Protective Effects of Chicken Bone Marrow-Derived Mesenchymal Stem Cells on a Mouse Model of Acute Lung Injury

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Protective effects of chicken bone marrow-derived mesenchymal stem cells on a mouse model of acute lung injury

Abstract

Objective: Mesenchymal stem cells (MSCs) were an emergent tool to treat acute lung injury (ALI) and its complications. We attempted to isolate, culture, and characterize chicken bone marrow-derived mesenchymal stem cells (BM-MSCs), investigate whether BM-MSCs protect against lipopolysaccharide (LPS)-induced ALI, and explore the related mechanisms.

Methods: Ninety-six male ICR (6 wks old) mice were divided into three groups: Con, LPS, and LPS + MSC. Mice in the LPS and Ex+LPS groups received 5 mg/kg LPS intratracheally to induce ALI. Histopathological micrographs showed morphological changes in the lung, heart, liver, kidney, and aorta. Lung permeability, bronchoalveolar lavage fluid (BALF) cell counts, oxidative stress parameters, and inflammatory cytokine levels in the BALF, plasma, and lung tissue were detected. Furthermore, gene expression levels for Toll-Like Receptor 4 (TRL4) and myeloid differentiation factor (Mdy88) were measured. Besides, a survival analysis was performed in sixty male ICR mice.

Results: BM-MSCs administration significantly increased the survival rate and decreased the histopathological severity of LPS-induced lung, liver, kidney, and aortic injury. BM-MSC administration improved LPS-induced pulmonary inflammation, pulmonary fibrosis, and oxidative stress injury. BM-MSC administration reduced

systemic inflammation and the infiltration of neutrophils in lung, liver, kidney, and heart tissues. In addition, BM-MSC administration reduced TLR4 and Mdy88 mRNA expression in mice with ALI.

Conclusions: Chicken BM-MSCs improved LPS-induced ALI and liver, kidney, heart, and aortic injury. Chicken BM-MSCs improved LPS-induced ALI, in part through TLR4/Myd88 signaling and inhibition of inflammation and oxidative stress injury. BM-MSCs improved LPS-induced pulmonary fibrosis via suppressing TGF- β and MMP-9. BM-MSCs reduced neutrophil content via suppressing CXCL-1, IL-8, and TNF- α .

Keywords: chicken, BM-MSCs, ALI, neutrophil inflammation, oxidative stress, TLR4/Myd88 signaling

1. Introduction

ALI, which is characterized by lung permeability and inflammation, leads to diffuse alveolar damage, lung edema, hypoxia, and respiratory failure, generally stemming from pneumonia, trauma, and sepsis^{1,2}. Although substantial progress has been made, ALI is still a substantial cause of morbidity and mortality worldwide³. Approved pharmacological treatments are not available for ALI, and the development of new and effective treatments is desperately needed.

The pathogenesis of ALI is complex. Inflammation plays a critical role in the pathogenesis of ALI^{4,5}. ALI is characterized by an uncontrolled inflammatory response involving inflammatory mediators, including IL-1 β , IL-6, IL-8, and TNF- α , and effector cells, among which neutrophils play a key role^{6,7}. Deleterious

accumulation of neutrophils is a critical mechanism underlying ALI⁸. Neutrophils release multiple factors including pro-fibrogenic factors including transforming growth factor-beta (TGF- β) and matrix metalloproteinase 9 (MMP-9)⁹. It was well documented that TGF- β and MMP-9 were important in the pathogenesis of pulmonary fibrosis^{10,11}. Hence, ALI may be accompanied by pulmonary fibrosis. Besides, deleterious accumulation of neutrophils within remote vital organs leads to collateral tissue damage and ultimately multiple organ failure because deleterious activation of neutrophils is a critical reason leading to host tissue injury and organ damage¹². Hence, ALI may be accompanied by extrapulmonary injuries, such as liver, kidney, heart, and aortic injury. Previous studies have demonstrated that ALI causes acute organ dysfunction, including dysfunction of the cardiovascular system¹³, which delivers oxygen, nutrients, inflammatory cells, and inflammatory cytokines to tissues, and seriously impairs organ function, but previous studies have ignored the effects of MSCs on the cardiovascular system in ALI models.

MSCs, which have a typical fibroblast-like morphology, enormous proliferative potential, and multilineage differentiation potential, are used to treat many autoimmune and inflammatory diseases, including ALI, acute respiratory distress syndrome (ARDS), and sepsis, due to their low immunogenicity and potent immunomodulatory and paracrine signaling capacities¹³⁻¹⁵. Previous studies have demonstrated that MSCs improved immune function by altering the number of immune cells, including neutrophils^{16,17}. Inflammatory mediators such as IL-1 β and IL-10, and effector cells, including neutrophils have been established as crucial

mechanisms of ALI, but their physiological functions in response to BM-MSC in mice with ALI remain unclear.

Toll-like receptor 4 (TLR4)/myeloid differentiation factor 88 (MyD88) signaling, which could be activated by LPS, constitutes a major part of the innate immune system^{18,19}. It has been confirmed that activation of TLR4/MyD88 signaling can lead to a variety of diseases including ALI²⁰. LPS led to an excessive inflammatory response via TLR4/MyD88 signaling, while it was not clear the effects of BM-MSCs on TLR4/MyD88 signaling in mice with ALI.

Chicken BM-MSCs offer multiple applicative advantages. MSCs can be isolated, cultured, and propagated easily in vitro. Compared with embryonic stem cells (ESCs), MSCs have a limited risk of tumor formation and do not pose an ethical controversy²¹. However, the vast majority of studies of MSCs mainly concentrate on cells from humans, mice, and rats, and numerous sources for these MSCs are not available and MSCs cannot be harvested at low cost. Compared with the species mentioned above, chicken embryos possess broader sources and are generally the cheapest choice at present. More importantly, the time needed for egg incubation is only 21 days, hence, tissues can be conveniently collected at specific stages of development. The timing of sampling exerts a substantial effect on the activity and proliferative capacity of MSCs²². Generally, if the timing of sampling was too early, tissues would not yet have formed, if the timing of sampling was too late, the viability of the MSCs would decrease significantly. Hence, this study would reduce the cost of MSC research and contribute to further studies and application of MSCs.

We attempted to provide a scientific foundation for the use of chicken BM-MSCs and identified whether BM-MSCs reduced ALI and extrapulmonary injury.

We aimed to identify whether BM-MSCs can prevent ALI-induced multiple-organ injury, including injury to the lung, liver, kidney, heart, and aorta. In addition, the underlying molecular mechanisms involved in the preventive effects of BM-MSCs against inflammatory parameters and the oxidative stress index in LPS-induced ALI have not yet been fully elucidated.

2. Materials and methods

2.1. Isolation, culture, and cryopreservation of chicken BM-MSCs

Bone marrow was obtained from 16-day-old chicken embryos. The connective tissues were carefully removed. Complete DMEM/F12 supplemented with 10% fetal bovine serum (FBS) was utilized to flush the marrow cavity. The resulting cell suspension was filtered through a 100- μ m mesh sieve and then centrifuged at 1200 r/min for 10 min. Then, the cell pellets were resuspended in complete DMEM/F12 containing 10% FBS, 2 mM L-glutamine, and 5 ng/mL bFGF, seeded into 6-well plates (10^4 cells per well) and cultured at 37 °C in a humidified incubator with 5% CO₂. At 48 h post-seeding, the supernatant was discarded and the cells were washed three times with PBS to remove the nonadherent cells and exfoliated tissue masses. Thereafter, the medium was replaced every 24-48 h. When the cells reached 80-90% confluence, 0.25% trypsin/EDTA was utilized to passage the BM-MSCs at a 1:2 dilution.

Cryopreservation of MSCs is essential for further research. When the BM-MSCs

reached 90% confluence, they were digested and harvested in 15-mL sterile centrifuge tubes. The cell pellets were resuspended in a freezing medium composed of 50% FBS, 40% DMEM/F12, and 10% DMSO. One milliliter of the resulting suspension (1×10^6 cells/mL) was transferred to 1.8-mL sterile cryogenic tubes followed by storage at $-80\text{ }^{\circ}\text{C}$ overnight, and then the BM-MSCs were transferred to liquid nitrogen tanks for long-term storage.

2.2. Flow cytometry

Using an established procedure, chicken BM-MSCs in the logarithmic phase were dissociated into a single-cell suspension. After counting, 1×10^6 cells were transferred to a sterile centrifuge tube, followed by centrifugation at 1200 r/min for 8 min. Precooled 70% ethanol was added dropwise to fix BM-MSCs, and then the cells were gently prepared as a single cell suspension, followed by incubation overnight at $4\text{ }^{\circ}\text{C}$. The BM-MSCs were incubated with PBS containing rabbit-derived primary antibodies against CD73, CD90, CD105, OCT-4, SOX-2, CD34, and CD45, followed by 3 washes for 5 min each. PBS containing FITC-labeled goat anti-rabbit IgG was incubated with MSCs in the dark for 60 min, followed by 2 washes for 10 min each. Finally, MSCs were subjected to flow cytometry analysis using a Beckman Coulter flow cytometer and the FACSscan program.

2.3. Identification of the multilineage differentiation potential of chicken BM-MSCs

2.3.1. Osteogenic differentiation of BM-MSCs

BM-MSCs were seeded in 12-well plates (2.0×10^5 cells/well). The medium was

replaced with an osteogenic differentiation medium when the cells reached 30% confluence. Half of the medium was replaced with fresh medium every 2 days. On days 14-21 of culture and differentiation, calcium deposition was identified by performing Alizarin Red staining. All information on the induction medium is listed in [Supplementary Material 1](#).

2.3.2. Adipogenic differentiation of BM-MSCs

Third-generation BM-MSCs were inoculated in 12-well plates at a density of 2.0×10^4 cells/well. The media were replaced with adipogenic induction and differentiation medium when cells were 30% confluent. Adipogenic induction and differentiation medium was refreshed with 50% fresh medium every other day for 21 days. Afterward, the accumulation of intracellular lipid droplets was visualized using Oil Red O staining.

2.3.3. Chondrogenic differentiation of BM-MSCs

For chondrogenic differentiation, third-generation BM-MSCs were cultured in the chondrogenic medium when they proliferated to 30% confluence. The chondrogenic medium was refreshed with 50% fresh medium every 2 days. On day 21 of culture, alcian blue staining was performed to confirm chondrogenic differentiation.

2.3.4. Neuroblastic differentiation of BM-MSCs

When 30% confluence was achieved, BM-MSCs were exposed to a neuroblastic differentiation medium, which was replaced with 50% fresh medium every 2 days. On day 21 of differentiation and culture, Nestin and MAP-2, which are all neural-specific markers, were detected using immunohistochemical staining. BM-MSCs were fixed

with ice-cold PBS containing 4% paraformaldehyde (PFA) for 15 min at room temperature, followed by 3 washes for 5 min each. The BM-MSCs were then permeabilized with 0.1% Triton X-100 for 30 min at room temperature, followed by 3 washes for 5 min each. Nonspecific binding sites were blocked with 10% sheep serum for 30 min at room temperature. After blocking, the BM-MSCs were incubated overnight at 4 °C with the following primary antibodies: FITC-rabbit Nestin antibody and FITC-rabbit MAP-2 antibody (1:200; Abcam, Cambridge, MA, USA). After incubation, the BM-MSCs were washed three times and incubated with PBS containing a FITC-conjugated goat anti-rabbit secondary antibody (1:100; Santa Cruz, CA, USA) for 2 h at 37 °C in the dark, followed by 3 washes for 5 min each. BM-MSCs were counterstained with 1 µg/mL DAPI in the dark for 15 min at room temperature. Eventually, the expression of cell surface markers and DAPI signals were visualized and imaged using a confocal microscope (Nikon TE-2000-E, Tokyo, Japan) equipped with a digital camera system (Nikon ZE-1-C1 3.70).

2.4. LPS-induced ALI model

All protocols used in this study were approved by the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). All experiments were performed in accordance with the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The authors have read the ARRIVE guidelines and the study was carried out in compliance with the ARRIVE guidelines.

Every effort was made to minimize suffering. All mice were anesthetized via intraperitoneal injection of pentobarbital (0.2 mg/kg). Thirty male ICR mice (6 weeks old, 18-21 g) were provided by Beijing HFK Biotechnology Co., Ltd. (Beijing, China). The conditions of the husbandry room were temperature at 22 ± 5 °C, relative humidity at $50\% \pm 10\%$, and the light cycle was 12 h/day. The body weight and health status of the mice were recorded. The mice were euthanized with isoflurane. The ALI model was established by administering an intratracheal injection of 5 mg/kg LPS (O55:B5, Sigma-Aldrich, St. Louis, MO, USA). Ninety-six mice were randomly assigned to one of three groups: (1) mice in the saline group (Sham) were intratracheally injected with an equal volume of normal saline to the volume of LPS; (2) mice in the LPS group (LPS) were intratracheally injected with 5 mg/kg LPS; and (3) mice in the LPS + MSC group (LPS + MSC) were intratracheally injected with 5 mg/kg LPS. Two hours later, the mice received 1×10^6 chicken BM-MSCs via tail vein injection. All mice were intraperitoneally injected with the anti-rejection drug cyclosporine once a day for one week before the experiment. The mice were euthanized at 6 h, 12 h, 24 h, and 48 h. Each group contained 8 mice.

2.5. Assessment of the survival rate

For survival analyses, 60 mice were randomly divided into three groups: (1) saline group (Sham), where the mice were intratracheally injected with the same volume of saline as the volume of LPS; (2) LPS group, where the mice were intratracheally injected with 12 mg/kg LPS; (3) LPS + MSCs group (LPS + MSC), where the mice were intratracheally injected with 12 mg/kg LPS and then received 1×10^6 chicken

BM-MSCs via a tail vein injection two hours later. ALL mice were intraperitoneally injected with the anti-rejection drug cyclosporine for one week before the experiment. The number of dead mice was recorded every 6 h for 48 h. Each group included 20 mice.

2.6. Collection of BALF and tissue samples

Blood samples were collected from the retro-orbital sinus and placed into a blood collection vessel containing an anticoagulant. The blood samples were centrifuged at 3000 r/min at 4 °C for 15 min. The upper serum layer was transferred to 1.5-mL EP tubes and frozen at -20 °C. After sampling, the tissues were immediately placed in an incubator filled with liquid nitrogen. Then, the tissues were transferred to liquid nitrogen tanks for long-term preservation. Mouse tissue samples of the appropriate size were fixed with 4% paraformaldehyde for 24 h.

Two milliliters of ice-cold PBS was utilized to collect BALF, the whole BALF was flushed four times, the output fluid was collected and centrifuged at 3000 r/min for 12 min at 4°C. The supernatant was transferred to 1.5-mL sterile EP tubes and immediately stored at -20 °C. The remaining lung tissues were preserved in a liquid nitrogen container.

2.7. Histopathology

For the histological analysis, 4% PFA-fixed tissue samples were fixed with 4% PFA for 24 h. All tissue samples were fixed with 4% PFA and embedded in paraffin. Following paraffin sectioning, 5-mm-thick sections were stained with Hematoxylin and eosin (HE) and Masson trichrome. Images were captured with an inverted

microscope and utilized to determine the degree of organ injury.

2.8. Detection of pulmonary permeability

The wet-dry weight ratio (W/D) of lung tissues and the protein content in the BALF were detected to quantify the degree of pulmonary permeability. Bibulous papers were utilized to absorb liquid and blood from the surface of the lung tissues, and then the wet weight of lung tissue was weighed. The lung tissues were dried until a stable dry weight was obtained. The wet/dry (W/D) ratio was calculated according to the following formula: $W/D = \text{wet weight of the left lower lobe} / \text{dry weight of the left lower lobe}$. A Bio-Rad protein assay kit was utilized to calculate the protein concentration in the BALF. Evans blue (Sigma, St. Louis, MO, USA) extravasation from the lung tissues was detected with a spectrophotometer.

2.9. Determination of the oxidative stress index

MDA, SOD, MPO, and GSH levels in the mice were detected using spectrophotometry (Bio-Tek Instruments Inc., software KC4 v3.0), as previously described according to the manufacturer's instructions.

2.10. Measurement of the serum levels of inflammatory factors

Based on the manufacturer's specifications, the serum levels of CXCL-1, TNF- α , IL-1 β , IL-8, and TNF- α were detected with mouse ELISA kits (Neobioscience).

2.11. Measurement of the BALF levels of inflammatory factors

Based on the manufacturer's specifications, the BALF levels of CXCL-1, IL-1 β , IL-1RN, IL-8, IL-10, TNF- α , MMP-9, TGF- β were detected with mouse ELISA kits (Neobioscience).

2.12. Measurement of markers of extrapulmonary injury

For histological analysis, liver, kidney, heart, and aortic tissue samples were fixed in 4% PFA for 24 h. Liver, kidney, heart, and aortic tissue samples were fixed in 4% PFA and embedded in paraffin. Following the paraffin section method, 5-mm-thick sections were stained with hematoxylin and eosin (HE). Images were captured with an inverted microscope.

According to the manufacturer's specifications, the levels of markers of liver disease (ALT and AST) and kidney injury (Urea and BUN) in serum were detected using commercial kits (Rsbio, Shanghai, China).

2.13. Quantitative real-time PCR

Total RNA was extracted from lung tissues using TRIzol (Promega, Madison, WI), and an Applied Biosystems 2720 Thermal Cycler was used to synthesize cDNAs. Real-time fluorescence quantitative PCR was used to measure gene expression levels. GAPDH was selected as the internal reference gene, and the $2^{-\Delta\Delta CT}$ method was used for the quantitative analysis. All primers were designed and synthesized by the Shanghai Sangon Biotech Company. [Supplementary Material 2](#) shows the murine PCR primer sequence information. Table 1 shows the PCR primer sequences used to amplify sequences from the mice.

2.14. Statistical analysis

All data are presented as the means $\pm$ SD ($\bar{x} \pm s$). The SPSS 20 software package was utilized to analyze differences among the three groups. One-way ANOVA was applied in this study. P values < 0.05 were considered statistically significant.

GraphPad Prism 7 software was used to prepare images.

3. Results

3.1. Isolation and characterization of chicken BM-MSCs

The cellular morphology of chicken BM-MSCs was determined at different passages (P1, P5, P15, and P25) (Fig. 1A, a-d). After culture under osteogenic induction conditions for 21 days, many calcium nodules appeared in the cytoplasm, and Alizarin red staining showed a positive reaction for calcified nodules in differentiated BM-MSCs (Fig. 1B-a). After culture under adipocytic induction conditions for 21 days, many lipid droplets formed in the cytoplasm, and the lipid droplets were successfully stained with Oil Red O (Fig. 1B-b). BM-MSCs displayed positive Alcian blue staining after incubation with chondrogenic medium for 21 days (Fig. 1B-c). Culture under neurogenic induction conditions for 21 days changed the cellular morphology from a spindle-shaped morphology to a multipolar and stellate morphology, and the BM-MSCs grew many branches and formed many synapses on the cell surface, as indicated by the arrows (Fig. 1B-d). The results of immunofluorescence staining revealed the expression of the markers of neural cells Nestin (Fig. 1C-a) and MAP-2 (Fig. 1C-b), while undifferentiated cells did not express Nestin (Fig. 1C-c) or MAP-2 (Fig. 1C-d). As shown in [Supplementary Material 3](#), the LMSC surface antigens CD73, CD90, CD105, Sox-2, and OCT-4 were positively expressed, while CD34 and CD45 were negatively expressed, as identified by flow cytometry.

3.2. BM-MSC administration reduced mortality

All sham-operated mice survived. Compared with the Sham group, the mortality rate of mice increased significantly after 12 mg/kg LPS administration ($P < 0.01$). Compared with the LPS group, BM-MSC administration markedly improved the survival rate of the mice (Fig. 2A) ($P < 0.05$). (n = 20 mice per group).

3.3. BM-MSC administration reduced systemic inflammation

After the administration of LPS, the serum levels of CXCL-1 (Fig. 2B) ($P < 0.05$), IL-1 β (Fig. 2C) ($P < 0.05$), IL-8 (Fig. 2D) ($P < 0.05$), and TNF- α (Fig. 2E) ($P < 0.05$) were significantly increased within 6 h and decreased at 12 h and 24 h compared to the Sham group. The administration of BM-MSCs noticeably prevented the increase in the levels of CXCL-1 ($P < 0.05$), IL-1 β ($P < 0.05$), IL-8 ($P < 0.05$), and TNF- α ($P < 0.05$) compared to the LPS group, indicating that BM-MSC administration reduced systemic inflammation.

3.4. BM-MSC administration attenuated oxidative stress injury in lung tissues

The levels of SOD (Fig. 2F), MDA (Fig. 2G), GSH (Fig. 2H), and MPO (Fig. 2I), indicators of oxidative stress injury, were detected. Compared with the Sham group, the levels of MDA ($P < 0.05$) and MPO ($P < 0.05$) were significantly increased, and the levels of SOD ($P < 0.05$) and GSH ($P < 0.05$) were significantly decreased after LPS administration. Compared with the LPS group, significant increases in the MDA ($P < 0.05$) and MPO ($P < 0.05$) levels and significant decreases in SOD ($P < 0.05$) and GSH ($P < 0.05$) levels were detected after the administration of BM-MSCs.

3.5. BM-MSC administration reduced lung injury

The histological assessment of lung tissues from the three groups revealed evidence of the degree of lung injury, inflammatory cell infiltration, and interstitial edema. The alveolar structure was intact and clear in the control group, and no inflammatory cells or blood cells appeared in the pulmonary alveolus. Compared with the lung tissues of normal mice (Fig. 3A, D, and G), the degree of lung injury, inflammatory infiltration, and interstitial edema increased after LPS administration (Fig. 3B, E, and H). Compared with the LPS group, treatment with BM-MSCs prominently prevented lung injury at each time point and significantly decreased the degree of lung injury, inflammatory cell infiltration, and interstitial edema (Fig. 3C, F, and I). The histological analysis indicated that the administration of chicken BM-MSCs relieved the degree of lung injury at each time point.

3.6. BM-MSCs attenuate neutrophil content in lung tissue

Micrographs showing the density of neutrophils in the lung tissue. Compared with the Sham group (Fig. 4A), LPS administration increased neutrophil content (black arrows) in lung tissues (Fig. 4B), but BM-MSCs administration prevented this increase in neutrophil content (Fig. 4C). Similarly, LPS administration significantly increased the number of neutrophils in lung tissues ($P < 0.01$), and compared with the LPS group, BM-MSC administration significantly decreased the number of neutrophils in lung tissues (Fig. 4D) ($P < 0.01$).

As shown in Table 1, the number of total cells and neutrophils in the BALF were significantly increased at 6 h after LPS administration ($P < 0.01$), and BM-MSCs played an essential role in preventing the increase in the number of total cells ($P <$

0.01) and neutrophils ($P < 0.01$) at 6 h after LPS administration. Furthermore, LPS and BM-MSCs had no effect on the number of lymphocytes ($P > 0.05$), macrophages ($P > 0.05$) or eosinophils ($P > 0.05$) in the BALF.

3.7. BM-MSC administration relieved pulmonary edema

Compared with the Con group, the LPS group showed a significantly increased total protein concentration in the BALF (Fig. 5A) ($P < 0.01$) and lung tissue W/D ratio (Fig. 5B) ($P < 0.01$) after the administration of LPS. BM-MSCs administration exerted a prominent preventative effect on the increases in total protein concentration in the BALF and lung tissue W/D ratio ($P < 0.01$), demonstrating that BM-MSCs administration reduced the degree of pulmonary edema. As shown in Supplementary Material 4, compared with the Sham group, LPS injection increased the Evans blue content ($P < 0.01$). Compared with the Sham group, BM-MSCs administration attenuated Evans blue content ($P < 0.01$).

3.8. BM-MSC administration reduced pulmonary inflammation

The administration of LPS markedly increased the expression levels of the CXCL-1 (Fig. 6A) ($P < 0.01$), IL-1 β (Fig. 6C) ($P < 0.01$), IL-8 (Fig. 6D) ($P < 0.01$), and TNF- α (Fig. 6J) ($P < 0.01$) mRNAs and markedly decreased the expression levels of the IL-1RN (Fig. 6B) ($P < 0.01$) and IL-10 (Fig. 6E) ($P < 0.01$) mRNAs in lung tissues. The administration of BM-MSCs markedly increased the expression levels of the IL-1RN ($P < 0.01$) and IL-10 ($P < 0.01$) mRNAs and markedly decreased the expression levels of the CXCL-1 ($P < 0.01$), IL-1 β ($P < 0.01$), IL-8 ($P < 0.05$), and TNF- α ($P < 0.01$) mRNAs in lung tissues.

As shown in [Table 2](#), our data observed an effect of LPS in increasing the levels of CXCL-1 ($P < 0.01$), IL-6 ($P < 0.01$), IL-8 ($P < 0.01$), IL-10 ($P < 0.01$), and TNF- α ($P < 0.01$) and decreasing the levels of IL-1RN ($P < 0.01$) in the BALF. Compared with the LPS group, BM-MSCs administration increased the levels of IL-10 ($P < 0.01$) and IL-1RN ($P < 0.05$) and decreased the levels of CXCL-1 ($P < 0.05$), IL-6 ($P < 0.05$), IL-8 ($P < 0.05$), and TNF- α ($P < 0.05$) in the BALF.

3.9. Effect of BM-MSCs on the gene expression levels of TLR4/Myd88 signaling pathway

LPS significantly activated the expression of the TLR4 ([Fig. 6I](#)) ($P < 0.01$) and Myd88 ([Fig. 6F](#)) ($P < 0.01$) mRNAs. Compared with the LPS group, BM-MSC administration inhibited the expression of TLR4 ($P < 0.01$) and Myd88 ($P < 0.01$) mRNAs in lung tissues.

3.10. BM-MSC administration reduced pulmonary fibrosis

LPS administration increased the gene expression levels of profibrotic factors MMP-9 ([Fig 6. G](#)) ($P < 0.01$) and TGF- β ([Fig 6. H](#)) ($P < 0.01$) compared to the Sham group, while BM-MSCs administration decreased the gene expression levels of TGF- β ($P < 0.01$) and MMP-9 ($P < 0.01$) compared to the LPS group.

As [Table 2](#) showed, LPS administration increased the levels of pro-fibrotic factors MMP-9 ($P < 0.01$) and TGF- β ($P < 0.01$) in the BALF compared to the Sham group, while BM-MSCs administration decreased the levels of MMP-9 ($P < 0.01$) and TGF- β ($P < 0.05$) in the BALF compared to the LPS group ($P < 0.05$).

Mason staining revealed evidence of the degree of pulmonary fibrosis. Compared

with the Sham group (Fig 7. A), the degree of pulmonary fibrosis increased after LPS administration (Fig 7. B). Compared with the LPS group, BM-MSCs administration decreased the degree of pulmonary fibrosis (Fig 7. C).

Sirius Red staining revealed evidence of collagen fiber deposition in the airway wall. Compared with the Sham group (Fig 8. A), LPS administration increased collagen fiber deposition in the airway wall (Fig 8. B). Compared with the LPS group, BM-MSCs administration decreased collagen fiber deposition in the airway wall (Fig 8. C).

3.11. BM-MSCs attenuated LPS-induced extrapulmonary injury

3.11.1. BM-MSCs attenuated aortic injury

In the Sham group, the aorta was uniformly stained and arranged regularly, the endothelium was smooth and organized, and the elastic fibers had a regular, wavy shape (Fig. 9A). After LPS administration, the endothelium was not smooth or regular, the elastic fibers of the media became sparse, and the elastic fibers lost their regular, wavy shape; furthermore, LPS administration significantly increased the aortic media thickness and significantly decreased the area of the elastic fibers (Fig. 9B). Compared with the LPS group, after BM-MSCs administration, mice in the LPS + MSC group showed a significantly increased elastic fiber area and significantly increased aorta media thickness; furthermore, in the LPS + MSC group, the shape of the endothelial elastic fibers and distribution of the elastic fibers were improved, but these fibers had not completely returned to their normal form (Fig. 9C).

As Table 3 showed, LPS administration increased the medial thickness of the aorta

($P < 0.01$) and decreased the medium membrane elastic fiber area ratio ($P < 0.01$) compared to the Sham group. BM-MSCs administration decreased the medial thickness of the aorta ($P < 0.05$) and increased the medium membrane elastic fiber area ratio ($P < 0.01$) compared to the LPS group.

3.112. BM-MSCs attenuated liver injury

The liver lobules of mice in the Sham group (Fig. 10A, D) were intact and clear, the cells were neatly arranged, the intercellular space was free of edema, the liver structure was clear and regular, and no symptoms of injury were observed. The liver lobules of mice in the LPS group were severely damaged, the liver cells were swollen, the intercellular space was absent, and a large amount of neutrophil (black arrow) infiltration was observed (Fig. 10B, E). The liver lobules of mice in the LPS + MSC group showed significantly less structural damage to the liver tissue, with clearer liver lobules and the infiltration of a small number of neutrophils (Fig. 10C, F).

As shown in Table 3, LPS administration increased the levels of markers of liver disease, ALT ($P < 0.01$) and AST ($P < 0.01$) compared with those in the Sham group, and BM-MSC administration decreased ALT ($P < 0.05$) and AST ($P < 0.05$) levels compared with those in the LPS group.

3.113. BM-MSCs attenuated kidney injury

The kidney tissues from mice in the Sham group were intact and clear, the cells were neatly arranged, the intercellular substance was free of edema, cortical tubular epithelial cells were well-shaped, and almost every epithelial cell contained intact nuclei (Fig. 11A). The kidneys of mice in the LPS group were severely damaged (Fig.

11B), the cells were swollen, and the intercellular space was absent, accompanied by the infiltration of a large number of neutrophils (white arrow) and hemocytes. Compared with the LPS group, the kidney tissues from mice in the LPS + MSC group displayed significantly less kidney tissues structural damage and a small amount of neutrophil infiltration (Fig. 11C), suggesting that BM-MSC administration reduced LPS-induced kidney injury.

As shown in Table 3, compared with the Sham group, LPS administration increased the levels of markers of kidney injury, Urea ($P < 0.01$) and BUN ($P < 0.01$), while Urea ($P < 0.05$) and BUN ($P < 0.05$) levels were significantly decreased in the LPS + MSC group compared with those in the LPS group.

2.114. BM-MSCs attenuated heart injury

In the Sham group, the myocardial tissue was uniformly stained; the myocardial fibers were arranged regularly; the cell morphology, size, and arrangement were normal; and the interstitial spaces were normal (Fig. 12A). In the LPS group, the myocardial tissue was disordered, myocardial degeneration and dissolution were observed, and a large number of inflammatory cells had infiltrated the muscle space (Fig. 12B). Compared with the LPS group, the LPS + MSC group showed less inflammatory cell infiltration, and the myocardial fiber tissue structure was basically normal. The distribution of muscle fibers was improved but had not completely returned to its normal form (Fig.12C).

4. Discussion

Here, we performed preliminary studies to solve some pivotal questions, such as

simple and effective methods for the isolation, culture, and proliferation of chicken BM-MSCs. Next, we aimed to characterize BM-MSCs by evaluating their colony-forming and multidirectional differentiation capabilities and the levels of specific surface markers. Next, we attempted to explore whether BM-MSC administration would exert a positive effect on protecting against LPS-induced ALI, and potential mechanisms, including inflammation, oxidative stress, and the underlying molecular mechanism, were studied.

Using bone marrow tissues obtained from 12-day-old chicken embryos, we separated and characterized a novel subset of stem cells from chicken bone marrow, termed BM-MSCs, which showed unique mesenchymal stem cell-like properties. These plastic-adherent cells exhibited a spindle-like morphology similar to fibroblasts and possessed potent self-renewal potential. BM-MSCs derived from the embryonic mesoderm were induced to differentiate not only into cells that have an endoderm origin, such as adipocytes, osteoblasts, and chondrocytes but also into cells with an ectodermal origin, such as neuroblasts, confirming that BM-MSCs have cross-embryonic layer differentiation potential and further supporting their stem cell-like properties. The directional differentiation of the BM-MSCs was determined by the presence of different stimuli. However, the mechanisms underlying the differentiation of MSCs are unclear. Hence, further research into the inducers and differentiation mechanisms is desperately needed, and these studies will be quite important for cell-based treatment and regenerative medicine.

LPS administration led to an excessive inflammatory response, pulmonary edema,

and the infiltration of inflammatory cells, which are the three main features of ALI^{23,24}. Treatment with BM-MSCs improved the excessive inflammatory response, pulmonary edema, and inflammatory cell infiltration. Based on these results, BM-MSCs are a potential treatment for LPS-induced ALI.

An excessive inflammatory response is a crucial component of the pathogenesis of ALI. We identified that the deleterious accumulation of neutrophils in lung tissues was a crucial component of the development of ALI, which is consistent with previous experimental results²⁵. We found that BM-MSC administration reduced the neutrophil content in the BALF and lung tissues. Thus, BM-MSCs improved ALI partly because BM-MSCs inhibited neutrophil infiltration. The underlying molecular mechanisms through which BM-MSCs reduced the neutrophil content were unclear. Previous studies have shown that CXCL1, IL-8, and TNF- α are chemotactic factors for neutrophils^{26,27}. Strikingly, we found that BM-MSCs reduced the expression levels of CXCL1, IL-8, and TNF- α mRNAs in lung tissues and the BALF levels of CXCL1, IL-8, and TNF- α . Therefore, BM-MSC administration inhibited neutrophil infiltration via suppressing the levels of CXCL1, IL-8, and TNF- α .

We found that MSCs modulated the inflammatory-anti-inflammatory balance because MSCs interacted with neighboring immune cells, including neutrophils, and altered the levels of inflammatory mediators^{28,29}. Various inflammatory cytokines, such as IL-1 β , IL-6, IL-8, and TNF- α , play prominent roles in the development of ALI³⁰. BM-MSC administration decreased the expression levels of CXCL1, IL-1 β , IL-6, IL-8, and TNF- α mRNAs and decreased the expression levels of IL-1RN and

IL-10 mRNAs in lung tissues in the present study. Hence, BM-MSCs modulated the inflammatory-anti-inflammatory balance because MSCs interacted with neighboring immune cells, including neutrophils, and altered the levels of inflammatory mediators.

Previous studies have found that the oxidant/antioxidant balance plays a prominent role in the pathogenesis of ALI and that LPS administration leads to oxidative stress injury. In the present study, we found that BM-MSCs have an antioxidant effect. We demonstrated that BM-MSCs administration downregulated the levels of MDA and MPO but upregulated the levels of SOD and GSH in mice with ALI. Hence, BM-MSCs attenuated oxidative stress injury and modulated the oxidative/antioxidative balance. Thus, BM-MSCs were a preventive tool for sepsis, and one of the mechanisms involved in this role is linked to an increase in antioxidant capacity.

Our data identified that LPS administration led to severe liver, kidney, heart, and aortic injury. We observed the deleterious accumulation of neutrophils within distant vital organs, leading to collateral tissues damage and ultimately multiple organ injury. The activation of neutrophils was a critical factor leading to host tissue injury and organ damage after LPS administration. The present study documented the abnormal accumulation of neutrophils in the lung, liver, kidney, and heart after LPS injection. BM-MSCs protected against LPS-induced liver, kidney, heart, and aortic injury partially because BM-MSCs reduced the neutrophil content. Previous studies ignored the effects of LPS and MSCs on the aorta. Generally, vascular injury occurs before the onset of pulmonary edema and inflammatory cell infiltration. Inflammatory cells, including neutrophils, attacked blood vessels first, and the vascular injury resulted in

increased lung permeability, inflammatory cell infiltration, and pulmonary edema. The protective effects of BM-MSCs on the aorta in acute lung injury mice were first demonstrated. We found that BM-MSC administration prominently prevented the increase in the total protein concentration in the BALF and W/D ratio of lung tissues induced by LPS. Based on these results, BM-MSCs protected against LPS-induced vascular injury.

Mason and Sirius Red staining revealed evidence of the degree of pulmonary fibrosis. Compared with the Sham group, the degree of pulmonary fibrosis increased after LPS administration. Compared with the LPS group, BM-MSCs administration decreased the degree of pulmonary fibrosis. LPS administration increased the levels of pro-fibrotic factors MMP-9 and TGF- β in the BALF, which was reversed by BM-MSCs administration. Similarly, LPS administration increased the gene expression levels of profibrotic factors MMP-9 and TGF- β compared to the Sham group in lung tissue, while BM-MSCs administration decreased the gene expression levels of TGF- β and MMP-9 compared to the LPS group. These evidence showed that BM-MSCs improved LPS-induced pulmonary fibrosis via suppressing TGF- β and MMP-9.

TLR4/Myd88 signaling plays a pivotal role in the development of ALI³¹. Our data demonstrated that LPS administration activated the expression of the TLR4 and Myd88 mRNAs. BM-MSC administration inhibited the expression of the TLR4 and Myd88 mRNAs in lung tissues. Hence, TLR4/Myd88 signaling pathway may be a new molecular mechanism through which BM-MSCs protect against ALI.

In contrast to traditional therapeutic methods, MSC-based therapy is a comprehensive intervention treatment. Previous studies have mainly focused on single factors and single pathways. The idea that one or two pathways exert a decisive role in ALI is not comprehensive and may explain why traditional methods for treating ALI are ineffective. However, MSC administration exerted a positive effect on ALI through multiple mechanisms simultaneously, including inhibition of an excessive inflammatory response and oxidative stress injury. In addition, BM-MSCs administration decreased vascular injury and lung edema. All of these factors combined improved the survival rate of ALI mice.

Collectively, chicken BM-MSCs serve as a potential alternative resource for stem cell therapy and exert a prominent effect on LPS-induced ALI and liver, kidney, heart, and aortic injury. One of the mechanisms involved in this process is linked to the increase in the antioxidant capacity and the increase in the immune response of the organism. BM-MSCs reduced the excessive and uncontrolled inflammatory response involving inflammatory mediators, including IL-1 β , IL-6, IL-8, IL-10, and TNF- α , and effector cells, among which neutrophils play a key role. BM-MSCs reduced the neutrophil content partially because BM-MSCs inhibited the expression of CXCL1, IL-8, and TNF- α . BM-MSCs alter the levels of inflammatory mediators partially by inhibiting TLR4/Myd88 signaling.

Declaration of Competing Interests

There are no competing interests with regard to the publication of this study.

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Figures and legends

Fig 1. Biological characteristics of BM-MSCs. **(A)** The cellular morphology of chicken BM-MSCs was determined at different passages P1 (a), P5 (b), P15 (c), and P25 (d). BM-MSCs were spindle-like cells. (magnification, x 40). **(B)** Detection of multilineage differentiation potential of BM-MSCs. Many calcium nodules appeared in the cytoplasm, and alizarin red staining showed a positive reaction for calcified nodules in BM-MSCs that differentiated under osteogenic induction conditions for 21 days (a). Many lipid droplets formed in the cytoplasm, and lipid droplets were successfully stained with Oil Red O in cells cultured under adipocytic induction conditions for 21 days (b). BM-MSCs were positive for Alcian blue staining after an incubation with chondrogenic medium for 21 days (c). The cellular morphology changed from a spindle-shaped morphology to a multipolar and stellate morphology, and the BM-MSCs grew many branches and formed many synapses (white arrows) on

the cell surface after culture under neurogenic induction conditions for 21 days (d). (magnification, x 40). (C) The results of immunofluorescence staining. The high expression of Nestin (a) and MAP-2 (b), markers of neural cells, in differentiated BM-MSCs were detected, while Nestin (c) and MAP-2 (d) were not detected in undifferentiated BM-MSCs. (magnification, x 40).

Fig 2. Assessment of survival rates, systemic inflammation, and oxidative stress injury. (A) Assessment of survival rates. The serum levels of CXCL-1 (B), IL-1 β (C), IL-8 (D), and TNF- α (E) were detected. The levels of SOD (F), MDA (G), GSH (H), and MPO (I), as indicators of oxidative stress injury, were detected. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ### $P < 0.01$ compared with the LPS group. Values are expressed as the means $\pm$ SD.

Fig 3. BM-MSC administration reduces lung injury. Histological assessment of lung tissues from the three groups revealed evidence of the degree of lung injury, inflammatory cell infiltration, and interstitial edema. Compared with the lung tissues of normal mice (**A, D, and G**), the degree of lung injury, inflammatory infiltration, and interstitial edema increased after LPS administration (**B, E, and H**). Treatment with MSCs significantly decreased the degree of lung injury, inflammatory cell infiltration, and interstitial edema (**C, F, and I**) at each point. (magnification, x 40).

Fig 4. The density of neutrophils in the lung tissue 6 h after LPS treatment. The microphotograph shows the density of neutrophils in the lung tissue. (A) Sham group, (B) LPS group, (C) LPS + MSC group, (D) the density of neutrophils in lung tissue. ** $P < 0.01$ compared with the Sham group. ## $P < 0.01$ compared with the LPS group, Values are expressed as the means $\pm$ SD. (magnification, x 400).

Fig 5. Effect of BM-MSCs on lung edema. (A) The wet/dry ratio of lung tissue. (B) Detection of total protein concentration. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ##

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Fig 6. BM-MSCs prevented lung injury via the TLR4/Mdy88 pathway 6 h after LPS treatment. The gene expression levels of CXCL-1 (A), IL-1RN (B), IL-1β (C), IL-8 (D), IL-10 (E), Mdy88 (F), MMP-9 (G), TGF-β (H), TLR4 (I), and TNF-α (J) were detected. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ## $P < 0.01$ compared with the LPS group. Values are expressed as the means $\pm$ SD.

Fig 7. BM-MSCs improved LPS-induced pulmonary fibrosis at 24 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. Masson staining revealed evidence of the degree of pulmonary fibrosis. (magnification, $\times 40$).

Fig 8. BM-MSCs improved LPS-induced pulmonary fibrosis in the airway wall at 24

h after LPS treatment. **(A)** Sham group. **(B)** LPS group. **(C)** LPS + MSC group. Sirius Red staining were performed to identify the collagen fiber deposition in the airway wall. (magnification, x 40).

Fig 9. BM-MSCs improved LPS-induced aortic tissues at 48 h after LPS treatment.

(A) Sham group. **(B)** LPS group. **(C)** LPS + MSC group. (magnification, x 400).

Fig 10. BM-MSCs improved LPS-induced liver injury at 48 h after LPS treatment. **(A,**

D) Sham group. **(B, E)** LPS group. **(C, F)** LPS + MSC group. (magnification, A, B,

and C x 40. D, E, and F x 400).

Fig 11. BM-MSCs improved LPS-induced kidney injury at 48 h after LPS treatment.

(A) Sham group. (B) LPS group. (C) LPS + MSC group. (magnification, x 40).

Fig 12. BM-MSCs improved LPS-induced heart injury at 48 h after LPS treatment. (A)

Sham group. (B) LPS group. (C) LPS + MSC group. (magnification, x 40).

Tables

Table 1. Cell counts in the BALF (cells/ml) at 6 h after LPS treatment.

Group	Con	LPS	LPS + MSC
Total cells in BALF	1.35 ± 0.21	8.91 ± 2.14**	6.98 ± 0.94##
Neutrophils in BALF	0.04 ± 0.01	7.24 ± 1.67**	5.84 ± 2.38##
Lymphocytes in BALF	0.04 ± 0.01	0.15 ± 0.03	0.15 ± 0.04
Macrophages in BALF	1.07 ± 0.38	1.32 ± 0.27	0.96 ± 0.16
Eosinophils in BALF	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.02

Increases in the numbers of total cells and neutrophils in the BALF were detected after the LPS injection, while BM-MSCs exerted a prominent effect on preventing the increase in the numbers of total cells and neutrophils in the BALF. Treatment with LPS and BM-MSCs did not alter the numbers of lymphocytes, macrophages, or eosinophils in the BALF. ** indicates that the difference in the data is very significant compared with the Sham group ## indicates that the difference in the data is very significant compared with the LPS group. Values are expressed as the means ± SD.

Table 2. Cytokine levels in the BALF (pg/ml) at 6 h after LPS treatment.

	Con	LPS	LPS + MSC
CXCL-1	9.24 ± 1.65	594.82 ± 128.67**	466.4 ± 83.7 [#]
IL-6	9.67 ± 2.33	1021.96 ± 297.69**	864.44 ± 130.75 [#]
IL-8	12.38 ± 3.54	365.72 ± 82.66**	251.49 ± 76.51 [#]
IL-1RN	54.15 ± 3.88	20.45 ± 10.79**	38.52 ± 15.17 [#]
IL-10	9.62 ± 1.64	495.31 ± 136.6**	660.4 ± 174.1 ^{##}
TNF-α	18.37 ± 16.38	343.82 ± 97.7**	211.45 ± 59.67 ^{##}
MMP-9	12.64 ± 2.39	98.47 ± 11.54**	48.34 ± 6.73 ^{##}
TGF-β	23.86 ± 5.29	132.98 ± 30.87**	103.41 ± 26.19 [#]

Our data observed an effect of LPS in increasing the levels of CXCL-1, IL-6, IL-8, IL-10, MMP-9, TNF-α, and TGF-β and decreasing the levels of IL-1RN in the BALF. Compared with the LPS group, BM-MSCs administration increased the levels of IL-10 and IL-1RN and decreased the levels of CXCL-1, IL-6, IL-8, MMP-9, TNF-α, and TGF-β in BALF. [#] $P < 0.05$ compared with the LPS group. ^{**} $P < 0.01$ compared with the Sham group. ^{##} $P < 0.01$ compared with the LPS group. Values are expressed as the means ± SD.

Table 3. The degrees of organ injury.

	Con	LPS	LPS + MSC

Alanine aminotransferase (U/L)	30.13 ± 7.51	148.33 ± 28.59 ^{**}	114.27 ± 25.48 [#]
Aspartate aminotransferase (U/L)	119.64 ± 14.96	180.67 ± 26.45 ^{**}	150.25 ± 25.31 [#]
Cre (μmol/L)	55.91 ± 11.98	214.39 ± 10.35 ^{**}	184.47 ± 15.49 [#]
BUN (mmol/L)	7.99 ± 1.64	13.56 ± 2.31 ^{**}	10.59 ± 2.16 [#]
Medial thickness of aorta	51.4 ± 10.12	65.46 ± 11.8 ^{**}	57.79 ± 11.8 [#]
Medium membrane elastic fiber area ratio	48.93 ± 9.24	27.7 ± 4.01 ^{**}	41.57 ± 8.34 ^{##}

LPS injection increased markers of liver damage ALT and AST and markers of kidney damage Cre and BUN, which was improved by a 4-week AE. LPS administration increased the medial thickness of the aorta and decreased the medium membrane elastic fiber area ratio, which was reserved by a 4-week AE. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ## $P < 0.01$ compared with the LPS group. Values are expressed as the means ± SD.

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Effect of BM-MSCs on lung edema. (A) The wet/dry ratio of lung tissue. (B) Detection of total protein concentration. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ## $P < 0.01$ compared with the LPS group. Values are expressed as the means $\pm$ SD.

Figure 6

BM-MSCs prevented lung injury via the TLR4/Mdy88 pathway 6 h after LPS treatment. The gene expression levels of CXCL-1 (A), IL-1RN (B), IL-1 β (C), IL-8 (D), IL-10 (E), Mdy88 (F), MMP-9 (G), TGF- β (H), TLR4 (I), and TNF- α (J) were detected. * $P < 0.05$ compared with the Sham group. # $P < 0.05$ compared with the LPS group. ** $P < 0.01$ compared with the Sham group. ## $P < 0.01$ compared with the LPS group. Values are expressed as the means $\pm$ SD.

Figure 7

BM-MSCs improved LPS-induced pulmonary fibrosis at 24 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. Mason staining revealed evidence of the degree of pulmonary fibrosis. (magnification, x 40).

Figure 8

BM-MSCs improved LPS-induced pulmonary fibrosis in the airway wall at 24 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. Sirius Red staining were performed to identify the collagen fiber deposition in the airway wall. (magnification, x 40).

Figure 9

BM-MSCs improved LPS-induced aortic tissues at 48 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. (magnification, x 400).

Figure 10

BM-MSCs improved LPS-induced liver injury at 48 h after LPS treatment. (A, D) Sham group. (B, E) LPS group. (C, F) LPS + MSC group. (magnification, A, B, and C x 40. D, E, and F x 400).

Figure 11

BM-MSCs improved LPS-induced kidney injury at 48 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. (magnification, x 40).

Figure 12

BM-MSCs improved LPS-induced heart injury at 48 h after LPS treatment. (A) Sham group. (B) LPS group. (C) LPS + MSC group. (magnification, x 40).

Supplementary Files

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