

ORIGINAL ARTICLE

Systemic Transplantation of Mesenchymal Stem Cells Modulates Endothelial Cell Adhesion Molecules Induced by Ovalbumin in Rat Model of Asthma

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Abstract— Achieving the optimal clinical outcome of mesenchymal stem cells (MSCs) is particularly dependent on fundamental understanding of therapeutic mechanisms. The current study was focused on the possible mechanisms by which rat bone marrow-derived mesenchymal stem cells (rBMMSCs) and/or conditioned media (CM) display broad immunomodulatory properties for ameliorating of asthma-related pathological changes. Male rats were divided equally into four experimental groups ($n = 6$): healthy rats received 50 μ l PBS intravenously (group C), sensitized rats received 50 μ l PBS intravenously (group OVA), sensitized rats received 50 μ l CM intravenously (group OVA + CM), and sensitized rats received 50 μ l PBS intravenously containing 2×10^6 rBMMSCs (group OVA + MSCs). After 2 weeks, the expression of interleukin (IL)-5, IL-12 and INF- γ , ICAM-1, and VCAM-1; pathological injuries; and the homing of MSCs into the lung tissues were assessed. Our results showed that systemic delivery of rBMMSCs, but not CM, returned the expression of IL-5, IL-12 and INF- γ , ICAM-1, and VCAM-1 and pathological injuries in the lung tissues of asthmatic groups to the near level of control group ($p < 0.001$ to $p < 0.05$). Moreover, rBMMSCs had potential to successfully recall to asthmatic niche in cell-administrated rats. However, no regulatory function was observed by MSC-CM. Collectively, our data notified the potency of MSCs in ameliorating OVA-mediated airway inflammation in a rat model of asthma presumably by regulating endothelial expression of leukocyte-selective cell adhesion molecules in lung tissue.

KEY WORDS: asthma; mesenchymal stem cells; interleukin; ICAM-1; VCAM-1.

INTRODUCTION

Asthma is a debilitating chronic lung disorder, affecting approximately 300 million individuals globally without any definite therapeutic approach [1]. Histopathological changes in the asthmatic lung tissues are mainly induced by the promotion of airway inflammation due to the shift in the Th1/Th2 balance toward a Th2-dominant phenotype [2, 3]. The first stage in the development of allergic inflammation is leukocyte adhesion to the vascular endothelium by physical interaction between cell adhesion molecules (CAMs) expressed on endothelial surfaces and their cognate ligands

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on circulating leukocytes [4]. It is well known that various CAMs are expressed on endothelium notably intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), playing critical roles in the pathogenesis of asthma by recruiting circulating lymphocytes to asthmatic sites [4, 5]. Following exposure to an allergen, the upregulation of Th2 cytokines potentiates VCAM-1 and ICAM-1 expression on endothelial cells, leading to continuous leukocyte recruitment to inflammatory niche, and finally aggravates asthmatic condition. Thus, the control of Th2-derived cytokines could result in a decreased VCAM-1 and ICAM-1 endothelial distribution [4, 6–8]. Commensurate with these descriptions, new emerging policies have been undertaken for the management of asthmatic changes to improve the imbalance of Th1/Th2 [9]. Due to the existence of an immune regulatory features of stem cells namely mesenchymal stem cells (MSCs), mainly orchestrated by paracrine mechanisms, this makes them as attractive candidate for a broad range of inflammatory disorders such as asthma [10–12]. Since MSCs secrete numerous factors such as IGF-1, LIF, SDF-1 α , and VEGF responsible for some of the therapeutic effects, so systemic use of MSC conditioned media (CM) may reflect the functional capability of these cells [10, 12]. The administration of CM could alone attenuate some of the disadvantages associated with direct transplantation of MSCs such as immune responses, tumorigenic risk, infectious agents, total costs, and long-term for stem cell expansion [13]. Increasing evidence in animal studies demonstrated that systemic direction of MSCs and/or CM could alleviate airway inflammation by modulating Th1/Th2 cytokines and suppressing airway recruitment of inflammatory cells [12, 14–16]. Although the immunomodulatory properties of MSCs have attracted significant interest, nevertheless, the precise mechanisms of immune regulation by MSCs are still largely unknown [17]. Until to the present time, there is not enough data on the immunomodulatory potency of MSCs related to the expression of VCAM-1 and ICAM-1 in the lung endothelium of OVA-challenged rats. In present study, we hypothesized that rat bone marrow MSCs (rBMSCs) and/or CM could be effective in alleviation of chronic asthmatic changes *via* the regulation of VCAM-1 and ICAM-1 mRNA expression in lung tissues.

MATERIALS AND METHODS

Experimental Design

Twenty-eight adult male Wistar rats, with the average weight of 200–250 g, were enrolled to the current study.

Four healthy rats were randomly used for extraction rBMSCs. The remaining 24 rats were equally divided into four groups (6 rats in each group) as follows:

- I. C group: healthy rats received intravenously 50 μ l PBS alone
- II. OVA group: sensitized rats received intravenously 50 μ l PBS alone
- III. OVA + CM group: sensitized rats received intravenously 50 μ l CM
- IV. OVA + MSC group: sensitized rats received intravenously 50 μ l PBS containing 2×10^6 rBMSCs.

In ovalbumin (OVA)-sensitized groups, rats were exposed to OVA for a period of 32 ± 1 days according to previously published articles [9, 18]. Shortly, each animal was given 1 mg ovalbumin (Sigma-Aldrich, USA) and 200 mg aluminum hydroxide (as adjuvant) intraperitoneally pre-dissolved in 1 ml saline on first and eighth days. From days 14 to 32 ± 1 , the sensitized rats were challenged for 5 min daily with aerosolized 4% OVA by using a nebulizer (CX3, Omron Co., Netherland) without any interruption. The recent procedure was done in a closed chamber with dimensions $30 \times 20 \times 20$ cm³. In a control group, rats received saline instead of ovalbumin in the same manner. One day after sensitization, the rats were subjected to the experimental procedure of cell suspension, CM or PBS treatment through the femoral vein [18]. In our study, all measurements were carried out 2 weeks after treatment (Fig. 1).

Culture and Isolation of rBMSCs

rBMSCs were isolated from healthy rats according to previous published protocols [18, 19]. In brief, the animals were euthanized by high doses of ketamine/xylazine combination, and after complete removal of femurs under aseptic conditions, the cellular contents of the medullary cavity were aspirated carefully with PBS containing 2% fetal bovine serum (FBS; Gibco, USA). Then, fresh bone marrow mononuclear cells were harvested by gradient centrifugation using Ficoll solution (gravity density, 1.073; Sigma, USA), at 400g for 20 min, and then washed twice by PBS. The enriched cells were resuspended in low-glucose content Dulbecco's modified Eagle's medium (DMEM/LG; Gibco, USA) supplemented with 20% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin (Biosera, UK). Finally, 1×10^5 cells were seeded in 6-well culture plates (SPL). After 24 h, the medium was replaced to exclude non-adherent cells. For cell subculture,

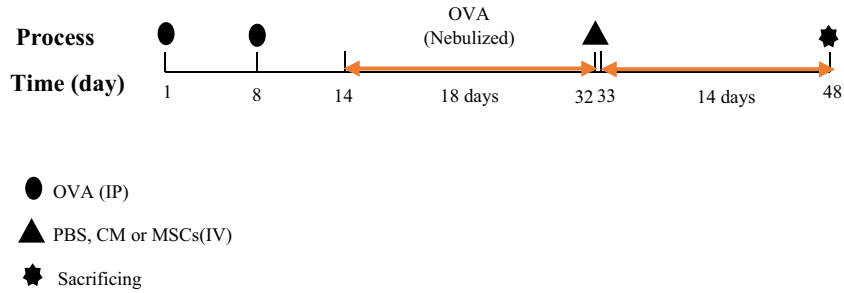


Fig. 1. Flow chart of experimental design. OVA; ovalbumin, IP; intraperitoneally; IV, intravenously.

rBMSCs were trypsinized at 70–80% confluency and subjected to subsequent analyses, at passages three to six.

Phenotypic Characterization of rBMSCs

rBMSCs were characterized by flow cytometry analysis by a panel of different monoclonal antibodies against cell surface markers, including FITC-conjugated anti-CD133, anti-CD44, and anti-CD34 as well as PE-conjugated anti-CD31 (all purchased from eBioscience, USA) [9, 20]. Isotype control antibodies were applied to exclude non-specific binding. Then, cells were trypsinized by using 0.25% trypsin-EDTA solution (Gibco, USA), washed by PBS, and blocked by 1% bovine serum albumin (BSA, Sigma, USA) for 15 min. Antibodies were added into 100 μ l of PBS containing 5×10^5 cells and incubated for the next 30 min at RT according to manufacturer's recommendations. Finally, cells were analyzed with a BD FACSCalibur flow cytometer system (USA) and Flow Jo software version 7.6.1. The percentages of CD34, CD31, CD44, and CD133 were calculated to determine purity of isolated rBMSCs.

Pre-labeling of rBMSCs with Cell Tracker

We used a non-invasive fluorescence dye to tag cells and track the recruitment of rBMSCs into the pulmonary parenchyma in sensitized rats [9, 18]. After detaching by enzymatic solution, cells were labeled with 2 μ M Cell Tracker™ CM-Dil at 37 °C for 20 min (Catalog No. C-7000; Molecular Probes, Invitrogen, USA). A total number of 2×10^6 CM-Dil pre-labeled cells in 50 μ l of PBS was administrated gently to each animal's femoral vein.

Conditioned Media Harvesting

Therapeutic effect of rBMSC secretome on OVA-induced asthma was examined by rBMSC-derived CM.

By 70–80% confluency, the exhausted DMEM/LG was discarded and cells washed three times with PBS. Subsequently, DMEM/LG-free serum was added to each flask [9, 18]. The supernatant was collected after 72 h, centrifuged at 400g for 5 min, and filtered by a 0.20- μ m-pore syringe filter. An approximately 50-fold concentrated solution was prepared by using centrifuge filter tubes with molecular weight cut-offs at 4 kDa (Catalog No.: 003099.125, Eppendorf, Germany). Total protein level in concentrated CM was determined using bicinchoninic acid assay (BCA assay, iNtRon, Korea). Over a 500 μ g/ml of protein content was detected in concentrated CM. An equal volume of CM with same protein content was used. We administrated 50 μ l of CM per case intravenously [18].

Total RNA Extraction and Real-Time PCR

The transcription levels of IL-5, IL-12 and INF- γ , ICAM-1, and VCAM-1 were quantitatively determined by real-time PCR assay. Primer sequences for each mRNAs were designed using Gene-Runner Software, version 3.05 (Table 1). In brief, the left lung tissue was quickly removed, soaked in normal saline, and immediately snap-frozen in liquid nitrogen. The extraction of total RNA from each lung tissue was performed using a total RNA extraction mini kit (YTA, Taiwan) [9, 18]. The integrity of each sample RNA was confirmed by a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, 19810 USA). Reverse transcription was done by a cDNA synthesis kit (YTA, Taiwan). cDNA was used as a template for separate assay for mRNA quantitative real-time PCR by using SYBR Green master mix (YTA, Taiwan). Real-time PCR reactions were carried out on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia). Quantitative gene expression data were normalized to the expression levels of housekeeping GAPDH gene. We used $2^{-\Delta\Delta CT}$ formula as a relative quantification strategy for qPCR

Table 1. Forward and Reverse Primer Sequences Used for Real-Time PCR

Target mRNA	Accession number	Primer sequence
GAPDH	NM_017008.4	F: 5'-CAA GTT CAA CGG CAC AGT CAA G-3' R: 5'-ATA CTC AGC ACC AGC ATC ACC-3'
IL-5	NM_021834	F: 5'-TGC TTC TGT GCT TGA ACG TTC TAA C-3' R: 5'-TTC TCT TTT TGT CCG TCA ATG TAT TTC-3'
IL-12	NM_022611.1	F: 5'-CCG ATG CCC CTG GAG AAA C-3' R: 5'-CCT TCT TGT GGA GCA GCA G-3'
INF-gamma	NM_138880	F: 5'-GAA AGC CTA GAA AGT CTG AAG AAC-3' R: 5'-GCA CCG ACT CCT TTT CCG CTT CCT-3'
ICAM-1	NM_012967	F: 5'-TGG AGG TGA CTG AGA AGT TGG-3' R: 5'-CAC AGT TAC TTG GTC CCC TTC-3'
VCAM-1	NM_012889	F: 5'-GTG TGT GAA GGA GTG AAT CTG G-3' R: 5'-CCA ACA GCA GCA CAT GTC AGA A-3'

data analysis. The results were expressed as fold change *versus* the relevant controls.

Tracking of CM-Dil-Labeled Cells in Lung Tissues

To monitor the existence of CM-Dil-tagged rBMMSCs in the pulmonary system, the isolated lung tissues were thoroughly embedded in Tissue Freezing Media (OCT), snap-frozen, and then sectioned at a thickness of 5 μ m using a cryostat apparatus. Sections were thawed at RT, rinsed twice with PBS (5 min each), and stained with 4', 6-diamidino-2-phenylindole (DAPI, 1 μ g/ml, Catalog No.: D9542, Sigma-Aldrich, USA) for background counterstaining [20].

Pathological Evaluation

The right lung of each rat was removed, fixed by fully immersion in 10% buffered formaldehyde solution (37%, Merck, Germany) for 1 week, embedded in paraffin, and cut into 4- μ m-thick sections using a microtome. All sections were carefully transferred to glass slides, stained with hematoxylin-eosin (H&E) solution for morphological evaluation under a light microscope [21]. The severity of lung injury in all experimental groups was calculated by using a semi-quantitative scoring system based on following pathological changes: alveolar membrane thickening, interstitial cell infiltration, mucus plug, and bronchial hyperplasia. Each change was scored from 0 to 3 as follows: absence of pathologic injuries = 0, patchy injuries = 1, local injuries = 2, and scattered injuries = 3.

Statistical Analysis

All quantitative results (mean $\pm$ SEM) were analyzed using a one-way ANOVA with Tukey–Kramer *post hoc* analysis. Histopathological scores were analyzed using Kruskal–Wallis test followed by a *post hoc* Mann–Whitney test. Statistical significance was set at $p < 0.05$.

RESULTS

Immunophenotype of MSCs

Flow cytometry analysis confirmed that expanded rBMMSCs obviously expressed the sternness surface markers CD44 and CD133, but were negative for CD34 and CD31 (Fig. 2).

rBMMSCs Were Recruited into Pulmonary Tissue

The presence of pre-labeled cells was determined in lung tissues from OVA + MSC group, indicating the potency of MSCs in homing to inflammatory sites (Fig. 3).

MSCs Effectively Modulated the VCAM-1 and ICAM-1 mRNA Expression in Asthmatic Niche

The expression levels of VCAM-1 and ICAM-1 in all the sensitized groups were significantly higher than those in the C group ($p < 0.001$). We notified a significant decrease in levels of VCAM-1 and ICAM-1 in the cell-treated group as compared with the other sensitized groups ($p < 0.05$ and $p < 0.001$ respectively). In addition, the pattern of ICAM-1 and VCAM-1 expression in the CM-treated group was more similar to that in the OVA group (Fig. 4).

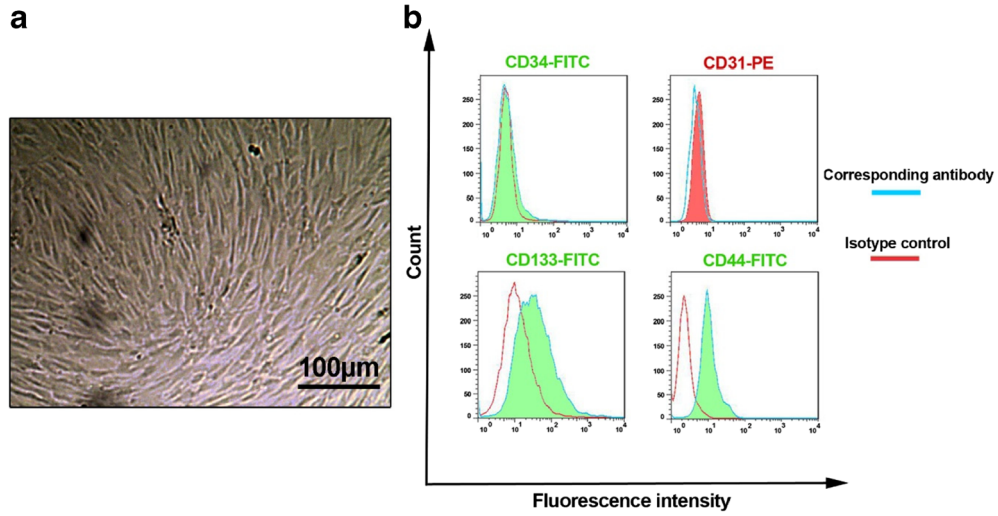


Fig. 2. Microphotomicrograph of rBMSCs at passage 3 (a). Phenotypic characterization of rBMSCs (b).

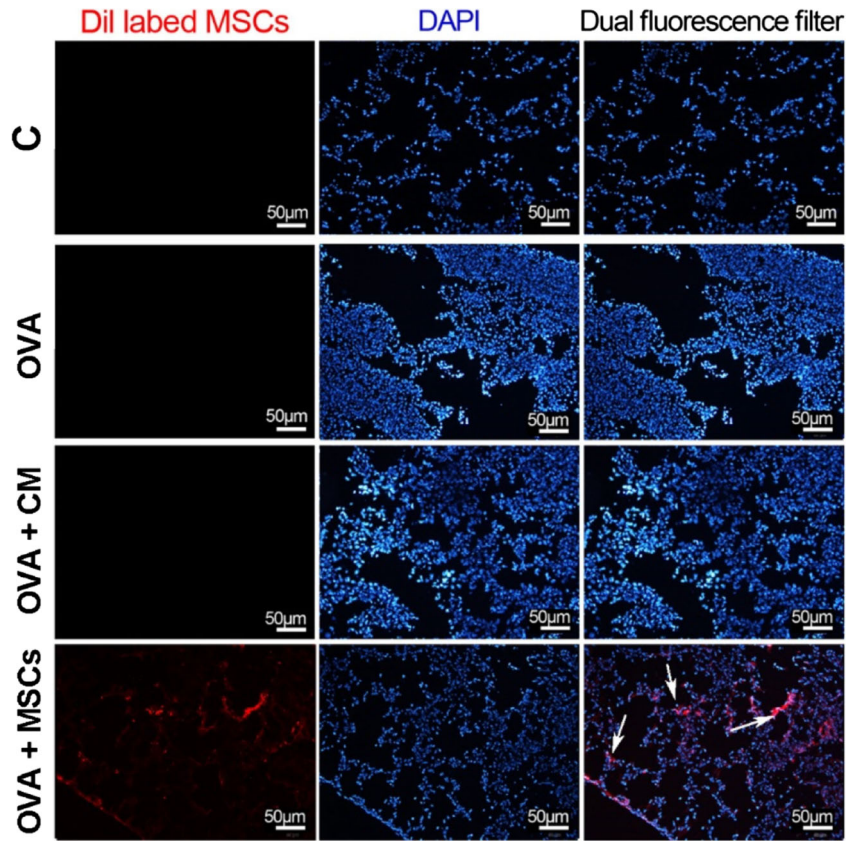


Fig. 3. Representative images of fluorescently tagged rBMSCs in lung tissues (a, b). The existence of rBMSCs was evident in all the sensitized rats given cells by systemic delivery. For background staining, the directed cells were stained with DAPI solution. Control animals received PBS (C), sensitized animals received PBS (OVA), sensitized animals received CM (OVA + CM), and sensitized animals received rBMSCs (OVA + MSCs).

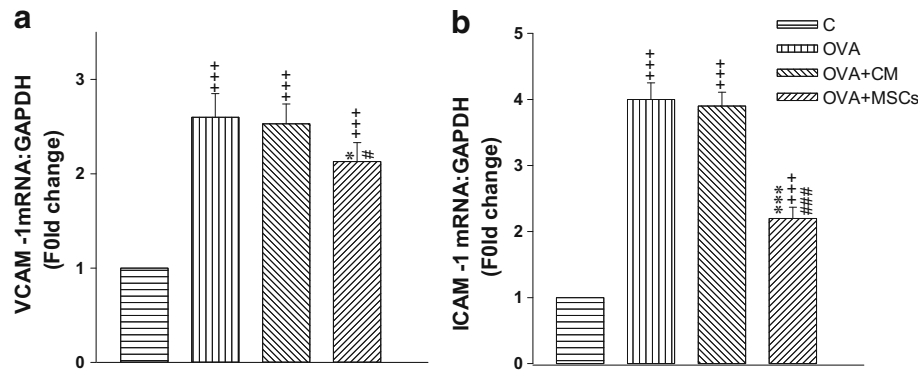


Fig. 4. Expression levels of VCAM-1 (a) and ICAM-1 (b) mRNA in the lung tissues of control animals received PBS (C), sensitized animals received PBS (OVA), sensitized animals received CM (OVA + CM), and sensitized animals received rBMMSCs (OVA + MSCs) (for each group, $n = 6$). Bars represent mean $\pm$ SEM. Statistical differences between control and different groups: $+++p < 0.001$. Statistical differences between OVA + CM and OVA + MSC vs OVA group: $*p < 0.05$ and $***p < 0.001$. Statistical differences between OVA + CM and OVA + MSC group: $#p < 0.05$ and $###p < 0.001$.

The Transcription Level of IL-5, INF- γ , and IL-12 Was Changed After MSC Recruitment to Asthmatic Niche

Quantitative transcript analysis of asthmatic lungs indicated a marked expression in the levels of IL-5 with decrease of INF- γ and IL-12 in comparison with the C group ($p < 0.001$). The expression of measured mRNA in cell-treated rats showed drastic difference compared with the other sensitized groups. In detail, the expression levels of IL-5 diminished significantly ($p < 0.01$), whereas INF- γ and IL-12 increased simultaneously ($p < 0.05$). In addition, no significant modulatory effects of CM injection were achieved in terms of IL-5, INF- γ , and IL-12 levels in asthmatic lungs (Fig. 5).

Ameliorating Effects of MSCs on Pathological Changes

Pathological injuries observed in the lung tissues of all the sensitized groups were significantly higher than those in the C group ($p < 0.001$ to $p < 0.01$). There was a significant decrease in the all pathological injuries in cell-treated rats in comparison with the other sensitized groups ($p < 0.01$ to $p < 0.05$). Calling attention, the pathological injuries in the CM-treated group were more similar to those in the OVA group (Fig. 6, Table 2).

DISCUSSION

Given the lack of definite therapeutic advantages and the progressive nature of the disease, new strategic approaches seem to be essential for facilitating the regeneration rate of asthmatic changes [9, 18]. It has been

demonstrated that systemic transplantation of MSCs and/or CM could significantly shift CD4⁺ T lymphocyte population from Th2 cells toward a Th1 phenotype, alleviating airway inflammation during lung injury [12, 14–16]. However, underlying mechanisms governed by MSCs during asthma have still not been fully addressed. Nonetheless, no enough data exist on the modulatory effects of MSCs on the expression levels of ICAM-1 and VCAM-1 in lung tissues of OVA-induced asthmatic rats. By choosing rBMMSCs and CM, we tried to address the possible mechanism for immunomodulatory properties of MSCs in attenuating chronic asthmatic changes *via* modulating ICAM-1 and VCAM-1 expression.

Rat models of asthma not completely, but considerably, mimic many of the features of human allergic asthma. Indeed, illumination of the underlying mechanisms directed by MSCs in rat model of asthma will promote clinical application of stem cells for asthmatic subjects as one of the most promising candidates for regenerative medicine field and cellular therapy [18, 22]. OVA with aluminum hydroxide, as an adjuvant, is one of the most common antigen used in inducing asthmatic changes in animal models and it is a suitable choice for the development of Th2-type allergic inflammatory response [23]. Therefore, the rats were enrolled to this study and exposed to OVA. Previously, Abreu and co-workers revealed that regenerative potential of stem cells diminished following sensitizing with OVA in mice compared to non-sensitized counterpart, and thereby, MSCs were isolated from normal rats [24]. Additionally, the paracrine effects of intravenously administrated rBMMSCs and relevant CM phase were individually investigated by monitoring the expression of IL-5, IL-12, INF- γ , VCAM-1, and ICAM-1 in asthmatic

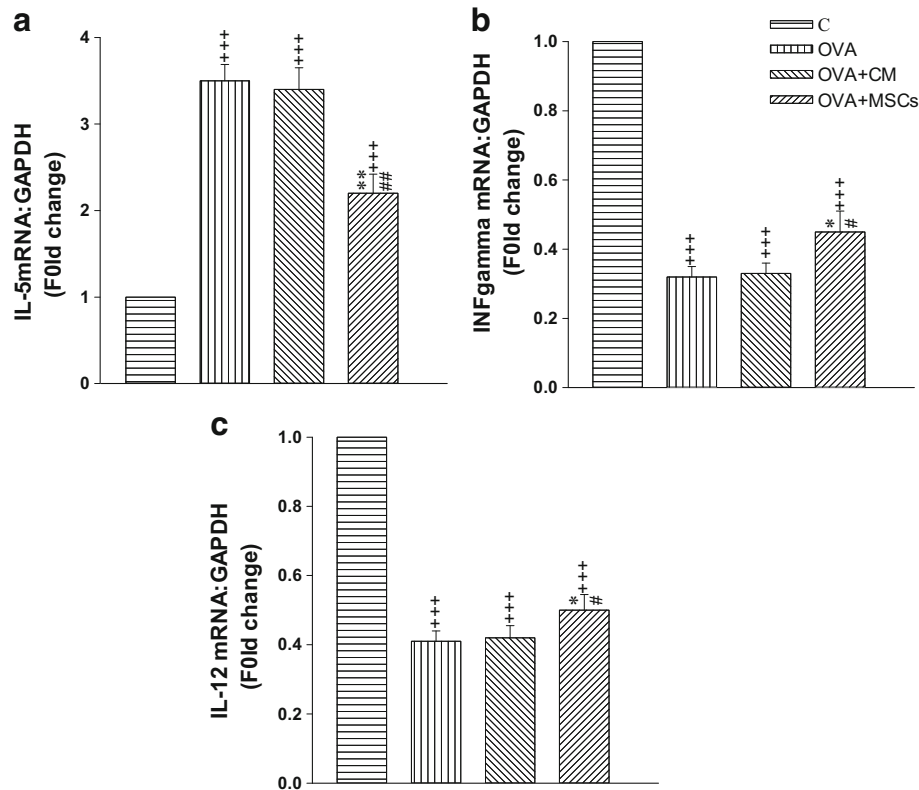


Fig. 5. Expression levels of IL-5 (a), INF-gamma (b), and IL-12 (c) mRNA in the lung tissues of control animals received PBS (C), sensitized animals received PBS (OVA), sensitized animals received CM (OVA + CM), and sensitized animals received rBMMSCs (OVA + MSC) (for each group, $n = 6$). Bars represent mean $\pm$ SEM. Statistical differences between control and different groups: +++ $p < 0.001$. Statistical differences between OVA + CM and OVA + MSC vs OVA group: * $p < 0.05$ and ** $p < 0.01$. Statistical differences between OVA + CM and OVA + MSC group: # $p < 0.05$ and ## $p < 0.01$.

pulmonary tissue. Our pathological results revealed that lesions observed in the lung tissue of sensitized rats were significantly higher than those in the control group, approving the rat model of asthma was established successfully [9]. Consistent with previous experiments, our findings notified a remarkable augmentation in the expression of IL-5, VCAM-1, and ICAM-1 coincided with a significant reduction in the expression of INF- γ and IL-12 in lung tissues of all sensitized animals compared to control group [3, 7]. An appropriate number of pre-labeled MSCs were identified in pulmonary tissue. Cell treatment was found to reduce pathological injuries by the modulation of interleukins and CAMs to near normal levels in sensitized rats. In contrary, MSC-CM did not yield any obvious effects in asthmatic lung tissues. In better words, systemic injection of rBMMSCs, but not CM, diminished significantly inflammation rate which was supported by our previous experimental findings [18]. Unlike to our results, some experiments showed that the systemic injection of MSC-CM could positively modulate the

inflammatory responses in experimental lung injury [11, 12]. The ineffectiveness of CM on the factors measured in present study could be possibly related to animal species, sensitization technique, the source, volume and time of CM administration, variety in content of secretome, and expansion conditions [18]. In support of our results, the secretome could easily be distributed in the whole body and not reach efficiently into the injured lung tissue. In addition, the presence of the stem cells provides an available source for the chronic secretion of soluble factors whereas the CM contains only a limited amount of agents [25, 26]. Further investigation must be done related to CM injection with high volume and recurrent doses to achieve possibly efficient results.

Regarding to asthma pathology and underlying immunoregulatory mechanism of MSCs, monitoring of Th1 (INF- γ , IL-12) and Th2 (IL-5) cytokine levels is crucial [3]. IL-5 plays a key role in the recruitment of eosinophils to inflamed sites in allergic asthma by regulating cell attachment to lung microvascular adhesion molecules [27]. On the other hand,

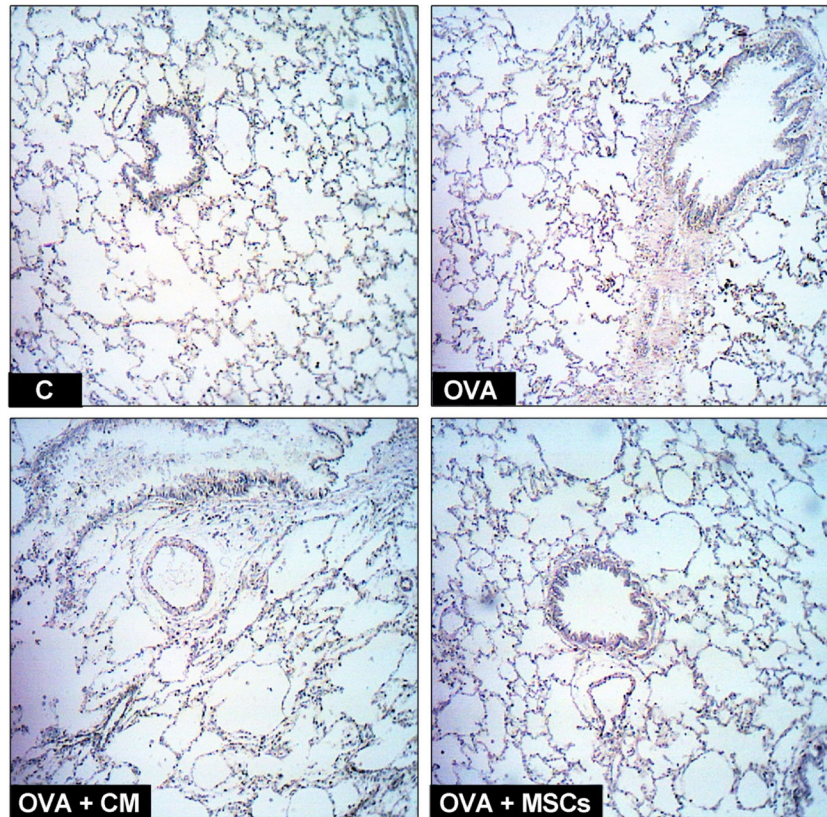


Fig. 6. Lung tissue microphotographs from different groups (magnification for each group; $\times 80$). Normal lung tissue in non-sensitized rats received PBS (C group). An alveolar membrane thickening, interstitial cell infiltration seen in sensitized rats received PBS (OVA group), mucus plug, bronchial hyperplasia in sensitized rats received CM (OVA + CM group), and slight interstitial cell infiltration and improved bronchial inflammation in sensitized rats received rBMSCs (OVA + MSC group).

INF- γ and IL-12 are involved in diminishing Th2-cell responses to confine the progress of asthma [3, 28]. Based on different reports, the close interaction of inflammatory cells

with endothelial layer plays an important role in asthma pathology *via* engaging adhesion molecules [5].

Table 2. Pathological Finding Scores in the Lung Tissues of Control Animals Received PBS (C), Sensitized Animals Received PBS (OVA), Sensitized Animals Received CM (OVA + CM), and Sensitized Animals Received rBMSCs (OVA + MSC) (for Each Group, $n = 6$). Statistical Differences Between Control and Different Groups: $^{++}p < 0.01$ and $^{+++}p < 0.001$. Statistical Differences Between OVA + CM and OVA + MSC *vs* OVA group: $^{*}p < 0.05$ and $^{**}p < 0.01$. Statistical Differences Between OVA + CM and OVA + MSC group: $^{#}p < 0.05$ and $^{##}p < 0.01$. Each Change Was Scored from 0 to 3 as Follow: Absence of Pathologic Injuries = 0, Patchy Injuries = 1, Local Injuries = 2, and Scattered Injuries = 3. The Scales Were Placed Inside the Parentheses, Representing the Minimal and Maximal Pathological Changes in Each Group

Pathological findings	Scores in groups (for each group, $n = 6$)			
	(minimum-maximum)			
	C	OVA	OVA + CM	OVA + MSC
Alveolar membrane thickening	(0–0)	(1–3) $^{+++}$	(1–3) $^{+++}$	(1–2) $^{+++} * \#$
Mucus plug	(0–0)	(2–3) $^{+++}$	(1–3) $^{+++}$	(1–2) $^{+++} ** \# \#$
Interstitial cell infiltration	(0–1)	(2–3) $^{+++}$	(2–3) $^{+++}$	(0–2) $^{++} * \#$
Bronchial hyperplasia	(0–0)	(2–3) $^{+++}$	(2–3) $^{+++}$	(1–2) $^{+++} * \#$

Among adhesion molecules participated in asthma inflammatory process, ICAM-1 and VCAM-1, belonged to the superfamily of immunoglobulins, are predominant and their expression is upregulated by pro-inflammatory cytokines, contributing to airway epithelial injury [5].

Pathological changes in asthmatic lungs are tightly associated with imbalance of Th1/Th2 cells and their secretome [9]. Notably, the upregulation of Th2 cytokines in allergic airways potentiates VCAM-1 and ICAM-1 expression on lung microvascular ECs, leading to continuous eosinophil entrance to inflammatory niche, and finally exacerbates asthmatic condition. Thus, the control of Th2 cytokines could result in a decreased VCAM-1 and ICAM-1 endothelial distribution [4, 6–8, 29]. In fact, targeting of ICAM-1 and VCAM-1 could add invaluable data for the treatment of allergic asthma regarding on Th2 cytokine levels. The dynamic changes of ICAM-1 and VCAM-1 expression on endothelium along with measured cytokines during pre- and post-MSC transplantation could show that the anti-inflammatory effects of MSCs occurred likely by restoring the Th1/Th2 balance *via* downregulating of ICAM-1 and VCAM-1 expression.

Limitations regarding current experiment are that the expressions of ICAM-1 and VCAM-1 cognate ligands on circulating leukocytes were not investigated. The CM content and existence of modulatory factors must be detected in the future investigations. In conclusion, data from this study support a notion that administration of rBMSCs to the circulatory system, unless CM, could be strategic approach in alleviating asthma pathophysiological changes, presumably by the regulating of our targeted endothelial cell adhesion molecule expression in OVA-sensitized male rats.

FUNDING INFORMATION

This study was supported by a grant from the Tuberculosis and Lung Disease Research Center of Tabriz University of Medical Sciences.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

Animal Ethics. All animals were treated humanely in compliance with the guidelines released by the National Institutes of Health for Laboratory Animals Care and Use (NIH Publication No. 85-23, revised 1996) and proved by the Laboratory Animal Ethics Committee of Tabriz University of Medical Sciences (No. IR.TBZMED.VCR.REC.1397.025).

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