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Marrow-derived mesenchymal stem cells regulate the inflammatory response and repair alveolar type II epithelial cells in acute lung injury of rats

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Abstract

Objective: We investigated the effect of untransplantable bone marrow-derived mesenchymal stem cells (BMSCs) in acute lung injury (ALI) and whether BMSCs attenuate damage of lipopolysaccharide (LPS) to alveolar type II epithelial cells (AECIIs).

Methods: ALI models were prepared by nebulizing LPS and then BMSCs were infused 1 hour later. We observed histopathological changes of lung tissue and evaluated inflammatory exudation by the wet/dry weight ratio, bronchiolar lavage fluid cell count, and protein concentration determination. Inflammatory and vascular factors were detected by immunohistochemistry and western blotting. For *in vitro* experiments, AECIIs were stimulated with 10 μg/mL LPS for 4 hours and then BMSCs were seeded in transit inserts to co-culture for 24 hours. The activity of AECIIs was detected.

Results: In the LPS + BMSCs group, histopathological examination showed that the degree of lung injury was significantly reduced compared with the LPS group. Protein expression of inflammatory and vascular factors was significantly lower with treatment. Optical density values and cell viability of the LPS + BMSCs group were significantly higher than those of the LPS group.

Conclusions: Untransplanted-BMSCs can inhibit the inflammatory response in ALI and promote repair of AECIIs. This might be due to substances secreted by BMSCs and interaction between these substances.

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Keywords
Acute lung injury (ALI), bone marrow-derived mesenchymal stem cells (BMSCs), inflammatory factor, vascular endothelial growth factor, alveolar type II epithelial cells (AECIIs), lipopolysaccharide

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Introduction

Acute lung injury (ALI) is a clinical syndrome characterized by alveolar–capillary injury, and this syndrome is clinically manifested as progressive aggravation of dyspnea and severe hypoxemia. Severe cases of ALI develop acute respiratory distress syndrome (ARDS). The morbidity and mortality of ALI are high, and the fatality rate is still as high as 30% to 40%.[1] The pathophysiological characteristics of ALI are reduced lung volume, reduced lung compliance, and imbalance of the ventilation/blood flow ratio.[2,3]

ALI/ARDS is a complex disease process, which can be divided into two stages of the early exudation stage and the fibrosis stage. Early local or distant tissue damage leads to a large release of inflammatory mediators, which causes diffuse alveolar epithelial cell damage. This process destroys the alveolar integrity, increases alveolar capillary permeability, and leads to increased protein exudation in the alveolar cavity, resulting in pulmonary edema.[2,3] ALI/ARDS is clinically characterized by progressive hypoxemia and respiratory distress. A major biological event in the pathogenesis of ALI that causes ARDS is activation of lung endothelial cells, which is triggered by a variety of inflammatory insults leading to barrier disruption and excessive accumulation of neutrophils.[4] At present, clinical treatment methods of ALI mainly include mechanical ventilation, application of antibiotics, and restriction of liquid volume, but their efficacy is poor, and its morbidity and mortality are still high.

A large number of studies on the mesenchymal stem cell (MSC) repair mechanism of tissues and organs have shown that MSCs have a paracrine effect of secreting protein, peptides, cell microvesicles, and exosomes.[5–8] These studies showed that MSCs mediated the immune response, which improved the cell survival rate and prevented tissue fibrosis. Bone mesenchymal stem cells (BMSCs) have the characteristics of differentiation potential, and are easy for extraction and *in vitro* culture with low immunogenicity.

This study aimed to determine whether MSCs have a therapeutic effect on ALI using animal and cell experiments. We established a model of ALI in juvenile Sprague–Dawley (SD) rats and used lipopolysaccharide (LPS) to attack alveolar type II epithelial cells (AECIIs).

Materials and methods

Experimental animals and cells

This study obtained approval from the Medical Ethical Committee of Shengjing Hospital of China Medical University (2016PS003K) on 14 January 2016. The experiment involved 144 male juvenile SD rats, which were aged 5 weeks and weighed 100 ± 10 g. The rats were purchased from Shenyang Changsheng Co., Ltd. (Benxi, Liaoning Province, China) and were raised in the specific pathogen free Animal Department of Shengjing Hospital, affiliated to China Medical University. In our study, the feeding environment of animals

was at a controlled temperature of 20°C to 25°C and 35% to 75% humidity with a 12-hour light/dark cycle.[9] At the end of the experiments, 5% chloral hydrate 0.6 mL/100 g was injected intraperitoneally, and tissue was taken after anesthesia. Green fluorescent protein-labeled SD rat BMSCs were purchased from a company with a quality inspection report (Guangzhou SaiYe Biotechnology Co., Ltd., Guangzhou, Guangdong Province, China). Rat AECIIs were purchased from China Cell Bank (Shanghai, China).

The main reagent

The reagents used in study were anti-vascular endothelial growth factor (VEGF) antibody (ab46154; Abcam, Cambridge, UK), von Willebrand factor (vWF) antibody (11778-1-AP; Proteintech Group Inc., Rosemont, IL, USA), anti-toll-like receptor (TLR)-4 antibody (ab30667; Abcam), anti-nuclear factor kappa B (NF-κB) p65 antibody (ab16502; Abcam), anti-interleukin (IL)-17A (ab134086; Abcam), anti-IL-17 rabbit pAb (WL02981; Shenyang Wanlai Biotechnology, Shenyang, Liaoning Province, China) and the Cell Counting Kit (E1CK-000208; EnoGene, Nanjing, Jiangsu Province, China) (Table 1).

Animal models and groups

Juvenile SD rats were randomly divided into the LPS group (model group), control group, and LPS + BMSCs group (treatment group) according to the random number table method. Each group was randomly divided into subgroups of 3 hours, 6 hours, 12 hours, 1 day, 2 days, 3 days, 7 days, and 14 days (n = 6). In the treatment group, atomization inhalation of LPS (5 mg/mL, 2 mL) (L-2630; Sigma, St Louis, MO, USA) was performed for preparation of the ALI juvenile rat model.[10] After 1 hour, BMSCs (1×10^7/kg, 1×10^7/mL) were injected via the tail vein. In the

Table 1. Key resources table.

Reagent or resource	Source	Identifier
Antibodies		
Rabbit polyclonal anti-VEGF	Abcam	ab46154
Rabbit polyclonal anti-vWF	Proteintech	11778-1-AP
Mouse monoclonalanti-TLR4	Abcam	ab30667
Rabbit polyclonal anti-NF-κB	Abcam	ab16502
Rabbit monoclonal anti-IL-17a	Abcam	ab134086
Rabbit polyclonalanti-IL-17	Shenyang Wanlai Biotechnology	WL02981
Rabbit PolyclonalGAPDH	Proteintech	10494-1-AP
Chemicals, Peptides, and Recombinant Proteins		
Lipopolysaccharide	Sigma	L-2630
Critical Commercial Assays		
Cell Counting Kit	EnoGene	E1CK-000208
Experimental Models: Cell Lines		
GFP-labeled SD rat bone marrow-derived mesenchymal stem cells	Guangzhou SaiYe Biotechnology	RASMX-01101
Rat alveolar type II epithelial cells(AECII)	China Cell Bank	N/A
Experimental Models: Organisms/Strains		
Sprague-Dawley Rats	Shenyang Changsheng	SCXK2015-0001
Software and Algorithms		
GraphPad Prism7	GraphPad Software, Inc.	N/A

model group, atomization inhalation of LPS (5 mg/mL, 2 mL for one animal) was performed for preparation of the ALI juvenile rat model. After 1 hour, the rats were injected with an equal volume of phosphate-buffered saline (PBS) via the tail vein. In the control group, saline inhalation (2 mL) was performed. After 1 hour, the rats were injected with an equal volume of PBS via the tail vein.

Cell experiment grouping

The following four groups were used. In Group A (Dulbecco's modified Eagle's medium [DMEM]), ACEIIs were cultured only with DMEM without any treatment. In group B (DMEM+BMSCs), ACEIIs were cultured with DMEM and BMSCs were added at the corresponding time point for co-culture. In group C (LPS), ACEIIs were cultured with DMEM and then LPS was added. In group D (LPS + BMSCs) ACEIIs were cultured with DMEM. After the addition of LPS, BMSCs were co-cultured at the corresponding time point. BMSCs were cultured in a culture medium containing 10% fetal bovine serum, mixed and inoculated in a culture bottle, and cultured in a 5% CO_2 cell incubator. AECIIs were stimulated with LPS at 10 μg/mL in groups C and D. The same volume of DMEM was used in groups A and B. After 4 hours, groups B and D were co-cultured with BMSCs in a 0.4-μm aperture branchial chamber. In groups A and C, cells were only inserted into a transit chamber without BMSCs. After culture for 24 hours, the AECII activity of each group was detected by using a cell proliferation and toxicity assay (cholecystokinin octapeptide [CCK-8]) (see more detail in the subsection CCK-8 cell proliferation and toxicity test below).

Detection of indicators

1. BALF cell count and BALF protein concentration measurement

The right lung main bronchus was ligated and tracheal intubation was performed. A total of 1.5 mL of PBS lavage was performed in the left lung. This was repeated three times, with a return rate of 90%. Cells were counted under a microscope. Lavage fluid concentrations were determined by the bicinchoninic acid method.

2. Determination of the lung wet weight/dry weight ratio

Whole lung tissue was used for blotting lung tissue surface water and blood stains by filter paper, and weighed as wet weight (W). The tissue was then dried for 48 hours to a constant weight (65°C constant temperature box) and weighed again as dry weight (D). The lung tissue W/D ratio was calculated.

3. Lung tissue histopathological and morphological observations

The middle of the right lung was fixed with 4% paraformaldehyde for 72 hours, dehydrated, cleared, paraffin-embedded, and cut into 4-μm sections. The sections were stained with hematoxylin for 3 minutes and eosin for 1.5 minutes, and were then dehydrated and sealed to transparency. Morphological changes in lung tissue were observed under a light microscope after hematoxylin–eosin staining. The pathological scoring criteria of ALI were as follows: (1) pulmonary capillary hyperemia (alveolar hyperemia); (2) intrapulmonary hemorrhage (erythrocyte effusion); (3) neutrophils were present in the vascular wall with interstitial accumulation or infiltration (neutrophil exudation or accumulation in the alveolar space); (4) alveolar wall thickening and/or transparent membrane formation.

According to the affected area, the following scores were recorded: 0 points (normal lung tissue, no lesions or very minor lesions); 1 point, minor lesions (25% lung tissue involvement); 2 points, moderate lesions (25% to 50% involvement); 3 points, severe damage (50% to approximately 75% involvement); 4 points, the most serious damage (>75% involvement). The four scores were added to obtain the total score.

Lung tissue immunohistochemistry

The middle of the right lung was fixed with 4% paraformaldehyde for 72 hours, dehydrated, cleared, paraffin-embedded, and cut into 4-μm sections. Sections were deparaffinized by xylene and dehydrated by gradient alcohol, and then repaired in antigen repair solution (0.4 g citric acid+3.0 g trisodium citrate+distilled water) in a fixed volume to 1000 mL by using the heating method of a microwave for 10 minutes. After adding 3% H_2O_2 deionized water for 30 minutes, serum was blocked for 30 minutes, and the first antibody was added and incubated at 4°C overnight. A biocatalyst secondary antibody and horseradish peroxidase streptococci workable solution were added and incubated for 30 minutes. Staining using 3, 3'-diaminobenzidine was then performed until the positive reaction was terminated. VEGF, NF-κB, and IL-17α in lung tissue were detected by image analysis with Image pro-Plus software (Media Cybernetics, Inc., Rockville, MD, USA). After the gray values were corrected, six fields were randomly selected from each specimen, and the optical density (OD) value was measured and the absorbance was analyzed.

Western blotting of lung tissue

A small piece of lung tissue was placed on ice. This tissue was weighed and placed in RIPA liquid and phenylmethylsulfonyl fluoride (99: 1) mixture at a ratio of 1: 5. The lung tissue was then cut up on the ice. The tissue was then homogenized, allowed to sit for 30 minutes, and centrifuged at 20,600 ×g for 15 minutes at 4°C. The supernatant was collected and protein concentrations were measured by the BCA method (BCA Protein Assay; Thermo Fisher Scientific, Waltham, MA, USA). According to the concentration curve, RIPA was diluted to the same concentration and then the protein loading buffer was added at a ratio of 4: 1. A total of 40 μg of protein was loaded onto a gel and 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed. The gel was transferred to a 0.22-μm polyvinylidenefluoride membrane for 90 minutes. Skimmed milk powder (5%) was then added with incubation for 2 hours. The membrane was washed in TBST three times for 10 minutes/time. The first antibody (glyceraldehyde-3-phosphate dehydrogenase [GAPDH] [1: 5000], VEGF [1: 1000], vWF [1: 500], TLR-4 [1: 1000], NF-κB [1: 1000], tumor necrosis factor [TNF]-α [1: 500], and IL-17A [1: 500]) was added and the membrane was incubated at 4°C overnight. The membrane was then rewarmed for 30 minutes, washed with TBST three times for 10 minutes/time, and incubated with secondary antibody for 2 hours at room temperature. The membrane was then washed with TBST three times for 10 minutes/time and detected by electrochemiluminescence. ImageJ software (http://imagej.nih.gov/ij) was used to analyze GAPDH, VEGF, vWF, NF-κB, TNF-α, and IL-17A bands, and the OD value was calculated for statistical analysis.

CCK-8 cell proliferation and toxicity test

Rat lung AECIIs that were grown in the log phase were inoculated into a 12-well plate at a density of 5×10^4/mL and 1 mL of cells was added to each well. After inoculation for 16 hours, the C and D groups were

stimulated with 10 μg/mL LPS. Groups A and B had the same amount of DMEM added. After 4 hours, groups B and D were indirectly co-cultured in 0.4-μm transit chambers plus BMSCs (5×10^4/well). Groups A and C were placed in a small chamber without BMSCs. After 24 hours of culture, the supernatant was carefully removed, and then 1 mL of DMEM and 100 μl of CCK-8 solution were added to each well. Culture was continued for 1 hour in the incubator and then the absorbance at 450 nm was measured. Cell viability (%) of each group was calculated as follows: (experimental group absorbance – blank group absorbance)/(control group absorbance – blank group absorbance) × 100%.

Statistical analysis

Statistical analysis and graphics were performed using GraphPad Prism7 software (GraphPad Software, Inc., San Diego, CA, USA). All data are expressed as mean ± standard deviation and multiple groups were compared by one-way analysis of variance. $P < 0.05$ was considered statistically significant.

Results

In this study, we first confirmed whether BMSCs were transplanted in lung tissue. At each time point, the rats were placed into a small animal imager for optical imaging small animals. The rats were quickly killed and their lung tissue was taken for frozen sectioning. A fluorescence microscope was used to observe any fluorescence. Unfortunately, there was no fluorescence in the tissue, which indicated that transplantation of BMSCs was not successful. Therefore, we carried out the following experiments to examine the possible effects of untranslatable BMSCs on lung injury.

BMSCs reduce the degree of lung injury in the early phase of ALI

The BALF cell count, BCA protein concentrations, and lung W/D ratio were significantly higher in the ALI model group than in the control group in the early phase of ALI (all $P < .05$). The BALF cell count, BCA protein concentrations, and lung W/D ratio were significantly lower in the BMSCs treatment group than in the ALI model group in the early phase of ALI (all $P < .05$) (Figure 1).

BMSCs improve pathological morphology of lung tissue

In the control group, the lung tissue of SD rats was generally reddish with no oozing. Under light microscopy, the alveolar shape

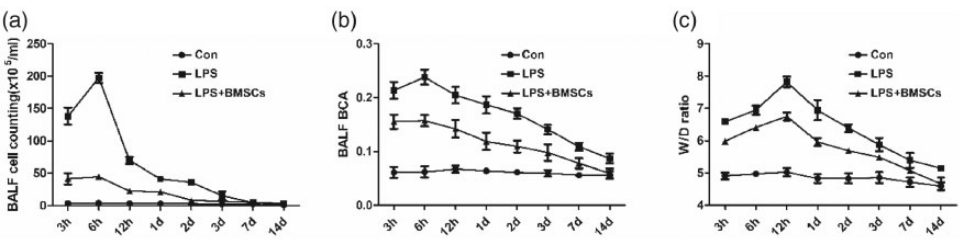

Figure 1. Changes in BALF cell counts (a), protein concentrations by the BCA method (b), and the W/D ratio of lung tissue (c) in each group. BALF: bronchiolar lavage fluid; BCA: bicinchoninic acid; W/D: wet weight/dry weight; Con: control; LPS: lipopolysaccharide; BMSCs: bone marrow-derived mesenchymal stem cells; d: days; h: hours. *$P < 0.05$ between the acute lung injury model group and the control group; #$P < 0.05$ between the treatment group and the acute lung injury model group.

was intact and smooth, the alveolar wall was not thickened, there was no obvious inflammatory exudation in the alveolar space, and the pulmonary interstitial area showed no congestion or edema (Figure 2a). In the ALI model group, lung tissue of SD rats showed a large oozing point. Under light microscopy, the normal shape of the alveoli had disappeared, the alveolar wall was thickened, and the alveolar capillaries had become congested. Additionally, the alveolar cavity showed hemorrhage and inflammatory cell infiltration, and there was pulmonary interstitial edema. The most obvious injury occurred at 12 hours (Figure 2b). A transparent film had formed. In BMSCs in the treatment group, the gross oozing point of the lung tissue of SD rats was decreased. The alveolar shape was intact compared with the ALI model group, and alveolar infiltration, hemorrhage, and edema of the lung tissue were alleviated. In the early stage of ALI, histopathology showed a tendency to be aggravated and then relieved (Figure 2c). The pathological score in the model group was significantly higher than that in the control group ($P < .05$ for all time points). The pathological score in the treatment group was significantly lower than that in the model group ($P < .05$ for all time points).

BMSCs reduce production of inflammatory factors

VEGF, NF-κB, and IL-17A expression as shown by immunohistochemistry was observed in the cytoplasm and cell walls of AECIIs and vascular endothelial cells (Figure 3a). VEGF expression in the model group was significantly higher than that in the control group ($P < .05$ for all time points). Furthermore, early VEGF expression in the treatment group was significantly lower than that in the model group ($P < .05$ for all time points) (Figure 3b). Immunohistochemistry of NF-κB and IL-17A expression in rat lung tissue showed a similar expression pattern to that of VEGF ($P < .05$ for all time points) (Figure 3c and d, Figure 4).

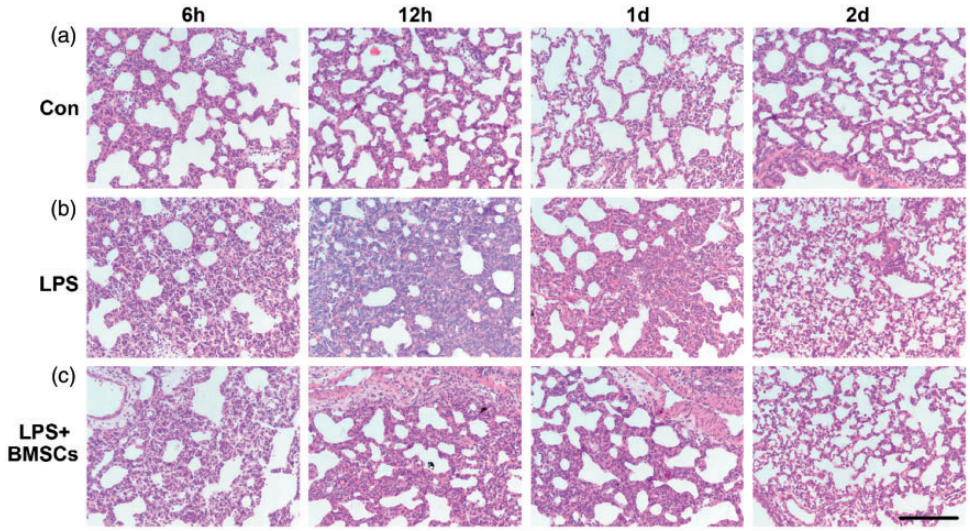

Figure 2. Immunohistochemical staining of rat lung tissue (×200) after different treatment times. (a) Control group; (b) acute lung injury model group; (c) treatment group. Scale bar=50 μm. Con: control; LPS: lipopolysaccharide; BMSCs: bone marrow-derived mesenchymal stem cells; d: days; h: hours.

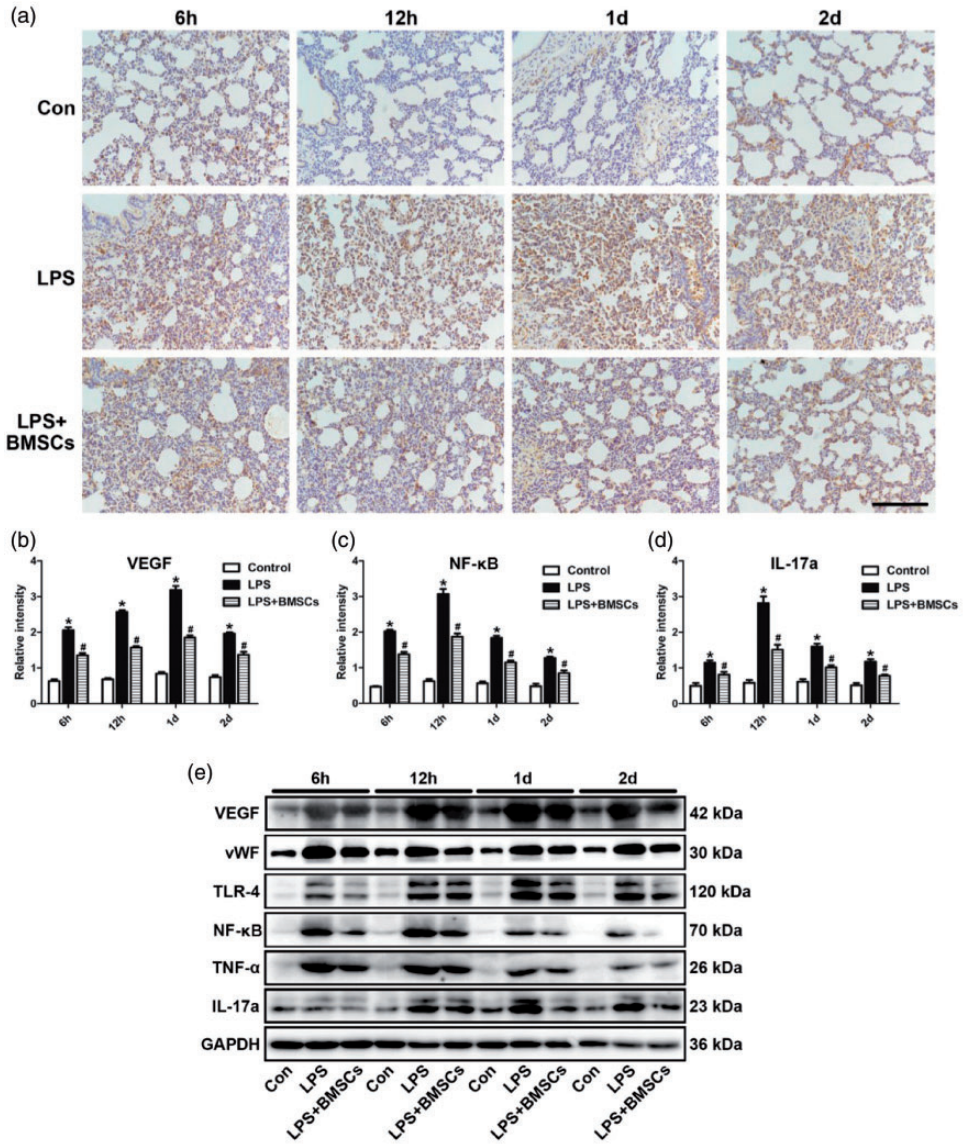

Figure 3. Protein expression levels of vascular factors and inflammatory factors after different treatment times. (a) Immunohistochemistry of VEGF in rat lung tissue (×200). Scale bar = 50 μm. (b–d) Immunohistochemical results for VEGF, NF-κB, and IL-17A protein levels in each group. (e) Western blot bands of VEGF, vWF, TLR-4, NF-κB, TNF-α, and IL-17A in each group. VEGF: vascular endothelial growth factor; Con: control; LPS: lipopolysaccharide; BMSCs: bone marrow-derived mesenchymal stem cells; d: days; h: hours; NF-κB: nuclear factor-kappa B; IL-17A: interleukin-17A; vWF: von Willebrand factor; TLR-4: toll-like receptor-4; TNF-α: tumor necrosis factor-α; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. *$P < 0.05$ between the acute lung injury model group and the control group; #$P < 0.05$ between the treatment group and the acute lung injury model group.

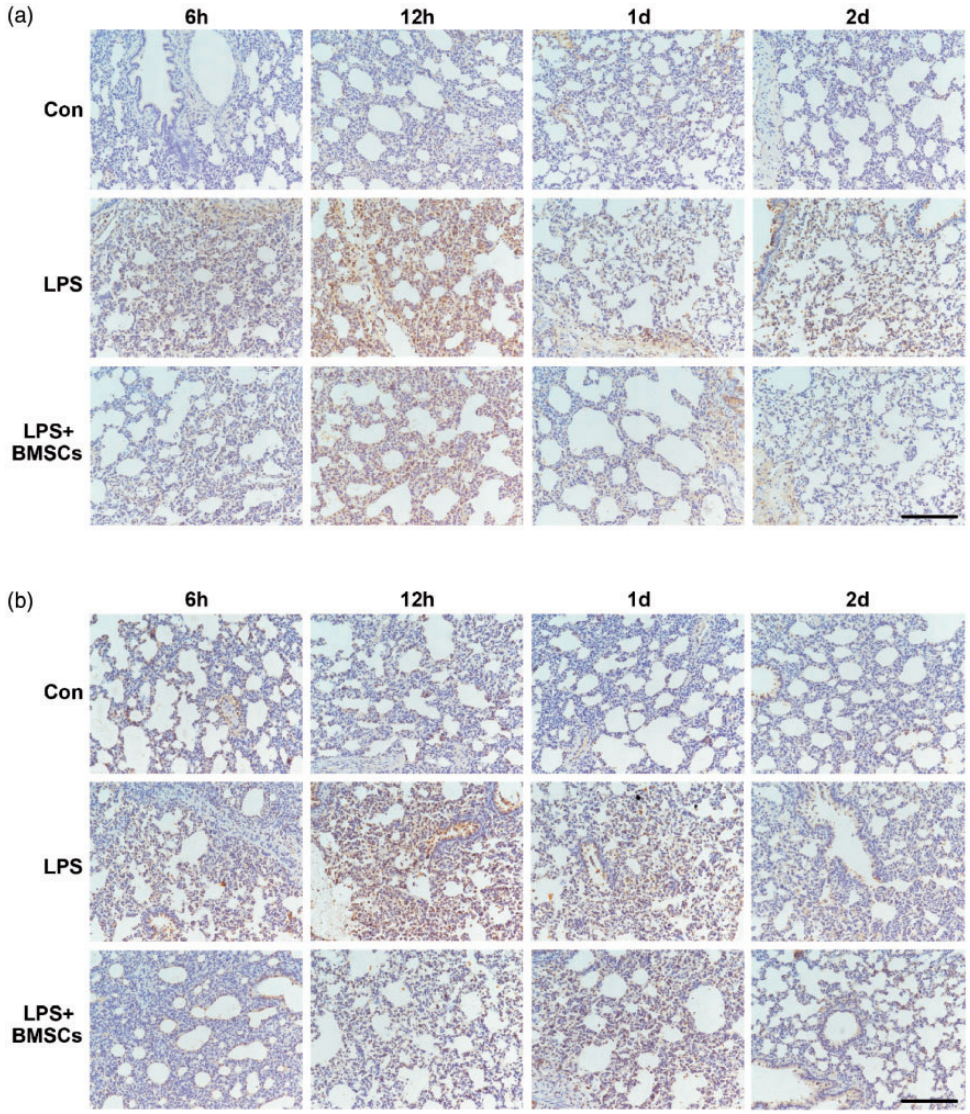

Figure 4. Immunohistochemistry of NF-κB and IL-17A. (a) Immunohistochemistry of NF-κB in rat lung tissue (×200). Scale bar = 50 μm. (b) Immunohistochemistry of IL-17A in rat lung tissue (×200). Scale bar = 50 μm. NF-κB: nuclear factor-kappa B; IL-17A: interleukin-17A; Con: control; LPS: lipopolysaccharide; BMSCs: bone marrow-derived mesenchymal stem cells; d: days; h: hours.

Western blotting showed that expression of VEGF, vWF, TLR-4, NF-κB, TNF-α, and IL-17A in the model group was significantly higher than that in the control group (all $P < .05$) (Figure 3e). Expression levels of these proteins in the model and treatment groups initially increased and then decreased over time. Early expression of these proteins was significantly lower in the treatment group compared with the model group ($P < .05$) (Figure 3e).

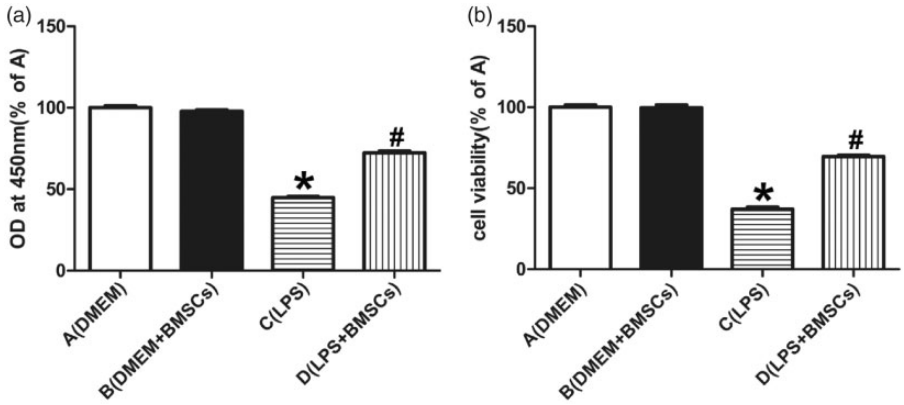

Figure 5. OD values at 450 nm (a) and the cell viability (b) in each group of cells by the cholecystokinin octapeptide assay. Cell viability (%) of each group = (experimental group absorbance − blank group absorbance)/(control group absorbance − blank group absorbance) × 100%. *$P < 0.05$ between groups A and C; #$P < 0.05$ between groups C and D. OD: optical density; DMEM: Dulbecco's modified Eagle's medium; LPS: lipopolysaccharide; BMSCs: bone marrow-derived mesenchymal stem cells.

BMSCs alleviate damage of AECIIs by LPS and promote their repair

To validate the function of untransplanted BMSCs, the absorbance (OD value) of different cell lines was detected by the CCK-8 assay. There was no significant difference in OD values and cell viability between group A (DMEM) and group B (DMEM+BMSCs), which suggested that BMSCs had no effect on normal AECIIs (Figure 5). The OD value of group C was significantly lower than that of group A ($P < .05$) (Figure 5a), which suggested that LPS caused AECII injury. The OD value of group D was significantly higher than that of group C ($P < .05$), which suggested that BMSCs alleviated the damage of AECIIs by LPS and promoted their repair. The results of cell viability were consistent with the OD values in these groups (Figure 5b).

Discussion

ALI is a clinically common disease. In addition to high morbidity and mortality, patients with ALI who are discharged from hospital are accompanied by physical, psychological, and cognitive impairments.[11,12] ALI/ARDS is a complex disease process, and direct and indirect alveolar damage can lead to a series of pathological changes. Additionally, reports have shown that mechanical ventilation and nutrition, and fluid therapy are related to progression of ALI.[13–15] At present, there are many methods for treating ALI in clinical practice, such as application of antibiotics, mechanical ventilation, and limiting the amount of intravenous fluid. However, the curative effect of these methods is poor and cannot effectively prevent progression of this disease. The latest data have shown that the mortality rate of ALI is still as high as 30%.[16] Therefore, determining for in-depth treatment of ALI is important.

MSCs are homologous cell populations with self-replication and multi-directional differentiation potential that are derived from different tissues and can proliferate *in vitro* to form adherence.[17] The therapeutic effects of MSCs in diseases involve various important organs of the heart, brain, liver, and bones, and some achievements

have been made in animal experiments.[18,19] MSCs can migrate to damaged tissues and inflammatory sites,[20] and can sense hypoxia, stimulate repair of endogenous tissue damage,[21] and regulate the immune response.[22] Lung tissue has structural particularities, an abundant capillary bed, high capacity, and low resistance. MSCs easily remain in the lungs after transplantation. Therefore, this allows favorable conditions for MSCs to treat respiratory diseases.[23] Reducing the inflammatory response is not only a method of treating ALI/ARDS, but is also extremely important for recovery of lung tissue.[24]

VEGF, which is a cytokine secreted by AECIIs, is expressed in many tissues (e.g., heart, lungs, kidney, and liver). VEGF is particularly abundant in lung tissue, and mainly produced by alveolar epithelial cells, bronchial gland cells, bronchial epithelial cells, and activated macrophages.[25] vWF is mainly synthesized by endothelial cells and is a high molecular weight plasma glycoprotein, and it is a relatively sensitive indicator of vascular endothelial cell damage.[26] VEGF and vWF may be involved in the pathological process of ALI (Figure 3a, b, e), but their specific expression during the development of ALI is not fully understood.

Some studies have shown that LPS binds to TLR-4 to activate a key pro-inflammatory transcription factor NF-κB. The TLR-4/NF-κB signaling pathway is involved in the pathogenesis of ALI.[27] LPS binding to TLR-4 can activate NF-κB, which plays an important role in regulating immune responses and induces the expression of various chemokines and pro-inflammatory cytokines, such as IL-1β, TNF-α, and macrophage inflammatory proteins.[28,29] Recent studies have shown that the TLR-4 signaling pathway is involved in the pathogenesis of non-infectious lung injury.[30,31] IL-17 is associated with inflammatory lung diseases. However, the expression and biological role of IL-17 in ALI are not completely clear. In ALI, IL-17 expression is increased, and expression of inflammatory cells and inflammatory factors can be promoted by activating extracellular signal-regulated kinase (ERK)-1/2 and NF-κB signaling pathways, further aggravating lung injury.[32] In the study, we found that BMSCs reduced the degree of lung tissue injury in ALI by reducing secretion of inflammatory cytokines (Figures 1, 2, and 3). However, we do not know exactly what substances BMSCs secrete to regulate the inflammatory response. *In vitro*, Wnt5a promotes migration of mouse MSCs and differentiation of MSCs into AECII cells via the Wnt/JNK pathway or Wnt/JNK and Wnt/PKC pathways alone.[33] The current study showed that BMSCs alleviated the damage of LPS on AECIIs, and promoted repair and regeneration of AECIIs, with no effect on normal AECIIs (Figure 4). Unfortunately, we did not detect the presence of Wnt5a or other exosomes in the co-medium in this study.

Lung tissue has lung MSCs, and lung mesenchymal cells with the LGR5 marker secrete a large number of Wnt5 molecules. This promotes formation of alveolar epithelial cells, while lungs with LGR6 markers in mesenchymal cells play an important role in repairing airway cells.[34] We did not observe any changes in pulmonary MSCs in damaged lung tissue after infusion of these cells. Therefore, we examined whether MSC infusion can affect changes in pulmonary MSCs in damaged lung tissue. Exosomes from MSCs and cardiomyocyte progenitor cells can promote angiogenesis through an extracellular matrix metalloproteinase inducer.[35] MSC-derived exosomes improve the inflammatory microenvironment of the myocardium by relying on macrophage activation mediated by the JAK2-STAT6 pathway, thereby reducing inflammatory cardiomyopathy.[36] Ohyashiki et al.[37] found that

vesicles derived from small endosome-derived vesicles containing micro RNAs regulate the microenvironment without direct contact with non-tumor cells. These authors also found that the human myeloid leukemia cell line K562 promotes angiogenesis by secreting exosomes containing large amounts of miR-92a. Whether BMSCs can promote repair of capillary endothelial cells in injured lung tissue by secreting some exosomes requires further investigation.

In our study, BMSCs without transplantation reduced the inflammatory response in ALI through a paracrine action, thereby alleviating lung injury (Figures 2 and 3) and promoting repair of AECIIs (Figure 4). This finding suggests that specific secretion of substances might play an important role in this process. BMSCs *in vitro* are differentiated into lung epithelial cells and pulmonary microvascular endothelial cells by first differentiating MSCs of lung tissue itself, or directly differentiating into lung epithelial cells and pulmonary microvascular endothelial cells. Royce et al.[38] showed that, in chronic allergic airway disease and a bleomycin-induced pulmonary fibrosis model, alveolar epithelial cell-exosomes reduced pulmonary inflammation and pulmonary fibrosis, while serelaxin enhanced the therapeutic effect of alveolar epithelial cell-exosomes. In our study, we showed that untransplanted MSCs mitigated ALI. This finding indicates that MSCs may regulate inflammation and reduce lung tissue damage by secreting certain substances. However, because the substances involved are unclear, further research needs to focus on clarifying the substances secreted by BMSCs and the mechanism involved.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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