Mesenchymal stem cells overexpressing interleukin-10 prevented allergic airway inflammation

Peng-Peng Kuang
Sun Yat-sen University First Affiliated Hospital

Xiao-Qing Liu
Sun Yat-sen University First Affiliated Hospital

Chan-Gu Li
Sun Yat-sen University First Affiliated Hospital

Bi-Xin He
Sun Yat-sen University First Affiliated Hospital

Ying-Chun Xie
Sun Yat-sen University First Affiliated Hospital

Zi-Cong Wu
Sun Yat-sen University First Affiliated Hospital

Cheng-Lin Li
Sun Yat-sen University First Affiliated Hospital

Xiao-Hui Deng
Sun Yat-sen University First Affiliated Hospital

Qing-Ling Fu (fuqingl@mail.sysu.edu.cn)
Sun Yat-sen University First Affiliated Hospital

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Abstract

Backgrounds:

Allergic airway inflammation is widely distributed worldwide and imposes a considerable burden on both society and affected individuals. This study aimed to investigate the therapeutic advantages of mesenchymal stem cells (MSCs) overexpressed interleukin-10 (IL-10) for the treatment of allergic airway inflammation, as both IL-10 and MSCs possess immunosuppressive properties.

Methods

Induced pluripotent stem cell (iPSC)-derived mesenchymal stem cells (MSCs) were engineered to overexpress IL-10 by lentivirus transfection (designated as IL-10-MSCs). The MSCs and IL-10-MSCs were administrated intravenously to the mice induced as allergic inflammation using ovalbumin (OVA), and the features of allergic inflammation, including inflammatory cell infiltration, Th cells in lung and T helper 2 cells (Th2) cytokine levels in bronchoalveolar lavage fluid (BALF) were examined. MSCs and IL-10-MSCs were co-cultured with CD4^+ T cells from patients with allergic rhinitis (AR), and the level of Th2 cells and the expression of corresponding type 2 cytokines were studied. RNA-sequence was employed to further probe the potential effects of MSCs and IL-10-MSCs on CD4^+ T cells.

Results

Stable IL-10-MSCs were established and characterized as high expression of IL-10. IL-10-MSCs significantly reduced inflammatory cell infiltration and epithelial goblet cell numbers in lung tissue of mice with allergic airway inflammation. Inflammatory cells and cytokine levels in BALF were also decreased after the administration of IL-10-MSCs. Moreover, IL-10-MSCs showed stronger capacity to inhibit the levels of Th2 after cocultured with CD4^+ T cells from patients of AR. Furthermore, we found the lower levels of IL-5 and IL-13 for IL-10-MSCs treated CD4^+ T cells, and the blockade of IL-10 significantly reversed the inhibitory effects of IL-10-MSCs. We further reported the mRNA profiles for CD4^+ T cells treated with IL-10-MSCs and MSCs, and in which IL-10 exerts an important role.

Conclusion

We identified that IL-10-MSCs showed good effects in the treatment of allergic airway inflammation, providing solid support for genetically engineered MSCs as a potential novel therapy for allergic airway inflammation.

1. Background
Allergic airway inflammation, including allergic rhinitis (AR) and asthma, is widely distributed worldwide and places a significant burden on individuals and society(1). Although bronchodilators and inhaled corticosteroids are widely used to suppress inflammation and relieve symptoms, these agents do not reverse the ongoing remodeling process. Allergen-specific immunotherapy is an effective treatment for common allergic conditions(2). However, there is still some limitation such as the risk of potential side effects including systemic allergic reactions. Therefore, novel therapeutic strategies are still required.

Interleukin (IL)-10 is a soluble anti-inflammatory cytokine produced by macrophages, regulatory T cells, and some other cell types that helps to regulate the immune response and prevent tissue damage(3, 4). Previous studies have shown that IL-10 can exert anti-inflammatory effects in a variety of diseases, including traumatic brain injury(5), stroke(6), spinal cord injury(5), obesity(7), and so on. Most importantly, IL-10 also plays a critical role in controlling allergic airway inflammation(8). Compared with nonasthmatics, there are low levels of IL-10 in bronchoalveolar lavage fluid (BALF) of patients with asthma (9), and low secretion of IL-10 from alveolar macrophages(10). Similarly, IL-10−/− mice develop enhanced allergic responses to a variety of allergens(11–13) and increased airway eosinophilic inflammation. These studies highlight the key role for IL-10 in the allergen-induced airway disease, and raise the possibility of IL-10 as a therapeutic agent for allergic airway inflammation. However, IL-10 protein has a short half-life of only about 1–2 minutes in vivo, then it is difficult to maintain an effective dose in clinic.

Mesenchymal stromal cells (MSCs) are multipotent stromal cells not only have the ability of self-replication, but also display a broad and profound immunomodulatory role in a variety of diseases(14). It has been reported that MSCs can migrate into damaged tissues under the stimulation of various inflammatory cytokines and release various cytokines (15) to exert therapeutic effects(16). Our previous study reported that MSCs derived from induced pluripotent stem cells (iPSCs) inhibited the differentiation of human monocyte-derived dendritic cells (DCs) by producing IL-10 and induced the generation of IL-10-producing regulatory DCs in the process of maturation(17). More importantly, we reported that iPSC-MSCs exhibited a higher proliferation rate with less cell senescence even more than passage 50(17), providing the possibility for undergoing genetic engineering such as upregulating expression of IL-10. Combined therapy with both MSCs and IL-10 has previously been shown to reduce inflammation in models of collagen-induced arthritis(18), experimental autoimmune myocarditis(19). There is a paucity of research addressing whether genetically engineered MSCs with IL-10 gene can alleviate allergic airway inflammation.

The present study aimed to investigate whether treatment with MSCs engineered to overexpress IL-10, could reduce inflammation, exhibit pro-immunomodulatory effects, and improve functional recovery in allergic airway inflammation.

2. Materials and methods

2.1.1 Subject
Blood samples from patients with AR (n = 19) were collected from The First Affiliated Hospital, Sun Yat-sen University. The eligibility criteria for patients with AR were determined based on the guidelines of the Initiative on Allergic Rhinitis and its Impact on Asthma: (1) history of nasal symptoms by nose itching, obstruction, sneezing, and rhinorrhea; (2) positive specific IgE. Patients were excluded from the study if they were pregnant, had received antihistamines or intranasal steroid treatments within the past month, or had taken oral steroids within the past three months. This study was approved by The Ethics Committee of The First Affiliated Hospital of Sun Yat-sen University, and informed consents were obtained from all participants.

2.1.2 Animal

Female BALB/c mice (16-20g), aged 4–6 weeks (n = 34) were purchased from GemPharmatech (Nanjing, China), and maintained in specific pathogen-free Animal Experimental Center of North Campus, Sun Yat-sen University. All procedures involving animals were performed according to the guidelines for animal experiments and were approved by the Ethics Committee of Sun Yat-sen University.

2.2 Establishment of IL-10MSCs

Human iPSC-MSCs were prepared as described in our previous study(17). The human IL-10 gene (ref. sequence NM_000572.2) plasmid was synthesized by Tsingke Biological Co. LTD (Guangzhou, China) and confirmed by sequencing. HEK-293T cells were transfected with packaging, envelope, and target plasmids using Lipo 3000 (ThermoFisher, Waltham, USA). Lentivirus-containing supernatant was collected at 48 and 72h, and iPSC-MSCs were infected with lentivirus and selected using puromycin (2 µg/ml) to obtain stable cells with IL-10 expressing (Fig. 1A).

2.3 Extraction of RNA and RTquantitative PCR

Briefly, the total RNA was extracted from MSCs and IL-10-MSCs using an RNA Quick Purification kit (ESscience, RN001). The complementary DNA (cDNA) was synthesized with PrimeScript RT Master Mix (Takara Bio Inc., Japan). The quantitative PCR was performed using the FastStart Universal SYBR Green Master kit (Roche, Mannheim, Germany) to detect the expression of IL-10. β-Actin was used as an endogenous reference. We calculated fold changes in gene expression normalized to β-Actin by the ΔΔCT method using Eq. $2^{-\Delta \Delta CT}$. The results were shown as fold changes compared to the control group. Primers designed for quantitative real-time PCR in this study are as follows: IL-10 (sense primers, 5’-GTTGTTAAAAGGAGTCTTTCTGCT-3’, and reverse primer, 5’-TTCCACAGGGAAGAAATCGATGA-3’); β-Actin (sense primers, 5’-AGAGCTACGAGCTGCCAGAC-3’, and reverse primer, 5’-AGCACTGTGTTGGCGTACAG-3’)

2.4 Western blot

The levels of IL-10 in IL-10-MSCs were examined using Western blot analysis. Equal amounts of each sample were loaded into the well of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. Proteins were separated on 10% SDS-PAGE gel and then transferred to a polyvinylidene difluoride membrane (Merck Millipore, IPVH00010). After that, the membranes were washed in TBST for 3
× 10 minutes and then blocked in 5% fat-free milk powder in TBST for 1.5 h. The diluted primary of antibodies as follows: rabbit anti-IL-10 (1:1000; Abcam, Cambridge, United Kingdom) and rabbit GAPDH (1:1000; Cell signaling, Danvers, MA) were added to the membrane and incubated overnight at 4°C. Membranes were then incubated at room temperature with a secondary antibody (1:4000, goat anti-rabbit IgG; Santa Cruz, Biotechnology, Dallas, TX) for 1 h. Finally, ECL blotting detection reagents were used to visualize membranes.

2.5 Mouse Model of Allergic Airways Inflammation

A mouse model of allergic airway inflammation was induced as our previous report(20). Briefly, mice were sensitized by intraperitoneal injection ovalbumin (OVA, grade V, Sigma) or phosphate-buffered saline (PBS) on days 0, 7, and 14, as shown in Fig. 2A. From day 21 to 27, mice were challenged daily with aerosolized 5% OVA or PBS for 30 minutes. The mice were randomly assigned using a random number generator to different groups: (1) PBS/PBS/PBS mice (n = 5) that were sensitized and challenged with PBS, followed by treatment with PBS on day 20; (2) OVA/OVA/PBS mice (n = 6) that were sensitized and challenged with OVA, then treated with PBS on day 20; (3) OVA/OVA/MSCs mice (n = 5) that were sensitized and challenged with OVA and then treated with MSCs on day 20; (3) OVA/OVA/IL-10-MSCs mice (n = 6) that were sensitized and challenged with OVA and then treated with IL-10-MSCs on day 20. On day 27, 4 hours after the last challenge, the mice were sacrificed. BALF was collected and further centrifuged at 400 g for 5 min. The supernatants of BALF were collected for the detection of IL-5 and IL-13, while the pellets were used for flow cytometry analyses of inflammatory cells. The left lobe of lung was collected for histopathologic evaluation. The remaining lung tissues were minced and digested for the isolation of lung cells following the procedures described in our previous reports(21) and used for flow cytometry analyses of Th2. Blinding is adopted in order to minimize bias and ensure objective assessment of the outcomes. During the allocation stage and the conduct of the experiment, the technicians assigning the mice to different groups, constructed the mouse models and administered the drugs. During the outcome assessment, the researchers assessing the outcomes or measuring the relevant parameters were blinded to the group allocation.

2.6 Induction of human Th2 cells and co-culture with IL-10-MSCs

Human peripheral blood mononuclear cells (PBMCs) were separated from patients with AR. Subsequently, CD4+ T cells were further purified by magnetic positive selection using MACS CD4 beads (MiltenyiBiotec, Bergisch Gladbach, Germany). To study the effects of IL-10-MSCs on the differentiation of Th2 cells, a total number of 2×10^5 CD4+ T cells were seeded in 24-well plate and co-cultured with or without 2×10^4 MSCs or IL-10-MSCs in T helper 2 cells (Th2) polarizing medium of IL-2 (10ng/ml), IL-4 (25ng/ml), Anti-CD3 (1µg/ml) and Anti-CD28 (1µg/ml). After 5 days, supernatants were collected for the analysis of the cytokine levels using ELISA, and the T cells were collected for flow cytometry analysis.
For the transwell culture experiments, iPSC-MSCs and IL-10-MSCs (5×10^4 cells/well) were plated into the lower chamber of 24-well transwell plates (Costar, Corning, NY, USA) with PBMCs (5 × 10^5 cells/well) cultured in the upper chamber. To investigate the role of IL-10 on the immunomodulatory effects of IL-10-MSCs, an anti-IL-10 monoclonal antibody (0.075 µg/mL; R&D Systems Europe) was used for the co-culture systems. After culturing for 3 days, supernatants in the transwell were collected for ELISA analysis.

2.7 ELISA

Levels of IL-10, IL-5 and IL-13 in the study were analyzed by using ELISA kits (Invitrogen, Waltham, Mass) according to the manufacturer's instruction.

2.8 Flow cytometric analysis

2.8.1 Determination of surface markers on MSCs

For the characterization of surface MSCs markers, a total of ~3×10^5 cells were harvested and resuspended in 50 µl PBS, followed by incubation with the monoclonal antibodies CD34-PE, CD45-APC, CD19-cy5.5, CD73-APC, CD90-APC, CD105-APC. After incubation at room temperature for 25 min, cells were washed and resuspended in washing buffer for flow cytometry analysis (BD FACS Aria; NJ, USA). FlowJo V10 software was used to analyze the data.

2.8.2 Flow cytometry analysis of T helper cells in lung tissues

Lung tissues were minced and incubated in D-Hank's buffer containing 1 mg/mL collagenase type IA (Life Technologies, Carlsbad, CA, USA) and 50 µg/mL DNaseI (Sigma, St. Louis, MO, USA) at 37°C for 1 h. The digested lung tissues were smashed against 70-µm strainers (Fisher Scientific, Pittsburgh, PA, USA) to obtain a cell suspension. The single lung cells were finally gotten and stimulated with phorbol myristate acetate (50 ng/mL, Sigma), ionomycin (1000 ng/mL, Sigma) and brefeldin A for 5 h. The cells were first stained with anti-CD4-PerCP-Cy5.5 (Biolegend, San Diego, CA, USA), Fixable Viability Dye-eF506 and then stained with anti-IFN-γ-PE, anti-IL-4-APC (e-Bioscience, San Diego, CA, United States). The cells were then analyzed using a flow cytometer (Beckman Coulter Gallios, Fullerton, CA, USA).

2.8.3 Flow cytometry analysis of BALF

For analysis of different inflammatory cells in mouse BALF, pellets of BALF were stained with antibodies to CD45-FITC, CD11b-APC-Cy7, CD64-PE, Ly-6G-Alexa 700, Siglec-F-Alexa 647, Ly-6C-PE-Cy7 following the gating strategies as reported previously(20).

2.8.4 Flow cytometry analysis of CD4^+ T cell

CD4^+ T cells of AR were first stained with anti-CD4-PerCP-Cy5.5 (Biolegend, San Diego, CA, USA) and Fixable Viability Dye-eF506. CD4^+ T cells were stimulated with phorbol myristate acetate, ionomycin, and
brefeldin for 5 hours, and then the cells were fixed, permeabilized, and stained with anti-IFN-γ-PE, anti-IL-4-APC (e-Bioscience, San Diego, CA, United States). After staining, the cells were analyzed using a flow cytometer (Beckman Coulter Gallios, Fullerton, CA, USA).

2.9 CD4⁺ T cell RNA sequence

After co-cultured with MSCs or IL-10-MSCs for five days, human CD4⁺ T cells were purified by magnetic positive selection using MACS CD4 beads (MiltenyiBiotec, Bergisch Gladbach, Germany). The control CD4⁺ T cells, and CD4⁺ T cells co-cultured with MSCs or IL-10-MSCs were referred to as “ctrl-CD4⁺ T”, “MSCs-CD4⁺ T” and “IL-10-MSCs-CD4⁺ T”, respectively. Total RNA was extracted from the three groups using TRIzol® Reagent (Magen) according to the manufacturer’s instructions. Only qualified samples can be used for library construction. Paired-end libraries were prepared using an ABclonal mRNA-seq Lib Prep Kit (ABclonal, China) following the manufacturer’s instructions. The library preparations were sequenced on an Illumina Novaseq 6000 (or MGISEQ-T7) and 150 bp paired-end reads were generated. All of the bioinformatics analyses were performed using an in-house pipeline from Shanghai Applied Protein Technology.

2.10 Statistical analysis

All the data were analyzed using GraphPad 8.0 (GraphPad Software, La Jolla, CA, USA) and all the results were expressed as Mean ± SEM. Statistical analyses were performed using unpaired or paired independent two-tailed Student’s t-test for single comparisons or one-way ANOVA with Tukey’s correction for multiple comparisons. A Kruskal-Wallis rank sum test followed by a Mann-Whitney U test was performed for comparisons of data with abnormal distribution. A P value less than 0.05 were considered statistically significant.

3. Results

3.1 Establishing stably overexpressing IL-10-MSCs

In this study, IL-10 was over-expressed in iPSC-MSCs by lentiviral transfection to generate IL-10-MSCs (Fig. 1A). Positive clones were selected with puromycin and passaged as stable cell lines. As shown in Supplementary Fig. 1, IL-10-MSCs maintained normal growth when 2 µg/mL puromycin selected for 24h or 48h, and the morphology of IL-10-MSCs was similar to naive iPSC-MSCs (referred to as “MSCs”). Then the expression of green fluorescent protein (GFP) in transfected cells was detected using immunofluorescence. The results showed that there was significant GFP expression on IL-10-MSCs and MSCs transfected with vector lentivirus (referred to as “GFP-MSCs”) compared to MSCs without transfection (Fig. 1B), indicating that lentivirus transfection was successful. Real-time PCR revealed that IL-10-MSCs expressed significantly higher levels of IL-10 mRNA (Fig. 1C). Moreover, we identified that IL-10-MSCs produced higher levels of IL-10 than MSCs (Fig. 1D). Similarly, we confirmed that IL-10 was successfully overexpressed in IL-10-MSCs using western blotting (Fig. 1E). These results suggested that we significantly got the stable IL-10-MSCs. We further analyzed the surface marker profile of MSCs. As
shown in Fig. 1F, both IL-10-MSCs and GFP-MSCs expressed high levels of surface markers CD73, CD90, and CD105, and low levels of surface markers CD34, CD19, CD45, which were consistent with MSCs.

### 3.2 IL-10-MSCs significantly ameliorated allergic airway inflammation in mice.

To investigate the effect of IL-10-MSCs on allergic airway inflammation, OVA-induced airway inflammation model was established, and MSCs or IL-10-MSCs were administrated 1 day before the challenge (Fig. 2A). We identified that there was higher level of IL-10 in serum after administration of IL-10-MSCs compared to that in MSCs or PBS groups at 24 hours (Fig. 2B). We observed enhanced lung inflammatory cell infiltration in the peribronchial and perivessel tissues, and more PAS positive cells in OVA/OVA/PBS group (referred to as "OVA group") compared to PBS/PBS/PBS group (Fig. 2C-E). However, the treatment of IL-10-MSCs dramatically alleviated peribronchial and perivessel inflammation and decreased mucus secretion in hyperplastic goblet cells as compared to the OVA group (Fig. 2C-E). There were lower levels for the inflammation scores in peribronchial and perivessel tissues in the IL-10-MSCs groups compared with the OVA group (Fig. 2C-E).

Next, we examined the effect of IL-10-MSCs on the OVA-induced inflammatory cell profiles in the BALF by flow cytometry. We found increased numbers of total cells, eosinophils, and neutrophils in BALF in OVA/OVA/PBS mice compared to PBS/PBS/PBS control mice. However, the administration of IL-10-MSCs significantly decreased the number of total inflammatory cells and eosinophils but not neutrophils in BALF (Fig. 3A-C). Additionally, we found higher levels of type 2 cytokines IL-5 and IL-13 in BALF in OVA group, and the administration of IL-10-MSCs dramatically downregulated their levels in BALF (Fig. 3D-E).

Moreover, we evaluated the proportion of Th2 cells in the lung. OVA/OVA/PBS mice displayed a significantly higher percentage of Th2, and IL-10-MSCs significantly reduced the levels of Th2 cells in the lung (Fig. 4A). In contrast, no differences were observed for the levels of Th1 between three groups (Fig. 4B). In total, these findings demonstrated that systemic administration of IL-10-MSCs was able to significantly prevent allergic airway inflammation in mice.

### 3.3 IL-10-MSCs inhibited the Th2 response for patients with allergic rhinitis

We next investigated the immunoregulatory effect of IL-10-MSCs on T cells using PBMCs derived from patients with AR. Purified CD4^+ T cells were polarized to Th2 and co-cultured with MSCs or IL-10-MSCs for 5 days (Fig. 5A). We confirmed that the purities of the CD4^+ T cells were more than 90% by the flow cytometry (Supplementary Fig. 5). We observed a significantly higher Th2 level under the polarizing stimulation, and the treatment with both types of MSCs significantly reversed the level of Th2 cells, and IL-10-MSCs exhibited a significantly better inhibitory effect than MSCs (Fig. 5B-C, P < 0.05). Similarly, we found that the levels of IL-13 and IL-5 were significantly increased in response to the polarizing condition, but decreased after co-culturing with MSCs and IL-10-MSCs. Importantly, there were lower levels of IL-13
and IL-5 after the administration with IL-10-MSCs compared with MSCs (Fig. 5D-E, P < 0.001). These results suggested that IL-10-MSCs exhibit high immunoregulatory capacity for allergic inflammation.

### 3.4 IL-10 was involved in the immunomodulation of IL-10-MSCs on the production of type 2 cytokines

We next investigated the possible mechanism of IL-10-MSCs on Th2 cells. MSCs were reported to exert their therapeutic effects via cell-cell contact and paracrine. Our previous studies have showed that iPSC-MSCs exerted their effects on DC differentiation through cell-to-cell contact(17) and on antigen-stimulated PBMCs from patients with AR via prostaglandin E2 (22). We next investigated the role of cell-to-cell contact and IL-10 in the effects of IL-10-MSCs on type 2 cytokines using PBMCs derived from patients with AR. Similarly as above, the high levels of IL-13 and IL-5 in response to Anti-CD3/Anti-CD28 were significantly decreased after co-cultured with IL-10-MSCs. We did not find any difference for the levels of IL-13 after PBMCs were co-cultured with IL-10-MSCs in a transwell system (Fig. 6A), suggesting that cell-cell contact may not be involved in the immunomodulation of IL-10-MSCs on production of type 2 cytokines. However, the administration of anti-IL-10 antibody dramatically reversed the lower levels of IL-13 treated with IL-10-MSCs (Fig. 6B). Similar results were found for the levels of IL-5(Fig. 6C, D). These data indicate that IL-10 played a major role in the IL-10-MSCs-mediated inhibition of IL-13 and IL-5 production.

### 3.5 The mRNA profiles for CD4+ T cells with the treatments of IL-10-MSCs and MSCs

In order to investigate possible functional differences in CD4+ T cells between MSCs and IL-10-MSCs group, we performed RNA sequence for ctrl-CD4+ T cells, MSCs-CD4+ T cells and IL-10-MSCs-CD4+ T cells. We found that there was significant difference for the mRNA profiles in CD4+ T cells after co-cultured with MSCs or IL-10-MSCs. In total, we identified 5325 and 6001 differentially expressed mRNAs between ctrl-CD4+ T cells and MSCs-CD4+ T cells, ctrl-CD4+ T cells and IL-10-MSCs-CD4+ T cells, respectively. Interestingly, there was only 71 differential mRNAs between MSCs-CD4+ T cells and IL-10-MSCs-CD4+ T cells (Fig. 7A). Among 71 differential mRNAs, 12 mRNAs were increased and 59 mRNAs were decreased in IL-10-MSCs-CD4+ T cells compared to those in MSCs-CD4+ T cells (adjusted P-value < 0.05) (Fig. 7B). IL-10 was the most significantly upregulated mRNAs in IL-10-MSCs-CD4+ T cells, suggesting that IL-10 levels in CD4+ T cells was increased in response to IL-10-MSCs treatment, which may also exert an inhibitory effect on Th2. The 30 mRNAs involved in regulating Th2 function were shown as Fig. 7C. Of them, in addition to IL-10, EPHA2 and PTX3 were upregulated in IL-10-MSCs-CD4+ T cells, which were reported to play an inhibitory role in Th2 function(23) or airway hyperresponsiveness(24). Using GO enrichment analysis, we found that the differentially expressed mRNAs were associated with the processes commonly associated with inflammation, such as immune responses, cytokines and its receptor signaling pathways, receptor-ligand activity. Similarly, the KEGG
pathway analysis has showed the cytokine-cytokine receptor interaction, Jak-Stat signaling pathway and Th1 and Th2 cell differentiation were involved in the above mRNA changes (Fig. 7D, E). These results further suggested that the treatment of IL-10-MSCs had more effects on cytokine production and interaction compared to MSCs.

4. Discussion

In this study, we introduced the IL-10 gene into human iPSC-MSCs to establish highly overexpressing IL-10-MSCs and confirmed its strong immunomodulation on allergic airway inflammation. We demonstrated that IL-10-MSCs significantly prevented the allergic airway inflammation in a mouse model, and decreased the function of type 2 cytokines for patients of AR. Our findings provide a novel therapeutic strategy for allergic airway disease based on IL-10-MSCs.

MSCs have been frequently considered as the candidate for immunotherapy due to the beneficial effects of MSC-based therapies to treat different pathologies. The therapeutic benefits of MSCs are thought to result from their immunomodulation and anti-inflammatory properties via soluble factors, which have been thoroughly investigated for the treatment of inflammatory disease both in vitro and in vivo(25). IL-10 is a key cytokine in controlling allergic airway inflammation. In allergic asthma, where pathological responses to inhaled allergens develop due to a failure of immune tolerance, a successful therapeutic strategy is associated with an increase of IL-10. This is the case for the glucocorticoid dexamethasone, which facilitates IL-10 production by human CD4 and CD8 T cells(26). In addition, severe steroid-resistant asthma is associated with a failure of IL-10 enhancement by patient cells in response to dexamethasone(27). Several strategies for enhancing IL-10 in the context of neurological diseases with immune components have also been developed(28). These include administration of recombinant IL-10, enhancement of IL-10 production by agonists, or delivery of IL-10 by viral vectors(4).

Due to the short half-life of recombinant IL-10, it was difficult to maintain a relatively stable concentration in the body when applied to humans(29). In our study, we used iPSC-MSCs as a carrier to establish IL-10-MSCs. In a mouse model, the data showed that administration of IL-10-MSCs could boost the serum level of IL-10 when compared with MSCs or PBS group. Compared to the half-life of IL-10 protein in vivo of about 1–2 min, mice injected with IL-10-MSCs can still maintain a higher serum level of IL-10 even after 24 hours. What’s more, we identified that human IL-10-MSCs were able to effectively attenuate lung pathology, decrease the levels of Th2 cytokines in the BALF and inhibit the activation of Th2 in the lung tissue.

Subsequently, we cocultured CD4+ T cells with MSCs or IL-10-MSCs. We found that there was a decreased level of Th2 in CD4+ T cells after co-culturing with MSCs and IL-10-MSCs, and the production of IL-13 and IL-5 in Th2 was significantly decreased. Most importantly, in our study, IL-10-MSCs demonstrated stronger inhibitory effects on T-cell differentiation than MSCs. These results are consistent with previous reports(22, 30). Coomes, Kannan (30) demonstrate that IL-10 directly limits Th2 cell differentiation and survival in vitro and in vivo. Ablation of IL-10 signaling in Th2 cells led to enhanced
Th2 cell survival and exacerbated pulmonary inflammation in a murine model of house dust mite allergy. Similarly, in the current studies, the immunosuppressive effect of IL-10-MSCs was able to be specifically reversed by the anti-IL-10 monoclonal antibody. Mechanistically, we performed RNA-sequence for CD4\(^+\) T cells to evaluate the different mRNAs related to Th2 regulation between MSCs and IL-10-MSCs. We found that IL-10 was the most significantly upregulated mRNAs in IL-10-MSCs-CD4\(^+\) T cells, suggesting that IL-10 levels in CD4\(^+\) T cells was increased in response to IL-10-MSCs treatment. Results of the study by Barrat, Cua (26) showed that vitamin D3 and Dexamethasone, induced human and mouse naive CD4(+) T cells to produce IL-10. Our data suggest that IL-10-MSCs treatment at least partially exerts the same effects as dexamethasone treatment. Both in vivo and in vitro, IL-10-MSCs have a better therapeutic effect than MSCs, further reinforcing the immunoregulatory effects of IL-10-MSCs.

Anti-inflammation strategy using anti-inflammatory cytokines is a promising method for treating allergic airway inflammation. Current MSC-based augmenting IL-10 therapy has achieved good efficacy in a variety of diseases. Gao, Huang (31) introduced the IL-10 gene into human umbilical-cord-derived MSCs (HUCMSCs), and HUCMSCs overexpressing IL-10 significantly enhanced functional recovery after spinal cord injury by directionally activating macrophages. Wang, Gao (32) constructed stable overexpressing IL-10 HUCMSCs which were applied to obesity mice, compared with HUCMSCs, IL-10-HUCMSCs treatment had much better anti-obesity effects including body weight reduction, greater glucose tolerance, less systemic insulin resistance, and less adipose tissue inflammation in HFD feeding mice. In addition, Peruzzaro, Andrews (33) also observed a significant improvement in fine motor function in rats that received transplants of bone marrow (BM)-MSCs engineered to overexpress IL-10. However, despite the availability of MSCs from umbilical cord or bone marrow, these cells have a limited proliferative capacity, a large variability in cell quality derived from different donors, and quickly lose their differentiation potential(34). All of these factors limit its therapeutic benefit, especially its clinical applications. In contrast, MSCs loaded with IL-10 derived from iPSCs, were reprogrammed from human urine cells. Urine cells can be easily obtained from most people, so it is a viable and non-invasive method for gathering an endless supply of human cells for reprogramming. Besides, the proliferation rate of the iPSC-MSCs was higher(35). More importantly, it has been reported that, compared to BM-MSCs, human iPSC-MSCs are less immunogenic and have a stronger immune privilege after transplantation(36). These results suggest that iPSC-MSCs could be a source of MSCs that are more readily available and acceptable, particularly for therapeutic applications. Therefore, our iPSC-MSC-based overexpression IL-10 therapy will have greater clinical translational potential.

The present study has several potential limitations that should be acknowledged. Firstly, our animal experiments only utilized a single dosage and a single administration of MSCs and IL-10-MSCs. This approach may not fully capture the dose-dependent responses and the long-term effects of these treatments. To gain a more comprehensive understanding of the underlying mechanisms and optimize the therapeutic approach, it would be beneficial to conduct further studies involving varying dosages and multiple intravenous administrations. Secondly, our study primarily focused on the preventive effects of MSCs and IL-10-MSCs in our model. This narrow focus limits the generalizability of the findings to
situations where IL-10-MSCs can be administered to the onset of symptoms. To expand the therapeutic applicability of IL-10-MSCs, it would be valuable to investigate the effects of administering IL-10-MSCs after the challenge. This additional research would provide a more comprehensive understanding of the potential benefits of IL-10-MSCs in treating allergic airway inflammation. Finally, findings in animal studies may not directly translate to humans due to inherent biological differences between species. It is crucial to replicate and validate findings from animal studies in human studies.

5. Conclusions

Taken together, our data suggest that iPSC-MSCs engineered to overexpress IL-10 exerted better therapeutic effects than MSCs alone in allergic airway inflammation. It indicates that upregulating IL-10 in MSCs is available to allergic airway inflammation treatment, providing new insights for cell-based therapeutic products in allergic diseases.

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AR</td>
<td>allergic rhinitis</td>
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<tr>
<td>BALF</td>
<td>bronchoalveolar lavage fluid</td>
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<td>BM-MSCs</td>
<td>bone marrow-mesenchymal stem cells</td>
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<td>DCs</td>
<td>dendritic cells</td>
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<td>GFP</td>
<td>green fluorescent protein</td>
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<td>PBMCs</td>
<td>peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>sulfate-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Th2</td>
<td>T helper 2 cell</td>
</tr>
</tbody>
</table>

Declarations
Ethics approval and consent to participate

This study involving human participants was approved by The Ethics Committee of The First Affiliated Hospital, Sun Yat-sen University. (Project title: The effects of exosomes derived from stem cells on immune cells. Approval number: No. 2019-331. Date of approval: Sep. 16, 2019). And The participants provided their written informed consent to participate in this study. This study adheres to the Declaration of Helsinki. The protocol of this study involving animals was reviewed and approved by the Ethics Committee of Laboratory Animal Center, Sun Yat-sen University (Project title: Extracellular vesicle-coated IL-10 derived from mesenchymal stem cells as a novel nano drug for the treatment of allergic airway inflammation. Approval number: SYSU-IACUC-2022-000948. Date of approval: June 23, 2022).

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request. The RNA sequence datasets presented in this study can be found in online repositories. The names of the repositories and accession numbers can be found below: SRA, Accession PRJNA1005638 (https://www.ncbi.nlm.nih.gov/sra).

Competing interests

The authors declare no conflicts of interest in this work.

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Author Contributions

PK helped in collection and assembly of data and manuscript writing. XL helped data analysis, discussion and manuscript writing. CL, BH, YX, ZW, CL and XD helped in the collection of data. QF helped in concept and design, data analysis and manuscript writing. All authors contributed to the article and approved the submitted version.

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References


the British Society for Allergy and Clinical Immunology. 2000;30(6):775-83.


15.


Figures
Figure 1

**Stable IL-10-MSCs were established.** A. The protocol of establishing IL-10-MSCs. B. GFP expressions in MSCs and IL-10-MSCs. C. The mRNA levels of IL-10 in MSCs and IL-10-MSCs. D-E. The levels of IL-10 protein in the supernatant of MSCs and IL10-MSCs under ELISA, and in the cells under Western blotting. The full-length blots are presented in Additional file: Fig. S2. MSCs: mesenchymal stem cells; MW:
molecular weight. F. The expression of surface markers of MSCs and IL-10-MSCs. Data presented as mean±SEM. ** P < 0.01, **** P < 0.0001.

**Figure 2**

The effects of IL-10-MSCs in eosinophilic allergic airway inflammation in mice. A. Schematic diagram for the mice model of eosinophilic airway inflammation and the administration of MSCs or IL-10-MSCs. B. The levels of IL-10 in serum mice 24h after intravenous injection of PBS (n=4), MSCs (n=4), IL-10-MSCs (n=4). C-E. H&E and PAS staining for lung tissues. BALF, bronchoalveolar lavage fluid; HE (Br), H&E-
stained bronchia; HE (Ve), H&E-stained vessels. Data are shown as mean ± SEM.* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

Figure 3

The effects of IL-10-MSCs on BALF in eosinophilic allergic airway inflammation in mice. **A-C.** The inflammatory cell profiles by flow cytometry in BALF. **D-E.** Levels of Th2-related cytokines in the BALF. Data are shown as mean ± SEM. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001.
Figure 4

The effects of IL-10-MSCs on Th2 in eosinophilic allergic airway inflammation. A. Th2 response in lung tissues with the treatment of MSCs or IL-10-MSCs. B. Th1 response in lung tissues with the treatment of MSCs or IL-10-MSCs. Data are shown as mean ± SEM.* * * P < 0.001, **** P < 0.0001.
Figure 5

The effects of IL-10-MSCs on Th2 response in the peripheral blood of patients with allergic rhinitis. 
A. Schematic protocol for isolation CD4+ T cell and co-culturing with MSCs or IL-10-MSCs. 
B-C. The levels of Th2 cells after co-cultured with MSCs or IL-10-MSCs (n=12). 
D-E. The levels of IL-13 and IL-5 in the supernatants were analyzed using ELISA. 
Data are shown as mean ± SEM. * P < 0.05, *** P < 0.01, **** P < 0.001.
Figure 6

IL-10 was involved in the immunomodulation of IL-10-MSCs on IL-13 and IL-5 production. A, C. The role of cell-cell contact in the effects of IL-10-MSCs on IL-13 and IL-5 production (n=4); B, D. The role of IL-10 in the effects of IL-10-MSCs on IL-13 and IL-5 production. Data are shown as mean ± SEM. * P < 0.05, ** P < 0.01, **** P < 0.0001.
Figure 7

mRNA profiling of CD4+ T cells with MSCs treatment. A. Venn diagram showing the differentially expressed mRNAs in CD4+ T cells, MSCs-CD4+ T cells and IL-10-MSCs-CD4+ T cells (n=3). B. Volcano plot showing differentially mRNAs between MSCs-CD4+ T cells and IL-10-MSCs-CD4+ T cells. C. Heatmap representation of differential mRNAs involved in IL-10-MSCs-CD4+ T cells and MSCs-CD4+ T cells. D-E. GO analysis and KEGG enrichment analysis of differential mRNAs between MSCs-CD4+ T cells and IL-10-MSCs-CD4+ T cells.

Supplementary Files

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