Effect of Intratracheal Administration of Adipose-derived Stromal Cells on Bleomycin-induced Lung Injury in a Rat Model

MASATO UJI, AKIRA NAKADA, TATSUO NAKAMURA, and KAZUTO HIRATA

MASATO UJI, AKIRA NAKADA, TATSUO NAKAMURA, and KAZUTO HIRATA. Effect of Intratracheal Administration of Adipose-derived Stromal Cells on Bleomycin-induced Lung Injury in a Rat Model. Osaka City Medical Journal. 2015, 61, 81-91

Osaka City Med. J. Vol. 61, 81-91, 2015

Effect of Intratracheal Administration of Adipose-derived Stromal Cells on Bleomycin-induced Lung Injury in a Rat Model

Masato Uji[1)], Akira Nakada[2)], Tatsuo Nakamura[2)], and Kazuto Hirata[1)]

Department of Respiratory Medicine[1)], Osaka City University, Graduate School of Medicine; and Department of Bioartificial Organs[2)], Institute for Frontier Medical Sciences, Kyoto University

Abstract

Background

Mesenchymal stromal cells (MSCs) have been intensively investigated in regenerative medicine. Among the different types of MSCs, adipose tissue-derived stromal cells (ASCs) can be obtained with relatively less invasive techniques. Since ASC administration is a candidate strategy for the treatment of refractory diseases including pulmonary fibrosis, we investigated whether intratracheal injection of ASCs had therapeutic potential against bleomycin (BLM)-induced lung injury in rats.

Methods

BLM was intratracheally administered to rats, and 1 week later ASCs were harvested. Two weeks after BLM treatment, ASCs or phosphate-buffered saline (PBS) were injected autologously into the rats via the trachea. A semi-quantitative histological evaluation was conducted to assess the injured lungs, followed by cell tracing at 3 or 6 weeks after BLM instillation.

Results

ASC administration did not affect the severity of lung damage on the third week after BLM exposure, but prevented further aggravation of the lung injury, as apparent on the sixth week. A fluorescent cell tracer revealed that the majority of ASCs did not appear to have penetrated inside the lung region injured by BLM on the third week after BLM instillation, but some of these cells sprouted deep into the thick distorted architecture of the injured lung on the sixth week after the BLM instillation.

Conclusions

The results of the present study suggest that ASCs may play a role in the prevention of ongoing aggravation of lung injury in the long term.

Key Words: Adipose tissue; Mesenchymal stromal cells; Intratracheal; Pulmonary fibrosis

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common form of idiopathic interstitial

Received September 5, 2014; accepted November 25, 2014.
Correspondence to: Masato Uji, MD.
Department of Respiratory Medicine, Osaka City University, Graduate School of Medicine,
1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan
Tel: +81-6-6645-3916; Fax: +81-6-6646-6067
E-mail: m2036283@med.osaka-cu.ac.jp

pneumonia[1,2]. It has been described as a gradually progressive disease, characterized by aggravation of respiratory symptoms, a steady decline in lung function, and gas exchange abnormalities over time. One of the major mechanisms driving IPF is deregulated wound healing in response to alveolar epithelial injury, involving exaggerated release of proinflammatory and profibrogenic factors[3,4]. Despite the expansion of scientific knowledge, the pathogenesis of IPF remains unclear. The basic therapeutic strategy involves the use of corticosteroids, alone or in combination with immunosuppressive, immunomodulatory, or antifibrotic agents, but so far the treatment has little impact on long-term survival[5-7].

Mesenchymal stromal cells (MSCs) of different cellular origins (umbilical cord, bone marrow, and other tissues) represent one of the most promising areas of research in terms of novel therapeutic strategies for the treatment of several chronic refractory diseases[8,9]. MSCs have been introduced into various animal model systems by intravascular injection, intratracheal instillation, or by bone marrow transplantation. Under certain circumstances, these cells can differentiate into epithelial cells, endothelial cells, neurocytes, chondrocytes, and myoblasts, possibly after migrating to damaged regions[10-12]. In addition, they have been observed to display immunomodulatory[13-16] and antifibrotic properties[17]. Collectively, these effects of MSCs are expected to modify and promote tissue repair and healing when administered exogenously, not only to lesions of pulmonary fibrosis and acute injury, but also to those of asbestosis, emphysema, and pulmonary hypertension[18-20]. Among the various MSCs, adipose-derived stromal cells (ASCs) have been identified as an alternative to bone marrow-derived MSCs because of their easy extraction, abundance of MSCs, and *ex vivo* expandability[21,22]. ASCs are known to be able to self-renew and differentiate into various mesenchymal cell types, and also to secrete significant levels of many potent growth factors and cytokines[23,24]. So far however, only a limited number of studies have investigated the effects of ASC administration on acute or chronic lung injury, including pulmonary fibrosis. Furthermore, previous studies have implanted the ASCs into target organs via the systemic circulation or intraperitoneal space[25-27]; no studies have investigated the effects of engraftment of ASCs via the respiratory tract.

The purposes of the present study were to observe the effects of intratracheal administration of ASCs on the lung tissue injury caused by bleomycin (BLM) instillation, and to investigate the possibility that ASC administration could modify the severity of the associated lung tissue damage. The BLM-induced lung injury model in rodents is commonly used to study the pathogenesis and treatment of pulmonary fibrosis[28]. BLM is an antibiotic agent with antitumor activity, but pulmonary fibrosis is a known side effect of BLM administration[29].

Materials and methods

Isolation and culture of rat ASCs

ASCs were obtained as previously described[26]. White adipose tissue (WAT) was collected under anesthesia induced by the intraperitoneal injection of sodium pentobarbital (3 mg/100g body weight). The anterior abdominal wall was opened 2 cm lateral to the spine, and WAT weighing from 3 to 7g was collected from the abdominal cavity. The WAT was washed twice with Dulbecco's minimal essential medium (DMEM) containing 10% fetal bovine serum and 1% antibiotic/antimycotic (Gibco, Los Angeles, Calif., USA), to remove blood cells. It was then chopped into pieces, and digested with 10 mL collagenase solution in a water bath at 37℃ for 60 min under continuous shaking. To make the collagenase solution, 100 mL of Hanks' balanced salt solution with calcium and magnesium

(Sigma, St. Louis, Mo., USA), pH 7.4, containing 4g of bovine albumin (fraction V), 300 mg of collagenase type Ⅷ (40% ammonium sulfate fraction from *Clostridium histolyticum*, Sigma) and 1.3 mg/mL of glucose were sterilized using a 0.22-μm filter. After collagenase treatment, the enzyme activity was neutralized with an equal volume of DMEM. The suspension was filtered through 250-μm nylon mesh and centrifuged at 300*g* for 5 min at room temperature to separate the pellet. The pellet was treated with red blood cell lysis buffer (Sigma) for 5 min at 37℃, then filtered through a 100-μm nylon mesh, followed by centrifugation (300*g* for 5 min at room temperature) to obtain a pellet of ASCs. The cells were cultivated in 75-cm^2 culture flasks, and the culture medium was changed twice a week. Upon reaching 60%-90% confluence, the ASCs were detached using 0.25% trypsin-ethylenediamine tetraacetic acid (EDTA) (Gibco), and divided 1:2. After 7 days in culture, the ASCs were isolated using trypsin, followed by centrifugation (300*g* for 5 min at room temperature) to obtain a pellet of ASCs, which was then suspended in phosphate-buffered saline (PBS), to be autologously injected back into the same recipient rats. The ASC suspension (1.0×10^7/0.5 mL) was infused via the trachea, under local anesthesia. Control rats received the same volume of PBS.

Bleomycin-induced lung injury model

A total of 10 mg BLM chloride (Nippon Kayaku, Japan) was dissolved in 1.0 mL of sterile 0.9% saline. Male Wistar rats over 24 weeks of age and weighing from 350 to 530g (Shimizu Laboratory Supply, Kyoto, Japan), were intratracheally administered 1.0 mL BLM/kg or 1.0 mL saline/kg under anesthesia induced by an intraperitoneal injection of sodium pentobarbital (3 mg/100g body weight), as described previously[26]. The relevant institutional animal care and use committee approved this study, and all experiments abided by the Principles of Laboratory Animal Care advocated by the Animal Experiment Committee of Kyoto University (2007).

Experimental groups

The schematic diagram of the experimental protocol was demonstrated in Figure 1. The rats were randomly assigned to 6 experimental groups. Group A included 6 animals that received intratracheal administration (IT) of BLM on day 0 and 0.5 mL PBS IT on day 14, and were sacrificed on day 21. Group B included 6 animals that received BLM IT on day 0 followed by ASC harvest on day 7, then received 1.0×10^7 ASC/0.5 mL PBS IT on day 14, and were sacrificed on day 21. Group C included 5 animals that received saline IT on day 0, PBS IT on day 14, and were sacrificed day 21. Group D included 6 animals that received BLM IT on day 0, PBS IT on day 14, and were sacrificed on day 42. Group E included 6 animals that received BLM IT on day 0, followed by ASC harvest on day 7, then received 1.0×10^7 ASC/0.5 mL PBS IT on day 14, and were sacrificed on day 42. Group F included 5 animals that received saline IT on day 0, PBS IT on day 14, and were sacrificed on day 42.

CM-DiI and 4',6-diamidino-2-phenylindole (DAPI) fluorescent labeling

For cell tracing, ASCs were labeled with the cell tracker CM-DiI (Invitrogen, Eugene, Oreg., USA) according to the manufacturer's protocol. Briefly, CM-DiI in dimethyl sulfoxide (DMSO) solution was diluted in PBS to obtain a concentration of 10 μg/mL. The ASCs were incubated with the dye solution for 3 min at 37℃ followed by 15 min at 4℃. The labeled ASCs were washed once with PBS, then cultured in DMEM at 37℃ under 5% CO_2 in humidified air. The labeling efficiency was confirmed to be greater than 90% by fluorescence microscopy. Nuclei were stained with DAPI (Sigma) on glass slides.

Preparation of lung tissues and histological evaluation via a semi-quantitative morphological index

On day 21 or 42 after BLM IT, rats were sacrificed by an intraperitoneal injection of an overdose

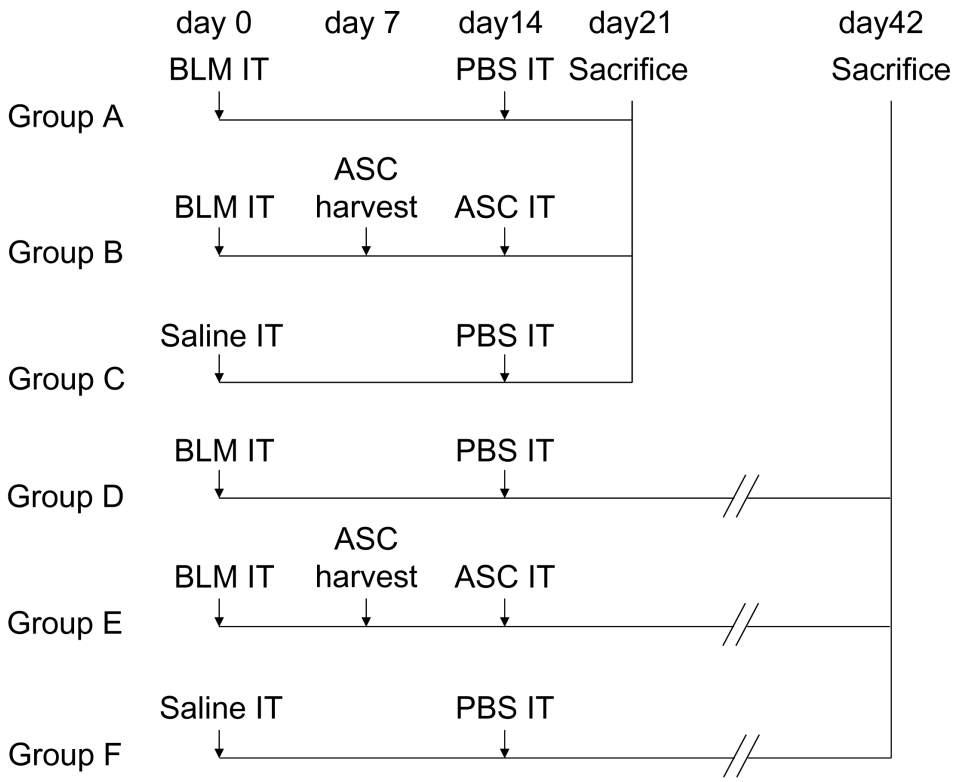

Figure 1. Schematic diagram of the experimental protocol. Animals in each group received BLM IT or normal saline IT on day 0. ASCs were harvested on day 7 in groups B and E. Animals in each group received ASCs IT or PBS IT on day 14, and were sacrificed on day 21 or day 42. BLM, bleomycin; IT, intratracheal administration; ASC, adipose tissue-derived stromal cell; and PBS, phosphate-buffered saline.

of sodium pentobarbital. Immediately after the animals were killed, each left lung was inflated at a constant pressure of 20 cm H_2O of 10% paraformaldehyde for 5 min, removed from each animal, and fixed in 10% paraformaldehyde for several days. For the histological examination, each left rat lung was transversely sectioned into seven pieces. The specimens were then dehydrated and embedded in paraffin. Four μm-thick sections were cut using a rotary microtome, placed on glass slides, deparaffinized, and sequentially stained with hematoxylin & eosin and Masson's trichrome stains. Thereafter, the sections were examined using a standard light microscope (BX-40, Olympus, Tokyo, Japan). Of six independently cut sections, the three most severely injured were selected for histological assessment via a semi-quantitative morphological index (SMI), without knowledge of the treatment groups, using a grading scheme reported by Lossos et al[30]. We used a fluorescence microscope (BZ-9000, Keyence, Tokyo, Japan) to examine the CM-DiI and DAPI signals. The SMI scores used for histological assessment were as follows: 0, normal lung; 1, minimal areas of inflammation, epithelial hyperplasia and fibrosis, usually limited to subpleural foci in just one or two sections; 2, more frequent lesions; 3, all three sections exhibit lung lesions which are not limited to subpleural foci; 4, extensive lesions in at least two of three sections; and 5, the majority of each of the three lung sections are affected by inflammation and fibrosis.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0.0.0 software for Windows (IBM Corporation, Somers, NY, USA). All data were analyzed non-parametrically. Upon detection of significant differences in SMI scores among multiple animal groups by the Kruskal-Wallis test, post-

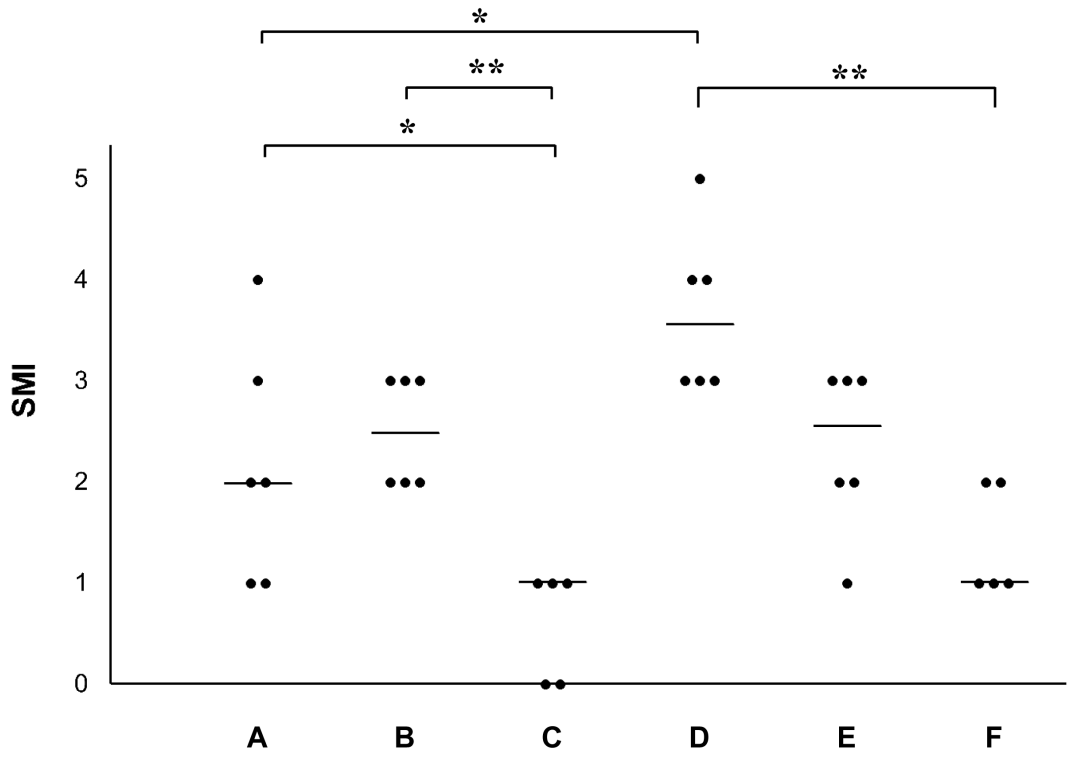

Figure 2. Comparison of the histological changes in lungs assessed by SMI after BLM and ASC IT. The SMI scores varied significantly among all six animal groups (p=0.001, Kruskal-Wallis test). BLM IT induced significant morphological changes, as evidenced by differences in SMI scores on day 21 between groups A and C, and on day 42 between groups D and F. The SMI scores of group D were significantly higher than those of group A. In contrast, there was no significant difference between the scores of the two ASC-treated groups (B and E). With regard to the groups evaluated on day 42, the SMI scores in group E (administered ASC IT) tended to be lower than those in group D (not administered ASC IT) (p=0.066).
Group A: BLM IT (day 0) + PBS IT (day 14), sacrificed on day 21.
Group B: BLM IT (day 0) + ASC harvest (day 7) + ASC IT (day 14), sacrificed on day 21.
Group C: Saline IT (day 0) + PBS IT (day 14), sacrificed on day 21.
Group D: BLM IT (day 0) + PBS IT (day 14), sacrificed on day 42.
Group E: BLM IT (day 0) + ASC harvest (day 7) + ASC IT (day 14), sacrificed on day 42.
Group F: Saline IT (day 0) + PBS IT (day 14), sacrificed on day 42.
SMI, semi-quantitative morphological index; BLM, bleomycin; ASC, adipose tissue-derived stromal cell; IT, intratracheal administration; and PBS, phosphate-buffered saline. *$p<0.05$, **$p<0.01$, Tukey-Kramer test. Horizontal bars indicate median values.

hoc pairwise comparisons were conducted using the Tukey-Kramer test, with the level of statistical significance set at $p<0.05$.

Results

Comparison of SMI scaling scores

The SMI scores varied significantly among all six groups (p=0.001, Kruskal-Wallis test) (Fig. 2). Compared with saline IT on day 0, BLM IT induced significant morphological changes as indicated by the differences in SMI scores on day 21 between groups A and C (p=0.030), and those on day 42 between groups D and F (p=0.001).

With regard to the groups receiving BLM IT on day 0 and subsequent PBS IT on day 14 (groups A and D), the SMI scores of group D evaluated on day 42 were significantly higher than those of group A evaluated on day 21 (p=0.029). In contrast, there was no significant difference between the SMI

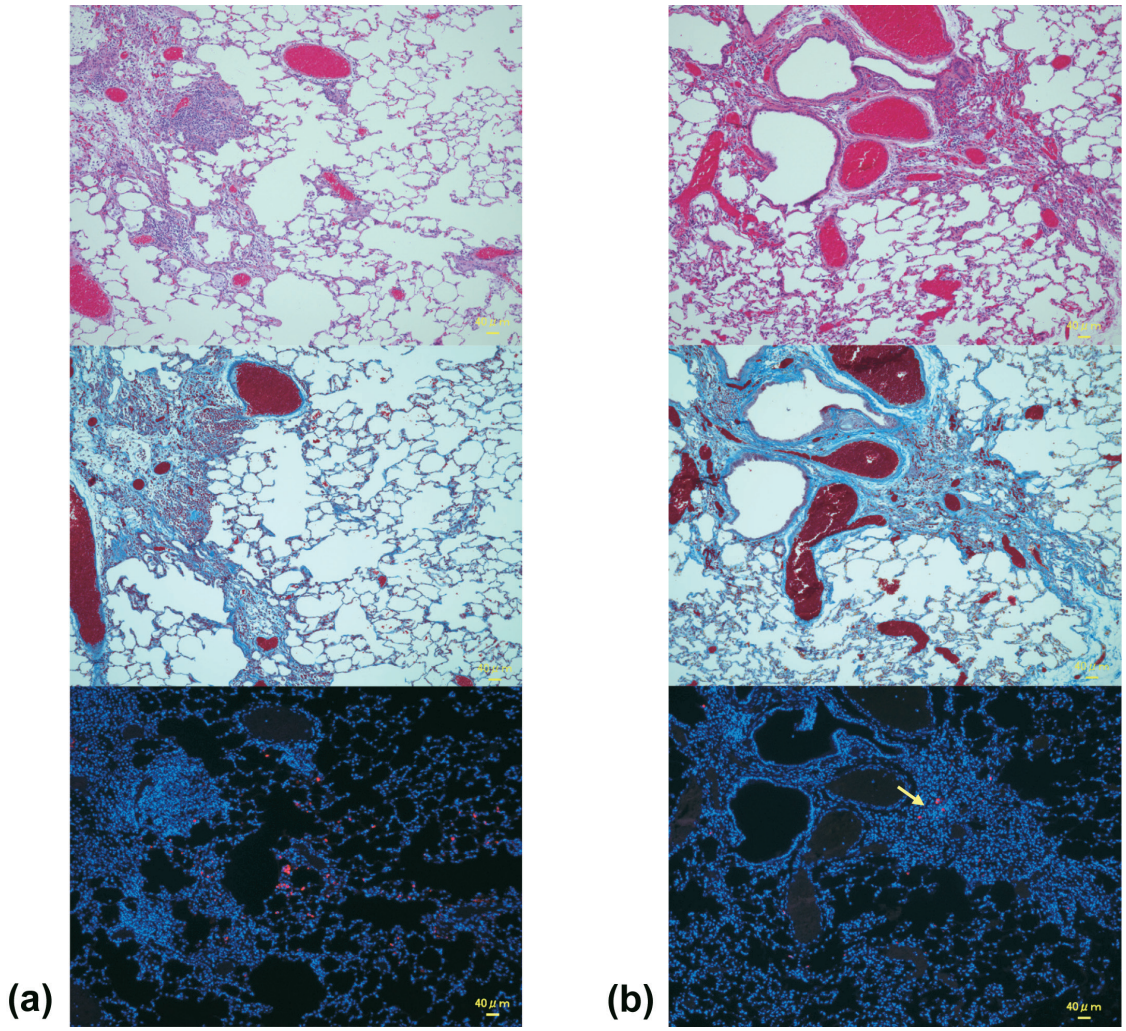

Figure 3. The distribution of ASCs which had accumulated in the fibrotic tissues observed at day 21 and day 42. The upper panels show the results of hematoxylin & eosin staining. The middle panels show the results of Masson's trichrome staining, where blue staining represents collagen deposition. The lower panels show the fluorescence detected. DiI-labeled ASCs fluoresced red. Nuclei were labeled with DAPI, and fluoresced blue. A representative photograph from group B is shown. The lower panel shows that some ASCs were found in the slightly injured area (central portion), and ASCs were scarce in the more severely injured area (left portion). A few ASCs were observed in the almost normal area (right portion) (a). A representative photograph from group E is shown. The lower panel shows that while the ASCs were reduced in number, some survived in clusters in the distorted architecture of the injured lung (yellow arrow, central portion), and others were seen in the almost normal tissue (b). ASC, adipose tissue-derived stromal cell; DAPI, 4',6-diamidino-2-phenylindole; BLM, bleomycin; and IT, intratracheal administration.

scores of the two ASC-treated groups (B and E), despite the SMI evaluations being conducted at the same time-points as those of groups A and D. When comparing the groups evaluated on day 42, the SMI scores in group E, which received ASC IT, tended to be lower than those of group D which did not receive ASC IT ($p=0.066$), in contrast to the lack of a significant difference between the SMI scores of groups A and B on day 21 after BLM IT.

Of the groups receiving simple saline IT and subsequent PBS IT, the SMI scores of group C on day 21 did not differ significantly from those of group F on day 42.

Distribution of CM-DiI-labeled ASCs in the lung

CM-Dil-labeled ASCs fluoresced red, and nuclei labeled with DAPI fluoresced blue. On day 21, ASCs were successfully dispersed in the lungs. However, the majority were scattered in the almost

normal areas, and only a limited number were found in the injured areas (Fig. 3a; a representative photograph from group B is shown). On day 42, although their number was reduced, the ASCs lay deep in the thick distorted architecture of the injured lung (Fig. 3b; a representative photograph from group E is shown).

Discussion

A growing body of evidence suggest that MSCs migrating to lesions produce favorable results, such as reduced inflammation and mortality, in a model of acute lung injury and fibrogenesis caused by BLM[31]. While the potential applications are increasing, recent research has highlighted new issues involving MSCs[32]. For instance, the optimal type of MSCs (*i.e.*, from umbilical cord blood, bone marrow, or other tissues), route of administration (bone marrow transplantation, injection to systemic circulation, or instillation to airways), timing (immediately after BLM exposure or several days later), and conditions of administration for repair and healing are not known. In addition, the mechanisms of repair and healing of disease by the administration of MSCs also remain to be determined. Before consideration is given to these questions, it is necessary to reflect on the typical time-course of tissue damage caused by BLM instillation.

A great deal is now known about the time-dependent changes in lung histopathology following a single dose of intratracheal instillation of BLM in rats[33]. Within the first 3 to 5 days, focal areas of intra-alveolar hemorrhage develop. Over the course of the first week, these changes give way to the appearance of atypical alveolar lining cells, and confluent interstitial inflammatory cell invasion. By the second week, the interstitial infiltrates are strongly associated with an increase in fibroblasts and the initiation of deposition of interstitial extracellular collagen. From the third week after instillation, the amount of collagen in the interstitial areas becomes increasingly prominent, and some condensation is evident. By 60 days, BLM delivered intratracheally may induce fibrosis that progresses or persists.

Based on the above-described time-course, the present study was primarily designed to administer WAT-derived ASCs to the lung at the second week after BLM instillation, when lung tissues are supposed to show initial fibrotic changes, and to examine whether the ASCs might affect the pathological processes that developed during the third to sixth week after instillation of BLM. The SMI scores showed that without ASC IT, the lung injury was more severe on the sixth week after BLM instillation than on the third week (groups A and D), consistent with the previous observations described above. ASC IT did not affect the severity of lung damage on the third week after BLM exposure (groups A and B). However, ASC administration tended to prevent further aggravation of the lung injury on the sixth week (group B vs E). Microscopic cell tracing labeled with CM-DiI revealed that while the majority of ASCs did not appear to penetrate inside the lung region injured by BLM on the third week after BLM instillation (Fig. 3a), some of these cells sprouted deep in the thick distorted architecture of the injured lung on the sixth week after BLM instillation (Fig. 3b). Given the prevention of further aggravation of the lung injury on the sixth week as assessed by the SMI scores in the present study, it might be that these migrating ASCs play a role in the preventive effect observed.

Unlike the present study, other morphometric studies have demonstrated that in early stages (within 1-2weeks), intravenously-administered MSCs successfully home to the lung in response to injury, adopt an epithelial-like phenotype, and diminish or abrogate the deleterious effects

of inflammation and collagen deposition in the lung tissue of mice challenged with BLM. The time-courses reported might depend on the experimental settings used. Most previous studies have investigated the effects of bone marrow-derived MSCs, and utilized an observation period ranging from 1 to 4 weeks after BLM exposure[31]. Several studies have assessed the effect of the administration of MSCs through the trachea. However, most of these did not involve the BLM-exposure model, but other disease models such as neonatal hyperoxic lung injury[34,35], bacterial exposure-induced acute lung injury[36,37], and pulmonary hypertension[20,38], and the morphometric observations were performed a few weeks after the injurious events. Accordingly, late-phase changes after injury cannot be predicted from evidence derived from the studies described above. Considering that the pathological changes after BLM exposure persist for over six weeks as described above, it is worthwhile monitoring changes over a longer time-frame than has been monitored in previous studies. In addition, some concerns have been raised about the potentially negative aspects of the administration of MSCs, because these cells can unexpectedly modulate surrounding tissues. For instance, a recent study showed reduced airway remodeling if stem cell factor (SCF) is blocked, and consequently bone marrow-derived MSCs are inhibited from migrating to the sites of injury[39]. Furthermore, another study has demonstrated that circulating fibrocytes are an indicator of poor prognosis with regard to the development of IPF[40]. In this sense, the real advantage of MSC transplantation should arguably not be judged solely on observations during the early stages of the tissue repair process, as has been done in previous studies. The present study investigated pathological changes 3 and 6 weeks after injury, and the findings provide new insight into the effects of intratracheal administration of MSCs on BLM-induced lung damage.

With regard to ASC, a few studies have investigated the effects of administration of ASCs on tissue injuries such as ischemia-reperfusion injury[25], BLM-induced lung injury in an animal model of pulmonary fibrosis[26,27], and acute respiratory distress syndrome[41]. In the study reported by Lee et al[27], a total of eight doses of BLM were intratracheally injected into mice every other week, and human ASCs were also administered repeatedly via intraperitoneal injection simultaneously during the latter 2 months of 4 months of BLM exposure. The lungs were harvested 2 weeks after the last dose (16 weeks after the first BLM exposure). The administration of ASCs, under this study design, ameliorated the inflammatory and remodeling changes in lung tissues induced by BLM instillation. Although the route and dose of administration of ASCs and the observation period differed from those of the present study, there are commonalities between Lee et al[27] and our study, in terms of the study designs focusing on the effects of ASCs on BLM-induced lung injury. With regard to the findings of Lee et al[27], a similar phenomenon seems to occur in injured lung following administration of ASCs via the trachea. However, the intratracheal approach seems more favorable, as ASCs are seeded directly in the airspace, compared with the intravenous or intraperitoneal approaches. Another point to note is the microenvironment formed by ASCs, resident lung cells, intercellular matrix, and secreted humoral factors, which interact and exert collective effects along the route of administration. Also, the microenvironment might constitute an area of settlement for newly migrating cells. For instance, it has been shown that MSCs block production of tumor necrotic factor (TNF)-α and interleukin (IL)-1, which reportedly mediate BLM-induced lung injury[14]. Similarly, ASCs not only possess the ability to self-renew and differentiate into various mesenchymal cell types, but also to secrete significant levels of many potent growth factors and cytokines[22-24]. Intraperitoneal administration of ASCs led to suppression of expression of transforming growth factor (TGF)-β[27]. Further studies are needed

to determine the cytological and biochemical microenvironment in animals with BLM-induced lung injury receiving intratracheal administration of ASCs.

There are some limitations to the present study. First, senescence of the animals used may have led to relative weakness of the repair process, compared with other experimental models[27]. However, since the incidence of IPF in humans is known to increase with age[42], it seems necessary to consider the effects of aging in recipient animals (or humans) on the activity of ASCs[43]. Second, while BLM was only injected once in the present study, multiple BLM instillations are known to increase lung damage[44]. Further studies are required, to determine the effects of intratracheal ASCs in animal models after multiple exposures to BLM. Third, in addition to the analyses of the cytological and biochemical microenvironments around the ASCs in injured tissues, the phenotypic characteristics of ASCs that successfully migrate into the fibrotic regions warrant further investigation.

In conclusion some of the ASCs administered intratracheally sprouted deep into the thick distorted architecture of the injured lung, not by the third week, but by the sixth week after BLM exposure. Given the prevention of further aggravation of the lung injury apparent on the sixth week, as assessed by SMI, it is possible that ASCs can play a preventive role against ongoing aggravation of existing lung injury. Further studies are needed to establish appropriate conditions and timing to facilitate sufficient migration of ASCs into injury sites, for the successful prevention of progression of fibrosis after lung injury, as well as to elucidate the physiological mechanisms involved.

References

1. American Thoracic Society; European Respiratory Society. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 2000;161:646-664.
2. American Thoracic Society; European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 2002;165:277-304.
3. Selman M, Pardo A. Idiopathic pulmonary fibrosis: misunderstandings between epithelial cells and fibroblasts? Sarcoidosis Vasc Diffuse Lung Dis 2004;21:165-172.
4. Selman M, King TE, Pardo A. Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med 2001;134:136-151.
5. Bjoraker JA, Ryu JH, Edwin MK, Myers JL, Tazelaar HD, Schroeder DR, et al. Prognostic significance of histopathologic subsets in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1998;157:199-203.
6. Flaherty KR, Travis WD, Colby TV, Toews GB, Kazerooni EA, Gross BH, et al. Histopathologic variability in usual and nonspecific interstitial pneumonias. Am J Respir Crit Care Med 2001;164:1722-1727.
7. Nicholson AG, Colby TV, du Bois RM, Hansell DM, Wells AU. The prognostic significance of the histologic pattern of interstitial pneumonia in patients presenting with the clinical entity of cryptogenic fibrosing alveolitis. Am J Respir Crit Care Med 2000;162:2213-2217.
8. Hare JM, Traverse JH, Henry TD, Dib N, Strumpf RK, Schulman SP, et al. A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction. J Am Coll Cardiol 2009;54:2277-2286.
9. Stripp BR, Shapiro SD. Stem cells in lung disease, repair, and the potential for therapeutic interventions: State-of-the-art and future challenges. Am J Respir Cell Mol Biol 2006;34:517-518.
10. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71-74.
11. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-147.
12. Prockop DJ, Gregory CA, Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci U S A 2003;100:11917-11923.
13. Rojas M, Xu J, Woods CR, Mora AL, Spears W, Roman J, et al. Bone marrow-derived mesenchymal stem cells

in repair of the injured lung. Am J Respir Cell Mol Biol 2005;33:145-152.
14. Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci U S A 2007;104:11002-11007.
15. Lee SH, Jang AS, Kim YE, Cha JY, Kim TH, Jung S, et al. Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis. Respir Res 2010;11:16.
16. Moodley Y, Vaghjiani V, Chan J, Baltic S, Ryan M, Tchongue J, et al. Anti-inflammatory effects of adult stem cells in sustained lung injury: a comparative study. PLoS One 2013;8:e69299.
17. Zhao F, Zhang YF, Liu YG, Zhou JJ, Li ZK, Wu CG, et al. Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on bleomycin-induced lung injury in rats. Transplant Proc 2008;40:1700-1705.
18. Spees JL, Pociask DA, Sullivan DE, Whitney MJ, Lasky JA, Prockop DJ, et al. Engraftment of bone marrow progenitor cells in a rat model of asbestos-induced pulmonary fibrosis. Am J Respir Crit Care Med 2007;176: 385-394.
19. Ribeiro-Paes JT, Bilaqui A, Greco OT, Ruiz MA, Marcelino MY, Stessuk T, et al. Unicentric study of cell therapy in chronic obstructive pulmonary disease/pulmonary emphysema. Int J Chron Obstruct Pulmon Dis 2011;6:63-71.
20. Baber SR, Deng W, Master RG, Bunnell BA, Taylor BK, Murthy SN, et al. Intratracheal mesenchymal stem cell administration attenuates monocrotaline-induced pulmonary hypertension and endothelial dysfunction. Am J Physiol Heart Circ Physiol 2007;292:H1120-1128.
21. Brzoska M, Geiger H, Gauer S, Baer P. Epithelial differentiation of human adipose tissue-derived adult stem cells. Biochem Biophys Res Commun 2005;330:142-150.
22. Cao Y, Sun Z, Liao L, Meng Y, Han Q, Zhao RC. Human adipose tissue-derived stem cells differentiate into endothelial cells in vitro and improve postnatal neovascularization in vivo. Biochem Biophys Res Commun 2005;332:370-379.
23. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation 2004;109:1292-1298.
24. Nakagami H, Maeda K, Morishita R, Iguchi S, Nishikawa T, Takami Y, et al. Novel autologous cell therapy in ischemic limb disease through growth factor secretion by cultured adipose tissue-derived stromal cells. Arterioscler Thromb Vasc Biol 2005;25:2542-2547.
25. Cai A, Qiu R, Li L, Zheng D, Dong Y, Yu D, et al. Atorvastatin treatment of rats with ischemia-reperfusion injury improves adipose-derived mesenchymal stem cell migration and survival via the SDF-1α/CXCR-4 axis. PLoS One 2013;8:e79100.
26. Uji M, Nakada A, Nakamura T. Intravenous administration of adipose-derived stromal cells does not ameliorate bleomycin-induced lung injury in rats. Open Journal of Regenerative Medicine 2013;2:39-45.
27. Lee SH, Lee EJ, Lee SY, Kim JH, Shim JJ, Shin C, et al. The effect of adipose stem cell therapy on pulmonary fibrosis induced by repetitive intratracheal bleomycin in mice. Exp Lung Res 2014;40:117-125.
28. B Moore B, Lawson WE, Oury TD, Sisson TH, Raghavendran K, Hogaboam CM. Animal models of fibrotic lung disease. Am J Respir Cell Mol Biol 2013;49:167-179.
29. Delaunois LM. Mechanisms in pulmonary toxicology. Clin Chest Med 2004;25:1-14.
30. Lossos IS, Izbicki G, Or R, Goldstein RH, Breuer R. The effect of suramin on bleomycin-induced lung injury. Life Sci 2000;67:2873-2881.
31. Weiss DJ, Bates JH, Gilbert T, Liles WC, Lutzko C, Rajagopal J, et al. Stem cells and cell therapies in lung biology and diseases: conference report. Ann Am Thorac Soc 2013;10:S25-44.
32. Brody AR, Salazar KD, Lankford SM. Mesenchymal stem cells modulate lung injury. Proc Am Thorac Soc 2010;7:130-133.
33. Thrall RS, McCormick JR, Jack RM, McReynolds RA, Ward PA. Bleomycin-induced pulmonary fibrosis in the rat: inhibition by indomethacin. Am J Pathol 1979;95:117-130.
34. Chang YS, Choi SJ, Ahn SY, Sung DK, Sung SI, Yoo HS, et al. Timing of umbilical cord blood derived mesenchymal stem cells transplantation determines therapeutic efficacy in the neonatal hyperoxic lung injury. PLoS One 2013;8:e52419.
35. Chang YS, Ahn SY, Jeon HB, Sung DK, Kim ES, Sung SI, et al. Critical role of vascular endothelial growth factor secreted by mesenchymal stem cells in hyperoxic lung injury. Am J Respir Cell Mol Biol 2014;51:391-399.
36. Gupta N, Su X, Popov B, Lee JW, Serikov V, Matthay MA. Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice. J

Immunol 2007;179:1855-1863.
37. Kim ES, Chang YS, Choi SJ, Kim JK, Yoo HS, Ahn SY, et al. Intratracheal transplantation of human umbilical cord blood-derived mesenchymal stem cells attenuates Escherichia coli-induced acute lung injury in mice. Respir Res 2011;12:108.
38. Jungebluth P, Alici E, Baiguera S, Le Blanc K, Blomberg P, Bozóky B, et al. Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. Lancet 2011;378:1997-2004.
39. Dolgachev VA, Ullenbruch MR, Lukacs NW, Phan SH. Role of stem cell factor and bone marrow-derived fibroblasts in airway remodeling. Am J Pathol 2009;174:390-400.
40. Moeller A, Gilpin SE, Ask K, Cox G, Cook D, Gauldie J, et al. Circulating fibrocytes are an indicator of poor prognosis in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2009;179:588-594.
41. Saito S, Nakayama T, Hashimoto N, Miyata Y, Egashira K, Nakao N, et al. Mesenchymal stem cells stably transduced with a dominant-negative inhibitor of CCL2 greatly attenuate bleomycin-induced lung damage. Am J Pathol 2011;179:1088-1094.
42. Navaratnam V, Fleming KM, West J, Smith CJ, Jenkins RG, Fogarty A, et al. The rising incidence of idiopathic pulmonary fibrosis in the U.K. Thorax 2011;66:462-467.
43. Faner R, Rojas M, Macnee W, Agusti A. Abnormal lung aging in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2012;186:306-313.
44. Degryse AL, Tanjore H, Xu XC, Polosukhin VV, Jones BR, McMahon FB, et al. Repetitive intratracheal bleomycin models several features of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2010; 299:L442-452.