



Bone marrow mesenchymal stem cells and condition media diminish inflammatory adhesion molecules of pulmonary endothelial cells in an ovalbumin-induced asthmatic rat model

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ABSTRACT

Objectives: Although excitations related to stem cell therapeutic outcomes have been highlighted enormously in asthma, the vast majority of works were conducted by researchers in animal models. Elucidating the mechanisms underlying the therapeutic effects of MSCs in asthmatic rats will provide a rational basis for assuring maximal safety of future clinical application of stem cells. In the current study, we sought to investigate the possible paracrine mechanism by which direct injection of MSCs and/or CM attenuate efficiently Th2-mediated inflammation in asthmatic lung tissues with the focus on ICAM-1 and VCAM-1 expression.

Methods: Male rats were divided into four experimental groups (n = 6); healthy rats received PBS intratracheally (group C), sensitized rats received PBS intratracheally (group S), sensitized rats received CM intratracheally (group S + CM), and sensitized rats received PBS intratracheally containing 2×10^6 rBMMSCs (group S + MSCs). Two weeks post-transplantation, the expression of interleukin (IL)-5, -12 and INF- γ , ICAM-1 and VCAM-1 were assessed along with pathological injuries and the homing of MSCs into the lung tissues.

Results: Our results showed CM, and notably rBMMSCs, returned the expression of IL-5, IL-12, INF- γ , ICAM-1, and VCAM-1 ($p < 0.001$ to $p < 0.05$) to the normal levels. Based on data, pathological injuries in pulmonary specimens of asthmatic rats were significantly attenuated ($p < 0.001$ to $p < 0.05$). Moreover, rBMMSCs had potential to successfully home to an asthmatic niche in cell-administrated rats.

Conclusions: Our data noted the potency of CM and especially MSCs in ameliorating pathological changes via intra-tracheal route presumably by targeting ICAM-1 and VCAM-1 in lung tissues in rat asthma model.

1. Introduction

The term asthma is generally devoted to a progressive respiratory disorder with a significant effect on socioeconomic conditions, accounting for about 240,000 annual deaths worldwide with limited therapeutic options (Keyhanmanesh et al., 2013; Peters et al., 2006). Structural changes in the lung tissues of asthmatic subjects are mainly contributed to the induction of Th2 type lymphocytes inflammatory response (Ahmadi et al., 2017; Chai et al., 2011). An allergic inflammation is primarily initiated by normal dynamic juxtacrine interaction between endothelial cells adhesion molecules (CAMs) and

provoking cognate receptors on circulating leukocytes (Lee et al., 2013). Various appropriate ligands for leukocyte populations particularly intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are weakly expressed on resting endothelial cells and mostly up-regulated by inflammatory cytokines in injured endothelium, playing crucial roles in developing allergic asthma via the extravasation of circulating leukocytes into asthmatic sites (Lee et al., 2013; Striz et al., 1999; van Buul et al., 2010). In response to an allergen, secretome of Th2 cells potentiates VCAM-1 and ICAM-1 expression in microvascular cells which leads to an untamed recruitment of leukocytes from the bloodstream to inflammatory niche

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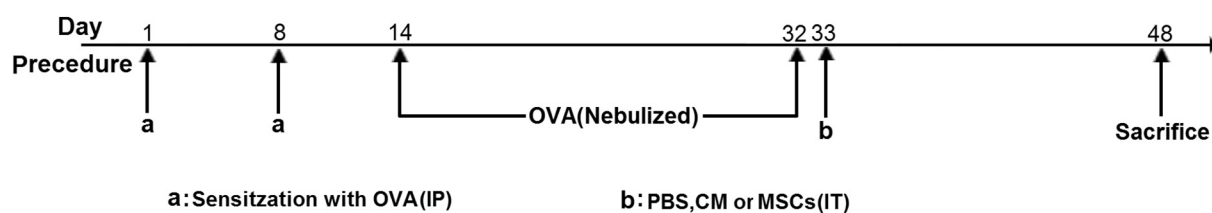


Fig. 1. Experimental design flow chart. OVA; Ovalbumin, IP; Intraperitoneally. IT; Intratracheally.

Table 1

Primer sequences used for real-time PCR analysis.

Gene name	Primer sequence (5'-3')	
	Forward	Reverse
IL-5	TGC TTC TGT GCT TGA ACG TTC TAA C	TTC TCT TTT TGT CCG TCA ATG TAT TTC
IL-12	CCG ATG CCC CTG GAG AAA C	CCT TCT TGT GGA GCA GCA G
INF-gamma	GAA AGC CTA GAA AGT CTG AAG AAC	GCA CCG ACT CCT TTT CCG CTT CCT
ICAM-1	TGG AGG TGA CTG AGA AGT TGG	CAC AGT TAC TTG GTC CCC TTC'
VCAM-1	GTG TGT GAA GGA GTG AAT CTG G-3'	CCA ACA GCA GCA CAT GTC AGA A
GAPDH	CAA GTT CAA CGG CAC AGT CAA G	ATA CTC AGC ACC AGC ATC ACC

and thereby exacerbate the asthmatic condition. Hence, the control of Th2-mediated immune responses could result in a significant reduction of endothelial molecules VCAM-1 and ICAM-1 to the near-normal levels and control cells migration rate (Lee et al., 2013; Striz et al., 1999). Regarding asthma pathophysiology, focusing on the control of the Th1/Th2 ratio in favor of Th1 is highly attended (Ahmadi et al., 2017). In line with this concept, the potent immunomodulatory plasticity of mesenchymal stem cells (MSCs), mainly mediated by paracrine mechanisms, has opened a promising therapeutic strategy for inflammatory disorders, peculiarly asthma (Kyurkchiev et al., 2014). Since some of the therapeutic effects of stem cells is due to their ability to secrete numerous growth factors, so it seems logical that a novel cell-

free therapy, MSC-derived conditioned media (CM), could be used an alternative therapeutic approach without any concern reported previously after cell transplantation such as immune compatibility, tumorigenic risk, emboli formation, infectious agents, total costs and long period for expansion and maintenance of stem cells (Vizoso et al., 2017). A great body of studies has reported that the direct injection of MSCs and/or relevant CM could improve airway inflammation by modulation of Th2-derived cytokines in allergic inflammation (Ahmadi et al., 2017; Urbanek et al., 2016). Indeed, although considerable facts related to immunomodulatory properties of MSCs have been gained in the field of regenerative medicine, however, the precise mechanisms of MSC-mediated immuno-regulatory effects in asthmatic niche have remained to be elucidated. Discovering the underlying mechanisms of MSCs in asthmatic rats will provide a rational basis for assuring the maximal safety of future clinical application of stem cells. Based on scientific literature, the immuno-modulatory effect of MSCs related to the expression of VCAM-1 and ICAM-1 has been neglected in the endothelium of asthmatic lungs. To our knowledge, our study is the pioneer attempt to highlight a possible paracrine mechanism by which direct injection of MSCs and/or CM attenuates efficiently Th2-mediated inflammation in lung tissues of asthmatic rats by focusing on modulating ICAM-1 and VCAM-1 expression.

2. Methods

2.1. Animal ethics

All Animals were cared humanely in compliance with the guidelines

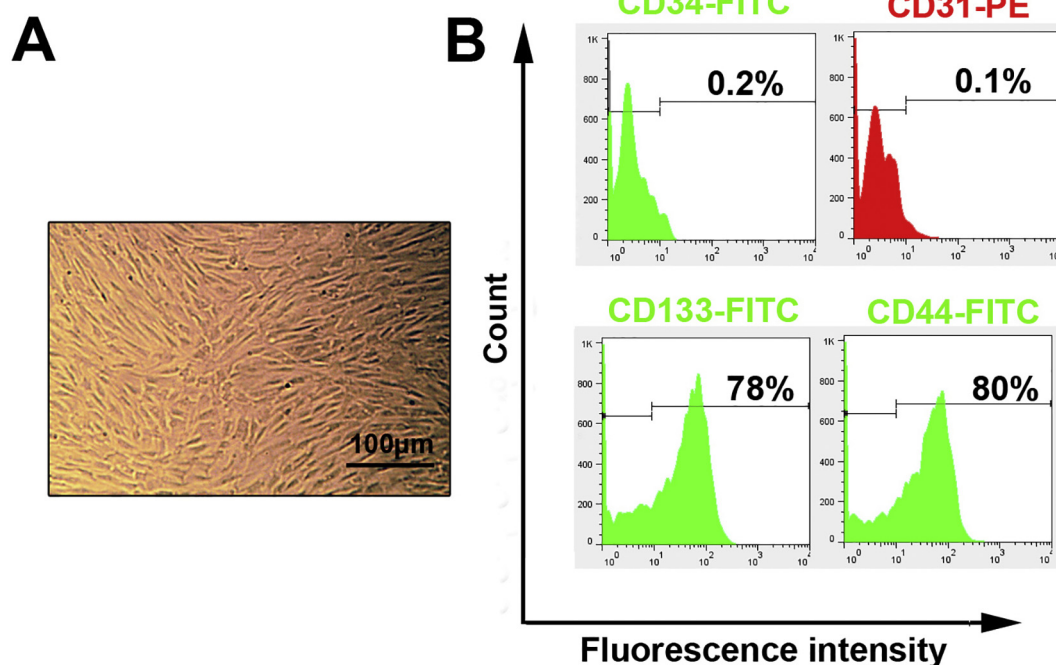


Fig. 2. Photomicrographs of rBMSCs in the third passage (A). phenotypic characterization of isolated rBMSCs (B).

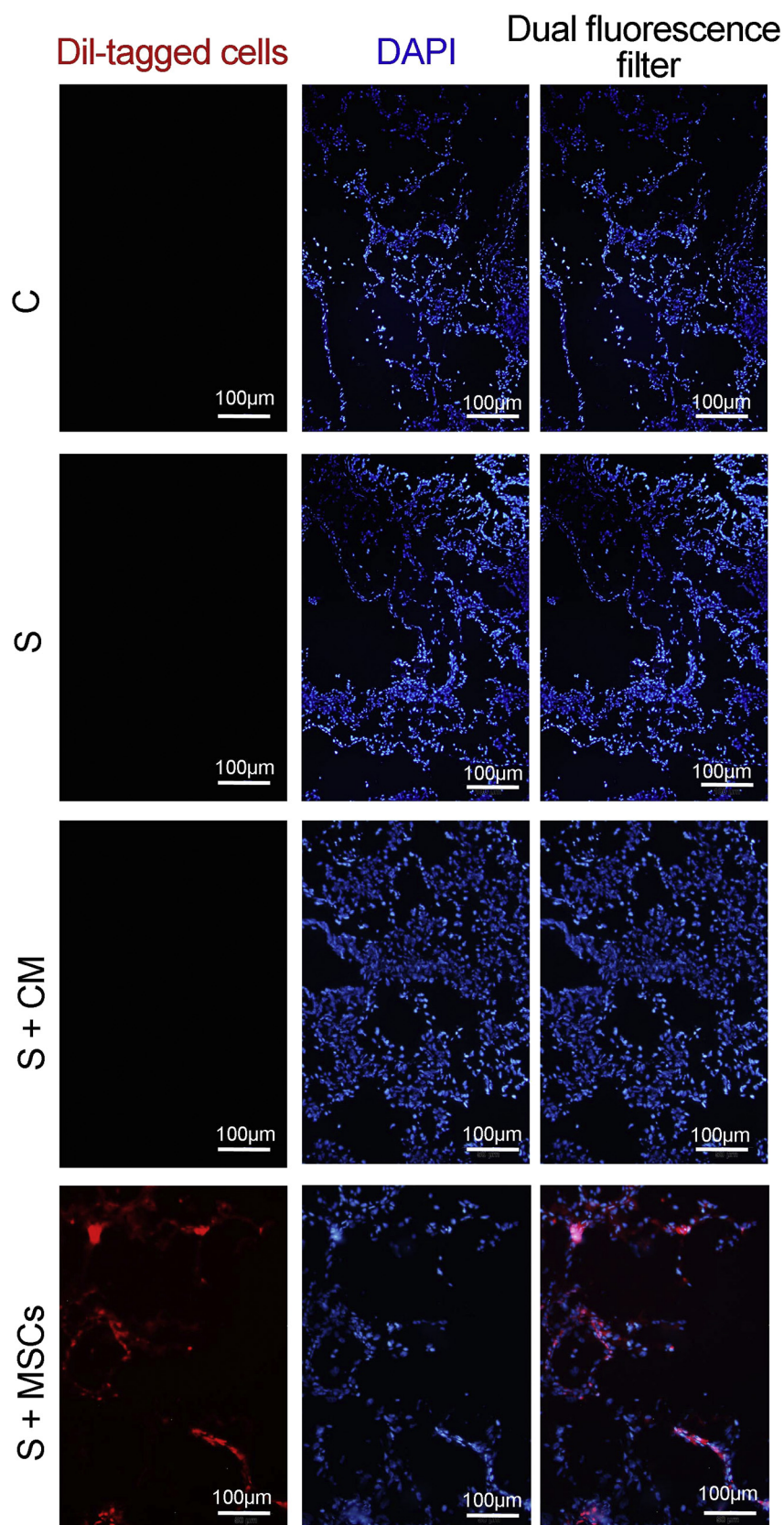


Fig. 3. Representative images of Fluorescent dye (CM-DiI, red)-labeled rBMSCs in lung tissues. The recruitment of rBMSCs to pulmonary tissue was detected in all sensitized rats with local delivery. For background staining, DAPI solution (blue) was used. Control animals received PBS (C), sensitized animals received PBS(S), sensitized animals received CM (S + CM), sensitized animals received rBMSCs (S + MSCs).

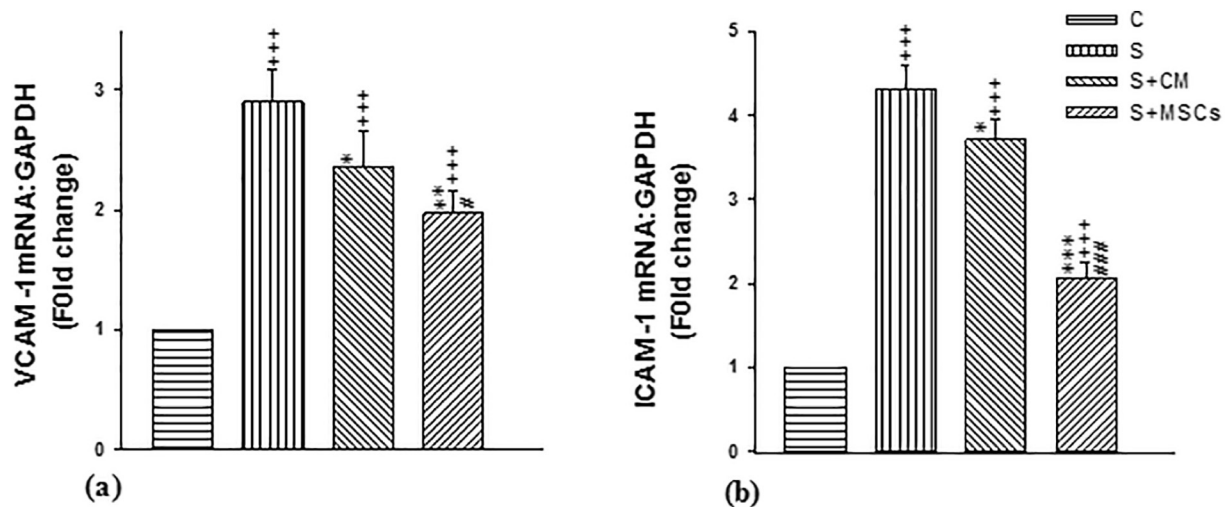


Fig. 4. The levels of VCAM-1 (a) and ICAM-1 (b) mRNA expression in the lung tissues of control animals received PBS (C), sensitized animals received PBS (S), sensitized animals received CM (S + CM), sensitized animals received rBMSCs (S + MSCs) (for each group, $n = 6$). Bars represent the mean $\pm$ SEM. Statistical differences between control and different sensitized groups: + + +; $p < 0.001$. Statistical differences between S + CM and S + MSCs vs. S group: *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$. Statistical differences between S + CM and S + MSCs groups: #; $p < 0.05$ and ###; $p < 0.001$.

released by the National Institutes of Health for Laboratory Animals Care and Use (NIH Publication No. 85-23, revised 1996) and proved locally by Laboratory Animal Ethics Committee of Tabriz University of Medical Sciences (No: IR.TBZMED.VCR.REC.1397.025).

2.2. Experimental groups

Twenty-eight healthy mature male Wistar rats (6–8 weeks old, initially weighing 200–250 g) were enrolled in the current experiment. Four rats were randomly used for isolation of rat bone marrow-derived MSCs (rBMSCs). The remaining 24 rats were equally assigned into four experimental groups (six animals in each group) as follows:

1. Healthy rats received intratracheally 50 μ l PBS alone (S group)
2. Sensitized rats received intratracheally 50 μ l PBS alone (S group)
3. Sensitized rats received intratracheally 50 μ l CM (S + CM group)
4. Sensitized rats received intratracheally 50 μ l PBS containing 2×10^6 rBMSCs (S + MSCs group).

2.3. Animal sensitization

In sensitized groups, animals were actively exposed to OVA for a period of 32 ± 1 days by using a standard protocol (Ahmadi et al., 2017; Ahmadi et al., 2018). Shortly, each rat was given intraperitoneally 1 mg OVA (Sigma-Aldrich, USA) and 200 mg aluminum hydroxide diluted in 1 ml sterile saline on days 1 and 8. From days 14 to 32, the sensitized rats were challenged intranasally with 4% OVA aerosol produced by a nebulizer (CX3, Omron Co., Netherland) in a special inhalation box with dimensions $30 \times 20 \times 20$ cm³ for 5 min daily. The control rats were treated with normal saline instead of OVA in the same manner. One day after the completion of sensitization procedure (on day 33), PBS, CM or stem cells were directly injected into the trachea slowly via a ventral neck dissection (Ahmadi et al., 2017). Two weeks after intratracheal administration (on day 48), all animals were sacrificed for the assessment of treatment efficacy (Fig.1).

2.4. Isolation and culture of rBMSCs

rBMSCs were pooled from the healthy rats according to standard protocols (Ahmadi et al., 2017; Ahmadi et al., 2016; Keyhanmanesh et al., 2018a, b). In brief, femurs were aseptically removed and bone marrow was flushed with PBS (2% fetal bovine serum, FBS; Gibco, USA)

by using an 18-gauge needle. Then, mononuclear cells layer was separated by Ficoll-hypaque® solution (Sigma, USA) for 20 min at 400g continuously. Freshly isolated cells were re-suspended in PBS and washed twice. For cell expansion, cells were maintained in low glucose content Dulbecco's modified Eagle's Medium (DMEM; Gibco, USA) including 20% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin (Biosera, UK). 1×10^5 cells/cm² were plated in 6-well culture flasks (SPL). The medium was changed every 3–4. At 70%–80% confluence, MSCs were trypsinized by Trypsin-EDTA solution (Gibco, USA). Cells between passages 3–6 were used in this study.

2.5. Immunophenotype characterization of isolated rBMSCs

The feature of MSCs was confirmed by flow cytometry by using a panel of antibody, including mouse anti-rat CD133 (FITC), CD44 (FITC), and CD34 (FITC) as CD31 (PE) supplied by eBioscience company (USA) (Ahmadi et al., 2017; Ahmadi et al., 2016). Isotype controls were also used for background staining and exclusion of false positive values. Cells were collected and blocked by 1% bovine serum albumin (BSA, Sigma, USA) for 15 min. 5×10^5 cells were incubated with 100 μ l PBS containing antibodies for 30 min at RT. Cells were analyzed with BD FACSCalibur Flow Cytometer apparatus (USA) and FlowJo software version 7.6.1.

2.6. rBMSCs staining with a fluorescent dye

A non-invasive fluorescence dye was employed to probe the recruitment of infused MSCs into the lung parenchyma of asthmatic rats. MSCs were labeled with 2 μ M fluorescent Cell Tracker™ CM-Dil at 37 °C for 20 min (Catalog No. C-7000; Molecular Probes, Invitrogen, USA). 50 μ l PBS with 2×10^6 CM-Dil pre-labeled cells was carefully administered by intratracheal approach (Ahmadi et al., 2017).

2.7. Preparation of conditioned media

To determine the possible paracrine effects of stem cells in the attenuation of asthmatic changes, CM was obtained from rBMSCs (Ahmadi et al., 2017; Ahmadi et al., 2016) Following 70–80% confluency, the medium was removed, cells rinsed three times with PBS and incubated with FBS-free DMEM/LG. After 72 h, the supernatants were centrifuged at 400g for 5 min and micro-filtered through 0.2 μ m-pore size syringe filter (GVS Filter Technology). In the current

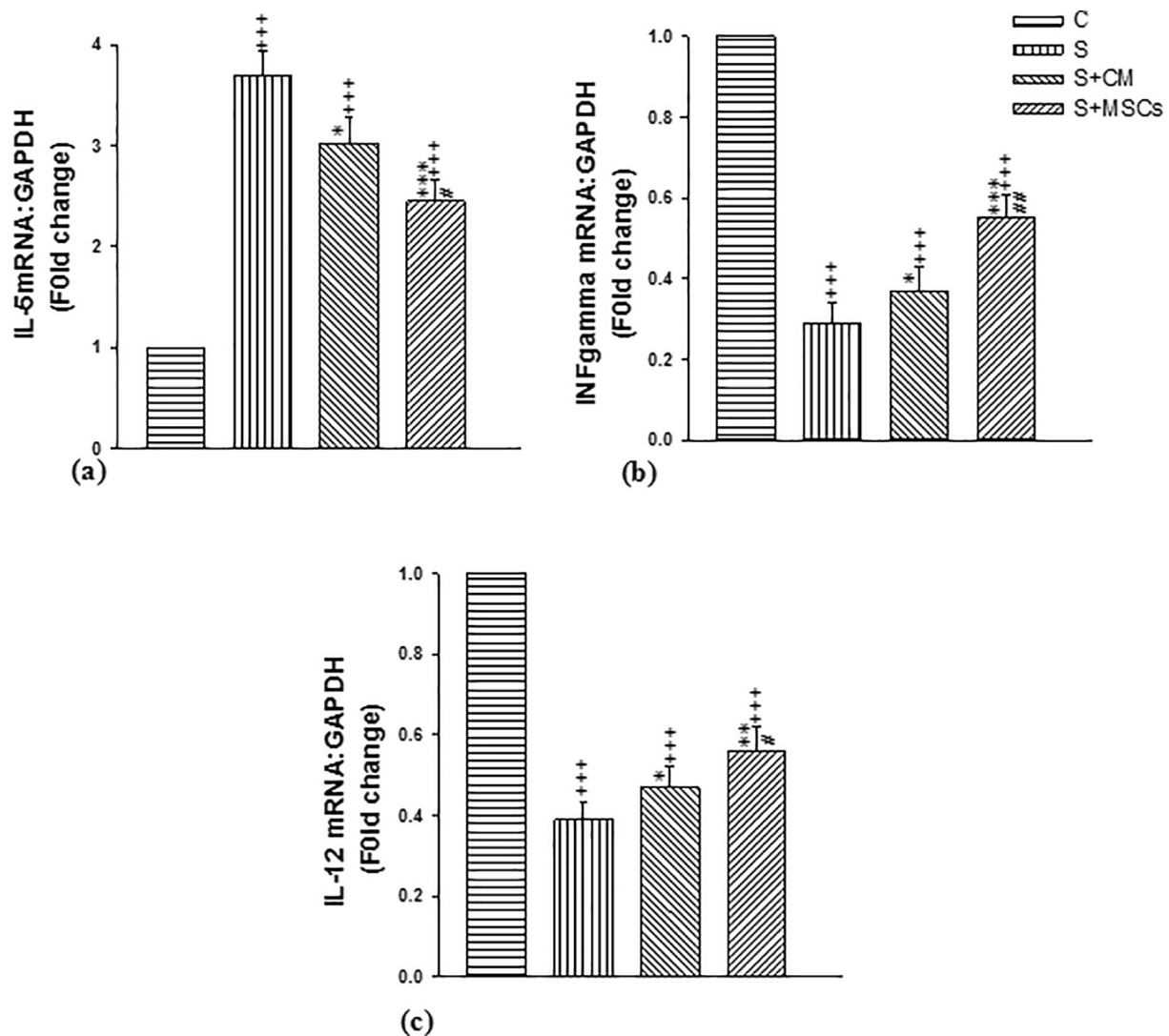


Fig. 5. The levels of IL-5(a) INF-gamma(b) and IL-12 (c) mRNA expression in the lung tissue of control groups received PBS (C), sensitized groups received PBS (S), sensitized groups received CM (S + CM), sensitized groups received rBMSCs (S + MSCs) (for each group, n = 6). Bars represent the mean $\pm$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

experiment, a 50-fold concentrated medium was used by filter tubes with a 4-kDa molecular weight cut-off (Catalog No.: 003099.125, Eppendorf, Germany). Protein level in CM was measured using the bicinchoninic acid assay (BCA assay, iNtRON, Korea). An amount of protein over 500 $\mu\text{g}/\text{ml}$ was confirmed in concentrated CM. Equal protein CM content was administrated with 50 $\mu\text{l}/\text{per rat}$ (Ahmadi et al., 2017).

2.8. Monitoring fluorescent MSCs in lung tissues

To ascertain the existence of CM-Dil-tagged rBMSCs in asthmatic tissues, samples were embedded in Tissue Freezing Media (OCT), snap frozen, and cut to 5- μm size sections using a Cryostat system. Next, after thawing at RT, slides were stained with DAPI (A blue fluorescent DNA-binding dye; 4', 6-diamidino-2-phenylindole, 1 $\mu\text{g}/\text{ml}$, Catalog# D9542, Sigma-Aldrich, USA) for nuclear staining.

2.9. Real-time PCR analysis

The expression of IL-5, IL-12 and INF- γ , ICAM-1 and VCAM-1 genes were determined by quantitative real-time PCR assay. Primer sequences

for each mRNA were designed with the aid of Gene-Runner Software, version 3.05 (Table 1). In brief, a part of left lung tissue was frozen in immediately in liquid nitrogen for total RNA isolation using YTA total RNA extraction mini kit (YTA, Taiwan) (Ahmadi et al., 2017; Ahmadi et al., 2016). The purity of extracted RNAs was detected photometrically by a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington DE 19810 USA). Each sample RNA was reverse-transcribed with cDNA Synthesis Kit (YTA, Taiwan). Each cDNA was used as a template for a 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). The expression levels of target genes were normalized to GAPDH as a control housekeeping gene and the relative fold changes were obtained using the $2^{-\Delta\Delta\text{CT}}$ method.

2.10. Pathological evaluation

The right lung of each rat was fixed in 10% neutral buffered formalin (37%, Merck, Germany), embedded in paraffin blocks, cut into 4- μm thick sections, stained with H&E and finally imaged by a light microscope. In all experimental groups, a 4-level semi-quantitative scaling

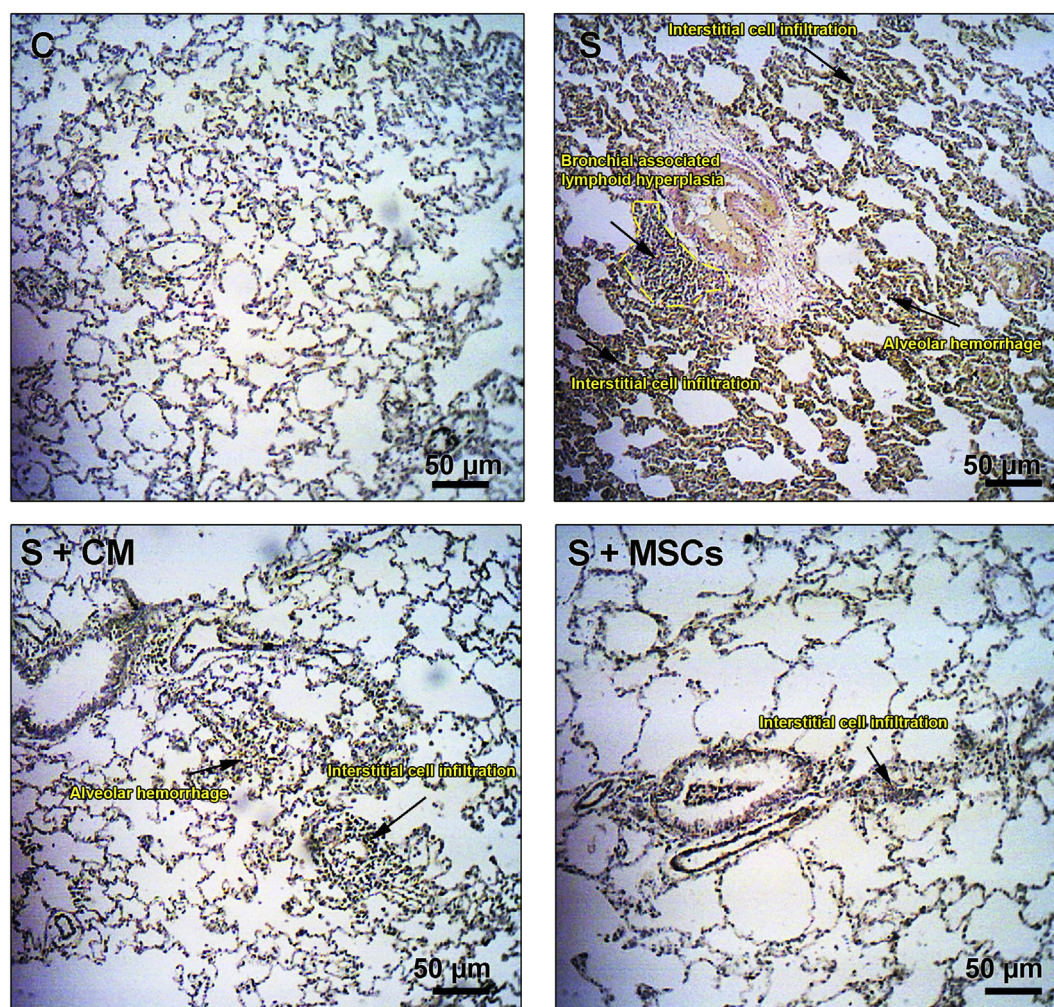


Fig. 6. Bright-field photographs of pulmonary tissue (magnification = X80). Normal lung tissue (C group). An alveolar hemorrhage, interstitial cell infiltration and bronchial associated lymphoid hyperplasia seen in sensitized rats received PBS (S group), a reduction in interstitial infiltration rate in sensitized rats received CM (S + CM group) and a prominent reduction of inflammatory cells infiltration coincided with diminished interstitial hemorrhage seen in sensitized rats received rBMMSCs (S + MSCs group).

Table 2

Pathological scales in the lung tissues of control animals received PBS (C), sensitized animals received PBS (S), sensitized animals received CM (S + CM), sensitized animals received rBMMSCs (S + MSCs) (for each group, n = 6). Statistical differences between control and different groups: ++, $p < 0.01$ and + + +, $p < 0.001$. Statistical differences between S + CM and S + MSCs vs. S group: *, $p < 0.05$, **, $p < 0.01$ and ***, $p < 0.001$. Statistical differences between S + CM and S + MSCs groups: #, $p < 0.05$ and ##, $p < 0.01$. The numbers were placed inside the parentheses, implying the minimal and maximal pathological score in each group.

Pathological findings	Scores in groups (for each group, n = 6)			
	(Minimum-maximum)			
	C	S	S + CM	S + MSCs
Alveolar hemorrhage	(0–0)	(2–3) +++	(1–3) +++*	(0–2) ++ ** #
Interstitial hemorrhage	(0–0)	(2–3) +++	(0–3) +++**	(0–2) +++ ** ##
Interstitial cell infiltration	(0–0)	(2–3) +++	(1–3) +++*	(1–2) ++ ** #
Bronchial hyperplasia	(0–1)	(2–3) +++	(1–2) +++	(0–2) ++ ***#

system was applied to evaluate the severity of lung injury based on following pathological findings: alveolar hemorrhage, interstitial cell infiltration, Interstitial hemorrhage, and bronchial hyperplasia. The numerical scores for each injury were determined from 0 to 3 as follows; Normal = 0; Patchy injury = 1; Local injury = 2; Scattered injury = 3.

2.11. Data analysis

All quantitative results were analyzed using a one-way ANOVA with Tukey–Kramer post hoc analysis and presented as mean $\pm$ SEM. Pathological scores were analyzed using the Kruskal–Wallis test followed by a post-hoc Mann–Whitney test. Significance was accepted at $p < 0.05$.

3. Results

3.1. MSCs characterization

Flow cytometry analysis revealed that rBMMSCs did not express the cell surface markers CD34 and CD31 but strongly expressed the markers CD44 and CD133 (Fig. 2).

3.2. Pre-labeled MSCs were recruited into asthmatic niche

Regarding the homing proficiency of MSCs to inflammatory sites, the presence of pre-labeled cells was successfully confirmed in S + MSCs group (Fig. 3).

3.3. CM and MSCs reduced the expression levels of VCAM-1 and ICAM-1 in OVA-sensitized rats

The level of VCAM-1 and ICAM-1 mRNAs was significantly high in asthmatic lungs more than C group ($p < 0.001$). The expression of VCAM and ICAM-1 in sensitized groups received MSCs or CM diminished significantly as compared with the S group ($p < 0.001$ to $p < 0.05$). In addition, the pattern of ICAM-1 and VCAM-1 expression in the cell-treated group was significantly lower than CM treated group ($p < 0.001$ and $p < 0.05$ respectively) (Fig. 4).

3.4. The transcription of IL-5, INF- γ , and IL-12 were normalized in lungs of CM and especially MSC treated rats

The level of IL-5, INF- γ , and IL-12 mRNA expression were changed significantly in asthmatic airways as compared to the control rats ($p < 0.001$). We showed a reduced expression in the level of factor IL-5 with an increase in INF- γ and IL-12 transcription in cell- and CM-treated rats as compared with S group ($p < 0.001$ to $p < 0.05$). Changes in the level of mRNAs were significantly more in rats received stem cells compared to the CM-treated counterpart ($p < 0.01$ to $p < 0.05$) (Fig. 5).

3.5. Pathological changes were attenuated in the presence CM and especially MSC

Pathological injuries in the asthmatic lung tissues were significantly higher than C group ($p < 0.001$ to $p < 0.01$). Pathological injuries were significantly alleviated by MSCs and relevant CM as compared with S group ($p < 0.001$ to $p < 0.05$). Of note, MSCs could ameliorate the asthmatic changes more than CM ($p < 0.01$ to $p < 0.05$) (Fig. 6, Table 2).

4. Discussion

Recent advances in the field of stem cell research have incontestably created new therapeutic opportunities against debilitating diseases that conventional approach had only marginal success such as asthma. Through continuing researches, it has been clarified that disproportionality of Th2/Th1 is the main cause of structural changes in the lung tissues of asthmatic subjects and a direct administration of MSCs and/or CM led to a shift from Th2 to Th1 responses and suppressing the recruitment of inflammatory cells to asthmatic airway via a paracrine mechanism (Ahmadi et al., 2017; Ge et al., 2013; Urbanek et al., 2016; Ahmadi, M., et al., 2018b). However, there are still largely unknown questions regarding the precise mechanisms of MSC-mediated immuno-regulatory effects in the asthmatic niche. Collectively, continued basic research for unraveling the underlying mechanisms directed by stem cells is clearly needed not only for identifying new targeted treatments but also for clinical application of MSCs (Bartosh et al., 2016; Wollert and Drexler, 2005). To the best of our knowledge, not enough data exist on the modulatory effects of MSCs on the expression levels of ICAM-1 and VCAM-1 in lung tissues of asthmatic rats. We, here, tentatively speculated that immune-modulatory effects of MSCs and/or relevant CM could, in part, be caused by interference with the transcription of the VCAM-1 and ICAM-1 gene in asthmatic lung endothelium. Due to specific anatomical and histological characteristics, lungs are a good candidate for MSCs and CM administration, certainly either by local or systemic routes (Wong et al., 2007). During the last decades; various controversial outcomes were

obtained regarding the route of MSCs or CM injection after lunge injuries (Ahmadi et al., 2017; Ahmadi et al., 2016; Ionescu et al., 2012; Tibboel et al., 2014; Trzil et al., 2014). In this experiment, the paracrine effects of intratracheally infused rBMSCs and/or relevant CM were investigated on the expression of IL-5, IL-12, INF- γ , VCAM-1 and ICAM-1 along with pathological changes in the lungs of sensitized rats. Similar to previous findings, a significant reduction in the expression of INF- γ and IL-12 was coincided with a remarkable augmentation in the pathological changes and expression of IL-5, VCAM-1 and ICAM-1 shown in the lung tissue of sensitized rats as compared with healthy rats, approving the success of model construction. Furthermore, the qualitative analysis revealed a large amount of pre-labeled MSCs was determined in lung tissues of cell treated asthmatic rats. Our data revealed that the local presentation of both MSCs and CM could significantly improve pathological injuries in OVA-sensitized rats by the modulation of interleukins and CAMs in lung tissues, although the improvement was more evident in the cell-treated group which was supported by our previous experimental findings (Ahmadi et al., 2017). In a recent experiment conducted by our group, we also discovered similar therapeutic effects driven by MSCs, but not CM, after systemic injection in the rat model of asthma (Keyhanmanesh et al., 2018a, b). Evidence points out stem cells can release soluble factors in a paracrine manner in normal condition or in response to different types of stimuli. It seems that one of the possible reasons for an explanation of such different results on the parameters measured through various experiments is the variety of soluble factors released by MSCs, and easy access of release factors to target sites (Kyurkchiev et al., 2014). Additionally, the secretome could easily bio-distribute in the peripheral tissues and organs at the proximity of the injection site and not reach efficiently into the target tissues. Finally, the presence of the stem cells provides a sustained and prolonged release source for the chronic secretion of soluble factors whereas the CM contains only a limited amount of agents (Sutsko et al., 2012). It seems that the injection of CM with high volume, recurrent doses and the culture in the presence of inflammatory agents to be more operative to yield a more therapeutic outcome. To investigate the mechanisms underlying the therapeutic effects of MSCs in asthmatic rats, monitoring of Th2 (IL-5) and Th1 (INF- γ , IL-12) cytokines levels is crucial (Ahmadi et al., 2017; Guo et al., 2014). Th2-originated IL-5 is a highly specific cytokine in the recruitment of eosinophils to an asthmatic niche by regulating cell attachment to lung microvascular adhesion molecules (Kips, 2001). Compared with Th2 cytokines, INF- γ and IL-12 suppressed Th2-driven inflammatory responses to confine the progress of allergic asthma (Guo et al., 2014). Leukocyte adhesion to vascular endothelial cells plays an important role in the initiation and progression of asthma pathology by employing various cell adhesion molecules mainly ICAM-1 and VCAM-1 (Just et al., 2003; Lee et al., 2013). Cell adhesion molecules have the potential to interact with the inflammatory cells from the circulation system and facilitate migration to the inflammatory niche (Lee et al., 2013). ICAM-1 and VCAM-1 belonged to the superfamily of immunoglobulins, are constitutively expressed at low levels in resting endothelial cells and up-regulated by Th2 cytokines following exposure to an allergen, facilitating continuous leukocytes migration to inflammatory tissue and finally exacerbates the asthmatic condition. The control of Th2-derived cytokines caused to a decreased VCAM-1 and ICAM-1 endothelial distribution (Lee et al., 2013; Striz et al., 1999; van Buul et al., 2010). In fact, targeting of ICAM-1 and VCAM-1 could be potentially a new therapeutic modality in allergic asthma regarding on Th2 cytokines levels. The dynamic changes of ICAM-1 and VCAM-1 expression on endothelium along with measured cytokines during pre- and post-MSC and CM transplantation show that the anti-inflammatory effects of MSCs governed likely by restoring the Th1/Th2 balance via down-regulating of ICAM-1 and VCAM-1 expression.

4.1. Limitations

Some limitations are related to the current experiment. The expression of ICAM-1 and VCAM-1 cognate ligands on circulating leukocytes was not investigated. Elucidation of both ligands and receptors orchestrating leukocyte-endothelial cell interaction can help us to precisely monitor the changes of inflammation pre-and post-treatment. It is better to immuno-phenotype MSCs recalled to the pulmonary tissue to show trans-differentiation into epithelial-like cells or other cell types. Accordingly, quantitative analyses enable us to efficiently foretell these changes. It is better to measure protein levels of both molecules and signaling pathways located at the downstream. Also, the CM content and total cytokine profiles were not fully-analyzed and the existence of modulatory factors must be addressed in the future investigations.

5. Conclusion

In conclusion, the results of this study shed a light that local administration of rBMSCs and CM could be effective in amelioration of asthmatic changes in rats presumably by the regulating the expression of endothelial cell adhesion molecules in OVA-sensitized male rats. However, the regenerative responses observed post local administrations of rBMSCs were higher than CM.

Acknowledgments

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