

RESEARCH ARTICLE

Mesenchymal stem cells combined with traditional Chinese medicine (qi-fang-bi-min-tang) alleviates rodent allergic rhinitis

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Abstract

Mesenchymal stem cells (MSCs) have been proved to exert anti-inflammatory effects and regulate immune reactions. Traditional Chinese medicine (TCM), qi-fang-bi-min-tang, is effective for some patients with allergic diseases. However, it remains unclear whether MSCs combined with TCM could benefit the treatment of allergic rhinitis (AR). In this study, we reported an additional effect of TCM (qi-fang-bi-min-tang) on the therapy of AR under MSCs treatment. Intriguingly, we observed that TCM-treated MSCs significantly inhibited the symptoms of AR and reduced the pathological changes of nasal mucosa in ovalbumin (OVA)-induced rats. The expression levels of interferon γ (IFN- γ), interleukin-17 (IL-17), and IL-4 were significantly decreased in the plasma of AR rats after injection of TCM-treated MSCs. TCM-treated MSCs reduced the levels of histamine secreted by mast cells and immunoglobulin E (IgE) secreted by plasma cells. In addition, we found that MSCs combined with TCM had a better therapeutic effect than TCM alone on AR in an OVA-induced mouse model. After OVA induction, MSCs combined with TCM significantly reduced the ratio of T helper type 1 (Th1), Th2, and Th17, but increased the proportion of Treg in the spleen of mice. Consistently, the expression levels of IFN- γ , IL-4, and IL-17 were significantly decreased, but transforming growth factor- β 1 was significantly increased in the plasma of AR mice after treated with TCM and MSCs. Our results from both rats and mice indicated that the effects of TCM combined with MSCs on the AR might be through regulating the secretion of Th1, Th2, and Th17 cytokines. This study suggested that TCM (qi-fang-bi-min-tang)-treated MSCs could be used in the clinical therapy of AR.

KEYWORDS

allergic rhinitis, inflammatory cytokines, mesenchymal stem cells, qi-fang-bi-min-tang, T help cells, traditional Chinese medicine

1 | INTRODUCTION

Allergic rhinitis (AR) is a chronic inflammatory disorder of the nasal airways, with an increasing challenge to public health worldwide, affecting nearly 30% of the world population.¹ AR is characterized by an imbalance of T helper type 1 (Th1) and Th2 cells² and can be induced after exposure to allergens via immunoglobulin E (IgE)-mediated hypersensitivity reactions. The classical symptoms of AR include watery rhinorrhea, nasal itching, nasal obstruction, and sneezing. People with AR may experience sleep disturbances, daytime fatigue, impaired school and work performance, decreased social functioning, headache, and irritability.³ Furthermore, 40% of patients with AR developed to asthma if the patients could not get effective therapy.^{4,5}

Treatment options for AR consist of allergen avoidance, pharmacotherapy, immunotherapy, and surgery.⁶ Available pharmacotherapy treatment includes antihistamines, corticosteroids, decongestants, leukotriene receptor antagonists, and anticholinergics.⁷ However, these drugs only alleviate allergic symptoms but fail to regulate the allergic reaction. Occasionally, the currently used drugs show side-effects, such as headache, throat irritation, and nasal dryness.⁸⁻¹⁰ Thus, it remains to be of a great requirement for the development of safer and more effective therapeutic agents. A recent study showed that immunotherapy could be an ideal option for patients who are refractory to pharmacotherapy as it reduced the reliance on pharmacotherapy.¹¹

The success of immunotherapy is based on the alteration of Th2 cells, which play a crucial role during the pathogenesis of AR.¹² Th2 cells are significantly increased during the process of AR. Simultaneously, activated by allergen, Th2 cells produce cytokines including interleukin-4 (IL-4), IL-5, IL-9, and IL-13, which modulate the responses of innate effector cells including eosinophils, basophils, and mast cells. These effector cells recruit more T helper cells into the allergic sections and promote the secretion of IgE.¹³ Allergen-specific IgE binds to high-affinity IgE receptor (FcεRI) on the surface of mast cells and basophils, which release allergic mediators including inflammatory cytokines and histamine, and cause the development of type I hypersensitivity.^{14,15} Therefore, regulation of Th2 cell could be a target for the therapy of AR.

Mesenchymal stem cells (MSCs) are plastic-adherent, spindle-shaped cells that highly express a panel of key markers including CD105, CD29, CD44, and CD90 but show a low level of CD45, CD31, and HLA-DR. MSCs can be isolated from multiple tissues including bone marrow, adipose tissue, placenta, dermis, umbilical cord blood, and the umbilical cord.^{16,17} The umbilical cord is a rich source of MSCs and easy to obtain. In recent years, MSCs have been reported to regulate the immune response in

many diseases by affecting T and B cell responses.¹⁸ Previously, our group reported that MSCs derived from umbilical cord were effective for the therapy of AR in a rat model.¹ In the current study, we aimed to improve the efficiency of MSC treatment by traditional Chinese medicine (TCM), qi-fang-bi-min-tang, which is reported to be effective on clinical patients with AR.¹⁹ Intriguingly, we observed that TCM (qi-fang-bi-min-tang) combined with MSCs had a significant therapeutic effect on AR by inhibiting the symptoms and reducing inflammatory cytokines both in rats and mice. Hence, TCM (qi-fang-bi-min-tang) combined with MSCs might be used as a promising therapy for AR.

2 | MATERIALS AND METHODS

2.1 | Cells

MSCs were expanded with high-glucose Dulbecco's-modified Eagle's medium (Gibco, Grand Island, NY), supplemented with 5% fetal bovine serum (Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin (Gibco), and growth factors (EGF, FGF, and PDGF).²⁰ Cultures were maintained at 37°C in a humidified atmosphere with 5% CO₂.

2.2 | Medications

Traditional Chinese medicine, qi-fang-bi-min-tang was provided by Youlin Li, a professor of the Center for Respiratory Diseases of China-Japan Friendship Hospital in China. The main ingredients of qi-fang-bi-min-tang are *Radix Astragali*, *Ramulus Cinnamomi*, *Radix Saposhnikoviae*, *Los Magnoliae*, *Rhizoma Atractylodis Macrocephalae*, *Rhizoma Zingiberis*, *Radix Paeoniae Alba*, and *Radix Glycyrrhizae*. The drug extraction process is water extraction and ethanol precipitation. The specific extraction process is briefly as follows: the ingredients of qi-fang-bi-min-tang were boiled with 10 times of water (by volume) for 1.5 hours. The liquid content was collected after filtration. Then the filtrate was concentrated to a relative density of 1.15 to 1.2 (60°C). After cooling to room temperature, ethanol was added into the concentrated filtrate with the final concentration of 75%. The ethanol was removed after the filtrate stands for 12 hours. The dry powder extract was dissolved in a ratio of 0.3 g per 1 mL of distilled of water. After filtering through a microporous membrane the TCM is ready to be used.

2.3 | Animals

Male Sprague-Dawley rats (160-180 g) and Balb/C mice were purchased from the Vital River Laboratories (Beijing, China). Animals were maintained in a pathogen-free room,

fed with an autoclaved pellet diet and water ad libitum. All animal experimental procedures were approved by the Animal Ethics Committee of Tsinghua University and were conducted in strict accordance with institutional guidelines. All efforts were made to minimize the number of animals used and to reduce their suffering.

2.4 | Immune phenotypic analyses of MSCs

To examine the expression of typical MSC surface markers,²¹ cells were analyzed by flow cytometry using anti-human antibodies against CD34, CD44, CD45, CD90, CD105, HLA-DR, CD182 (CXCR2), CD184 (CXCR4), CD195 (CCR5), and CD197 (CCR7) (antibodies were purchased from Biolegend or eBioscience). Positive cells were counted and compared to the signal of corresponding immunoglobulin isotypes.

2.5 | Multilineage differentiation assays in vitro

Differentiation experiments were performed to demonstrate the ability of MSCs differentiated into adipocytes and chondrocytes in vitro. MSCs were plated in 24-well plates at a density of 3000 cells/well. The specific conditional medium was added 24 hours later. MSCs at passage 5 were cultured under the conditional media (STEMCELL Technologies Inc, Canada) to induce the differentiation of adipocytes or chondrocytes according to the manufactures' protocol. Oil Red O and Alcian Blue staining were used to show adipocytes and chondrocytes, respectively.

2.6 | Cell proliferation assay by cell counting kit-8

Cell proliferation assay was performed using cell counting kit-8 (CCK-8; Dojindo, Japan) to examine cell proliferation ability according to the manufacturer's instruction. MSCs were seeded at a density of 1000 cells/well in 96-well culture plates overnight and then incubated with different concentrations of TCM for 72 hours. Then 10 μ L of CCK-8 solution was added to each well and incubated for 4 hours at 37°C. Finally, the absorbance was measured at 450 nm with a microplate reader (model 680; Bio-Rad).

2.7 | Ovalbumin-induced AR models

Rats were sensitized by ovalbumin (OVA) using a standard protocol.²² Briefly, OVA (Sigma-Aldrich) and aluminium hydroxide diluted in saline (1 mg/mL + 30

mg/mL + 1 mL) were used to sensitize the rats three times via injection in 5 sites (double back toes, bilateral inguinal subcutaneous region, and abdominal cavity, with 0.2 mL for each site) on Days 1, 5, and 10. The control rats were injected with the same amount of saline. From Days 15 to 35, the sensitized rats were intranasally challenged by daily droppings with OVA diluted with sterile physiological saline (50 μ L/nostril, 100 mg/mL OVA). The rats in normal group were intranasally challenged by daily dropping with saline. After the OVA-induced AR model was successfully established, rats were randomly divided into three groups (n = 12 per group): (a) AR model group, (b) MSC treatment group, (c) TCM-treated MSC treatment group. An amount of 5×10^6 MSCs, pretreated with 2% TCM, were suspended in 0.3 mL saline and intraperitoneally injected to each rat. After the last OVA challenging, blood and tissues were collected. In another AR model with OVA-induced mice, animals were randomly divided into four groups including normal group, AR model group, TCM+MSC treatment group, and TCM treatment group. The AR induction process in AR mice is the same as that in rats. For the groups with TCM treatment, mice were given water containing 20% TCM. An amount of 2×10^6 MSCs suspended in 0.2 mL saline were intraperitoneally injected to each mouse in MSC treatment group.

2.8 | Evaluation of nasal symptoms

The nasal symptoms were observed as the frequency of sneezing and nasal rubbing motions, which were counted for 10 minutes, after the final nasal challenge with OVA into the bilateral nasal cavities. Rats in the normal group were given the same amount of saline in the same manner. The final nasal challenge was performed by one operator.

2.9 | Histological analysis of nasal mucosa

At the end of the experiment, rats were killed and the blood was collected from the abdominal aortic artery after isoflurane anesthesia, and the nasal mucosa was separated and fixed in 4% paraformaldehyde for 48 hours. The tissues were routinely paraffin-embedded, sectioned at the 4- μ m thickness and stained with hematoxylin and eosin (H&E) to examine histological alternation of the nasal mucosa.

2.10 | Cytokines detection

Levels of tumor necrosis factor- α (TNF- α), IL-17, interferon γ (IFN- γ), IL-4, histamine, and IgE in rats plasma were

measured by enzyme-linked immunosorbent assay (ELISA) (TNF- α and IL-17 ELISA kits were from eBioscience; IL-4 and IFN- γ ELISA kits were from MULTI SCIENCES, China; IgE ELISA kit was from GenWay Biotech, Inc; histamine ELISA kit was from ADI) according to the manufacturer's instructions. Levels of IL-4, IFN- γ , IL-17, and transforming growth factor- β 1 (TGF- β 1) were measured with Ready-set-go ELISA kits (eBioscience), and IL-6 and TNF- α were examined with ELISA kits from BioLegend following the manufacturer's instructions. Absorbance was measured at 450 nm by a microplate reader (model 680; Bio-Rad).

2.11 | Statistical analysis

The results were performed using GraphPad Prism software (version 5.0). The differences among the groups were analyzed by one-way analysis of variance, followed by the Tukey test. Data are presented as mean $\pm$ SD from at least two experiments. $P \leq .05$ was considered as statistical significance.

3 | RESULTS

3.1 | Characterization of MSCs

To characterize the homogeneity of the cultured MSCs, we performed fluorescence-activated cell sorting (FACS) analyses on the levels of specific markers of MSCs. The results showed that 100% of MSCs expressed CD105 and CD44 (Figure 1A; a and b). The proportion of CD90 was 99.9% (Figure 1A; c). These results suggested that the isolated MSCs were almost of the feature of stem cells. On the other hand, we observed that the cultured MSCs expressed low levels of CD34 and CD45, with only 5.1% and 0.6% positive rates, suggesting that the cells were of little hematopoiesis (Figure 1A; d and e). Furthermore, we observed that only 1% of MSCs expressed HLA-DR, a marker for immune surveillance, suggesting that the MSCs owned little or no immune rejection (Figure 1A; f). In addition, MSCs was evaluated for the ability of adipogenic and chondrogenic differentiation in vitro. After 21 days of induction in the conditional culture medium, adipogenesis and chondrogenesis were analyzed by Oil Red O and Alsin Blue staining, respectively (Figure 1B). The data showed that MSCs differentiated into adipocytes and chondrocytes effectively, suggesting that MSCs maintained multiple differentiation potential.

As MSCs have a feature of homing to the site of inflammation, we determined to examine the levels of chemokine receptors on the cells for the homing ability. FACS analyses showed that MSCs expressed detectable but low chemokine receptors including CD184, CD182, CD197,

and CD195 (Figure 1C). These results suggested that MSCs functions not through its homing ability. To produce enough cells, MSCs were cultured under a conditional medium for 20 generations. Of note, we found that the markers of CD105, CD44, CD90, CD34, CD45, and HLA-DR maintained quite consistent from generations 5 to 20 during the culture of MSCs (Figure 1D). These results suggested that the MSCs were isolated and remained very stable features of stemness and immune surveillance, owing to the quality of the therapy application.

3.2 | The influence of TCM (qi-fang-bi-min-tang) treatment on the proliferation and phenotype of MSCs

To explore a suitable concentration of TCM (qi-fang-bi-min-tang) for the treatment of MSCs, different dosages of TCM were used to treat the cells. We observed that treatment with a ratio of 1:500 or 1:1000 of TCM significantly promoted the proliferation of MSCs, whereas only a slight enhancement was observed when treated with TCM at a dose of 1:50 (Figure 2A). Since the function of TCM on the proliferation of MSCs was comparable between the ratio of 1:500 and 1:1000, 1:500 of TCM was chosen in our experiments. To characterize whether TCM treatment influences the levels of specific markers of MSCs, a FACS analysis was conducted to the MSCs treated with TCM at a ratio of 1:500 for 72 hours. We observed that the MSCs markers remained at similar levels after TCM treatment (Figure 2B, comparing with Figure 1A and 1D). These results suggested that TCM treatment had little influence on the characteristic markers of MSCs. Interestingly, we found that the frequency of CD182 and CD184, two receptors for chemokines, were significantly increased while CD197 and CD195 were unchanged after the treatment with TCM (Figure 2C, comparing with Figure 1C), suggesting that TCM treatment may impact the homing capacity of MSCs. These result indicated that TCM may influence the ability of MSCs homing to inflammatory sites.

3.3 | TCM (qi-fang-bi-min-tang)-treated MSCs inhibited the symptoms of AR in rats

To investigate the role of TCM-treated MSCs on AR, rats were treated with OVA and aluminum hydroxide. The number of sneezing and nose rubbing motions were counted in 10 minutes after challenging rats with OVA in the nostril. The process of AR induction, including the time, frequency, and dosages of cell injected, is showed as Figure 3A. The results showed that injected MSCs significantly decreased the number of nose sneezing and rubbing induced by OVA. Notably, TCM-treated MSCs possessed a

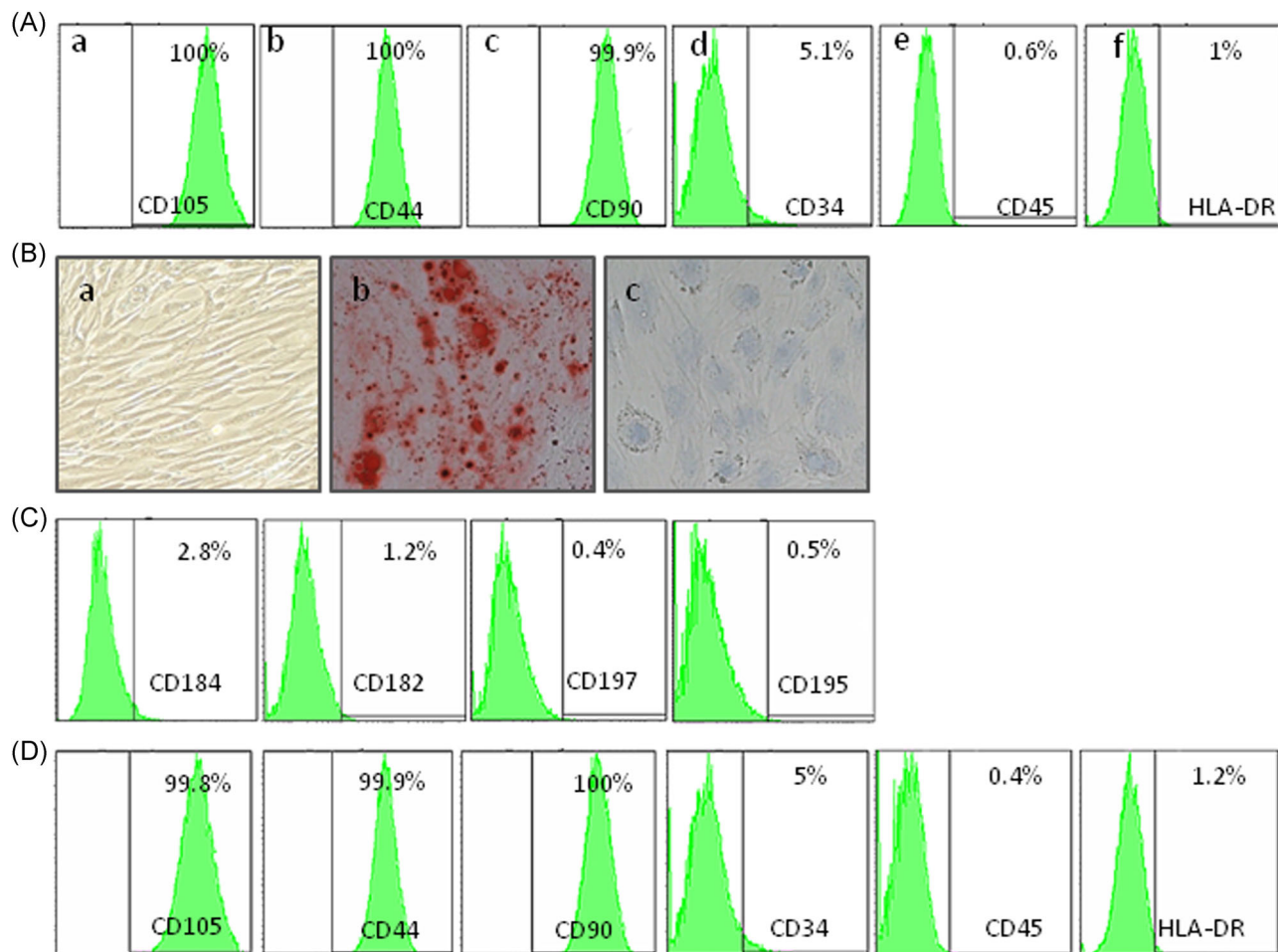


FIGURE 1 The characteristics of MSCs. A, Immunophenotypic analysis of MSCs isolated from human umbilical cords at passage 5 assessed by flow cytometry. Positive cells were compared with the signal of isotype controls. B, The differentiation ability of MSCs in vitro. (a) MSCs displayed a fibroblastic morphology. (b) Under adipogenic conditions, MSCs were induced to differentiate into adipocytes, as indicated by the positive Oil red O staining. (c) MSCs were induced to differentiate into chondrocytes and stained positive by Alcian blue as observed by light microscopy (×100). C, The expression of chemokine receptors on passage 5 of MSCs were examined by FACS. D, The expression of stem cell markers on passage 20 of MSCs assessed by FACS. FACS, fluorescence-activated cell sorting; MSC, mesenchymal stem cell

better therapeutic effect than MSCs alone in reducing the number of sneezed and rubbed nose motion (Figure 3B and 3C). These results indicated that MSCs, especially after the treatment with TCM, played a therapeutic role in acute AR. Taken together, these results suggest that TCM treatment is beneficial to the therapy of MSCs on AR.

The effect of MSCs on the histological changes in nasal mucosa was evaluated by H&E staining. Remarkable changes of epithelial disruption, mucosa exfoliation, and eosinophils infiltration were observed in the nasal mucosa of model rats (Figure 3D). Intriguingly, MSCs treated with or without TCM (qi-fang-bi-min-tang) showed significant therapeutic effects on the histology changes of the nasal mucosa. Especially, TCM-treated MSCs significantly protected the nasal mucosa from the damage of OVA challenge, which was evaluated by an analysis of the histology score (Figure 3E). These results indicated that

TCM treatment strengthened the protection role of MSC on nasal mucosa in OVA-induced AR rats.

3.4 | TCM (qi-fang-bi-min-tang)-treated MSCs reduced the expression of proinflammatory cytokines in the plasma of AR rats

To examine whether MSCs influence the production of proinflammatory mediators in the process of AR, cytokines including IFN- γ , IL-17, IL-4, and TNF- α were measured by ELISA. Our results showed that IFN- γ (Figure 4A) and IL-17 (Figure 4B) were remarkably increased in rats induced with OVA, but were significantly decreased following the treatment with MSCs. Moreover, TCM-treated MSCs exhibited further efficacy in reducing the levels of IFN- γ and IL-17 in comparison

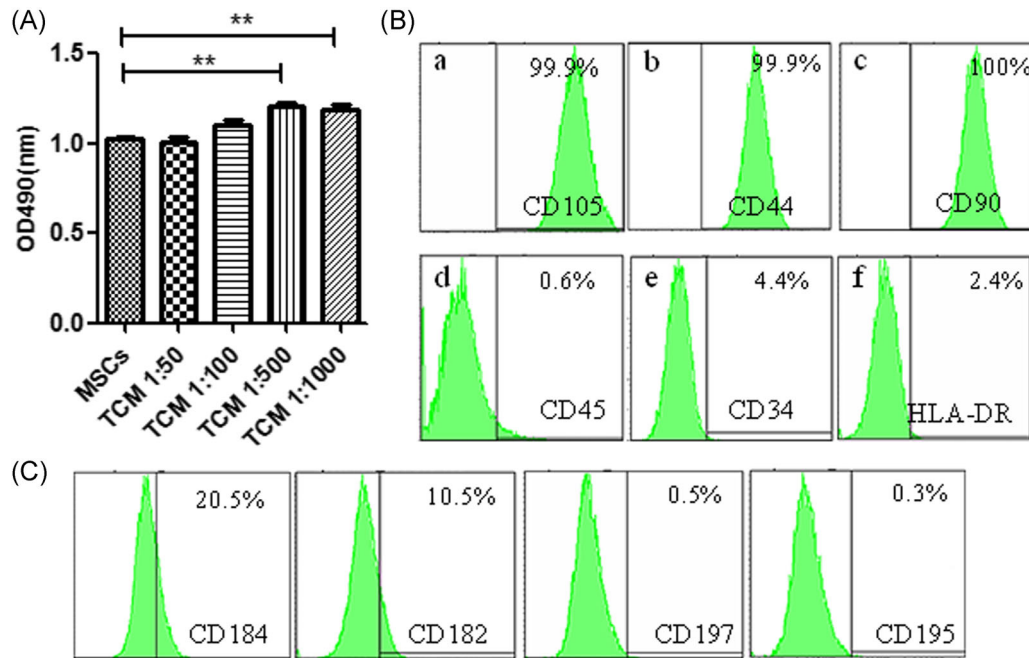


FIGURE 2 The influence of TCM (qi-fang-bi-min-tang) treatment on the MSC proliferation and the expression of MSC markers. A, The influence of TCM treatment on the proliferation of MSCs. MSCs, treated with or without different doses of TCM, were seeded into a 96-well plate at 1000 cells/well, cultured for 72 hours. The proliferative capacity of MSCs was evaluated using the CCK-8 kit. Data represent mean $\pm$ SD. * $P < .05$, ** $P < .01$. B, The effect of TCM treatment on immunophenotypic of MSCs was evaluated by FACS. C, TCM treatment promoted the expression of chemokine receptors on MSCs of passage 5. CCK-8, cell counting kit-8; FACS, fluorescence-activated cell sorting; MSC, mesenchymal stem cell; TCM, Traditional Chinese medicine

with MSCs alone. On the other hand, both MSCs and TCM-treated MSCs reduced the levels of IL-4 (Figure 4C) and TNF- α (Figure 4D) induced by OVA, although no significant difference was observed between MSCs alone and TCM-treated MSCs groups. Taken together, these results suggested that TCM-treated MSCs might possess a better anti-inflammation and immune-regulation potency than MSCs alone by targeting Th1 and Th17 cells, but not macrophages in the process of AR.

3.5 | TCM (qi-fang-bi-min-tang)-treated MSCs reduced the expression of IgE and histamine in AR rats

To further examine whether MSCs play a role in anti-inflammation and immune-regulation in the process of AR, the levels of antigen-induced IgE and inflammatory mediator histamine in the plasma of rats were measured by ELISA. The results showed that MSCs significantly decreased the level of IgE stimulated with OVA in rats, and a further decrease was observed in the TCM-treated MSC group (Figure 4E). In addition, TCM-treated MSCs reduced the level of histamine released by mast cells induced by OVA, whereas MSCs alone showed no significant difference in reducing the expression of histamine (Figure 4F). Altogether, these results further

indicated that TCM-treated MSCs exhibit increased anti-inflammation and immune-regulation effects in comparison with MSCs alone in AR therapy.

3.6 | TCM (qi-fang-bi-min-tang) combined with MSCs restored abnormal immune response in AR mice

The effect of MSCs together with TCM on AR was further validated in OVA-induced mouse model. To verify whether MSCs combined with TCM affects lymphocyte ratio, proportions of T cell, B cell, and helper T cell subset were measured by FACS after exposure to OVA in mice. The results showed no significant difference in the proportion of spleen T cells and B cells in each group of mice (Figure 5A and 5B). However, while the percentage of Th1, Th2, and Th17 were increased under OVA induction, MSCs combined with TCM significantly reduced the ratio of Th1 (Figure 5C), Th2 (Figure 5D), and Th17 (Figure 5E). Simultaneously, MSCs combined with TCM effectively increased the Treg ratio as OVA-induced decreased the ratio (Figure 5F).

The alteration of immune cell populations in mice echoes the change of cytokines in rats. Consistently, we find that MSCs combined with TCM exhibited a better efficacy in reducing the levels of TNF- α (Figure 6A), IL-6

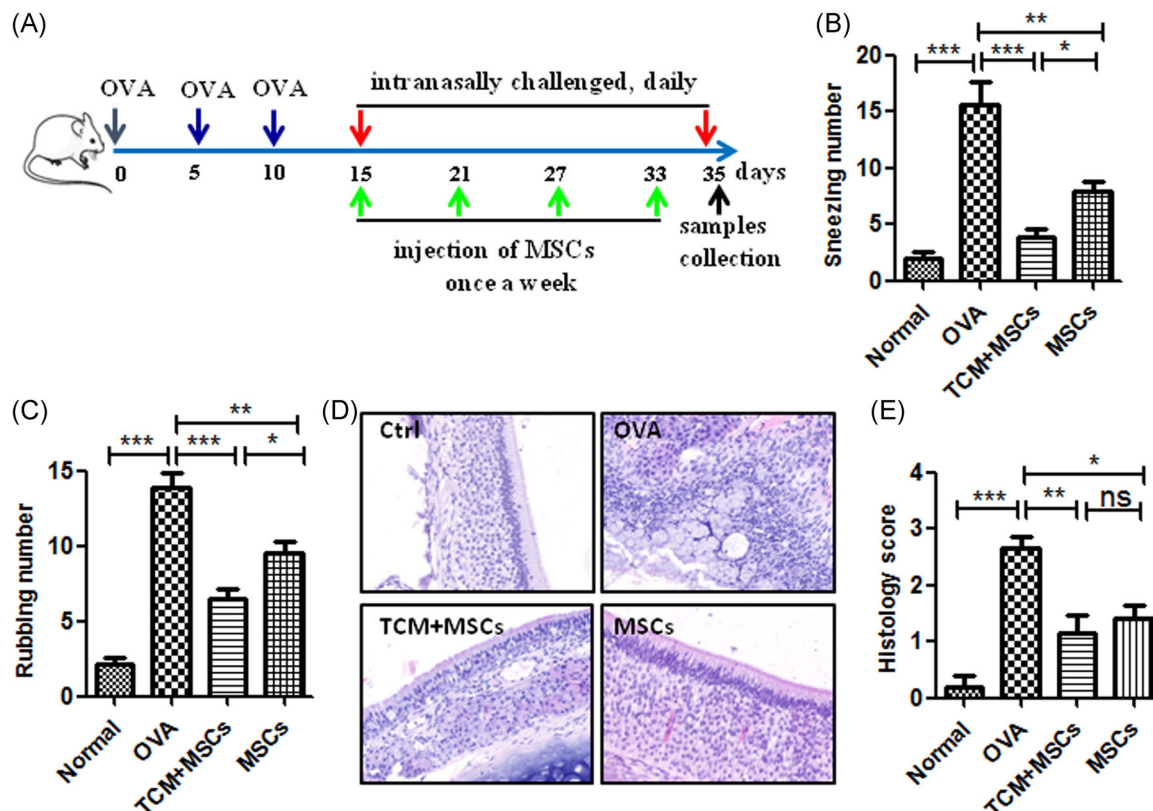


FIGURE 3 The influence of TCM (qi-fang-bi-min-tang)-treated MSCs on AR rats. A, The experimental protocol for AR rats and treatment procedure with TCM-treated MSCs. B, The frequency of sneezing was counted in the normal and AR rats administrated with or without TCM-pretreated MSCs. MSCs were cultured with medium containing 2% of TCM. Before injecting MSCs, the medium containing TCM was removed, washed twice with PBS, and then injected into rats. C, The frequency of nasal rubbing motions was measured in the normal and AR rats administrated with or without TCM-pretreated MSCs. D, Histological changes of the nasal mucosa stained with H&E (original magnification $\times 100$). E, Quantification of the tissue damage using the standard histology scoring system ($n = 12$). Data represent mean $\pm$ SD. AR, allergic rhinitis; H&E, hematoxylin and eosin; MSC, mesenchymal stem cell; ns, not significant; OVA, ovalbumin; PBS, phosphate-buffered saline; TCM, Traditional Chinese medicine. * $P < .05$, ** $P < .01$, and *** $P < .001$

(Figure 6B), IFN- γ (Figure 6C), IL-4 (Figure 6D), and IL-17 (Figure 6E) in comparison with TCM alone in OVA-induced AR mouse. In addition, MSCs combined with TCM treatment significantly increased the serum level of anti-inflammatory factor TGF- $\beta 1$ in AR mice (Figure 6F). Taken together, MSCs combined with TCM alleviated AR mainly by affecting the proportion of helper T cells.

4 | DISCUSSION

The prevalence of AR has been increasing worldwide. Although current treatments for AR provide effective symptomatic relief, there are still concerns over side-effects and the efficacies during the recovery of the pathological process. Therefore, it remains urgent to develop new therapeutic strategies with minimal side-effects. Many Chinese herbs have been proven effective in the treatment of allergic diseases including AR.^{23,24} TCM (qi-fang-bi-min-tang) is a clinical decoction in the Center of Respiratory

Diseases of China-Japan Friendship Hospital in China for the treatment of patients with AR. It was reported that TCM (qi-fang-bi-min-tang) were markedly effective to 76 of 100 cases of clinical patients with AR.¹⁹ TCM is effective in treating AR, but its effectiveness is not 100%. Therefore, we strived to find a way to improve the effectiveness of AR therapy. Our group reported that MSCs were effective for the therapy of AR in a rat model. Therefore, we intended to determine if MSCs could enhance the therapeutic effect of TCM (qi-fang-bi-min-tang). Our results in both rat and mouse models consistently demonstrated that TCM has an additional effect on MSC treatment of AR.

Over the past decade, MSCs have been applied in numerous preclinical and clinical studies because of their unique immunomodulatory and anti-inflammatory capabilities.²⁵ Others also observed that MSCs have a potent and effective therapeutic role on several autoimmune diseases including inflammatory arthritis, experimental autoimmune encephalomyelitis, systemic lupus erythematosus nephritis, graft-versus-host disease, and inflammatory bowel diseases,

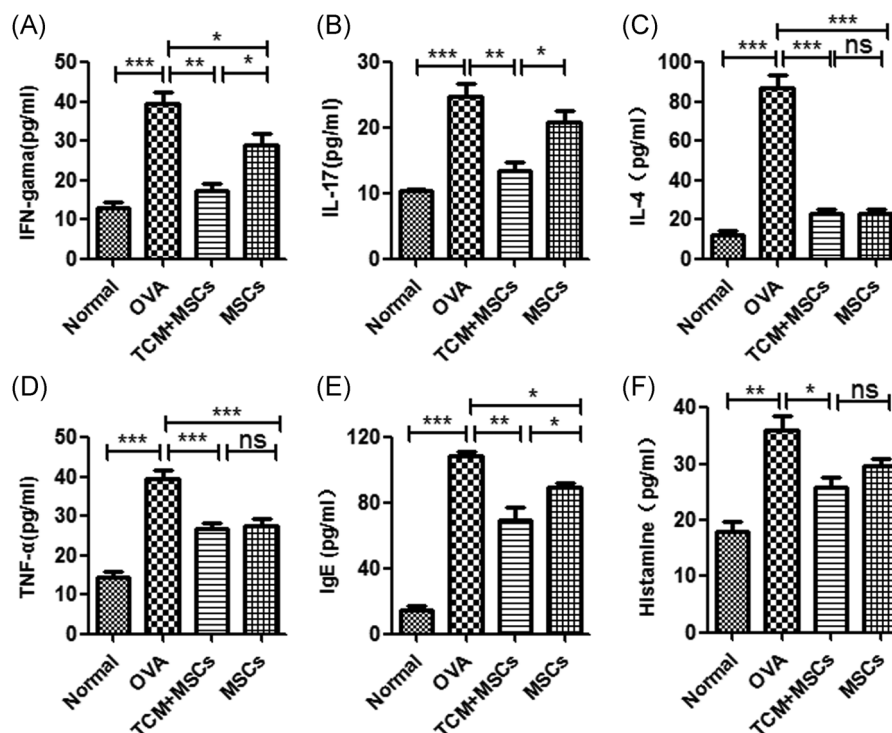


FIGURE 4 TCM (qi-fang-bi-min-tang)-treated MSCs decreased inflammatory cytokines and IgE in AR rats. Inflammatory cytokines secreted by Th1, Th17, Th2, macrophages, IgE, and histamine in the plasma of rats were measured by ELISA. Total of 12 rats in each group was examined. Levels of proinflammatory cytokines including (A) IFN- γ , (B) IL-17, (C) IL-4, and (D) TNF- α in AR rats induced with or without OVA were measured by ELISA. E, TCM-treated MSCs dramatically reduced the production of IgE in the plasma of the AR rats. F, TCM-treated MSCs significantly attenuated the level of histamine in AR rats. Data represent mean $\pm$ SD. AR, allergic rhinitis; ELISA, enzyme-linked immunosorbent assay; IFN- γ , interferon- γ ; IgE, immunoglobulin E; IL, interleukin; MSC, mesenchymal stem cell; ns, not significant; OVA, ovalbumin; TCM, Traditional Chinese medicine; TNF- α , tumor necrosis factor- α . * P < .05, ** P < .01, and *** P < .001

etc.²⁵⁻²⁹ In our previous study, we found that MSCs significantly ameliorated the symptoms and reduced the damage of nasal mucosa in acute AR rats.¹ However, no study is reported on whether TCM is able to improve the efficiency of MSCs. Therefore, our current study provided a new way for therapy for immune diseases.

We found that TCM treatment did not alter the immunophenotype of MSCs. Specifically, TCM did not change the proportion of CD105, CD29, CD44, CD90, CD45, CD34, and HLA-DR. Therefore, we speculated that the treatment of MSCs by TCM has no effect on the properties of stemness. However, after treatment with TCM, chemokine receptors, especially CD182 and CD184 were significantly increased. Since the expression of CD182 and CD184 indicates the ability of cell homing, we speculated that the treatment of MSCs by TCM could increase the homing capacity of MSCs to inflammatory sites. This may explain why TCM-treated MSCs were more effective in alleviating AR.

Previous studies have demonstrated that T-cell immunity plays an essential role in the pathogenesis of AR. Th17 cells are a new T-cell subset that functions in promoting inflammation and autoimmune diseases. It has been

reported that upregulation of Th17 cells increases the risk of AR.³⁰ Th17 cells mainly secrete IL-17, an important effector cytokine, which promotes the production of chemokines and causes local inflammation responses.³¹ In the present study, we found that MSCs significantly decreased the level of IL-17 in AR rats induced with OVA, and TCM treatment further decreased the IL-17 production. In addition, we found that MSCs combined with TCM treatment significantly reduced the ratio of Th17 and the secretion of IL-17 in OVA-induced AR mice, with a better therapeutic effect. We speculated that TCM treatment might further enhance the inhibitory effects of MSCs on AR progression through regulating the Th17 differentiation. As TNF- α , which was secreted by macrophages, contributed to Th17 formation in mice and humans,³² we speculated that TCM might inhibit the Th17 differentiation through inhibiting TNF- α production from macrophages. However, there was no significant change in serum TNF- α level in rats or mice compared with treatment with MSCs or TCM alone. We concluded that TCM treatment has no effect on macrophage activity. Therefore, our results demonstrated that TCM treatment enhanced the ability of MSCs on the regulation of Th17 cells rather than macrophages.

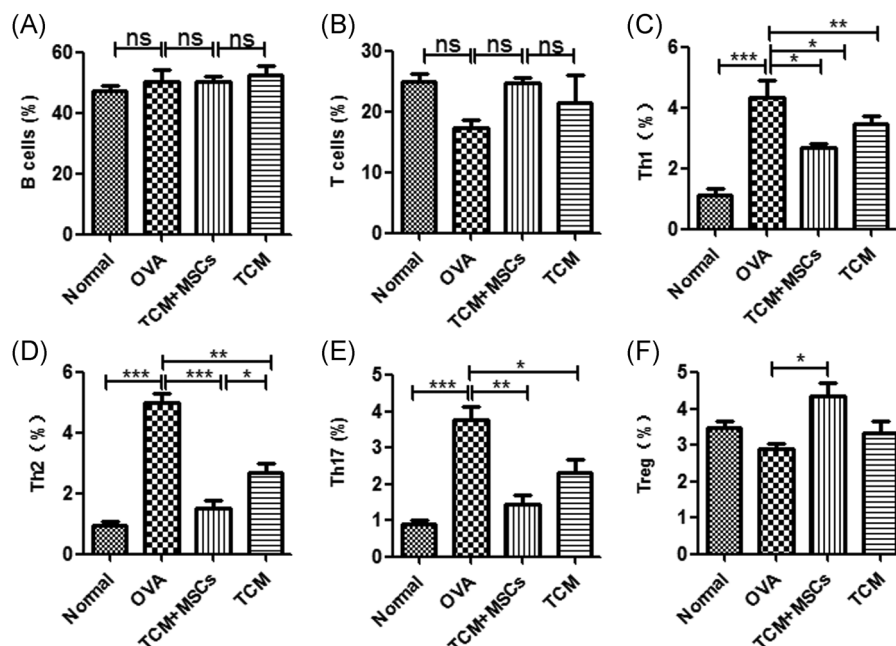


FIGURE 5 TCM (qi-fang-bi-min-tang) combined with MSCs restored the proportion of abnormal immune cells in AR mice. Flow cytometric analyses were performed to analyze B cells ($CD19^+CD3^-$), T cells ($CD3^+CD19^-$), Th1 ($CD4^+IFN-\gamma^+$), Th2 ($CD4^+IL-4^+$), Th17 ($CD4^+IL-17^+$), and Treg cells ($CD25^+Foxp3^+$) from spleen tissues of mice treated with or without OVA. Percentage of (A) B cells, (B) T cells, (C) Th1, (D) Th2, (E) Th17, and (F) Treg cells was measured. The ratio of Th1, Th2, Th17, and Treg cells was calculated by gating on CD4-positive population. Data represent mean $\pm$ SD. AR, allergic rhinitis; IFN- γ , interferon γ ; MSC, mesenchymal stem cell; OVA, ovalbumin; ns, not significant; TCM, Traditional Chinese medicine; Th, T helper type. * $P < .05$, ** $P < .01$, and *** $P < .001$

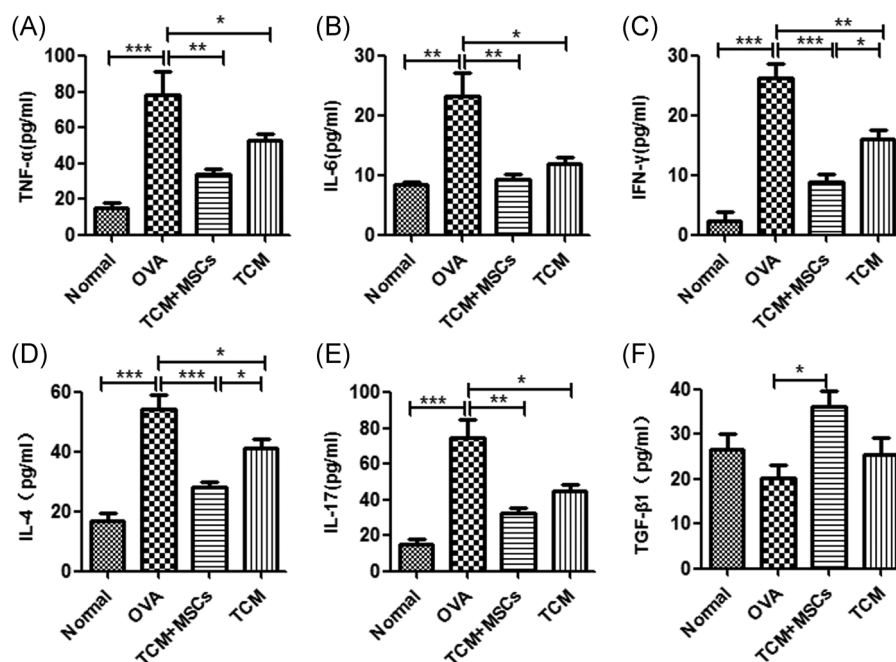


FIGURE 6 TCM (qi-fang-bi-min-tang) combined with MSCs decreased proinflammatory cytokines in AR mice. At the end of the experiment, blood was collected from mice of normal control, AR model, treatment with TCM combined with MSCs and treatment with TCM alone for the examination of inflammatory cytokine levels by ELISA. Different cytokines including (A) TNF- α , (B) IL-6, (C) IFN- γ , (D) IL-4, (E) IL-17, and (F) TGF- β 1 were measured. Data represent mean $\pm$ SD. AR, allergic rhinitis; ELISA, enzyme-linked immunosorbent assay; IFN- γ , interferon γ ; IL, interleukin; MSC, mesenchymal stem cell; OVA, ovalbumin; TCM, Traditional Chinese medicine; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α . * $p < .05$, ** $p < .01$, *** $p < .001$

Th1 and Th2 cells are critical in the development of allergic diseases. Th2 cells secrete cytokines including IL-4, IL-5, and IL-13, causing the production of allergen-specific IgE and the release of histamine from mast cells.^{33,34} We found that the levels of IL-4, IFN- γ , and histamine in the plasma of AR rats were strikingly decreased after the injection of MSCs. However, no significant difference in the expression of IL-4 and histamine were observed after TCM treatment. Notably, TCM-treated MSCs further decreased the level of IFN- γ . These results suggest that the therapeutic effect of MSCs on AR may be due to the regulation of Th2 cells. However, the treatment of TCM combined with MSCs might influence Th1 cells, instead of Th2 and mast cells. It has been widely accepted that the pathological changes of AR in nasal mucosa are mediated by IgE.³⁵ We found that TCM-treated MSCs exhibited a further efficacy in alleviating the pathological changes and reducing the IgE level in AR rats in comparison with MSCs alone. Therefore, IgE might be the target of TCM combined with MSCs therapy for AR. In addition, we found that the levels of IFN- γ in the plasma of AR rats and mice were strikingly decreased after treatment with TCM. Furthermore, TCM combined with MSCs further decreased the level of IFN- γ . Therefore, the therapeutic effect of MSCs combined with TCM may be through affecting Th1 and the inflammatory factors it produces. Taken together, TCM combined with MSCs might alleviate the process of AR mainly by inhibiting the production of proinflammatory cytokines secreted by Th1 and Th17.

In conclusion, we found that MSCs combined with TCM treatment alleviates AR symptoms by decreasing the production of inflammatory cytokines and rebalancing helper T cell populations, especially Th1 and Th17. Therefore, MSCs combined with TCM therapy could be used as an effective treatment for patients with AR in the future.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

YXF and ZJ Chang designed the research. YXF performed the research and analyzed the data. YHK and JL helped in the experiments. YXF, ZJC, MDL, and YW wrote the manuscript. YW, YYW, and FLR helped analyze the data. YLL provided TCM prescription. JN extracted TCM.

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