Analysis of the expression changes of IL-17+ γδ T cells and Treg cells in bone marrow mesenchymal stem cells targeted therapy for allergic rhinitis

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Abstract. – **OBJECTIVE:** Bone marrow mesenchymal stem cells (BMSCs) have immunomodulatory and therapeutic effects on immune system diseases. This study intends to assess the regulatory effect of BMSC targeted therapy on the IL-17+ γδ T cells and Treg cells in allergic rhinitis (AR).

MATERIALS AND METHODS: BALB/c mice were sensitized by ovalbumin (OVA), while BMSCs were injected intravenously before sensitization and followed by an analysis of nasal symptoms, inflammation, cytokines, and immunoglobulins. BMSCs were co-cultured with peripheral blood mononuclear cells for 3 days to test Foxp3+ expression, IL-17+ γδ T and Foxp3+Treg cell ratio, and cytokines secretion.

RESULTS: After intranasal administration of BMSCs, nasal symptoms and inflammatory infiltration in mice were significantly alleviated, accompanied by reduced OVA-specific IgE in serum. BMSCs significantly inhibited the activity of T lymphocytes, increased TGF-β1 level, decreased IL-17A level, promoted Treg proliferation, and suppressed the proliferation of IL-17+ γδ T cells.

CONCLUSIONS: BMSC targeted therapy can be used to treat AR by regulating Treg cells to correct IL-17+γδ T cell immune imbalance and is expected to be an effective treatment method for AR.

Key Words:
   IL-17+ γδ T cells, Treg cells, BMSC, AR.

Introduction

At present, the AR treatment mainly includes avoiding contact with susceptible allergens, anti-inflammatory, antihistamine, and other symptomatic treatments, but the effect is unideal[1-5]. Allergen-specific immunotherapy (AIT) is the only etiological treatment for allergic diseases approved by the WHO[6-11]. Drug treatment can reduce allergic symptoms. Allergen immunotherapy can be used in patients resistant to conventional therapies[12-14]. The desensitizers currently used in clinics are mainly derived from allergens' crude extracts, featured as complex components, poor stability, difficulty in standardization, and serious side effects. Immunotherapy includes regular injections of allergen vaccines to trigger allergen tolerance[15-17]. However, it is limited by the types of allergens. Moreover, long-term treatment cycles usually lead to poor clinical compliance[18]. Therefore, it is particularly important to develop new, safe, and effective methods to prevent AR.

The immune regulation ability of BMSCs is shown in several allergic disease models, which might be used to treat immune diseases[19-23]. BMSCs are considered a new method for the treatment of AR as they can reduce eosinophils infiltration and control CD4+ T cell response[24-27]. Since BMSCs are easily obtained[28-30], we intend to assess the regulatory effect of BMSC targeted therapy on the expression of IL-17+ γδ T cells and Treg cells in AR.

Materials and Methods

AR Mouse Animal Model

AR mouse model has been established *via* intraperitoneal injection of 25 µg OVA as described

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previously[16]. After 28 days, the mice were sacrificed by cervical dislocation under anesthesia.

Mice were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Children's Hospital of the Chongqing Medical University.

BMSC Treatment of AR Mouse Model

BMSCs were purchased from Lonza Company (CH-4002 Basel, Switzerland) as T25 cell culture flasks (second generation) and cultured in complete DMEM/F12. BMSCs were administrated into mice on the 18th, 19th, and 20th days. The sensitized mice were assigned into PBS group and sensitized group which were injected with PBS after the OVA challenge; and BMSC group, which was injected with BMSC on the basis of the OVA challenge.

Symptoms of Nasal Allergy and Inflammatory Cell Infiltration

On the 27th day after treatment of each group, within 10 minutes after last administration of nasal egg, frequency of sneezing and nasal friction were recorded. Inflammatory cell infiltration was detected by nasal irrigation fluid.

OVA-Specific IgE Measurement

24 hours after the last OVA challenge, blood was taken from the mouse eyeballs. The egg specific IgE level was measured by ELISA kit.

MTT Analysis of Cell Proliferation

In the quantitative proliferation experiment, after stimulating proliferation, the number of cells was plated in a conventional medium, and the digested cells were respectively inoculated in the corresponding culture equipment according to the designed density to analyze the effect of cell proliferation. The cells were washed, attached to a 24-well plate, and then incubated in a medium containing 5% CO_2. The cells in each group were treated for 48h after treatment according to the various conditions. The cell proliferation evaluation was analyzed on the 6th day using an automatic cell counter, and the absorbance was analyzed by a spectrometer with a wavelength of 495 nm.

Real-Time PCR

RNA was isolated and cDNA was then synthesized, followed by qPCR using Universal SYBR qPCR Green Mix (Bio-Rad, 1000 Alfred Nobel Drive, Hercules, CA, USA). The relative expression was evaluated by drawing a standard curve and standardizing the difference in Ct. The primer sequences were listed in Table I.

ELISA Analysis

The above-mentioned culture supernatant was collected to measure the secretion of BDNF by ELISA. The results were expressed in pg/mL.

Flow Cytometric Analysis

The data were analyzed by FlowJo software (version 7.6).

Statistical Analysis

Statistics are carried out in accordance with the statistical methodology and analyzed with statistical software. SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software was applied for data analysis. Measurement data was presented as mean ± standard deviation and compared by t test or one-way ANOVA. $p<0.05$ was depicted as significant difference.

Results

Analysis of Nasal Symptoms Caused by BMSC Targeted Therapy of AR

The frequency of sneezing and nasal friction in each group of mice was recorded separately so as to analyze the changes of BMSC targeted treatment of AR on nasal symptoms. The frequency of sneezing and friction in mice with AR increased ($p<0.05$), while BMSC targeted therapy for AR significantly reduced the frequency of sneezing and nasal friction in mice ($p<0.05$) (Figure 1).

Table I. Primer sequence.

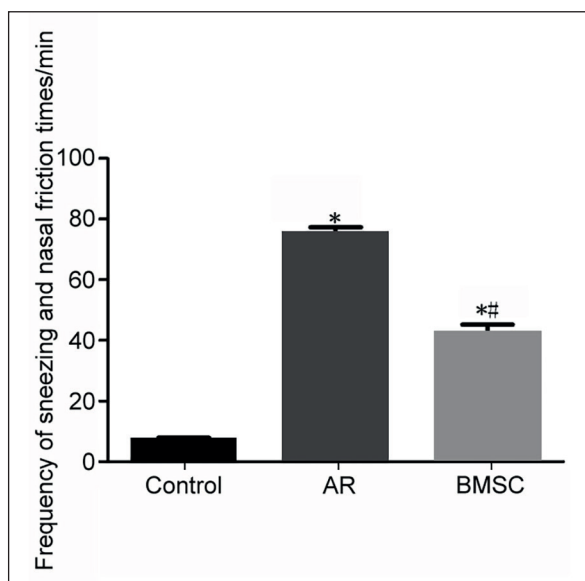| Gene	Forward 5′-3′	Reverse 5′-3′
GAPDH	AGTATGTTGTCACCGCTGG	TAACCTGTCTATACGGAGGGT
Foxp3	GCGGGATGAGGGGTCA	GACACGTTCTGTCGAGGT

Figure 1. Analysis of changes in nasal symptoms of BMSC targeted therapy for AR. Compared with the control group, the frequency of sneezing and nasal friction in mice with AR increased (*$p<0.05$); BMSC targeted therapy for AR can significantly reduce the frequency of sneezing and nasal friction in mice, compared with the AR group ($^\#p<0.05$).

Analysis of BMSC Targeted Treatment on Inflammatory Infiltrating Cells

Eosinophilic inflammatory infiltrating cells in nasal cavity of mice with increased ($p<0.05$), while BMSC targeted therapy for AR significantly reduced inflammatory infiltrating cells in mice ($p<0.05$) (Figure 2).
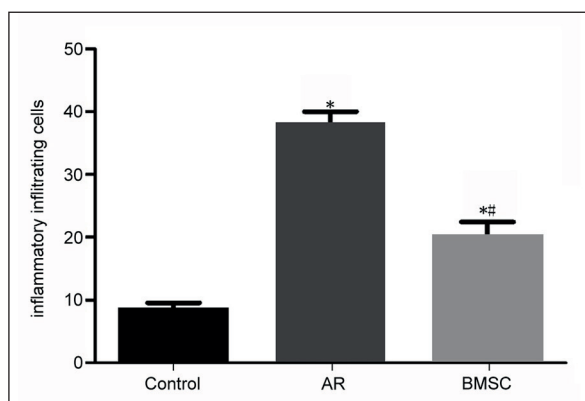

Figure 2. Analysis of BMSC targeted treatment of inflammatory infiltrating cells in AR. Compared with the control group, inflammatory infiltrating cells in the nasal cavity washing fluid of mice with AR increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly reduce the decrease in inflammatory infiltrating cells in mice, compared with the AR group ($^\#p<0.05$).
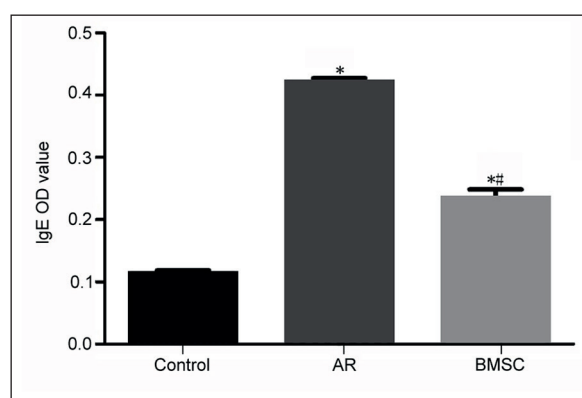

Figure 3. Analysis of serum IgE levels in BMSC targeted therapy for AR. Compared with the control group, the serum IgE level of mice with AR increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly reduce the serum IgE level, which is comparable to AR group comparison ($^\#p<0.05$).

Analysis of Serum IgE Levels in BMSC Targeted Therapy for AR

The level of IgE in the serum of mice with AR increased ($p<0.05$), and BMSC targeted therapy for AR significantly reduced the level of IgE in the serum ($p<0.05$) (Figure 3).

The Effect of BMSC Targeted Therapy on the Proliferation of T Lymphocytes

PHA stimulation significantly promoted T lymphocyte proliferation ($p<0.05$), while BMSC targeted treatment inhibited its promotion of T lymphocyte proliferation ($p<0.05$) (Figure 4).
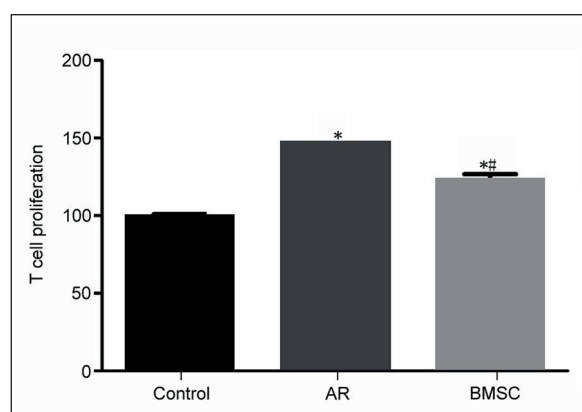

Figure 4. The effect of BBMSC targeted therapy for AR on the proliferation of T lymphocytes. Compared with the control group, PHA stimulation can significantly promote the proliferation of T lymphocytes, and the difference is statistically significant (*$p<0.05$); BMSC targeting treatment inhibits the promotion of T lymphocyte proliferation under PHA stimulation, and the PHA group comparison ($^\#p<0.05$).
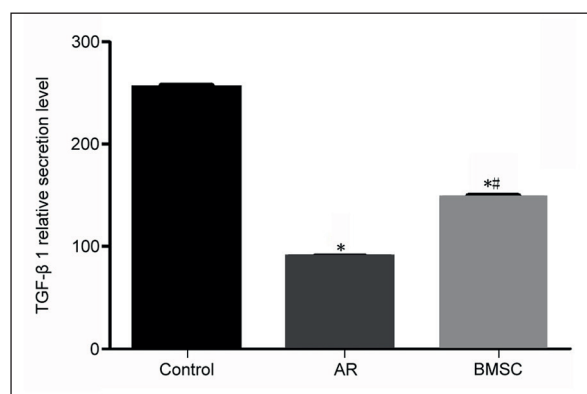
Figure 5. The effect of BMSC targeted therapy on serum TGF-β1 levels in AR. The TGF-β1 concentration unit is pg/ml; compared with the control group, the TGF-β1 level in the serum of AR mice is reduced, and the difference is statistically significant (*$p<0.05$); BMSC targeted therapy for AR can be significant promote the level of TGF-β1 in serum, compared with AR group, (#$p<0.05$).

The Effect of BMSC Targeted Therapy on Serum TGF-β1 Level in AR

TGF-β1 level in mice with AR decreased ($p<0.05$), while BMSC targeted therapy for AR significantly increased the level of TGF-β1 ($p<0.05$) (Figure 5).

The Effect of BMSC Targeted Therapy on Serum IL-17A Level in AR

The level of interleukin-17A in the serum of mice with AR increased ($p<0.05$), and BMSC targeted therapy for AR significantly reduced the level of interleukin-17A ($p<0.05$) (Figure 6).
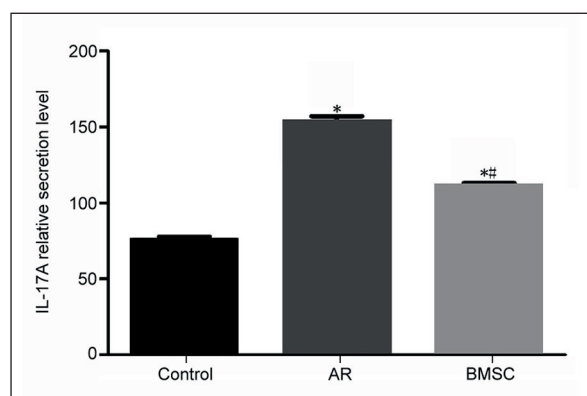
The Effect of BMSC Targeted Therapy on Serum IL-10 level in AR

The level of interleukin-10 in the serum of mice with AR upregulated ($p<0.05$), and BMSC targeted therapy for AR significantly declined the level of interleukin-10 ($p<0.05$) (Figure 7).

Effect of BMSC Targeted Therapy on IL-17+ γδ T Cells

IL-17+ γδ T cells in AR increased ($p<0.05$), while BMSC targeted therapy for AR significantly inhibited IL-17+ γδ T cells ($p<0.05$) (Figure 8).

Effects of BMSC Targeted Therapy on Treg Cells

Treg cells in AR decreased ($p<0.05$), while BMSC targeted therapy for AR significantly promoted Treg cells ($p<0.05$) (Figure 9).

Effect of BMSC Targeted Therapy on Foxp3 Expression in Mice with AR

Foxp3 expression in the nasal tissues of mice with AR decreased ($p<0.05$), while BMSC targeted therapy for AR significantly promoted Foxp3 in the nasal tissues ($p<0.05$) (Figure 10).

Discussion

AR is a common and frequently occurring disease in clinical practice[23]. At present, symptomatic treatment based on histamine and other anti-inflammatory drugs cannot achieve satisfactory results. We transplanted BMSCs into
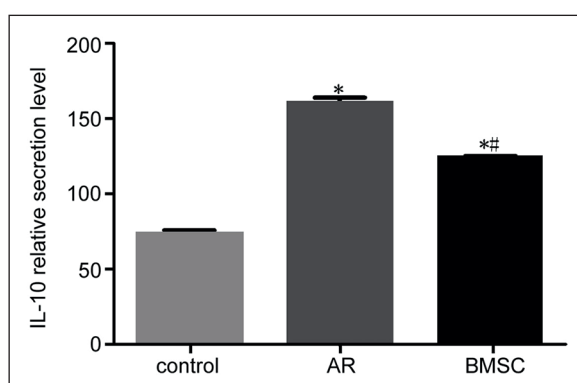

Figure 6. The effect of BMSC targeted therapy on serum IL-17A levels in AR. Compared with the control group, the IL-17A level in the serum of mice with AR increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly reduce the level of IL-17A in the serum. Comparison of AR group (#$p<0.05$).

Figure 7. The effect of BMSC targeted therapy on serum IL-10 levels in AR. Compared with the control group, the IL-10 level in the serum of mice with AR increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly reduce the level of IL-10 in the serum. Comparison of AR group (#$p<0.05$).
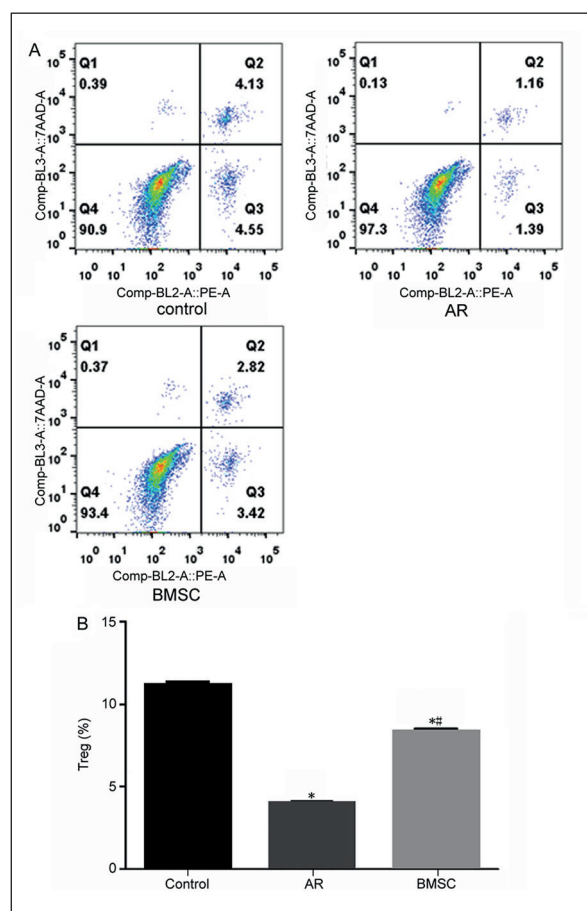
Figure 8. The effect of BMSC targeted therapy on the level of IL-17+ γδ T in AR. A flow cytometry analysis of the effects of BMSC targeting treatment on IL-17+ γδ T cells in AR mice; statistical analysis of the effects of BBMSC targeting treatment on IL-17+ γδ T cells in AR mice, compared with the control group, IL-17+ γδ T cells in AR mice increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly inhibit IL-17+ γδ T cells, compared with the AR group (#$p<0.05$).

AR mouse models to assess BMSCs' effect on IL-17+ γδ T cells and Treg cells after AR treatment. AR is featured as a decrease in the ratio of helper T cell subsets[31-33]. In this study, we observed that BMSCs promote the proliferation of regulatory T cells, inhibit the proliferation of IL-17+ γδ T cells, and reduce nasal-related inflammation symptoms. BMSCs can significantly increase the level of TGF-β1, reduce the level of IL-17A, and reduce IgE production in mice. Foxp3 level increased after BMSC injection, reflecting the immunosuppressive effect from another side.
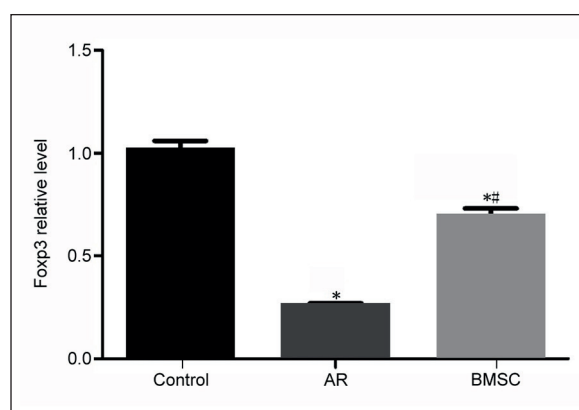
Figure 9. The effect of BMSC targeted therapy on Treg levels in AR. A flow cytometry analysis of the effect of BMSC targeted therapy on Treg cells in mice with AR; statistical analysis of the impact of BBMSC targeted therapy on Treg cells in mice with AR. Compared with the control group, Treg cells in mice with AR Increase, the difference is statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly inhibit Treg cells, compared with the AR group (#$p<0.05$).

Some studies have confirmed that BMSCs can increase the Th1/Th2 ratio, which is consistent with studies on adipose mesenchymal stem cells[34]. Th17 cells were involved in the infiltration of neutrophils and macrophages in AR[35-37]. Studies have shown that BMSC has a significant inhibitory effect on Th17 cells *in vitro*. Our results show that the IL-17A secretion level in the serum of AR mice after BMSC transplantation is
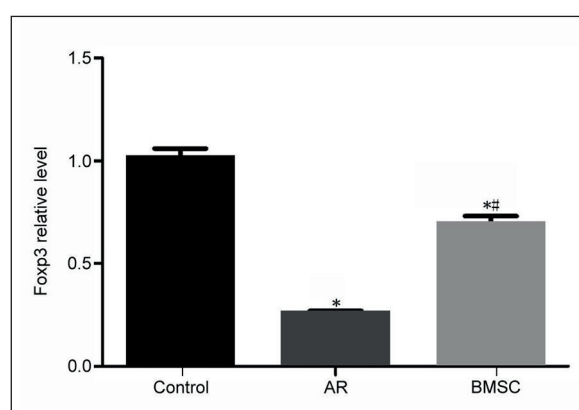

Figure 10. The effect of BMSC targeted therapy on Foxp3 expression in AR. Compared with the control group, the expression of Foxp3 in mice with AR increased, and the difference was statistically significant (*$p<0.05$); BMSC targeted therapy for AR can significantly inhibit the expression of Foxp3, compared with the AR group (#$p<0.05$).

significantly reduced, indicating that BMSCs can inhibit γδ T cells in AR mice and delay progression. In addition, Treg cells inhibit the inflammatory process[38-41]. Many studies have shown that after using BMSC in asthma models, the number and activity of Treg cells are up-regulated[42-44]. It is well known that BMSCs suppress the immune response by inhibiting the proliferation of T cells[45]. Compared with the OVA group, Foxp3 mRNA level in lymphocytes in BMSC group was significantly increased, which provides evidence for BMSCs to exert anti-inflammatory effects through Foxp3+Treg amplification in the AR mouse model. The soluble factor TGF-β1 is considered to be the main inducer of Treg cell during T cell differentiation[46]. Wang et al[47] have shown that neutralizing TGF-β1 can eliminate the immunosuppressive effect of ASC. This study shows that BMSCs enhance the frequency of CD4+CD25+Foxp3+Treg, suggesting that the change of TGF-β1 may be a potential way for BMSCs to promote Treg expansion.

Conclusions

We showed that BMSC targeted therapy can treat AR by regulating Treg cells to correct the immune imbalance of IL-17+ γδ T cells, and it is expected to become a cell therapy program for the treatment of related allergic diseases.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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