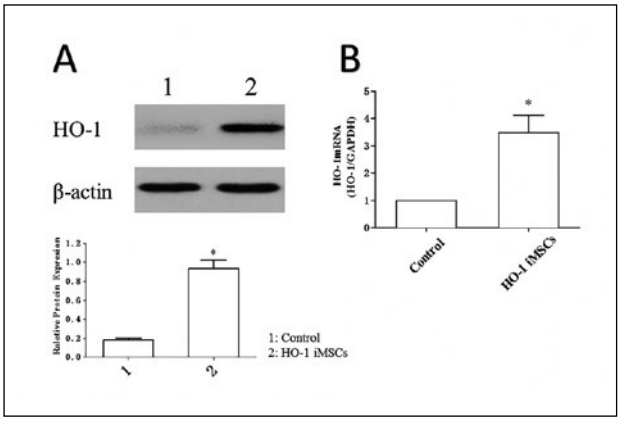

Figure 3: The expression of HO-1 in cells.
*The expression of HO-1 protein (A) and mRNA (B) in HO-1 iMSCs was significantly upregulated compared with iMSCs group (*P<0.05).*

HO-1 expression in nasal mucosa and spleen

To test if the administration of HO-1 iMSCs could enhance HO-1 expression in vivo. The HO-1 protein expression in nasal mucosa and spleen was measured by western blot. The results showed that HO-1 protein expression in nasal mucosa and spleen was increased in OVA+iMSCs and OVA+HO-1 iMSCs groups compared with the control group. Meanwhile, the expression of HO-1 in OVA+HO-1 iMSCs group was higher than the OVA+iMSCs group in both nasal mucosa and spleen (Figure 4).

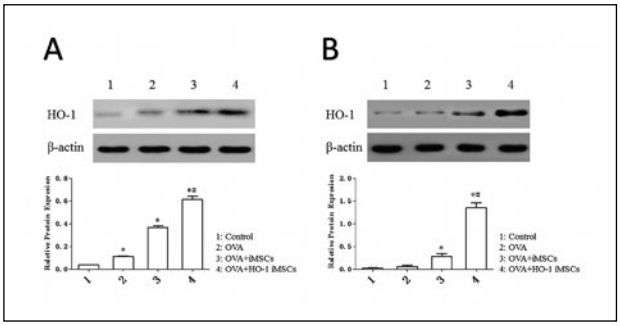

Figure 4: The expression of HO-1 in nasal mucosa and spleen.
*(A). Western blot analysis of HO-1 protein expression in nasal mucosa of four different groups. The HO-1 expression in OVA+HO-1 iMSCs group was higher than the other groups (B). The pattern of HO-1 protein expression in spleen was similar with the nasal mucosa group. *P<0.05 compared with the control group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, n=5.*

Allergic analysis of nasal symptoms

To assess the anti-inflammatory effects of HO-1 iMSCs on allergic symptoms, we counted the numbers of sneeze per hour in control, OVA, OVA+iMSCs and OVA+HO-1 iMSCs groups. The sneezing frequency in OVA group was significantly higher than the control group.

However, the frequency of sneeze was dropped by tail vein injection of OVA+iMSCs or OVA+HO-1 iMSCs, suggesting the improvement of allergic symptoms by these treatments. More importantly, in comparison to OVA+iMSCs group, the frequency of sneeze was further decreased in the presence of OVA+HO-1 iMSCs (Figure 5).

Secretion of immunoglobulin

We also tested the expression of OVA-specific IgE by ELISA in the four groups to confirm the anti-allergic effect of HO-1 iMSCs.

The level of OVA-specific IgE was significantly higher in OVA groups than control group. The injection of iMSCs and HO-1 iMSCs had a significant tendency to reduce the secretion of OVA-

specific IgE. This tendency was more obvious in HO-1 iMSCs group than iMSCs group (Figure 6).

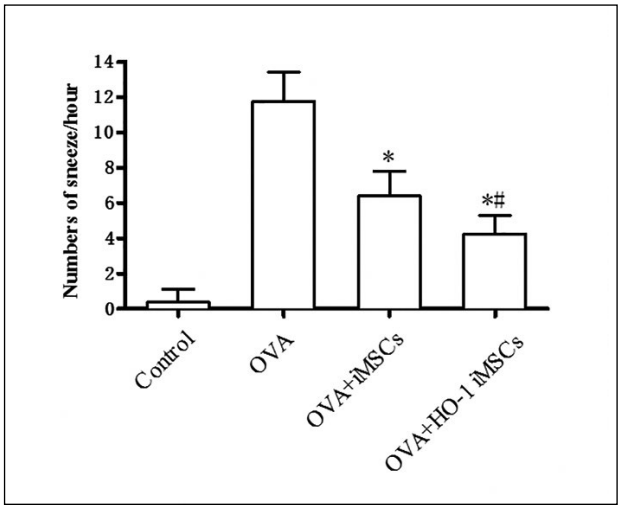

Figure 5: The frequency of sneeze. The number of sneeze per hour was decreased in HO-1 iMSCs group not only compared with the OVA group but also with the OVA+iMSCs group.
**P<0.05 compared with the OVA group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, n=5.*

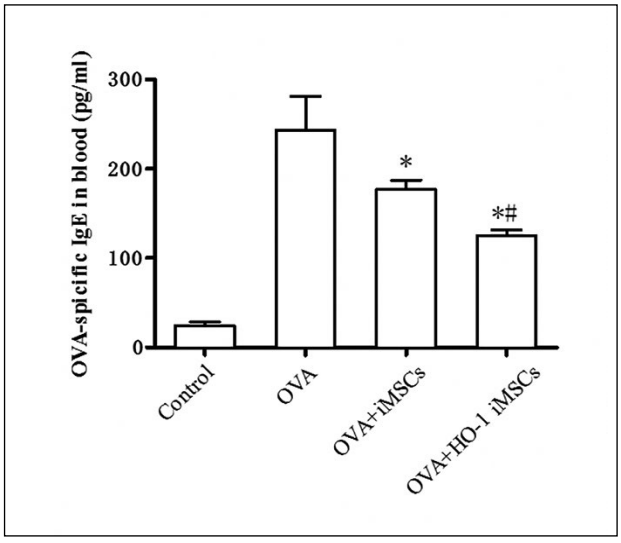

Figure 6: OVA-specific IgE in different groups. The levels of OVA-specific IgE in blood was determined by ELISA analysis. Consistent with the inhibition of the frequency of sneeze, the levels of OVA-specific IgE was decreased in OVA+iMSCs and OVA+HO-1 iMSCs groups compared with OVA group. Importantly, the further decrease was observed in the OVA+HO-1 iMSCs group.
**P<0.05 compared with the OVA group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, n=5.*

CD25 and Foxp3 expression in spleen tissue

To test the influence of HO-1iMSCs on Tregs in our model, the change of CD25 and Foxp3 in spleen was tested by western blot and real-time PCR. Both protein and mRNA of CD25 and Foxp3 were significantly decreased after OVA challenge compared with the control group. However, the administration of HO-1 iMSCs followed OVA challenge increased the level of CD25 and Foxp3. No difference of CD25 or Foxp3 was observed between the OVA+iMSCs and OVA groups (Figure 7 and 8).

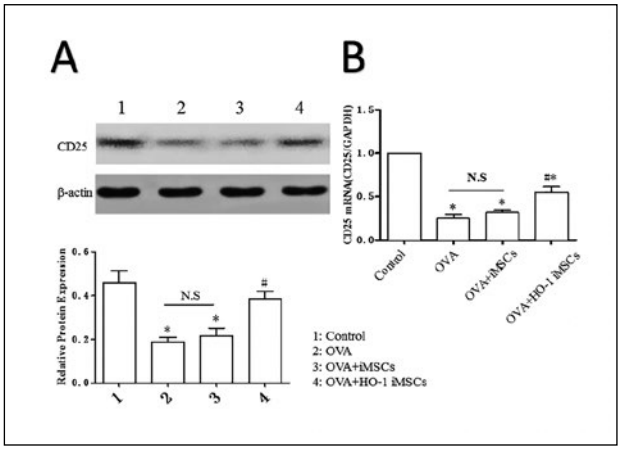

Figure 7: CD25 expression in spleen tissue. The change of CD25 was tested by western blot and real-time PCR. (A) CD25 protein was significantly decreased after OVA sensitization compared with its control group. The administration of HO-1 iMSCs significantly elevated the levels of CD25. No influence was observed in the iMSCs groups compared with the OVA group. (B) The expression of CD25 mRNA was similar with CD25 protein.
**P<0.05 compared with the control group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, N.S.: no significant difference, n=5.*

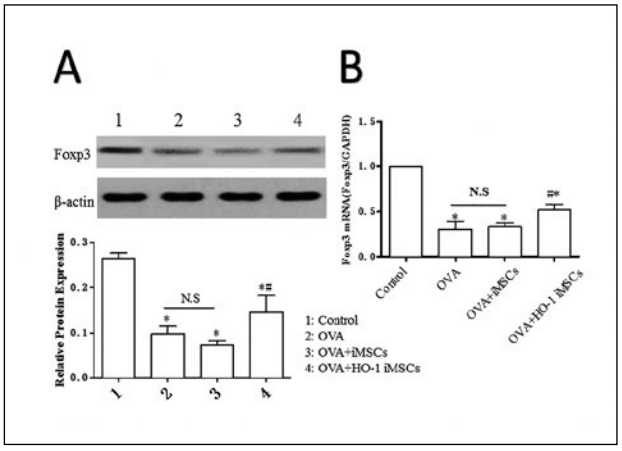

Figure 8: Foxp3 expression in spleen tissue. The change of Foxp3 was tested by western blot and real-time PCR. (A) The protein Foxp3 was decreased after OVA treatment compared with the control group. The administration of HO-1 iMSCs significantly elevated the levels of Foxp3. No influence was observed in the iMSCs groups compared with the OVA group. (B) The expression of Foxp3 mRNA was similar with Foxp3 protein.
**P<0.05 compared with the control group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, N.S.: no significant difference, n=5.*

IL-10 expression

We further examined the level of IL-10 in blood by ELISA. Our results showed that the expression of IL-10 was significantly declined in OVA group in comparison with those in the control group. However, such decline was markedly inhibited by the administration of HO-1 iMSCs (Figure 9). This tendency was in accordance with the expression of CD25 and Foxp3 shown above (Figure 7 and 8).

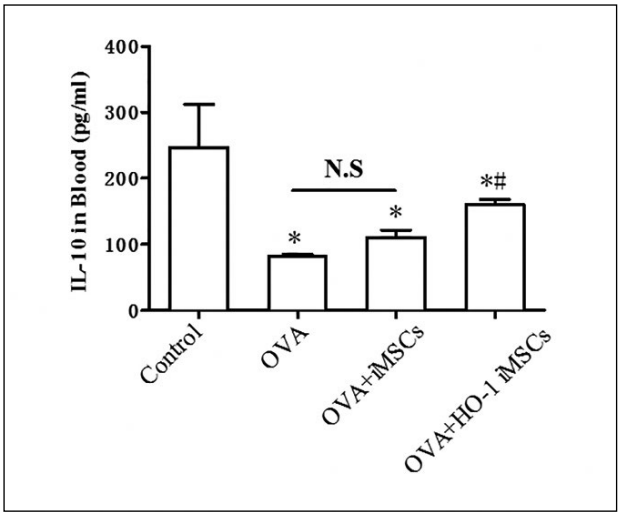

Figure 9: The level of IL-10 in different groups. The levels of IL-10 in blood was determined by ELISA analysis. Consistent with the expression pattern of CD25 and Foxp3, the levels of IL-10 was decreased in OVA and OVA+iMSCs groups and no difference was observed between them. The administration of OVA+HO-1 iMSCs elevated the level of IL-10 compared with the OVA+iMSCs group.

**P<0.05 compared with the control group, #P<0.05 OVA+HO-1 iMSCs group vs. OVA+iMSCs group, n=5.*

Discussion

In this study, we proposed a modified MSCs therapy strategy for treating AR. The results demonstrate that our strategy is technically feasible and HO-1 iMSCs has stronger immunomodulatory effect compared with their counterparts in vivo. To the best of our knowledge, this is the first study to test the anti-allergic effect of HO-1 iMSCs in a mouse model of AR. The immunomodulatory function of MSCs make them a promising candidate for allergic diseases therapy. However, there are several potential limitations of using MSCs, including their limited capacity to proliferate. To overcome this deficiency, we transduced the reversible immortalization system expressing SV-40 into MSCs, which made MSCs immortally in vitro[15]. According to our results, the phenotype of the modified MSCs was not changed, and the expression of HO-1 was successfully upregulated in vitro and in vivo. Immortalized human fetal bone-marrow derived cells were recently described to represent a suitable, safe and effective source for suicide gene delivery in anti-tumor therapeutic approaches, as demonstrated in vitro and in an immunodeficient animal model[14]. Murine immortalized cell line derived from the calvaria of newborn mice was currently established for bone biology studies[15, 16]. In line with these studies, our results demonstrate that iMSCs might provide an ideal source for their therapeutic utility in the disease of AR. MSCs attracted interest in stem cell therapy attributed to their immunomodulatory ability and their potential to differentiate into different kinds of tissues[7]. HO-1 is implicated to have protective effects in the diseases associated with oxidative stress, inflammation and apoptosis[17] and is involved in the modulatory effect of MSCs[12]. HO-1 MSCs has been reported to modulate the immune response in the situations such as liver transplantation[18]. Furthermore, Li and Mougiakakos reported the overexpressed HO-1 in MSCs could induce Treg[12], which possess a variety of immunomodulatory effects in allergic diseases[19]. However, the immunomodulatory effect of HO-1 MSCs in vivo has not been reported. Our in vivo study firstly demonstrates that the allergic symptom and allergen specific IgE were further reduced in HO-1 iMSCs group compared with iMSCs group in the mouse model of AR. These results imply that HO-1 iMSCs may be a better stem therapy approach than MSCs for AR patients.

Although previous studies suggest that Treg cells have a potential role in the prevention of allergic pathology, the role of Tregs in MSCs immunomodulation is still controversial. While several studies indicated that MSCs indirectly modulate the immune response via Tregs, others reported that no role for Tregs in this process[20]. In our model, the expression of $CD25^+$ $Foxp3^+$ Treg in HO-1 iMSCs group was higher than the control group, but interestingly, no significantly increased expression of $CD25^+$ $Foxp3^+$ Treg was observed in the iMSCs group. These results suggest that the expansion of $CD25^+$ $Foxp3^+$ cells are involved in the HO-1 iMSCs mediated immunosuppression and also imply that the immunomodulatory mechanism may be different in iMSCs and HO-1 iMSCs groups. Further studies are needed to fully understand this difference. Meanwhile, our study revealed that the increased expression of $CD25^+$ $Foxp3^+$ Treg in HO-1

iMSCs therapy was accompanied with the elevated IL-10. This is in line with the findings of recent studies that IL-10 and Treg are associated with the anti-inflammatory effect caused by HO-1[21].

The exact mechanisms of the anti-allergic effect of HO-1 iMSCs are likely to be more complex and remain to be elucidated. Further studies are required to gain deeper insights into these mechanisms. Taken together, in this study, we proposed a modified MSCs therapy strategy for AR therapy with HO-1 iMSCs. Our results demonstrate that this new strategy not only facilitate our operation with MSCs in vitro, but also improve the immunomodulatory effect of MSCs in vivo. These findings may provide a new strategy for AR therapy in the future.

References

1) Bernstein DI, Schwartz G, Bernstein JA. Allergic Rhinitis: Mechanisms and Treatment. Immunol Allergy Clin North Am. 2016; 36: 261-278.
2) Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). Allergy; 2008, 86: 8-160.
3) Uccelli A, Moretta L, Pistoia V. Immunoregulatory function of mesenchymal stem cells. Eur J Immunol. 2006; 36: 2566-2573.
4) Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E,et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood. 2005; 106: 1755-1761.
5) Djouad F, Fritz V, Apparailly F, Louis-Plence P, Bony C, Sany J, et al. Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor-alpha in collagen-induced arthritis. Arthritis Rheum. 2005; 52: 1595-1603.
6) Sun YQ, Deng MX, He J, Zeng QX, Wen W, Wong DS, et al. Human pluripotent stem cell-derived mesenchymal stem cells prevent allergic airway inflammation in mice. Stem Cells. 2012; 30: 2692-2699.
7) Zhao N, Liu Y, Liang H, Jiang X. Bone marrow-derived mesenchymal stem cells reduce immune reaction in a mouse model of allergic rhinitis. Am J Transl Res. 2016; 8: 5628-5636.
8) Yang C, Li J, Lin H, Zhao K, Zheng C. Nasal mucosa derived-mesenchymal stem cells from mice reduce inflammation via modulating immune responses. PLoS One. 2015; 10: e118849.
9) Zhu W, Chen J, Cong X, Hu S, Chen X. Hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells. Stem Cells. 2016; 24: 416-425.
10) Shibahara S, Yoshizawa M, Suzuki H, Takeda K, Meguro K, Endo K, et al. Functional analysis of cDNAs for two types of human heme oxygenase and evidence for their separate regulation. J Biochem. 1993; 113: 214-218.
11) Chabannes D, Hill M, Merieau E, Rossignol J, Brion R, Soulillou JP, et al. A role for heme oxygenase-1 in the immunosuppressive effect of adult rat and human mesenchymal stem cells. Blood. 2007; 110: 3691-3694.
12) Li JG, Zhuan-sun YX, Wen B, Wu H, Huang FT, Ghimire HB, et al. Human mesenchymal stem cells elevate CD4+CD25+CD127low/- regulatory T cells of asthmatic patients via heme oxygenase-1. Iran J Allergy Asthma Immunol. 2013, 12: 228-235.
13) Mougiakakos D, Jitschin R, Johansson CC, Okita R, Kiessling R, Le Blank K, et al. The impact of inflammatory licensing on heme oxygenase-1-mediated induction of regulatory T cells by human mesenchymal stem cells. Blood. 2011; 117: 4826-4835.
14) Lee WY, Zhang T, Lau CP, Wang CC, Chan KM, Li G, et al. Immortalized human fetal bone marrow-derived mesenchymal stromal cell expressing suicide gene for anti-tumor therapy in vitro and in vivo. Cytotherapy. 2013; 15: 1484-1497.
15) Nakamura-Ota M, Hamanaka R, Yano H, Adachi S, Sumiyoshi H, Matsuo N, et al. A new murine osteoblastic cell line immortalized with the SV40 large T antigen. Cell Tissue Bank. 2014; 15: 373-380.
16) Wu LA, Feng J, Wang L, Mu YD, Baker A, Donly KJ, et al. Development and characterization of a mouse floxed Bmp2 osteoblast cell line that retains osteoblast genotype and phenotype. Cell Tissue Res. 2011; 343: 545-558.
17) Zhao Y, Zhang L, Qiao Y, Zhou X, Wu G, Wana L, et al. Heme oxygenase-1 prevents cardiac dysfunction in streptozotocin-diabetic mice by reducing inflammation, oxidative stress, apoptosis and enhancing autophagy. PLoS One. 2013; 8: e75927.
18) Wu B, Song HL, Yang Y, Yin ML, Zhang BY, Cao J, et al. Improvement of Liver Transplantation Outcome by Heme Oxygenase-1-Transduced Bone Marrow Mesenchymal Stem Cells in Rats. Stem Cells Int. 2016: 9235073.
19) Rhoades C, Thomas F. Capnography: beyond the numbers. Air Med J. 2002; 21: 43-48.
20) Najar M, Raicevic G, Fayyad-Kazan H, Bron D, Toungouz M, Lagneaux L, et al. Mesenchymal stromal cells and immunomodulation: A gathering of regulatory immune cells. Cytotherapy. 2016; 18: 160-171.
21) Xia ZW, Xu LQ, Zhong WW, Wei JJ, Li NL, Shao J, et al. Heme oxygenase-1 attenuates ovalbumin-induced airway inflammation by up-regulation of foxp3 T-regulatory cells, interleukin-10, and membrane-bound transforming growth factor- 1. Am J Pathol. 2007; 171: 1904-1914.

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