Original Article

The effect of human adipose-derived stem cells on lipopolysaccharide-induced acute respiratory distress syndrome in mice

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Background: Acute respiratory distress syndrome (ARDS) is a type of acute respiratory failure in critically ill patients. Recently, several treatment modalities have been proposed for ARDS, but it still has a high mortality rate. In general, the role of mesenchymal stem cells (MSCs) in controlling inflammatory responses has been studied in various immune-associated diseases in humans and animals. However, only a few studies reported adipose-derived stem cells (ASCs), which are easier to isolate, are currently emerging as an attractive treatment option in ARDS. Therefore, in this study, we investigated the therapeutic effects of human ASCs and the regulation of inflammatory responses in an ARDS mouse model.

Methods: In the ARDS model, lipopolysaccharide (LPS) (5 mg/kg) was administered via the intra tracheal injection method. The mice were divided into the following four groups: (I) saline + medium; (II) saline + ASCs (2×10^5); (III) LPS + medium; (IV) LPS + ASCs. The ARDS observation time was divided into short and long term. LPS administration increased the concentration of proinflammatory cytokines, which was a consistent systemic inflammatory response.

Results: LPS/ASC group showed less neutrophil infiltration and less alveolar hemorrhage or congestion than did the LPS group. The lung injury scores of the LPS/ASC group were lower than those of the LPS group (3.8±0.9 *vs.* 6.8±1.1; P=0.03) at day 2. Compared to the LPS group, LPS/ASC group showed reduced collagen deposition around the vessels and fibrosis accompanied by alveolar septal or interstitial thickening and lower MPO levels than did the LPS group (453.2±26.2 *vs.* 670.2±65.9 pg/mL; P<0.01) at day 7.

Conclusions: ASC therapy can inhibit neutrophil recruitment, which shows trend of reducing short term lung injury (day 2) and affecting fibrosis in long term (day 7). Further studies are warranted to understand the mechanism and improve the therapeutic effect of ASCs.

Keywords: Acute respiratory distress syndrome (ARDS); lipopolysaccharide (LPS); adipose-derived stem cells (ASCs); stem cell

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Introduction

Acute respiratory distress syndrome (ARDS) is an acute respiratory failure in critically ill patients (1). ARDS is characterized by bilateral diffuse alveolar injury, profound inflammation, increased vascular permeability, and alveolar flooding after exposure to a rapid inflammatory reaction that has various causes (1). In the early stages, endogenous pro-inflammatory cytokines, coagulation pathway hyperactivity, or their imbalance could lead to mortality if multi organ failure or circulatory collapse occurs. The extent and duration of these dysfunctions are related to the morbidity and mortality of ARDS (2,3).

ARDS is known to progress pathologically in three stages. The initial exudation stage involves edema and inflammation that develop about a week after lung injury. The subsequent fibroplasia stage lasts for about 4 weeks, and the final fibrosis stage involves the restoration of the lung, accumulation of mature collagen, and progression of pulmonary fibrosis (4). However, recent studies have shown that vigorous collagen synthesis occurs from the beginning of ARDS (5), and a correlation exists between the presence of procollagen peptide III and a fatal outcome (6). Therefore, the processing of fibrosis is considered the early period of ARDS.

Over the past several decades, significant advances have been made in understanding the various causes, mechanisms, and pathophysiology of ARDS owing to several randomized clinical trials (7-9). In particular, low tidal volume ventilation (7), the use of positive end expiratory pressure (8), and conservative fluid management have contributed to the reduction of mortality (9). However, the mortality rate of patients with ARDS is still high at 30–50% (10,11); hence, new therapeutic strategies are needed to reduce the mortality rate. Mesenchymal stem cells (MSCs) are non-hematopoietic stromal cells with low immunogenicity that secrete multiple paracrine factors, including endothelial and epithelial growth factors, anti-inflammatory cytokines, and antimicrobial peptides (12-16). Studies have indicated that the use of MSCs could be a therapeutic modality for ARDS. However, harvesting bone marrow is a painful procedure, and low numbers of MSCs are present in the harvested bone marrow, which can be imposed a load on some patients with severe complications (17).

For clinical application, an alternative source of MSCs is the adipose tissue that is abundant and easily accessible, and yields large numbers of adipose-derived MSCs (ASCs), which are easier to isolate via less-invasive procedures. Compared to other tissues, ASCs can be obtained from a large amount of adipose tissue lipoaspirates and can easily be increased in number, thereby helping secure a large number of MSCs that can produce clinically effective doses (18). In addition, studies have shown that ASCs share immune-enhancing properties with bone marrow-derived stem cells (17-19). However, only a few studies reported adipose-derived stem cells (ASCs), which are easier to isolate, are currently emerging as an attractive treatment option in ARDS and studied acute short term effect only.

The purpose of this study was to investigate whether human ASCs have a therapeutic effect in the short term (day 2) and in long term (day 7) of lipopolysaccharide (LPS)-induced ARDS in a mouse model.

Methods

Mice

C57BL/6 mice (7-week-old males weighing 21–23 g; OrientBio, Gyeonggi-do, Korea) were used in this study. All mouse care and animal experiments were performed in accordance with the protocols approved by the Laboratory of Animal Research of Asan Medical Center (Seoul, Korea, IACUC No. 14-01-027). Monitoring was performed every 24 h (n=5 in all groups).

Purchase and cultivation of stem cells

Human ASCs (StemPRO® Human Adipose-Derived Stem Cells; Thermo Fisher, MA, USA) were purchased and subcultured according to the manufacturer's manual. Four to five passages of human ASCs were harvested and administered.

ARDS mouse model and ASC transplantation

We divided the mice into four groups: control group; LPS (Escherichia coli, serotype 055:B5; Sigma-Aldrich, MO, USA)-induced ARDS (LPS group); ASCs only (ASC group); and human ASCs following LPS-induced ARDS (LPS/ASC group). Ketamine (70 mg/kg; Yuhan Pharma, Seoul, Korea; Narcotics Handling permission: Seoul 574) and xylazine (9 mg/kg; Bayer Korea Ltd., Seoul, Korea) were used to anesthetize the mice.

In the LPS/ASC group, after the mice were anesthetized, LPS was injected into the trachea at a dose of 5 mg/kg in saline to induce ARDS, and human ASCs (2×10^5 cells in

0.1 mL of the medium; Gibco™, MA, USA) were intravenously injected into the tail vein 4 h later. The LPS group received the medium instead of human ASCs. The ASC group was injected with ASCs (2×10^5 cells in 0.1 mL of the medium) after saline intratracheal (IT) injection IT. The control group was intravenously injected with the medium after saline IT injection.

The short term ARDS model mice were sacrificed 2 days after LPS injection. The long term ARDS fibrosis model mice were injected the same dose of LPS repeatedly on days 0 and 3 (20). They were sacrificed after 7 days.

Tissue and bronchoalveolar lavage fluid (BALF) collection

For tissue harvesting, the lungs were perfused with normal saline. The right hilum was identified, tied off, and removed with the separate lobes, which were frozen immediately in liquid nitrogen and stored at −80 ℃. The trachea was then isolated, and using a blunt-tip needle and syringe, the remaining left lung was inflated with 4% paraformaldehyde by using a 25-cm pressure column. The trachea was then tied off and the lung was removed for fixation overnight in formalin, followed by embedding in paraffin. We collected the BALF for cell analysis and cytokine measurement and lung tissue for histological analysis.

Neutrophil count and protein content measurement in the BALF

The protein content of the BALF was measured using the bicinchoninate quantification method. After Diff-Quick staining (Richard-Allan Scientific™, CA, USA), the number of neutrophils was counted using the method described by Everhart *et al*. (21). We counted 500 cells in a ×400 field of view under an optical microscope, thereby calculating the cell numbers and neutrophil percentage.

Measurement variable

Tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, and IL-17 were measured using an enzyme-linked immunosorbent assay (ELISA) kit. Myeloperoxidase (MPO) was measured using the DuoSet® ELISA kit. Vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF)-1 were measured using a Luminex reading program with a Luminex Screening and Performance assay kit (all kits from R&D Systems Inc., MN, USA).

Histological analysis

The fixed lungs were randomly cut, cleaned, dehydrated, embedded in paraffin, cut into 5-μm-thick sections, and examined. The severity of lung injury was determined using these paraffin-embedded lung sections stained with hematoxylin and eosin, and the connective tissue around the cells was observed using Masson's trichrome staining. Lung injury was assessed by one pathologist who was blinded to the grouping of the mice. Histological variables associated with ARDS were examined, and each variable was given a grade from 0 to 3. The following variables were assessed: (I) intra-alveolar infiltration of neutrophils; (II) interstitial infiltration of neutrophils; (III) perivenous infiltration of neutrophils; (IV) pulmonary congestion; and (V) alveolar hemorrhage (total score, 0–15) (22). Lung fibrosis was evaluated using trichrome-stained lung sections by determining the degree of increased thickness of the interalveolar septa and the presence of fibrotic lesions with total or subtotal distortion of the parenchymal architecture compared with that of the normal architecture (23).

Data processing and analysis

All results are expressed as mean ± standard error of the mean. Differences among groups were assessed using one-way analysis of variance or the Kruskal-Wallis rank analysis of variance. Differences between pairs were assessed using Student's t-test or the Mann-Whitney test. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA), with the significance level set at $P<0.05$.

Results

ASC treatment attenuated neutrophil infiltration in the lung tissue of the LPS-induced ARDS model at day 2

The lung tissue of the control and ASC groups had normal structures such as alveolar septa, alveolar lumen, and capillary vessels (*Figure 1A,B*); however, the lung structures were not preserved in the LPS group (*Figure 1C*). In contrast, the LPS/ASC group showed less neutrophil infiltration and less alveolar hemorrhage or congestion than did the LPS group (*Figure 1D*). The lung injury scores of the LPS/ASC group were lower than those of the LPS group (3.8±0.9 *vs.* 6.8±1.1; P=0.03) (*Figure 1E*).

In the LPS/ASC and LPS groups, the total cell count increased in the BALF at day 2 [$(116.3\pm9.3)\times10^4$

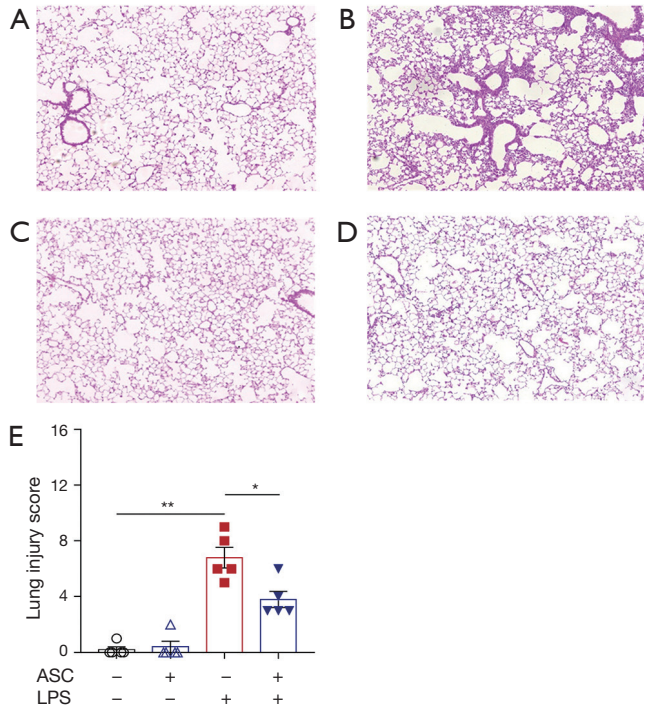

Figure 1 Adipose-derived stem cells (ASCs) attenuate lung injury in lipopolysaccharide (LPS)-induced acute respiratory distress syndrome at the short term. Lungs from each experimental group are fixed in 4% paraformaldehyde at 48 ℃ for histological evaluation using hematoxylin and eosin staining. (A) In the control and (B) ASC groups, the normal pulmonary structures, such as the alveolar septa, alveolar lumen, and capillaries, are well preserved. Inflammatory cell infiltration is not observed. (C) In the LPS group, the pulmonary structures, such as the alveolar septa and alveolar lumen, are not preserved. Perivascular edema and intrapulmonary hemorrhage are observed. Inflammatory cell infiltration, perivascular cuffing, and floating phagocytes are observed in the alveolar lumen. (D) In the LPS/ASC group, the alveolar septa and alveolar lumen are relatively well preserved. Intrapulmonary hemorrhage is less prominent than that in the lungs of the LPS-treated mice. Perivascular cuffing is also less prominent than that in the lungs of the LPS-treated mice. Inflammatory cell infiltration is less observed (magnification, ×100). (E) Compared to the LPS group, the LPS/ASC group shows lower lung injury scores (LPS/ASC, 3.8±0.9 *vs.* LPS, 6.8±1.1; *P<0.05). No difference is observed between the control and ASC groups. However, the lung injury scores are significantly different between the control and LPS groups (control, 0.2±0.3 *vs.* LPS, 6.8±1.1; **P<0.01). Five mice per each group were used. (A) Control group; (B) ASC group, ASCs only; (C) LPS group, LPS-induced acute lung injury; (D) LPS/ASC group, ASC injection following LPS-induced acute lung injury; (E) lung injury score at 2 days.

vs. (133.7±15.5)×10^4/mL; P=0.22]. Although ASC administration resulted in a reduced total cell count at day 2 compared to those in the LPS group, this did not reach statistical significance. The neutrophil percentage and count in the BALF of the LPS/ASC group showed a decreasing tendency compared to those in the LPS group [66.2%±3.8% *vs.* 75.3%±2.9%; P=0.05 and (75.7±8.7)×10^4 *vs.* (101.1±13.8)×10^4/mL; P=0.09, respectively] (*Figure 2*).

The increase in cell count in the BALF was accompanied by a significant increase in the levels of inflammatory mediators at day 2. Cytokine levels tended to decrease slightly but did not show any statistical significance between the LPS/ASC and LPS groups: TNF-α (73.7±21.7 *vs.* 102±15.3 pg/mL; P=0.22); IL-1β (55.3±14.5 *vs.* 47.2±5.4 pg/mL; P=0.55); IL-6 (566.5±253.8 *vs.* 877±211.5 pg/mL; P=0.15); IGF-1 (2.5±0.8 *vs.* 3.6±0.8 ng/mL; P=0.39); VEGF (474.4±170.2 *vs.* 607.7±77.7 pg/mL; P=0.69); HGF (689.9±183.6 *vs.* 785.9±74 pg/mL; P=0.84); and MPO (1±0.1 *vs.* 1.2±0.3 ng/mL; P=0.84) (*Figure 3*).

ASC treatment attenuated interstitial fibrosis in the LPS-induced ARDS model at day 7

At day 7, the LPS group showed inflammatory cell infiltration, mild alveolar septa, and interstitial thickening (*Figure 4A*). However, in the LPS/ASC group, the alveolar septa and alveolar lumen were relatively well preserved, and inflammatory cell infiltration was observed in the alveolar space and interstitium. Interstitial thickening was not observed (*Figure 4*).

Masson's trichrome staining showed inflammatory cell infiltration and mild thickening in the alveolar septa and interstitium of the LPS group, with prominent perivascular collagen and suspicion of fibrosis (*Figure 5A*). However compared to the LPS group, the LPS/ASC group showed reduced collagen deposition around the vessels and fibrosis accompanied by alveolar septal or interstitial thickening (*Figure 5*).

The total cell count in the ASC group was also higher than those in the control group at day 7. The total cell count in the BALF was not different between the LPS/ASC and LPS groups [(24.3±3.7)×10^4 *vs.* (21±0.6)×10^4/mL; P=0.13]. The percentage and count of neutrophils were also not different between the LPS/ASC and LPS groups [19.6%±3.4% *vs.* 19.2%±4.8%; P=0.69 and (4.6±0.8)×10^4 *vs.* (3.7±1)×10^4/mL; P=0.31, respectively].

The LPS/ASC group had lower MPO levels than did the LPS group (453.2±26.2 *vs.* 670.2±65.9 pg/mL; P<0.01).

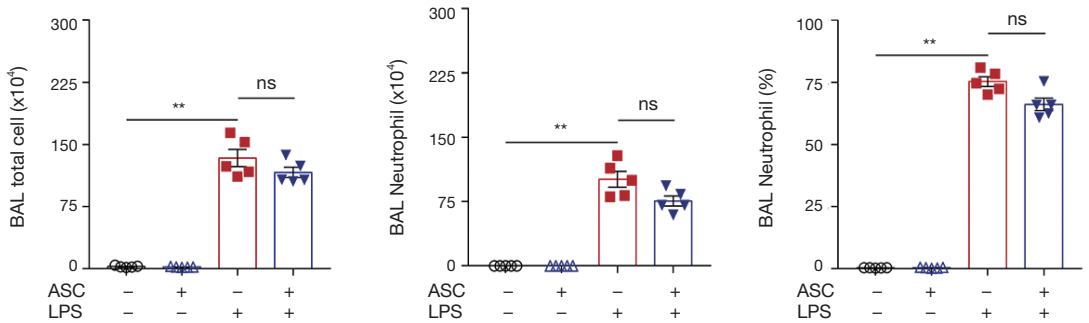

Figure 2 The effect of adipose-derived stem cells (ASCs) on the total cell count and percentage of neutrophils in the bronchoalveolar lavage fluid (BALF) at the short term. ASC injections tend to decrease the cell count in the BALF in mice [lipopolysaccharide (LPS)/ASC, (116.3±9.3)×10^4 *vs.* LPS, (133.7±15.5)×10^4/mL; P=0.22]. The LPS group has a higher total cell count in the BALF than does the control group [control, (2.6±0.7)×10^4 *vs.* LPS, (133.7±15.5)×10^4/mL; **P<0.01]. The count and percentage of neutrophils show similar decreases: neutrophil count [LPS/ASC, (75.7±8.7)×10^4 *vs.* LPS, (101.1±13.8)×10^4/mL; P=0.09]; neutrophil percentage (LPS/ASC, 66.2%±3.8% *vs.* LPS, 75.3%±2.9%; P=0.05). Compared to the control group, the LPS group shows more significant increases in neutrophil count [control, (0±0)×10^4 *vs.* LPS, (101.1±13.8)×10^4/mL; **P<0.01] and neutrophil percentage (control, 0.4%±0.1% *vs.* LPS, 75.3%±2.9%; **P<0.01). Values are mean ± standard deviation of five mice per group.

However, the concentration of IL-17 was not significantly different between the LPS/ASC and LPS groups (6.0±0.1 *vs.* 6.5±0.2 pg/mL; P=0.60) (*Figure 6*).

Discussion

In this study, we investigated the effects of human ASCs in an LPS-induced ARDS mouse model, as well as the relevant inflammatory factors and histological changes. The injection of ASCs resulted in a tendency of decrease in the percentage of neutrophils in the BALF and an improvement in histological assessment of lung injury at the short term. In addition, a decrease in MPO was observed in the long term of acute lung injury, as was a decrease in fibrosis on histological analysis. However, the decrease in inflammatory cytokine levels was not significant and the treatment effect was minimal.

The pathogenesis of ARDS involves lung endothelial injury, alveolar epithelial injury, and the accumulation of protein-rich fluid and cellular debris in the alveolar space (1). Among these, LPS-induced acute lung injury is caused by the interactions of IL-1β, IL-6, and TNF-α, which are inflammatory cytokines secreted by LPS-activated neutrophils, alveolar macrophages, and vascular endothelial cells, that are mainly involved in this mechanism (1,24). The mechanism of acute lung injury caused by neutrophils is important, and in this experiment, we observed that the epithelial and vascular barriers were destroyed to induce alveolar flooding. The most important factors in the LPS-induced ARDS model are neutrophils (25,26), which secrete a large amount of reactive oxygen species (27). ARDS reduced when LPS or TNF was administered to animal models with artificially removed neutrophils (25,28,29).

We observed a decrease in neutrophil infiltration in the short term injury model after ASC treatment (day 2); moreover, the slight thickening of the alveolar septum and interstitium was reduced and the deposition of collagen around the veins was less pronounced. In addition, ASC treatment resulted in a tendency of decrease in the neutrophil percentage in the BALF at the initial stage. ASC treatment substantially reduced LPS-induced lung injury and neutrophil infiltration as shown by hematoxylin and eosin staining. Because neutrophil activation and transmigration into the alveolar space and lung interstitium play crucial roles in the development of ARDS (30), reducing neutrophil infiltration by administering ASCs may have a clinically relevant therapeutic potential. Several studies mainly for bone marrow derived stem cell, a few ASC, as summarized in *Table 1*, have reported the ability of human ASC to reduce inflammation and lung injury. Previous studies for ARDS were done mainly for bone marrow derived stem cells not for ASC and studied only acute effect. There are few studies for effects of ASC and subacute effects of ASC in ARDS model. However, our study did not show any significant effects of human ASCs on cytokines and growth factors, such as IL-1β, IL-6, or

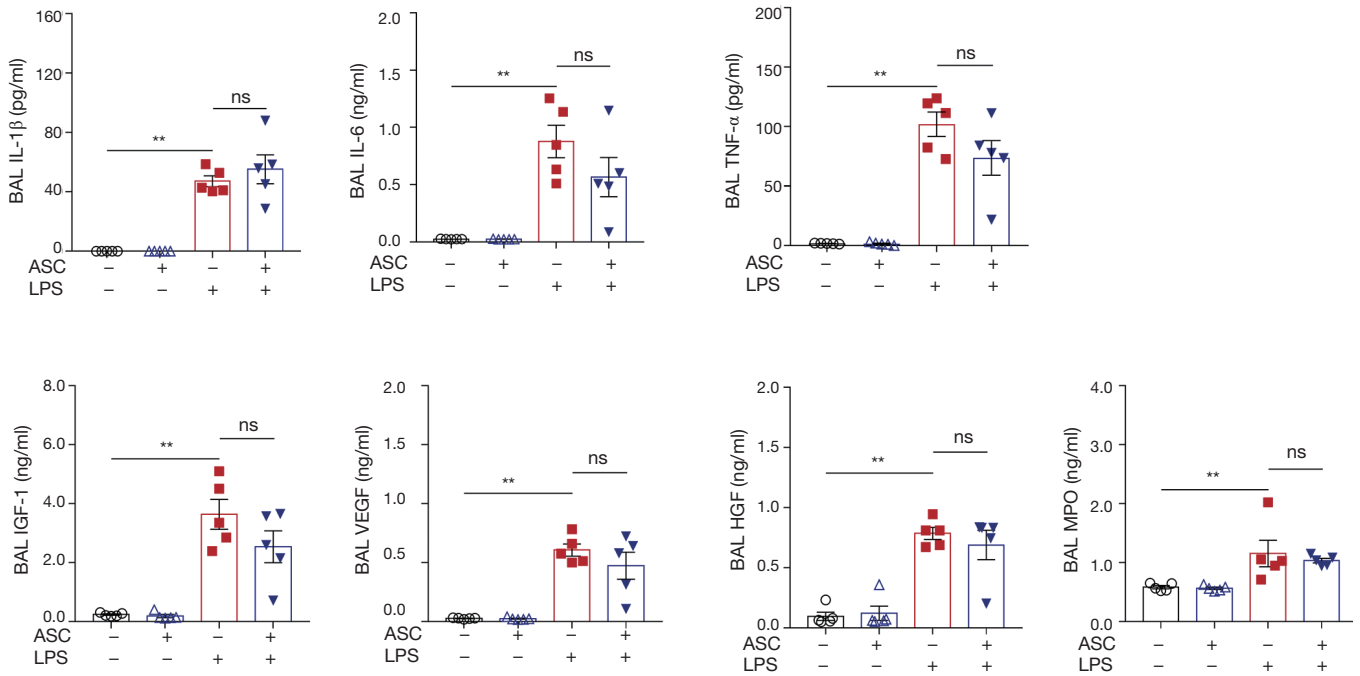

Figure 3 The effect of adipose-derived stem cells (ASCs) on bronchoalveolar lavage fluid (BALF) cytokine levels in mice at the short term. The levels of BALF interleukin (IL)-1β show no effects of ASC treatment [lipopolysaccharide (LPS)/ASC, 55.3±14.5 *vs.* LPS, 47.2±5.4 pg/mL; P=0.55]; the LPS group shows more significant increases than does the control group (control, 0.2±0.3 *vs.* LPS, 47.2±5.4 pg/mL; **P<0.01). Tumor necrosis factor (TNF)-α levels are not significant, but are lower in the LPS/ASC group than in the LPS group (LPS/ASC, 73.7±21.7 *vs.* LPS, 102±15.3 pg/mL; P=0.22); the LPS group shows more significant increases than does the control group (control, 1.7±0.2 *vs.* LPS, 102±15.3 ng/mL; **P<0.01). Similarly, no difference is observed in the levels of IL-6 (LPS/ASC, 566.5±253.8 *vs.* LPS, 877±211.5 pg/mL; P=0.15) and insulin-like growth factor (IGF)-1 (LPS/ASC, 2.5±0.8 *vs.* LPS, 3.6±0.8 ng/mL; P=0.39). Nevertheless, the LPS group shows more significant increases than does the control group in the levels of IL-6 (control, 25.7±1 *vs.* LPS, 877±211.5 ng/mL; **P<0.01) and IGF-1 (control, 0.2±0.1 *vs.* LPS, 3.6±0.8 ng/mL; **P<0.01). In addition, the levels of vascular endothelial growth factor (VEGF) (LPS/ASC, 474.4±170.2 *vs.* LPS, 607.7±77.7 pg/mL; P=0.69), hepatocyte growth factor (HGF) (LPS/ASC, 689.9±183.6 *vs.* LPS, 785.9±74 pg/mL; P=0.84), and myeloperoxidase (MPO) (LPS/ASC, 1±0.1 *vs.* LPS, 1.2±0.3 ng/mL; P=0.84) show minimal differences between the LPS/ASC and LPS groups. The LPS group shows more significant increases than does the control group in the levels of VEGF (control, 29±3.66 *vs.* LPS, 607.7±77.7 ng/mL; **P<0.01), HGF (control, 96.6±51.1 *vs.* LPS, 785.9±74 ng/mL; **P<0.01), and MPO (control, 0.6±0.1 *vs.* LPS, 1.2±0.3 ng/mL; **P<0.01). Values are mean ± standard deviation of five mice per group.

TNF-α, and showed only partial effects on acute lung injury in the mouse model of lung injury.

Zhang *et al.* showed that the administration of ASCs by oropharyngeal aspiration from mice in the acute lung injury mouse model improved lung injury and inflammatory response, and that anti-inflammatory effects mediated by IL-6 were useful in acute lung injury (35,38). We used human ASCs instead of mouse ASCs; hence, we suggest the use of heterologous ASCs and the different route of ASCs contributed to this discrepancy in our study.

Traditionally, ARDS has been known to progress over time with distinctly different features of inflammation, fibroplasia, and fibrosis, but recent research has shown that these concepts have changed significantly. Fibrosing alveolitis starts from the fibroplasia stage even before the occurrence of ARDS fibrosis (39,40), and the genes involved in inflammation and repair are co-expressed in the very early stage of ARDS (41); moreover, several common mediators are involved in these processes. We observed that MPO concentration in the BALF was significantly lower in the ASC group than in the LPS group in the long term (day 7), where the fibroplasia stage became. In addition, the

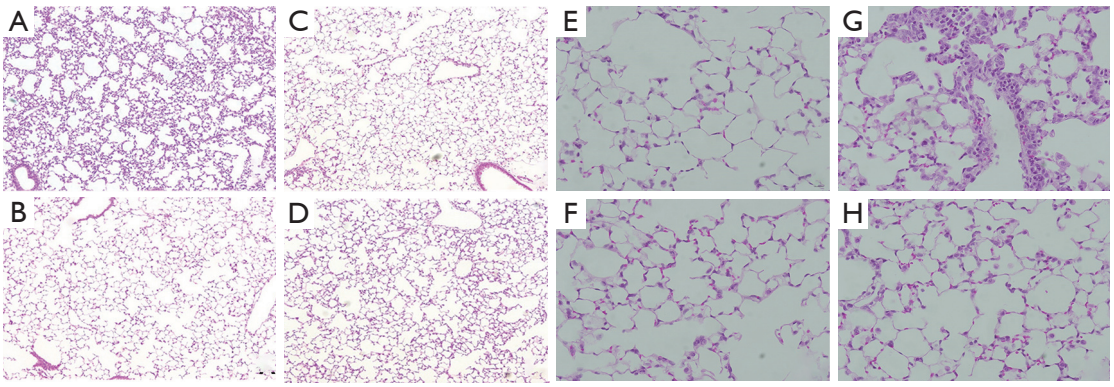

Figure 4 Histologic assessment of the effects of human adipose-derived stem cells (ASCs) on lipopolysaccharide (LPS)-induced lung injury at the long term, (A,B,C,D) ×100 and (E,F,G,H) ×400. Histologic evaluation is performed on hematoxylin and eosin-stained lung sections. In (B,E) the control and (C,F) ASC groups, the normal pulmonary structures, such as the alveolar septa, alveolar lumen, and capillary, are well preserved. Inflammatory cell infiltration is not observed. (A,G) In the LPS group, inflammatory cell infiltration is observed in the alveolar space and interstitium. Mild thickening in the alveolar septa and interstitium is suspected. (D,H) In the LPS/ASC group, the alveolar septa and alveolar lumen are relatively well preserved, and inflammatory cell infiltration is observed in the alveolar space and interstitium. Interstitial thickening is not observed. Five mice per each group were used. (B) Control group; (C) ASC group, ASCs only; (A) LPS group, LPS-induced acute lung injury; (D) LPS/ASC group, ASC injection following LPS-induced acute lung injury.

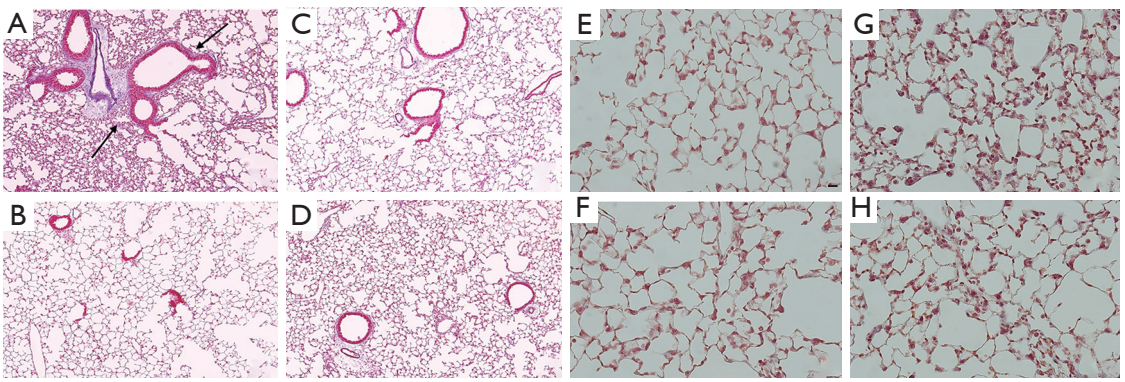

Figure 5 Human adipose-derived stem cells (ASCs) reduce fibrosis, as revealed by Masson's trichrome staining, in lipopolysaccharide (LPS)-induced lung injury at the long term, (A,B,C,D) ×100 and (E,F,G,H) ×400. Lungs fixed in 4% paraformaldehyde sections are again fixed in Bouin's solution and stained with Weigert's iron hematoxylin solution and Biebrich scarlet-acid fuchsin solution. Then, the sections are treated with phosphomolybdic and phosphotungstic acids and are stained again with aniline blue. The (B,E) control group and (C,F) ASC group have well-preserved normal pulmonary architectures. (A,G) Inflammatory cell infiltration and mild thickening are observed in the alveolar septa and interstitium in the LPS group, together with prominent perivascular collagen. (D,H) Compared to the LPS group, the LPS/ASC group shows collagen deposition around the vessels and fibrosis accompanied by reduced alveolar septal or interstitial thickening. Five mice per each group were used. (B) Control group; (C) ASC group, ASCs only; (A) LPS, LPS-induced acute lung injury; (D) LPS/ASC, ASC injection following LPS-induced acute lung injury.

fibrosis associated with peripheral collagen deposition and the alveolar septa or interstitium was reduced in the ASC group at the time of histological fibroplasia. The reduction in the levels of fibrogenic mediators resulted in improved lung remodeling. This might have an effect on fibrosis.

Fortunately, no significant adverse effects were found in the alveolar tissues. Neither the ASC group nor the control group showed any inflammatory reaction or swelling in

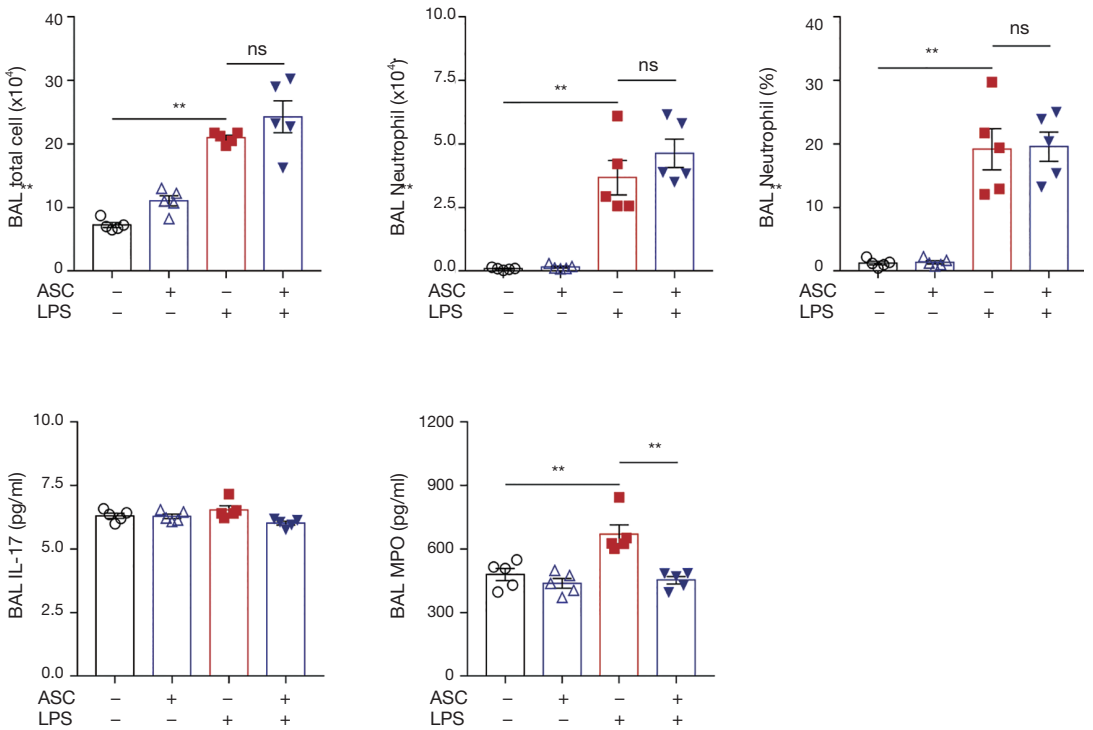

Figure 6 The effects of adipose-derived stem cell (ASC) treatment on lipopolysaccharide (LPS)-induced acute respiratory distress syndrome (ARDS) at the long term. In the late stage of ARDS, the total cell count in the bronchoalveolar lavage fluid (BALF) is not different between the LPS/ASC and LPS groups [LPS/ASC, $(24.3\pm3.7)\times10^4$ *vs.* LPS, $(21\pm0.6)\times10^4$/mL; P=0.13]. The comparison between the LPS and control groups shows a significant difference in the total cell count in the BALF [control, $(7.3\pm0.6)\times10^4$ *vs.* LPS, $(21\pm0.6)\times10^4$; **P<0.01]. Similarly, neutrophil count and percentage in the BALF are not different between the LPS/ASC and LPS groups [LPS/ASC, $(4.6\pm0.8)\times10^4$ *vs.* LPS, $(3.7\pm1)\times10^4$/mL; P=0.31; LPS/ASC, 19.6%±3.4% *vs.* LPS, 19.2%±4.8%; P=0.69, respectively]. Compared to the control group, the LPS group shows a higher neutrophil count [control, $(0.1\pm0)\times10^4$ *vs.* LPS, $(3.7\pm1)\times10^4$; **P<0.01] and neutrophil percentage (control, 1.3%±0.4% *vs.* LPS, 19.2%±4.8%; **P<0.01). However, the level of myeloperoxidase (MPO) significantly decreases in the LPS/ASC group than in the LPS group (LPS/ASC, 453.2±26.2 *vs.* LPS, 670.2±65.9 pg/mL; **P<0.01). The LPS group has higher MPO levels than does the control group (control, 480.3±42.3 *vs.* LPS, 670.2±65.9 pg/mL; **P<0.01). Interleukin (IL)-17 level is not different between the LPS and LPS/ASC groups (LPS/ASC, 6.0±0.1 *vs.* LPS, 6.5±0.2 pg/mL; P=0.60). No significant difference is observed in IL-17 levels between the control and LPS groups (control, 6.3±0.2 *vs.* LPS, 6.5±0.2 pg/mL; P=0.65). Values are mean ± standard deviation of five mice per group.

the alveoli. However, this does not show an obvious effect of ASCs. This finding suggests that the number of cells required for treatment is unknown, or that pretreatment is required to activate the function of the administered heterologous ASCs (37).

A recent systematic meta-analysis evaluated studies on the efficacy of stem cell administration in animal models of ARDS with preclinical-stage acute lung injury (42). This analysis found that death due to acute lung injury was associated with an odds ratio of 0.24 (95% confidence interval, 0.18–0.34), thereby showing the positive effects of stem cell therapy. However, studies on the administration of heterologous ASCs in acute lung injury models are relatively scarce, and these studies did not yield significant results in the meta-analysis (42). Chien *et al.* showed that human orbital fat-derived stem cells showed a decrease in lung injury and cytokine levels, which may be associated with their better dividing potential because they are stem cells derived from orbital fat and not subcutaneous adipose tissue (34,43).

Although the several parameters and histology of lung injury and fibrosis show trends in attenuation, there

Table 1 Summary of mesenchymal stem cell of lung injury in mouse model

Injury model	MSC source	MSC delivery	Major finding	Reference
Murine bleomycin	Murine BMMSC 5×10^5	Intravenous 6 h after injury	↓ Pro-inflammatory cytokines, ↑ GM-CSF, G-CSF	Rojas *et al*. (15)
Murine intratracheal LPS	Murine BMMSC 7.5×10^5	Intratracheal 4 h after injury	↑ Survival, ↓ pro-inflammatory cytokines, ↑ anti-inflammatory cytokine (IL-10)	Gupta *et al*. (13)
Murine intratracheal LPS, ALIp, intraperitoneal LPS, ALIexp	Murine BMMSC 1×10^5	Intravenous 24 h after injury	↓ Lung elastance, alveolar collapse, ↓ collagen fiber content, TGF-β, VEGF in ALIp. D2 ↓ Pro-inflammatory cytokines & chemokines, ↓ TIMP-1 & ↑ MMP-8 in ALIp, D7 ↓ IGF, VEGF in ALIexp	Maron-Gutierrez *et al*. (31)
Murine OA LPS	Human BMMSC 5×10^5	OA 4 h after injury	↓ Pro-inflammatory cytokines & chemokines, ↑ TNFα-induced protein 6 (TSG-6) expression	Danchuk *et al*. (32)
Murine intratracheal LPS	Human BMMSC 1.5×10^6 and MSC derived microvesicles	Intratracheal and intravenous 12 h after injury	↓ Neutrophil, MIP-2, ↓ edema by a KGF mechanism	Zhu *et al*. (33)
Murine intratracheal LPS	Human OFSC* 3×10^5	Intravenous 20 min after injury	↓ Neutrophil, MIP, IL-12, ↓ TLR4/CD14 signaling-iNOS, TGF-β	Chien *et al*. (34)
Murine OA LPS	Mouse & Human ASC, 7.5×10^5	OA 4 h after injury	↓ Neutrophil, MPO in all ASC, ↓ IL-1β & ↑ IL-10 in mASC	Zhang *et al*. (35)
Murine OA P. aeruginosa	Mouse ASC 1×10^6	OA 1 h after injury	↓ Neutrophil, MPO, ↑ phagocytosis of macrophage by inhibiting PGE2 signaling	Mao *et al*. (36)
Murine intranasal LPS	Human ASC & sST2-hASC, 1×10^6	Intravenous 6 h after injury	↓ Neutrophil, ↓ TNFα, IL-6, MIP-2 in sST2-hASC	Martínez-González *et al*. (37)

LPS, lipopolysaccharide; MSC, mesenchymal stem cell; BMMSC, bone marrow-derived stem cell; ASC, adipose-derived stem cells; OA, oropharyngeal aspiration; ALIp, pulmonary acute lung injury; ALIexp, extrapulmonary acute lung injury; OFSC, orbital fat-derived stem cell.

are some limitations in this study. It failed to detect the significant effects of ASCs on inflammatory cytokines and growth factors in mouse lung injury. It might be related to the different MSC delivery by oropharyngeal aspiration, or inadequate MSC dosage. More basically, the relative lack of human MSC-mediated process might be likely to be due to limitations of their immune privilege and differential priming of human MSC in mice.

In terms of the evaluation of lung fibrosis, collagen deposition in this study was not clear, it is needed the detailed and quantitative measurement including total collagen fiber in lung tissue.

However, our study suggests the possibility of human ASC treatment in lung fibrosis including inflammation in ARDS mice model. Further studies are needed to overcome these limitations of interspecies stem cell treatment. Moreover, the current study findings are meaningful in that human ASCs were injected into an LPS-induced acute lung injury mouse model to observe the fibrosis and exudative phases. Although this study did not reveal the role of fibrosis-related mediators, it showed a decrease in fibrosis histologically. Therefore, further studies are warranted to understand the effects of ASCs in ARDS.

Conclusions

Human ASC therapy may inhibit neutrophil recruitment, weaken early lung injury, and even affect fibrosis. However, the effectiveness of the treatment is minimal, and further studies are warranted to understand the mechanism and improve the therapeutic effect of ASCs.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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