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Bone marrow-derived mononuclear cell therapy attenuates silica-induced lung fibrosis

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ABSTRACT: **This study tests the hypothesis that bone marrow-derived mononuclear cell (BMDMC) therapy may reduce lung inflammation and fibrosis leading to an improvement in respiratory mechanics in a murine model of silicosis.**

52 female C57BL/6 mice were randomly assigned into four groups. In the silica group (SIL), silica suspension (20 mg/50 μL in saline) was intratracheally instilled. In the control animals, 50 μL saline was administered intratracheally. At 1 h, the control and SIL groups were further randomised, receiving BMDMC (2×10^6 *i.v.* control–cell and SIL–cell) or saline (50 μL *i.v.* control and SIL). BMDMC were obtained from male donor mice. At day 15, lung mechanics, histology, and the presence of Y chromosome, interleukin (IL)-1β, IL-1α, IL-1 receptor antagonist (IL-1RN), IL-1 receptor type 1, transforming growth factor (TGF)-β and caspase-3 mRNA expressions in lung tissue were analysed.

In the SIL–cell group, the fraction area of granuloma, the number of macrophages and the collagen fibre content were reduced, yielding improved lung mechanics. The presence of male donor cells in lung tissue was not confirmed using detection of Y chromosome DNA. Nevertheless, caspase-3, IL-1β, IL-1α, IL-1RN and TGF-β mRNA expression diminished after cell therapy.

In conclusion, BMDMC acted on inflammatory and fibrogenic processes improving lung function through paracrine effects.

KEYWORDS: **Cell therapy, collagen, elastance, macrophages**

Silicosis is a pneumoconiosis that involves formation of nodules and destruction of large areas of the lung leading to impaired gas exchange and pulmonary function, which may result in respiratory failure. Despite extensive efforts, no available therapy has been shown to halt or efficiently reverse this disorder [1–3].

Adult bone marrow-derived cells (BMDC) act as progenitor cells in replacing and/or repairing injured tissues [4] in experimental models of fibrosis [5–7]. Recently, we analysed the role of intratracheal instillation of BMDC in a model of silicosis and observed only a mild improvement in lung mechanics [8], which may be attributed to: 1) the pathogenesis of silicosis; 2) the timing of BMDC injection (early or late in the course of lung injury); and 3) the type of cell and route of administration. Therefore, we tested the hypothesis that early, intravenous and a single dose of bone marrow-derived mononuclear cells (BMDMC) may avoid mechanical changes in the lung in a murine model of silica-induced fibrosis. For this purpose, lung static elastance, resistive and viscoelastic pressures, histology, and inflammatory and fibrogenic mediators were measured 15 days after silica instillation.

METHODS

This study was approved by the Ethics Committee of the Carlos Chagas Filho Institute of Biophysics, Health Sciences Centre, Federal University of Rio de Janeiro (Rio de Janeiro, Brazil). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences (Washington, DC, USA).

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Animal preparation and experimental protocol

91 C57BL/6 mice (72 females and 19 males, 20–25 g) were kept under specific pathogen-free conditions in the animal care facility of the Laboratory of Pulmonary Investigation and Cellular and Molecular Physiology, Federal University of Rio de Janeiro. 32 female mice were used to evaluate lung mechanics and histology, and mRNA expression of caspase-3, interleukin (IL)-1β, IL-1α, IL-1 receptor antagonist (IL-1RN), IL-1 receptor type 1 (IL-1R1) and transforming growth factor (TGF)-β, while Y chromosome DNA detection was performed in the remaining 40. All animals were randomly assigned to four groups. In the silica group (SIL), mice received intratracheal injection of 20 mg of silica crystals (particle size: 80% between 1–5 μm; Sigma Chemical, St. Louis, MO, USA) suspended in saline solution (total volume 50 μL), while saline (50 μL *i.t.*) was instilled in the control group (C). BMDMC from male mice (2×10^6 cells·50 μL^{-1} of saline) were injected intravenously (C–cell and SIL–cell groups) 1 h after saline or silica administration.

Extraction of BMDMCs

Bone marrow cells from 14 male C57BL/6 mice (20–25 g) were aspirated from the femur and tibia by flushing the bone marrow cavity with Dulbecco's modified Eagle's medium (DMEM; Life Technologies®, Grand Island, NY, USA). After a homogeneous cell suspension was achieved, the cells were centrifuged ($400 \times g$ for 10 min), re-suspended in DMEM and added to Ficoll-Hypaque (Histopaque 1083; Sigma Chemical), centrifuged again and supplemented with sterile PBS. Cells were counted in a Neubauer chamber with Trypan Blue for evaluation of viability. Cell characterisation was performed by flow cytometry using specific antibodies.

Lung mechanics

15 days after administration of saline or silica, the animals were sedated (diazepam 1 mg *i.p.*), anaesthetised (thiopental sodium 20 mg·kg^{-1} *i.p.*), tracheotomised, paralysed (vecuronium bromide 0.005 mg·kg^{-1} *i.v.*) and ventilated with a constant flow ventilator (Samay VR15; Universidad de la Republica, Montevideo, Uruguay) with the following parameters: frequency of 100 breaths·min^{-1}; tidal volume (V_T) of 0.2 mL; and fraction of inspired oxygen of 0.21. The anterior chest wall was surgically removed and a positive end-expiratory pressure of 2 cmH_2O applied. After a 10-min ventilation period, lung mechanics were computed and at the end of the experiments (~30 min), lungs were prepared for histology.

Lung static elastance ($E_{st,L}$), resistive ($\Delta P_{1,L}$) and viscoelastic ($\Delta P_{2,L}$) pressures were measured by the end-inflation occlusion method [9]. All data were analysed using ANADAT data analysis software (RHT-InfoData, Inc., Montreal, Quebec, Canada).

Histology

A laparotomy was performed immediately after the determination of lung mechanics and heparin (1,000 IU) was injected intravenously in the vena cava. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, yielding a massive haemorrhage that quickly killed the animals. The right lung was fixed with 10% buffered formaldehyde solution and paraffin embedded. 4-μm thick slices (three per lung) were cut and stained with haematoxylin and eosin.

Lung morphometric analysis was performed using an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines (known length) coupled to a conventional light microscope (Olympus BX51; Olympus Latin America-Inc., São Paulo, Brazil). The area fraction of granuloma was determined by the point-counting technique across 20 random non co-incident microscopic fields at a magnification of ×200. Polymorphonuclear, mononuclear and total cells in lung parenchyma and granuloma were evaluated at ×1,000 magnification and determined by the point-counting technique [10].

Collagen fibres (picrosirius method) were quantified in both alveolar septa and granuloma [11]. Immunohistochemistry was performed with the conventional avidin-biotin-peroxidase histochemical technique using a goat anti-rat biotinylated antibody (BA-4001; Vector Laboratories, Burlingame, CA, USA) for biotinylated Bandeiraea simplicifolia lectin 1 (BSL-1, B1205; Vector Laboratories) which labels macrophages [12, 13]. In each slide, 15 different microscopic fields were randomly selected and quantification (×200 magnification) was carried out with the aid of a digital camera (Evolution MP kit Media Cybernetics, Silver Spring, MD, USA) coupled to a light microscope (Eclipse 400; Nikon, Tokyo, Japan). High-quality (2,048 × 1,536 pixels) images were obtained using the Image Pro Plus 4.5.1 software (Media Cybernetics). The thresholds for collagen fibres were established after the contrast was enhanced up to a point at which the fibres were easily identified as birefringent bands. The area occupied by collagen fibres was determined by digital densitometric recognition. Bronchi and blood vessels were carefully avoided during the measurements. The area occupied by fibres was divided by tissue area and expressed as fraction area of collagen fibre. Positive cells (macrophages) were counted and divided by lung parenchyma and granuloma area and expressed as fraction area of macrophages.

Y chromosome DNA detection

Quantification of murine Y chromosome in lung tissue was achieved by quantitative real-time PCR at days 1, 3, 7 and 15. Briefly, DNA was purified in a 600-μL solution of 0.2% sodium dodecyl sulfate/proteinase K (300 μg·mL^{-1}), extracted with an equal volume of phenol/chloroform/isoamyl alcohol. After centrifugation, the aqueous phase was transferred to a new tube and DNA was precipitated with two volumes of ethanol 100%. DNA was resuspended and quantified in a nanodrop spectrophotometer. 5 ng of DNA were used in a real-time PCR reaction with the SYBR Green detection kit run in a 7000 Sequence Detection System thermocycler according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The following PCR primers were used: forward: 5′-TCATCGGAGGGCTAAAGTG-3′; reverse: 5′-CAACCTTCTG CAGTGGGAC-3′. Primer sequences were defined using primer3 software based on the Mus musculus sex-determining region of the Chr Y (Sry) gene (GenBank accession number NM_011564; National Institutes of Health, Bethesda, MD, USA). These primers amplify an 88 bp product. The relative amount of total DNA was calculated as a ratio ($2-\Delta C_t$) of Sry and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers for GAPDH were: forward: 5′-CCACCAACT GCTTAGCCC-3′; reverse; 5′-GACACCTACAAAGAAGGGT CCA-3′, 145 bp [8].

Expression of caspase-3, IL-1β, IL-1α, IL-1RN, IL-1R1 and TGF-β

Quantitative real-time RT-PCR was performed to measure the relative levels of expression of inflammatory and fibrogenic mediators. Central slices of left lung were cut, collected in cryotubes, quick frozen by immersion in liquid nitrogen and stored at -70°C. Total RNA was extracted from the frozen tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations. RNA concentration was measured by spectrophotometry in Nanodrop® ND-1000. First-strand cDNA was synthesised from total RNA using an M-MLV Reverse Transcriptase Kit (Invitrogen). PCR primers for the target gene were purchased from Invitrogen. Relative mRNA levels were measured with a SYBR Green detection system using ABI 7500 real-time PCR (Applied Biosystems). All samples were measured in triplicate. The relative amounts of caspase-3, IL-1β and TGF-β expression were calculated as a ratio ($2^{-\Delta C_t}$) of the study gene and the control gene (GAPDH). Primers used and PCR product size were as follows. Caspase-3: forward 5′-TACCGGTGGAGG CTGACT-3′ and reverse 5′-GCTGCAAAGGGACTGGAT-3′, 104 bp; IL-1β: forward 5′-GTTGACGGACCCCAAAAG-3′ and

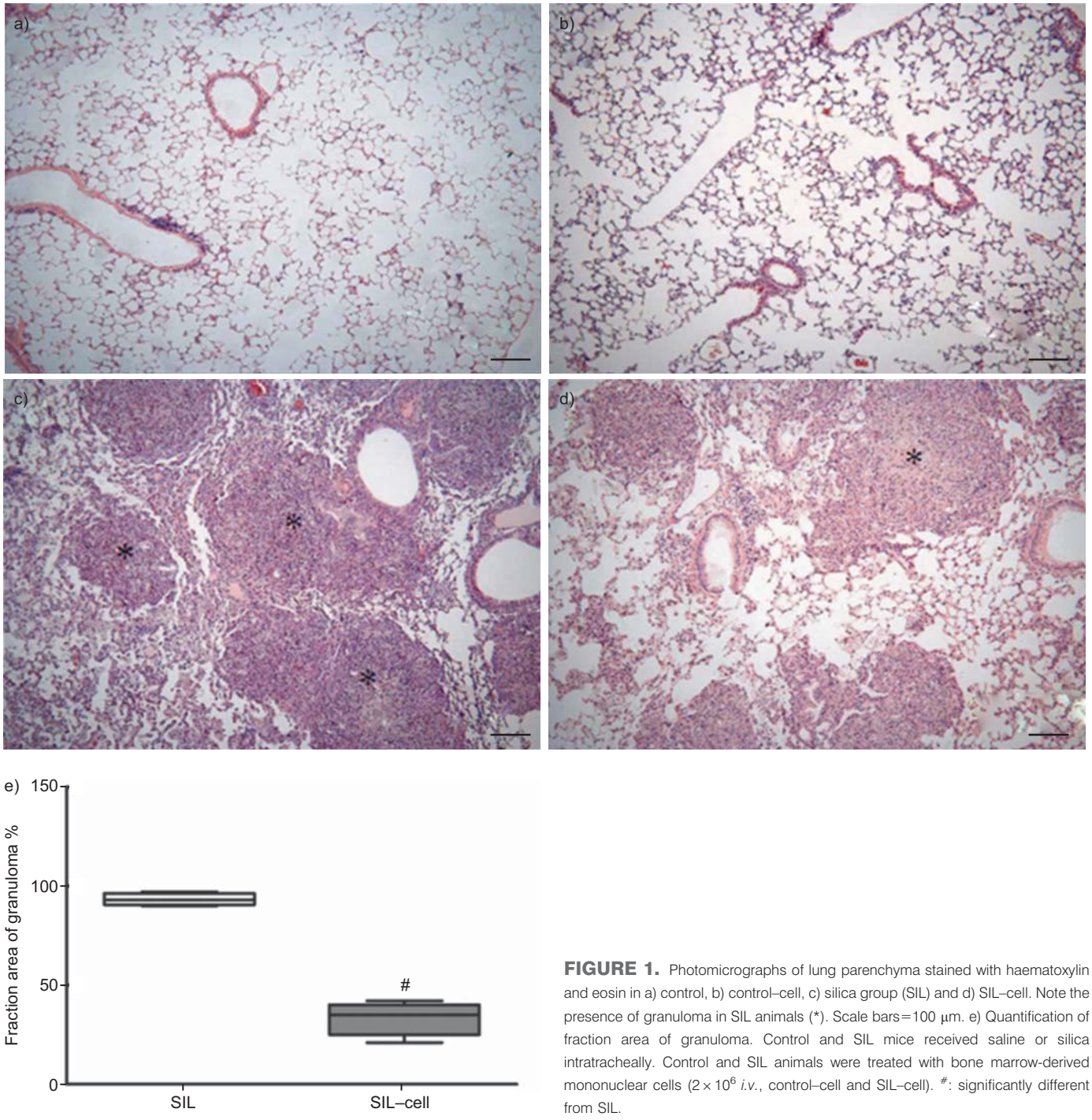

FIGURE 1. Photomicrographs of lung parenchyma stained with haematoxylin and eosin in a) control, b) control–cell, c) silica group (SIL) and d) SIL–cell. Note the presence of granuloma in SIL animals (*). Scale bars=100 μm. e) Quantification of fraction area of granuloma. Control and SIL mice received saline or silica intratracheally. Control and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, control–cell and SIL–cell). #: significantly different from SIL.

reverse 5′-GTGCTGCTGCGAGATTTG-3′, 93 bp; IL-1α: forward 5′-TCAACCAAACTATATATCAGGATGTGG-3′ and reverse 5′-CGAGTAGGCATACATGTCAAATTTTAC-3′, 102 bp; IL-1RN: forward 5′-AACCACCAGGGCATCACATA-3′ and reverse 5′-CCTCTTGCCGACATGGAATA-3′, 150 bp; IL-1R1: forward 5′-GAGTTACCCGAGGTCCAG-3′ and reverse 5′-GAA GAAGCTCACGTTGTC-3′, 66 bp; TGF-β: forward 5′-ATACGC CTGAGTGGCTGTC-3′ and reverse 5′-GCCCTGTATTCCGTCT CCT-3′, 77 bp; and GAPDH: forward 5′-AACTTTGGCATTGTG GAAGG-3′ and reverse 5′-GTCTTCTGGGTGGCAGTGAT-3′, 62 bp [8].

Statistical analysis

The normality of the data (Kolmogorov–Smirnov test with Lilliefors′ correction) and the homogeneity of variances (Levene median test) were tested. If both conditions were satisfied, differences between the groups were assessed by two-way ANOVA followed by Tukey's test. The comparison between the SIL and SIL–cell groups was performed using the unpaired t-test or Mann–Whitney U-test for parametric and nonparametric data, respectively. Data are presented as the mean±SEM or median (25th–75th percentiles) as appropriate. In all tests the significance level was set at 5%. Statistical analyses were performed using SigmaStat 3.1 (Jandel Scientific, San Rafael, CA, USA).

RESULTS

BMDMC effects on survival, lung inflammation and collagen accumulation

The pool of BMDMC intravenously injected were characterised by flow cytometry showing the following composition: total lymphocyte (4.18%) (CD45+, CD11b-, CD29- and CD34-); T lymphocyte (2.13%) (CD45+, CD3+ and CD34-); T-helper lymphocyte (0.47%) (CD4+ and CD8-); T cytotoxic (1.66%) (CD4- and CD8+); monocytes (2.76%) (CD45+, CD29+, CD14+, CD38+, CD11b-, CD34- and CD3-); granulocyte (78.7%) (CD45+, CD11b+, CD38+, CD34-, CD29-, CD14-, CD34- and CD3-); haematopoietic progenitors (0.48%) (CD34+); and other progenitors (9.13%).

The animal survival rate was 100% in the control group and 53% in the SIL group. BMDMC injection yielded a 79% survival rate ($p<0.05$).

Histological evaluation of the silica-treated mice showed interstitial and alveolar oedema, and granulomatous nodules with large accumulations of inflammatory cells. The treatment with BMDMC yielded a significant reduction in the area fraction of granuloma (144%) (fig. 1). However, no significant changes were observed in polymorphonuclear (PMN) and mononuclear (MN) cells in the granuloma. Conversely, silica-treated mice presented a higher number of PMN and MN cells in lung parenchyma compared with the control group. BMDMC therapy led to a reduction in PMN and MN cells (table 1).

In the SIL group, the number of BSL-1-positive macrophages was increased in lung parenchyma and granuloma. BMDMC therapy resulted in a significant reduction of the amount of BSL-1-positive macrophages in lung parenchyma with no significant changes in the granuloma (fig. 2).

The amount of collagen fibres in the alveolar septa and granuloma reduced after BMDMC therapy in silica-treated mice (fig. 3).

BMDMC effects on lung engraftment and IL-1β, IL-1α, IL-1RN, IL-1R1, TGF-β and caspase-3

Y chromosome DNA was not detected in lung tissue at days 1, 3, 7 and 15 (supplementary material).

IL-1β, IL-1α and IL-1RN expressions were higher in SIL compared to the control group (3.45-, 3.37- and 4.40-fold, respectively) (fig. 4). BMDMC significantly reduced IL-1β, IL-1α and IL-1RN mRNA expressions. IL-1R1 mRNA expression remained unaltered independent of silica or BMDMC administration.

Caspase-3 (fig. 5a) and TGF-β (fig. 5b) mRNA expressions in lung tissue were higher in SIL compared to the control group (2.48- and 3.51-fold, respectively). BMDMC therapy significantly reduced caspase-3 and TGF-β mRNA expressions to control values.

Lung mechanics

There was no significant difference in flow and V_T among the groups. $E_{st,L}$, $\Delta P_{1,L}$ and $\Delta P_{2,L}$ pressures were similar in the control and C–cell groups. In the SIL group, $E_{st,L}$, $\Delta P_{1,L}$ and $\Delta P_{2,L}$ were higher (177%, 150% and 177%, respectively) than in the control group. BMDMC significantly inhibited mechanical changes in the lung (fig. 6).

DISCUSSION

In the current study, we observed that early intravenous therapy with BMDMC led to a reduction in the area fraction of granuloma, the number of macrophages in the alveolar septa, and collagen fibre content resulting in an improvement in $E_{st,L}$, $\Delta P_{1,L}$ and $\Delta P_{2,L}$ pressures. These beneficial effects were shown to be not associated with engraftment but can be attributed to paracrine effects reducing caspase-3, IL-1β, IL-1α, IL-1RN, IL-1R1 and TGF-β mRNA expression.

Animal studies have shown that even a single exposure to crystalline silica can lead to pulmonary morpho-functional

TABLE 1 Total and differential cellularity in lung parenchyma and granuloma

	PMN %	MN %
Lung parenchyma		
Control	0.43±0.11	29.75±0.38
Control–cell	1.67±0.51	28.09±2.00
Silica group	5.95±0.65#	35.02±0.71#
Silica group–cell	2.63±0.67	27.00±0.75
Granuloma		
Silica group	8.35±0.81	28.36±0.65
Silica group–cell	7.57±1.05	27.91±2.14

Data are present as mean±SD. n=5 animals per group. PMN: polymorphonuclear cell; MN: mononuclear cells. Control and silica group mice received saline (50 μL) or silica (20 mg silica/50 μL saline) intratracheally, respectively. Control and silica group animals were treated with saline or bone marrow-derived mononuclear cells (2×10^6 *i.v.*). #: significantly different from control.

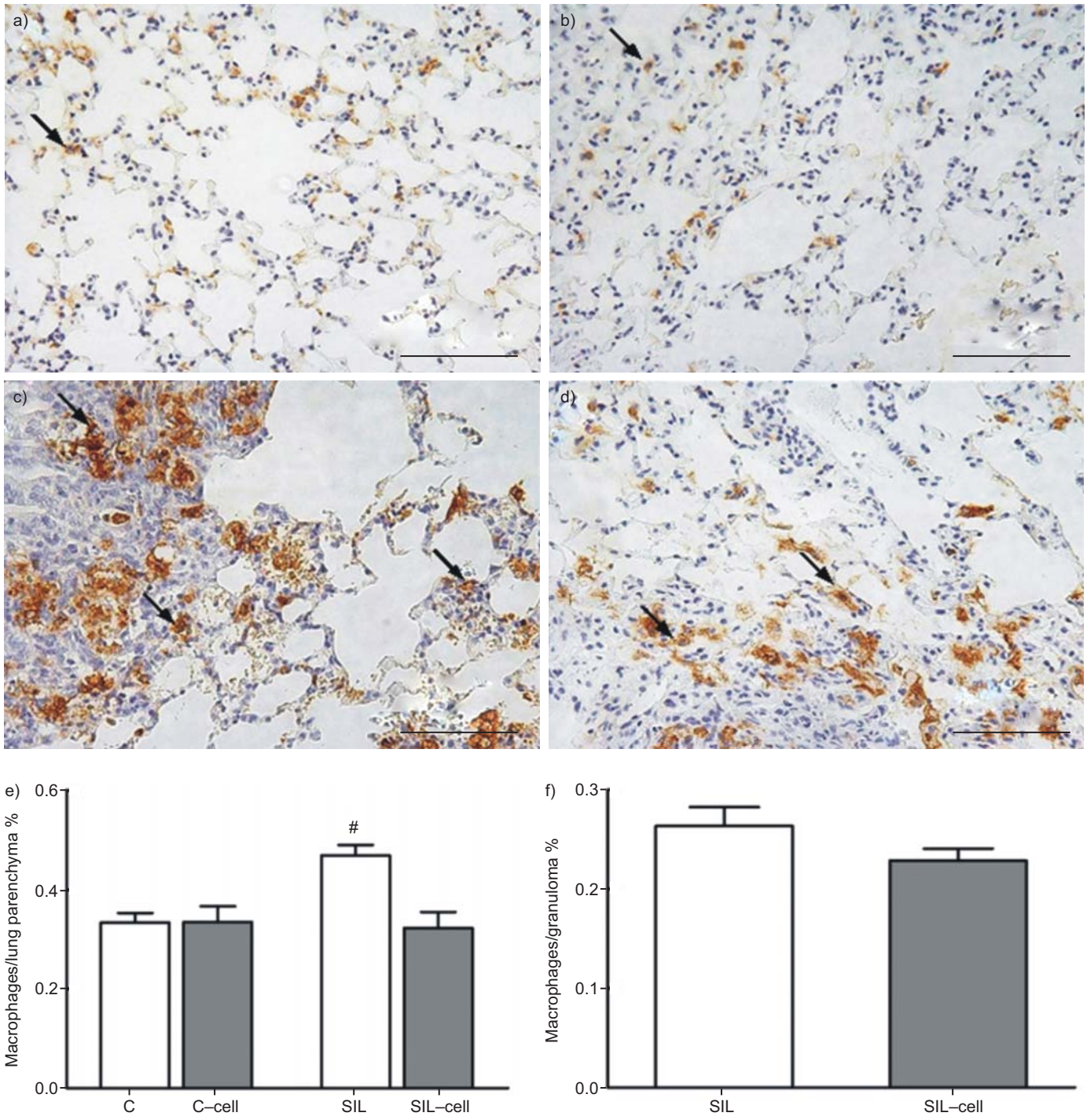

FIGURE 2. Photomicrographs of lung parenchyma after lectin histochemistry in a) control (C), b) C–cell, c) silica group (SIL) and d) SIL–cell. Note the macrophages in the lung parenchyma and the granuloma (arrows). Scale bars=100 μm. Quantification of macrophages in e) the lung parenchyma and f) the granuloma. C and SIL mice received saline or silica intratracheally. C and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, C–cell and SIL–cell). Data are presented as mean±SD. n=5 animals per group. #: significantly different from C.

changes at day 15 mimicking the clinical setting [14, 15]. Silica animals presented increased $E_{st,L}$, $\Delta P_{1,L}$ and $\Delta P_{2,L}$ pressures. The changes observed in $\Delta P_{1,L}$ could be attributed to the intrabronchial cellular infiltration obstructing the lumen. The increase in $E_{st,L}$ and $\Delta P_{2,L}$ pressure may be associated with the presence of granulomatous nodules, increased number of cells in the alveolar septa and granuloma, alveolar collapse, distortion of patent alveoli and interstitial oedema in accordance with previous studies on silica-treated BALB/c mice [14–16]. The SIL group also showed an increase in the number of macrophages in the granuloma and lung parenchyma, which may release inflammatory cytokines such as IL-1 [17],

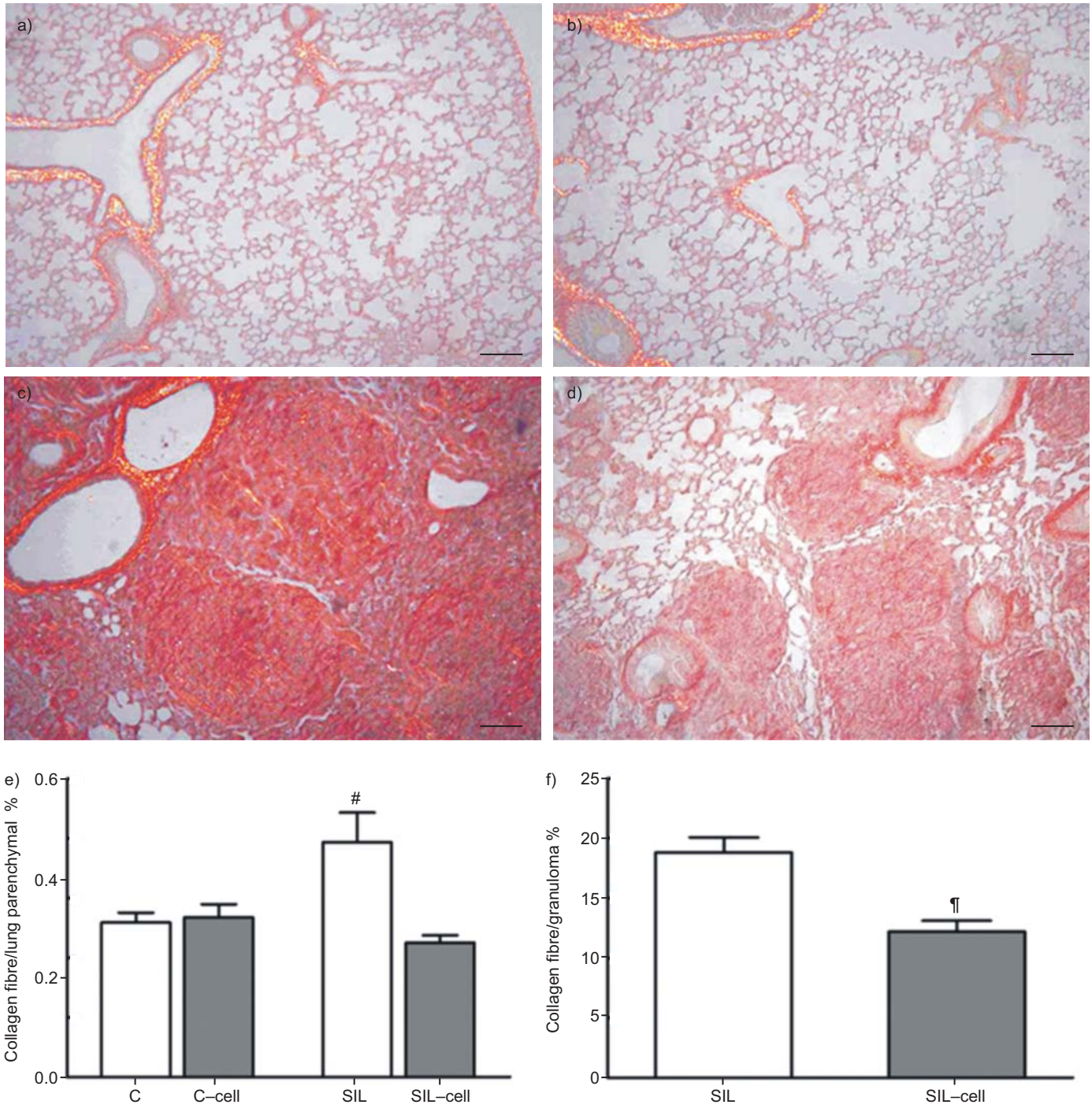

FIGURE 3. Photomicrographs of lung parenchyma stained with picrosirius under polarisation in a) control (C), b) C–cell, c) silica group (SIL) and d) SIL–cell. Scale bars=100 μm. Collagen fibre content in e) alveolar septa and f) the granuloma. C and SIL mice received saline or silica intratracheally. C and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, C–cell and SIL–cell). Data are presented as mean±SEM. n=5 animals per group. #: significantly different from C; ¶: significantly different from SIL.

yielding a recruitment of inflammatory cells into the alveolar septa [17, 18]. The IL-1 family includes IL-1α, IL-1β and IL-1RN, which bind to the same receptor, IL-1R1 [19, 20]. In this context, the increase in levels of IL-1 expression in lung tissue was correlated with the development of pulmonary fibrosis. BMDMC therapy reduced IL-1α and IL-1RN mRNA expressions in accordance with the study by ORTIZ *et al.* [19], who observed a decrease of IL-1α and IL-1RN mRNA expressions after mesenchymal stem cell administration in a murine model of bleomycin-induced injury. Conversely, IL-1R1 mRNA expression remained unchanged after silica and/or BMDMC injection. Concurrent to the inflammatory process, the exposure to silica

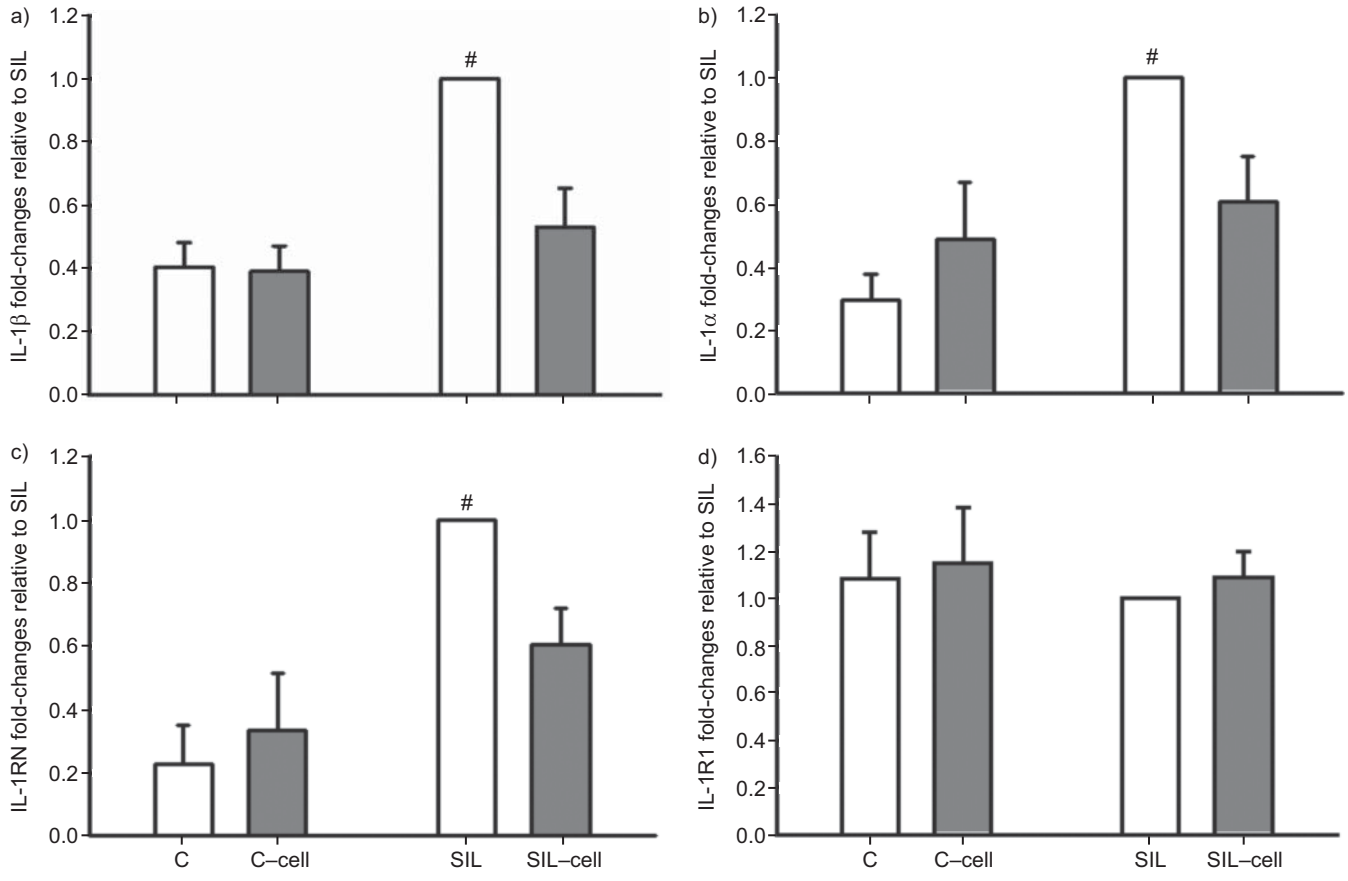

FIGURE 4. Real-time PCR analysis of a) interleukin (IL)-1β, b) IL-1α, c) IL-1 receptor antagonist (IL-1RN) and d) IL-1 receptor type 1 (IL-1R1) mRNA expressions. Control (C) and silica group (SIL) mice received saline or silica intratracheally. C and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, C–cell and SIL–cell). Data are presented as mean±SEM. n=5 animals per group. #: significantly different from C.

also initiates apoptosis [18, 21], increasing caspase-3 expression (fig. 4) [22]. Apoptosis has also been implicated in the trigger of the remodelling process [23], increasing TGF-β expression (fig. 4), which can influence mesenchymal cell migration, proliferation and extracellular matrix deposition [23, 24]. Therefore, collagen fibre content increased in alveolar septa and granuloma (fig. 3). BMDMC reduced the inflammatory process decreasing the number of macrophages which have a critical role in silica-induced lung fibrosis, and reducing IL-1β expression in lung tissue. The reduction of IL-1β mRNA

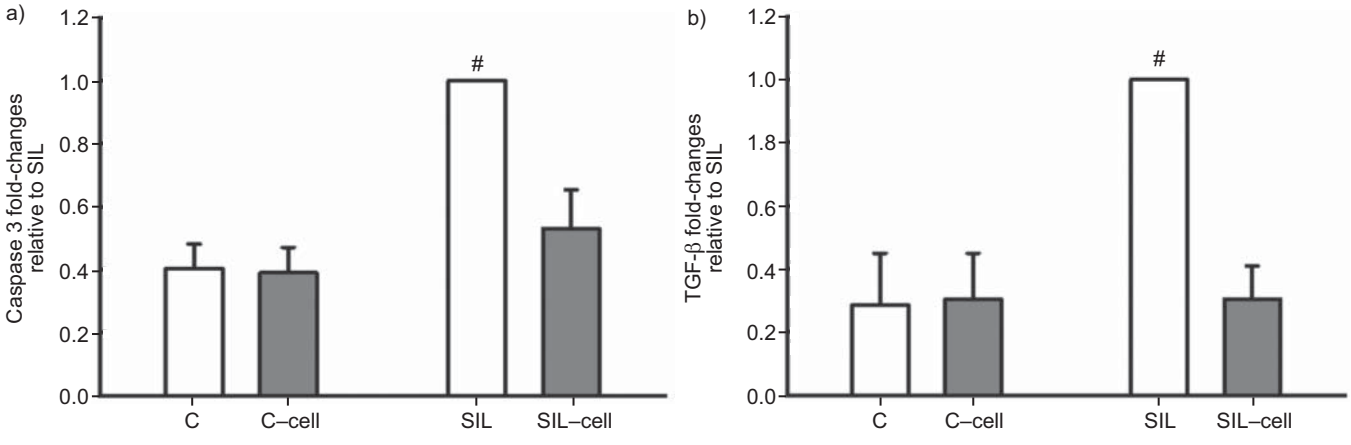

FIGURE 5. Real-time PCR analysis of a) caspase-3 and b) transforming growth factor (TGF)-β mRNA expressions. Control (C) and silica group (SIL) mice received saline or silica intratracheally. C and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, C–cell and SIL–cell). Data are presented as mean±SEM. n=5 animals per group. #: significantly different from C.

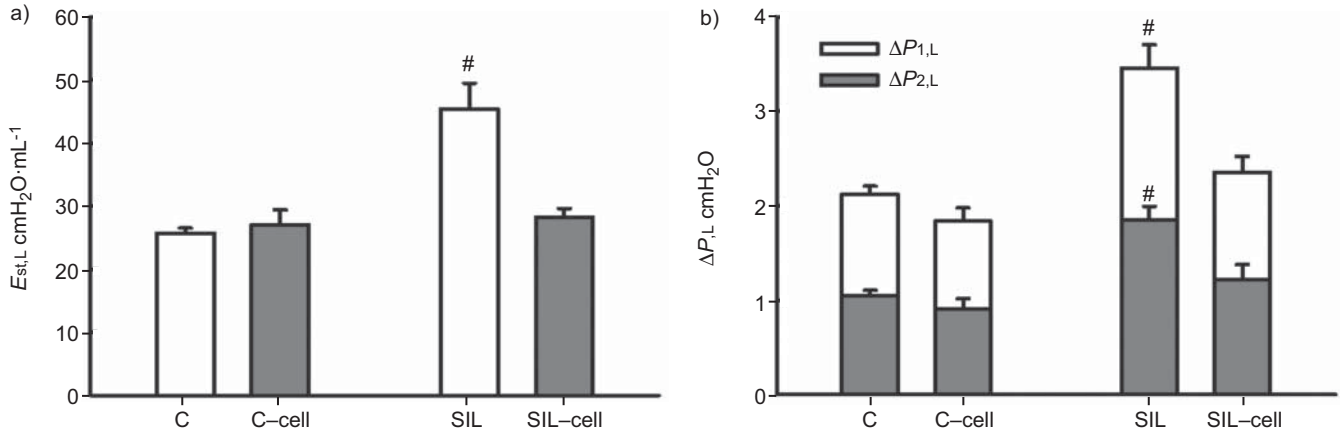

FIGURE 6. a) Lung static elastance ($E_{st,L}$) and b) resistive pressure ($\Delta P_{1,L}$) and viscoelastic/inhomogeneous ($\Delta P_{2,L}$) pressure. Control (C) and silica group (SIL) mice received saline or silica intratracheally. C and SIL animals were treated with bone marrow-derived mononuclear cells (2×10^6 *i.v.*, C–cell and SIL–cell). Data are presented as mean±SEM. n=5 animals per group. #: significantly different from C.

expression may be related to the decrease in the number of macrophages in lung parenchyma [2]. Pro-inflammatory cytokines, such as IL-1β, play a key role in the development of silicosis by regulating the mediators that are responsible for the persistence of inflammation and development of fibrosis [25]. In addition, IL-1β has been implicated in the deposition of collagen [26]. In this context, BMDMC reduced the amount of collagen fibre in the alveolar septa and granuloma, and TGF-β mRNA expression. Furthermore, apoptotic mechanisms have been implicated in silica-induced pathogenesis [2, 14, 16]. Caspase-3 expression was reduced in treated mice.

Previous studies have suggested that BMDCs have therapeutic potential for lung fibrosis [5, 6]. These studies used mesenchymal stem cells (MSC) which require cell-culture processes, yielding some disadvantages related to culture conditions that are detrimental for cell transplantation and the risk of contamination and immunological reactions [27]. Recently, haematopoietic stem cells have been recognised as progenitors for several cell types (endothelial, epithelial, myocytes and neurons) and have been able to reduce lung injury in murine model of fibrosis [28]. Therefore, in our study bone marrow mononuclear cells, which are known to contain both haematopoietic and MSC, were chosen to treat silica-induced lung fibrosis. Furthermore, the majority of stem cells are trapped inside the lungs following intravenous infusion, with the BMDMC passage increased 30-fold compared with MSCs [29].

In a previous study, ORTIZ *et al.* [5] showed that MSC therapy decreased lung inflammation and the levels of hydroxyproline in bleomycin-induced fibrosis. ROJAS *et al.* [6] have also described a reduction in the expression of some cytokines (IL-1β, IL-2, and IL-4) and the repair of bleomycin-injured lungs. In a murine model of asbestos-induced pulmonary fibrosis, SPEES *et al.* [7] were able to identify rare BMDC with the phenotype of type II pneumocytes. All these studies described an engraftment lower than 5% [5, 6]. In this context, LASSANCE *et al.* [8] reported that intratracheal instillation of BMDCs improved lung mechanics and histology independent of the ability of cells to engraft in the lung in a murine model of silicosis. In silicotic animals, the use of fluorescent techniques to evaluate homing, such as green fluorescent protein-positive cells and fluorescence *in situ* hybridisation analysis, is not feasible because silica crystal is highly birefringent. Therefore, in the present study, male DNA amplification by real-time PCR was performed [5, 6], and donor cells could not be detected in treated mice at days 1, 3, 7 and 15. The decrease in lung fibrosis by BMDMC therapy shown here could be ascribed to paracrine effects reducing the release of inflammatory and fibrogenic mediators.

In conclusion, the beneficial effects of early intravenous BMDMC therapy may be independent of bone marrow cell homing into the lung, but mediated through a down-regulation of inflammatory and fibrogenic responses to silica. Therefore, collagen fibre content was reduced followed by a reorganisation of lung parenchyma and improvement in pulmonary mechanics. These findings are the first evidence that early administration of BMDMC therapy may have beneficial effects after silica-induced lung injury. Therefore, the present experimental model system could be used as starting point for more focused research in the future.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

None declared.

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