Systemic human orbital fat-derived stem/stromal cell transplantation ameliorates acute inflammation in lipopolysaccharide-induced acute lung injury

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Objective: Acute lung injury results in acute respiratory distress syndrome. There is no standard therapy for acute respiratory distress syndrome but supportive care. Stem cells offer a new therapeutic potential for tissue regeneration as a result of their self-renewal, multipotency, and paracrine capabilities. The objective of this study is to investigate the effects and the mechanisms of systemic human orbital fat-derived stem/stromal cell transplantation on lipopolysaccharide-induced acute lung injury.

Design: Prospective, randomized, controlled study.

Setting: University-affiliated research institute.

Subjects: Male BALB/c mice.

Interventions: Twenty-five micrograms lipopolysaccharide in 50 μL sterile saline or 50 μL of sterile saline was delivered through intratracheal injection. Twenty mins later, the animals were further randomized into subgroups that received either a tail vein injection of 3 × 10^6 orbital fat-derived stem/stromal cells in 50 μL phosphate-buffered saline or 50 μL phosphate-buffered saline.

Measurements and Results: Low immunogenicity and immune-tolerated of orbital fat-derived stem/stromal cells were observed in this xenotransplanted model. Orbital fat-derived stem/stromal cells significantly reduced lipopolysaccharide-induced pulmonary inflammation, which was evidenced by a decrease in total protein concentration and neutrophil counts in alveolar fluid through bronchoalveolar lavage, reduced endothelial and alveolar epithelial permeability as well as neutrophil (Ly6G-expressing cells) and macrophage (CD68-expressing cells) infiltration. Lipopolysaccharide-induced expression of CD14, inducible nitric oxide synthase, and transforming growth factor-β in lung tissue was significantly inhibited by orbital fat-derived stem/stromal cells. Orbital fat-derived stem/stromal cells not only reduced the circulation numbers of macrophages and neutrophils (CD11b-expressing cells), but also decreased systemic proinflammatory chemokine levels such as macrophage inflammatory protein-1-γ, B-lymphocyte chemoattractant, interleukin-12, and subsequent circulation helper T cell (CD4-expressing cells) numbers. Furthermore, few human orbital fat-derived stem/stromal cells were detectable in the recipient lung after acute inflammation subsided.

Conclusions: Systemic orbital fat-derived stem/stromal cell transplantation was effective in modulating inflammation during acute lung injury. The therapeutic effect was attributed to the inhibition of acute inflammatory responses. (Crit Care Med 2012; 40:000–000)

KEY WORDS: acute lung injury; inflammation; lipopolysaccharide; macrophage; orbital fat-derived stem/stromal cells

Acute respiratory distress syndrome (ARDS), a severe complication of acute lung injury (ALI), is the major morbidity and mortality in critically ill patients (1–3). The pathomechanism of ALI/ARDS is characterized by a severe acute inflammatory response in lung parenchyma (3). Infection is the leading cause of ALI/ARDS (3). Lipopolysaccharides (LPS), the endotoxin derived from bacteria, is able, to its ability, to induce marked acute pulmonary inflammation. Experimental administration of LPS, either through intravenous injection or intratracheal injection, is used to create acute lung inflammation in animal models (4–8). Currently, the principal management of ALI/ARDS is through supportive care (9). Facilitating tissue repair without fibrotic change by attenuating local inflammatory reaction is the goal of successful treatment of ALI/ARDS (10). Therefore, a therapeutic strategy that specifically decreases local inflammation and promotes lung tissue repair would be highly valuable in treating ALI/ARDS.

Stem cells possess self-renewal, multipotency, and a paracrine capacity. These offer a therapeutic potential for tissue regeneration (11). Among stem cells,
bone marrow-derived mesenchymal stem cells (BM-MSCs) are known to be potent immune modulators (12). Therefore, cell-based therapy is seemingly a promising treatment for ALI (13). Recent evidence suggests that BM-MSCs decrease systemic and local inflammatory response and improve the mortality rate after ALI (14–18). However, the mechanism behind the effect of BM-MSCs on ALI is controversial. BM-MSCs reportedly engraft into injured lung parenchyma and differentiate into lung epithelial cells to repair the injured lungs (16, 19, 20). Other reports have shown that BM-MSCs inhibit endotoxin-induced acute lung inflammation without lung engraffment and differentiation (14, 15). In addition, BM-MSCs overexpressing Angiopoietin-1 result in further improvement in both alveolar inflammation and permeability on ALI (17, 18). Furthermore, BM-MSCs possess direct antimicrobial activity through the secretion of the antimicrobial peptide LL-37 (21).

For clinical application of stem cell transplantation, isolation of BM-MSCs requires bone marrow aspiration, which is a moderate-risk procedure (22). The greatest limitation of isolating MSCs from aspirated bone marrow is the low cell numbers obtained from a limited marrow volume (23). Alternately, adipose tissue is an abundant source of MSCs (24). In our previous study (25), a plat- form was established to isolate multipotent stem cells from a minimal volume (0.5–1 mL) of human orbital adipose tissue. Human orbital fat-derived stem/stromal cells (OFSCs) were negative for CD34, CD31, CD45, and CD106 but highly positive for CD29, CD90, and CD105. This finding was consistent with those for BM-MSCs. Both the growth kinetics and trilineage differentiation ability of OFSCs were comparable with BM-MSCs. In addition, OFSCs exhibited a higher epithelial differentiation potential than adipose-derived stem cells isolated from subcutaneous fatty tissues (25).

According to the findings, this study hypothesizes that OFSCs rescue ALI through their multipotency and anti-inflammation potential. The objective of this study was to evaluate the therapeutic effect and molecular mechanism of systemic OFSC transplantation in treating LPS-induced ALI in vivo.

MATERIALS AND METHODS

Animal Model of LPS-Induced ALI. Six-wk-old BALB/c male mice were purchased from BioLASCOS (BioLASCOS Taiwan Co., Ltd., Taiwan). Animals were maintained in the animal facility at Taipei Medical University. All experimental protocols were approved by the animal use and care committee of Taipei Medical University. LPS was generated from Esche- richia coli O55:B5 (Sigma-Aldrich, St. Louis, MO). Figure 1A shows the details of the experimental procedures. Briefly, 8- to 10-wk-old mice were first anesthetized through an intra-peritoneal injection of pentobarbital (40 mg/kg). Anesthetized animals were restrained on a surgical board at an angle of approximately 40°, and a small skin incision was made in the neck. Thereafter, the trachea was exposed by a blunt dissection and ALI was induced by an intratracheal injection of pentobarbital (40 mg/kg). Anesthetized animals were restrained on a surgical board at an angle of approximately 40°, and a small skin incision was made in the neck. Thereafter, the trachea was exposed by a blunt dissection and ALI was induced by an intratracheal injection of pentobarbital (40 mg/kg). Anesthetized animals were restrained on a surgical board at an angle of approximately 40°, and a small skin incision was made in the neck. Thereafter, the trachea was exposed by a blunt dissection and ALI was induced by an intratracheal injection of pentobarbital (40 mg/kg). Anesthetized animals were restrained on a surgical board at an angle of approximately 40°, and a small skin incision was made in the neck. Thereafter, the trachea was exposed by a blunt dissection and ALI was induced by an intratracheal injection of pentobarbital (40 mg/kg).

Isolation and Culture of OFSCs. Isolation and culture of the OFSCs were performed as described previously (25). In brief, during blepharoplasty, redundant orbital fat tissue was removed from the intraorbital cavity. All samples were removed with the informed con- sent of the subjects and followed the regulations of the Institutional Review Board of Taipei Medical University–Wan Fang Hospital. Adipose tissues were fragmented, digested, and filtered. After centrifuging the fluid, cells from the resulting pellet were plated in non-coated tissue culture flasks (BD Biosciences, Franklin Lakes, NJ) and maintained in Mesen Pro Medium (Invitrogen, Carlsbad, CA).

OFSC Transplantation. Various dosages of OFSCs from 1 × 10^3 to 1 × 10^6 cells in 50 μL of phosphate-buffered saline (PBS) were tested through tail vein injection into the ALI mice. In this study, the optimal dosage (3 × 10^5 OFSCs; see Supplemental Fig. 1 [Supplemental Digital Content 1, http://links.lww.com/CCM/A365]) was chosen and administered through the tail vein 20 mins after being challenged with LPS. Animals were euthanized 3 days after LPS exposure with an injection of sodium pentobarbital (120 mg/kg). Lung tissues were removed and divided into two portions: one for immunohistochemistry or fluorescence detection and the other for Western blot analysis. All experiments were repeated at least twice with a minimum of five mice in each group.

Antigenicity of OFSCs. The surface phenotype profiling of OFSCs was detected using a cell-based assay (BD lypolate; BD Bioscience). Serum samples were collected before and after an intravenous injection of 3 × 10^5 OFSCs or PBS. The serum levels of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) were measured using enzyme-linked immunosorbent assay kits (E Bioscience, Inc., San Diego, CA) as per the manufacturer’s instructions and analyzed using an enzyme-linked immunosorbent assay reader (Spectra MAX 250; Spectra Devices, Sunnyvale, CA).

Western Blot Analysis. Lung extracts were lysed, and the lysates were prepared as previously described (26). Western blot analysis was performed using primary antibodies against inducible nitric oxide synthase (iNOS) (0.1 μg/mL), TNF-α (0.1 μg/mL), tissue growth factor-β (TGF-β) (0.125 μg/mL), and CD14 (0.1 μg/mL).
Figure 2. Host inflammatory response to orbital fat-derived stem cells (OFSCs). Mice serum was collected before and after transplantation of OFSCs (10^7 /kg) or phosphate-buffered saline for 12, 24, 48, and 72 hrs. OFSCs did not trigger the serum level of tumor necrosis factor-α (TNF-α) (A) and interferon-γ (INF-γ), except at 24 hrs after transplantation (B). Values are mean ± se of five animals (analysis of variance with Tukey’s post hoc tests at 95 confidence intervals; different letters represent different levels of significance, n = 5).

Table 1. Immunocharacteristics of mesenchymal stem cells and orbital fat-derived stem cells

<table>
<thead>
<tr>
<th>Major Histocompatibility Complex I</th>
<th>CD40</th>
<th>CD80 (B7–1)</th>
<th>CD86 (B7–2)</th>
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<tbody>
<tr>
<td>Mesenchymal stem cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Orbital fat-derived stem cells</td>
<td>+</td>
<td>+</td>
<td>+</td>
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μg/mL), Lymphocyte Antigen 6 Complex, Locus G (Ly6G) (0.5 μg/mL) (Abcam, Cambridge, MA), or CD68 (0.9 μg/mL) (Epitomics, Burlingame, CA). The density of protein bands was assessed using a computing densitometer with Image-Pro plus software (Media Cybernetics, Inc., Bethesda, MD).

Histopathology. Paraffin-embedded lungs were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin. The histopathology of the lungs was evaluated and blindly scored by a pathologist using a light microscope. The severity of LPS-induced ALI was determined according to the following: 1) alveolar congestion; 2) hemorrhage; 3) infiltration or aggregation of neutrophils in airspaces or vessel walls; and 4) thickness of alveolar wall/hyaline membrane formation. Each criterion was graded according to a 5-point scale, as previously reported (27). A total lung injury score was calculated as the sum of the four criteria.

Lung Wet/Dry Weight Ratio Analysis. The lung wet/dry ratio was determined by dividing the wet weight by the dry weight (28).

Immunohistochemistry and Fluorescence Detection. For immunohistochemical staining, tissue sections were incubated with anti-iNOS (2 μg/mL), anti-TCP-β (5 μg/mL), or anti-CD14 (2 μg/mL) (Abcam) for 2 hrs. The staining was detected using the streptavidin–biotin peroxidase complex method with the DAB Peroxidase Substrate Kit (SK-4100; Vector Laboratories) and counterstained with hematoxylin. For fluorescence staining, Green CMFDA-labeled OFSCs (5-chloromethylfluorescein diacetate; Invitrogen, Carlsbad, CA) and blue fluorescence from 4',6-diamidino-2-phenylindole-labeled cell nuclei were monitored using a Zeiss Axioskop microscope with a CCD camera (Carl Zeiss, Inc., Thornwood, NY). Ten fields were randomly selected at ×20 magnification, and the CMFDA-positive (CMFDA⁺) and CMFDA-negative (CMFDA⁻) cells were counted. Blind analyses were performed.

Evaluation of Bronchoalveolar Lavage Fluid. Bronchoalveolar lavage fluid was collected for neutrophil cell counts and total protein concentration measurement as previously described (28).

Flow Cytometric Analysis. Whole blood samples were collected at 3 or 10 days after LPS or after LPS plus OFSC instillation. Erythrocytes were removed using an ACK lysing buffer (GIBCO, Grand Island, NY) before incubation for 30 mins with adequate PE-conjugated monocyte CD11b or IgG2b isotype control antibody (BD Biosciences), fluorescein isothiocyanate-conjugated monocyte CD4, or IgG2a isotype control antibody (eBioscence). The blood samples were further analyzed for immunofluorescence intensity by flow cytometry (FACS Calibur; BD Biosciences). Data were analyzed using Cell Quest Software (BD Biosciences).

Inflammatory Cytokine Array. Serum samples were collected from mice treated with LPS or with LPS plus OFSCs for 6 hrs or 3 days. The differential serum inflammatory cytokine levels between the LPS group (n = 5) and the LPS with OFSC group (n = 5) were analyzed using commercially available RayBio Mouse Inflammation Antibody Array 1.1 (Ray-Biotech, Inc., Norcross GA) according to the manufacturer’s protocol.

Statistical Analysis. Values are shown as means ± se. Statistical analysis was performed using the Statistical Package for Social Science software, version 16 (SPSS Inc., Chicago, IL). Data comparisons were performed with the Student’s t test when two groups were compared. One- or two-way analysis of variance analysis followed by Tukey’s post hoc test or the Bonferroni test were used when more than three groups were analyzed. The ALI score (given as a median) was analyzed with the nonparametric Wilcoxon’s rank sum test. Differences were considered significant at the 95% confidence interval when p < .05.

RESULTS

Figure 1A illustrates the study design. LPS or saline was delivered through an intratracheal injection. Systemic OFSC or PBS transplantation was performed 20 mins after intratracheal injection. Blood samples were collected 1 day before intratracheal injection and before euthanasia. Three days after LPS administration, BALB/c mice were euthanized for pathologic examination. The morphology of the OFSCs was spindle-shaped, fibroblast-like cells (Fig. 1B).

OFSCs Were Immune Tolerated in a Xenotransplanted Model. To evaluate the in vivo immunogenicity of OFSCs, the serum levels of TNF-α and INF-γ were monitored. The level of TNF-α (Fig. 2A) or INF-γ (Fig. 2B) was not significantly changed by the OFSCs, except for a transient increase in the IFN-γ level at 24 hrs after OFSC transplantation (Fig. 2B). In addition, immunophenotyping of OFSCs using a cell-based array showed that the OFSCs expressed Major Histocompatibility Complex class I molecules, whereas the antigen-presenting molecule (Major Histocompatibility Complex class II) and costimulating factors (CD40, CD80, and CD86) were undetectable on the cell surface (Table 1), which is similar to the phenotype of BM-MSCs (29).

OFSCs Reduced LPS-induced ALI by Ameliorating Local Inflammation. Hematoxylin and eosin staining of lung sections demonstrated that saline induced a mild inflammatory response (Fig. 3A–C), whereas LPS triggered severe lung inflammation (Fig. 3D–F) 3 days after intratracheal injection. In PBS-treated mice, LPS induced marked infiltration in the interstitial spaces, alveolar hemorrhagic congestion, and swelling of the
Figure 3. Orbital fat-derived stem cells (OFSCs) diminished lipopolysaccharide (LPS)-induced acute lung inflammatory injury. Histopathology of the lung are shown at ×40 (for A, D, G), ×200 (for B, E, H), and ×400 (for C, F, J) magnification. The high-power fields (×200 and ×400) are the magnified fields in the black boxed area in the left panel. A–C, Intratracheal saline injection induced minor lung inflammation. D–F, Intratracheal LPS injection triggered severe lung inflammation. G–I, Systemic OFSC transplantation significantly ameliorated LPS-induced acute lung inflammation. The quantitative results of Western blot analysis revealed that LPS increased CD68 (J) and Ly6G (K) expression, whereas OFSCs significantly decreased the amount of CD68 and Ly6G in lung parenchyma after LPS stimulation. Results (J) and (K) were analyzed using Student’s t test; *p < .05 as compared with the LPS treatment group.

Figure 4. Orbital fat-derived stem cells (OFSCs) reduced the severity of lung injury and endothelial/epithelial permeability. A. After 72 hrs, mice that received OFSCs had significantly less excess lung water that was triggered by lipopolysaccharide (LPS) than mice that received phosphate-buffered saline (PBS). Protein (B) and neutrophil counts (C) in bronchoalveolar lavage (BAL) fluid were significantly reduced in OFSC-treated mice. Results were analyzed by two-way analysis of variance with a Bonferroni test at 95% confidence intervals, (ns) nonsignificant, *p < .05, n = 5. W/D, wet/dry ratio.
Table 2. Lung injury score in mice with lipopolysaccharide-induced acute lung injury

<table>
<thead>
<tr>
<th>Measure</th>
<th>Saline/PBS</th>
<th>LPS/PBS</th>
<th>LPS/Orbital Fat-Derived Stem Cells</th>
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<tr>
<td>Lung injury score</td>
<td>1 (0–2)</td>
<td>5.5 (4–7)*</td>
<td>2.5 (1–3)*</td>
</tr>
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PBS, phosphate-buffered saline; LPS, lipopolysaccharide.

*p < .05 compared between saline/PBS versus LPS/PBS groups; \( p < .05 \) compared between LPS/PBS versus LPS/orbital fat-derived stem cells groups. \( p \) values for nonparametric Wilcoxon rank sum test are given.

This is the first study to demonstrate that systemic administration of OFSCs significantly decreased LPS-induced acute lung inflammation through the inhibition of LPS-triggered macrophage and neutrophil recruitment, local infiltration, and the activation of the Toll-like receptor 4/CD14-mediated signaling pathway. These findings suggest that OFSC transplantation may serve as a new therapeutic strategy for treating ARDS.

Immune modulation activity and the lack of Major Histocompatibility Complex class II expression (38, 39) result in the immune-tolerance of BM-MSCs (40, 41) in the context of allogenic transplantation. This study demonstrated that OFSCs isolated from a minimal volume (0.5–1 mL) of redundant orbital fatty tissue, similar to BM-MSCs, did not express Major Histocompatibility Complex class II, CD40, CD80, and CD86 (Table 1). OFSCs did not induce the elevation of TNF-\( \alpha \) and IFN-\( \gamma \), except for a transient increase in the IFN-\( \gamma \) level at 24 hrs after OFSC transplantation (Fig. 2). Those findings supported that OFSCs were of low immunogenicity and were immune-toleranced in this xenotransplanted model.

Although the immune phenotyping of BM-MSCs and OFSCs are almost identical, the immunomodulation and the anti-inflammatory ability of OFSCs in vivo are unclear. This study showed that OFSCs possessed anti-inflammatory capacity by inhibiting acute lung inflammatory response, including macrophage infiltration (Figs. 3–7). In the first few days after tissue injury, macrophage infiltration and their activation aggravate local inflammation through an inflammatory cytokine storm (42). LPS triggers macrophage activation through binding to Toll-like receptor 4/CD14 to mediate inflammatory protein release. These proteins include iNOS (30) and TGF-\( \beta \) (43), which are two critical cytokines in the pathogenesis of ALI (31, 32, 44). Inducible NOS produces cytotoxic oxidant, and TGF-\( \beta \) increases pulmonary endothelial and epithelial permeability (44, 45). We found that OFSCs abrogated the LPS-induced increased pulmonary endothelial and epithelial permeability (Fig. 4), the activation of the Toll-like receptor 4/CD14 signaling pathway, the produc-
tion of iNOS and TGF-β (Figs. 5 and 6), and the recruitment of macrophage (Figs. 3 and 7). These data suggest that OFSCs ameliorate LPS-induced acute lung injury, at least in part, by targeting macrophage-mediated inflammation. In addition, macrophages and neutrophils have also reportedly enhanced pulmonary endothelial and epithelial permeability (46). In our study, OFSCs attenuated both macrophage and neutrophil infiltrations (Figs. 3 and 4), which illustrates their powerful anti-inflammation ability during ALI.

In addition, systemic OFSC transplantation not only inhibited systemic macrophage and neutrophil recruitment (Fig. 7F), but also reduced serum levels of pro-inflammatory cytokines triggered by LPS (Figs. 7A–H). Localized and systemic macrophage inflammatory protein-1γ, B-lymphocyte chemoattractant, and IL-12 levels are reportedly elevated during the early stage of ALI (47, 48). Macrophage inflammatory protein-1γ is secreted by macrophages and induces the chemotaxis of CD4+ T lymphocytes and neutrophils (34, 35). B-lymphocyte chemoattractant is secreted by monocytes, lymphocytes, and dendritic cells (49) and can be detectable in serum when tissue inflammation occurs (36, 50). B-lymphocyte chemoattractant is a potent chemokine to attract B and T lymphocytes to the areas of infection and inflammation (51). IL-12 secreted by monocytes, macrophages, and neutrophils is a critical regulatory cytokine that stimulates type 1 helper T cells to release IFN-γ in response to bacterial products (48, 52, 53). In our study, systemic OFSC transplantation reduced serum levels of macrophage inflammatory protein-1γ (Fig. 7D), B-lymphocyte chemoattractant (Fig. 7E), IL-12 (Fig. 7F), and subsequent helper T cells recruitment (Fig. 7H), indicating that OFSCs rescued the damaged lung from further T cell-related complications.

It has been reported in the literature that in murine models of LPS-induced ALI, intravenous instillation of BM-MSCs reduced the inflammatory response; however, from 1 to 14 days after LPS treatment, the engraftment rate was variable and extremely low (0.1% to 9%) (15, 17, 18). Similarly, we observed a small degree of OFSC pulmonary engraftment (Fig. 8; Supplemental Fig. 2 [Supplemental Digital Content 2, http://links.lww.com/CCM/A366]). The average percentage of CMFDA+ cells in the lung was <1% in the randomly selected fields. Despite the low engraftment rate, OFSC administration led to a dramatic improvement in lung condition (Fig. 3; Table 2). These data suggested the therapeutic effect of OFSCs may be primarily from their paracrine effects.

OFSCs are advantageous over BM-MSCs because they can be isolated from a minimal volume (0.5–1 mL) of orbital fat tissues. Such a procedure produces a much lower risk and is more effective than the isolation MSCs from BM aspirates. Furthermore, TNF-α (17), IL-1β,
Figure 7. Orbital fat-derived stem cells (OFSCs) reduced systemic proinflammatory cytokines and immune cell recruitment. Differential proinflammatory cytokine levels were measured using a cytokine array and the array data were quantified to generate a protein profile (histogram) (A–H). OFSCs significantly decreased the serum level of macrophage inflammatory protein-1-γ (D) after lipopolysaccharide (LPS) treatment for 6 hrs but increased the interleukin-6 (IL-6) level (B) at the same time point. After LPS treatment for 72 hrs, OFSCs significantly decreased the serum level of B-lymphocyte chemoattractant (E) and IL-12 (F). OFSCs did not alter IL-1β, IL-10 (B, F), and tumor necrosis factor-α (D, H). LPS induced the elevation of CD11b+ (I) and CD4+ (J) -expressing cells in the circulation, whereas OFSCs significantly decreased CD11b+ and CD4+ populations after 3 and 10 days of LPS stimulation, respectively. Results (A–H) were analyzed using Student’s t test; *p < .05 as compared with the LPS treatment group; results (I–J) were analyzed using one-way analysis of variance with Tukey’s post hoc tests at 95% confidence intervals; different letters represent different levels of significance, n = 5. BLC, B-lymphocyte chemoattractant; GCSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; CD390L, cluster of differentiation 30L; IL, interleukin; I-TAC, interferon-inducible T-cell alpha chemoattractant; KC, keratinocyte-derived chemokine; LIX, lipopolysaccharide induced CXC chemokine; MCP-1, macrophage chemokine 1; MIG, monokine induced by gamma-interferon; MIP-1α, macrophage inflammatory protein-1α; MIP-1α, macrophage inflammatory protein-1γ; SDF-1, stromal cell-derived factor-1; TCA-3, thymus-driven chemotactic agent 3; TECK, thymus expressed chemokine; TIMP-1, tissue inhibitor of metalloproteinase; TNF, soluble tumor necrosis factor receptor; TNF-α, tumor necrosis factor-α.
and IL-6 (15) have been considered the targets of BM-MSCs in the regulation of inflammation during LPS-induced ALI. In contrast to BM-MSCs, TNF-α as well as IL-1β (Figs. 5D and 7B, D, F, and H) were unaltered by OFSCs. The level of circulating IL-6 increased after OFSC treatment (Fig. 7B). IL-6 reportedly possesses proinflammatory and anti-inflammatory properties (54). Recently, BM-MSCs have also been reported to mediate their immunosuppressive effects through an IL-6-dependent pathway (55). However, the role of IL-6 in OFSC-produced anti-inflammatory effects in ALI requires further investigation. Similar to BM-MSCs (15), OFSCs did not stimulate circulating IL-10 production, but attenuated circulating neutrophil recruitment in an ALI model. These data suggest that underlying molecular mechanisms between OFSC and BM-MSC inflammation inhibition in ALI are not exactly the same.

Limitations. First, the immune response to LPS-induced ALI in mice may not be identical to infectious ALI in humans. Besides, the LPS-induced ALI in mice may have not fully reproduced the complexity of clinical ALI/ARDS in patients. Therefore, the anti-inflammation ability of OFSCs may need to be further validated using other clinically relevant models such as the cecal ligation and puncture, sepsis, and pneumonia. Third, further studies to define the optimal timing of stem cell transplantation, the therapeutic effect of multiple transplantation as well as the molecular mechanisms underlying the effects of OFSCs on macrophage regulation are necessary.

CONCLUSION

We demonstrated that the systemic administration of multipotent OFSCs diminished acute LPS-induced lung inflammation by inhibiting macrophage and neutrophil-associated inflammatory responses. OFSCs were immune-tolerated in the host and possessed immune modulation ability in vivo. Our findings have provided a novel therapeutic strategy for ALI by developing a cell-based therapy using orbital fat-derived stem/stromal cells.

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Figure 8. Several of the orbital fat-derived stem cells (OFSCs) engrafted into the injured lung over the first 3 days. OFSCs were labeled with the cell tracing dye CMFDA (green) before injection. Nuclei were stained with 4′,6-Diamidino-2-phenylindole (DAPI) (blue). White arrows indicated labeled OFSCs. A, Several CMFDA-labeled OFSCs were observed in the lung parenchyma from lipopolysaccharide (LPS)-injured mice 3 days after OFSC transplantation. B, No CMFDA signal was detectable in the lung parenchyma from LPS-injured mice without OFSC transplantation. Original magnification: ×100.


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