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Bone marrow-derived mononuclear cells vs. mesenchymal stromal cells in experimental allergic asthma

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ABSTRACT

We compared the effects of bone marrow-derived mononuclear cells (BMMCs) and mesenchymal stromal cells (MSCs) on airway inflammation and remodeling and lung mechanics in experimental allergic asthma. C57BL/6 mice were sensitized and challenged with ovalbumin (OVA group). A control group received saline using the same protocol. Twenty-four hours after the last challenge, groups were further randomized into subgroups to receive saline, BMMCs (2×10^6) or MSCs (1×10^5) intratracheally. BMMC and MSC administration decreased cell infiltration, bronchoconstriction index, alveolar collapse, collagen fiber content in the alveolar septa, and interleukin (IL)-4, IL-13, transforming growth factor (TGF)-β and vascular endothelial growth factor (VEGF) levels compared to OVA-SAL. Lung function, alveolar collapse, collagen fiber deposition in alveolar septa, and levels of TGF-β and VEGF improved more after BMMC than MSC therapy. In conclusion, intratracheal BMMC and MSC administration effectively modulated inflammation and fibrogenesis in an experimental model of asthma, but BMMCs was associated with greater benefit in terms of reducing levels of fibrogenesis-related growth factors.

1. Introduction

Asthma is a chronic inflammatory disease of the airways (Murphy and O'Byrne, 2010) associated with structural changes such as subepithelial fibrosis, mucous metaplasia, wall thickening, smooth muscle cell hypertrophy and hyperplasia, myofibroblast hyperplasia, vascular proliferation, and extracellular matrix abnormalities (Al-Muhsen et al., 2011). These changes accelerate decline in lung function despite treatment with inhaled corticosteroids. Therefore, new strategies that can hasten the repair process and attenuate airway inflammation and remodeling are warranted.

Several recent studies have investigated the impact of bone marrow-derived mononuclear cells (BMMCs) (Abreu et al., 2011) or mesenchymal stromal cells (MSCs) (Firinci et al., 2011; Goodwin et al., 2011; Ou-Yang et al., 2011; Kapoor et al., 2012) in experimental allergic asthma. Each has specific advantages. BMMCs can be used in autologous transplantation, on the same day of harvesting, and avoid common complications such as graft-versus-host disease, whereas MSCs exhibit multilineage differentiation potential and immune-privileged features and enable allogenic use (Mathieu et al., 2009; Lu et al., 2011). So far, however, no study has evaluated the effects of cell type in cell therapy of experimental asthma. Furthermore, most cell therapies have been studied at the onset of the remodeling process; there are no data on the effects of cell therapy once the remodeling process of asthma is already established. Within this context, the present study sought to investigate and compare the therapeutic effects of BMMCs or MSCs on lung mechanics and histology, collagen fiber content in the airway and alveolar septa, and levels of cytokines and growth factors in lung tissue in a murine model of experimental allergic asthma.

2. Materials and methods

This study was approved by the Ethics Committee of the Health Sciences Center, Federal University of Rio de Janeiro.

2.1. Extraction and characterization of BMMCs and MSCs

BMMCs and MSCs were obtained from male C57BL/6 mice (weight 20–25 g, $n = 5$ per group) and administered on the day of collection or after 3 passages, respectively. Bone marrow cells

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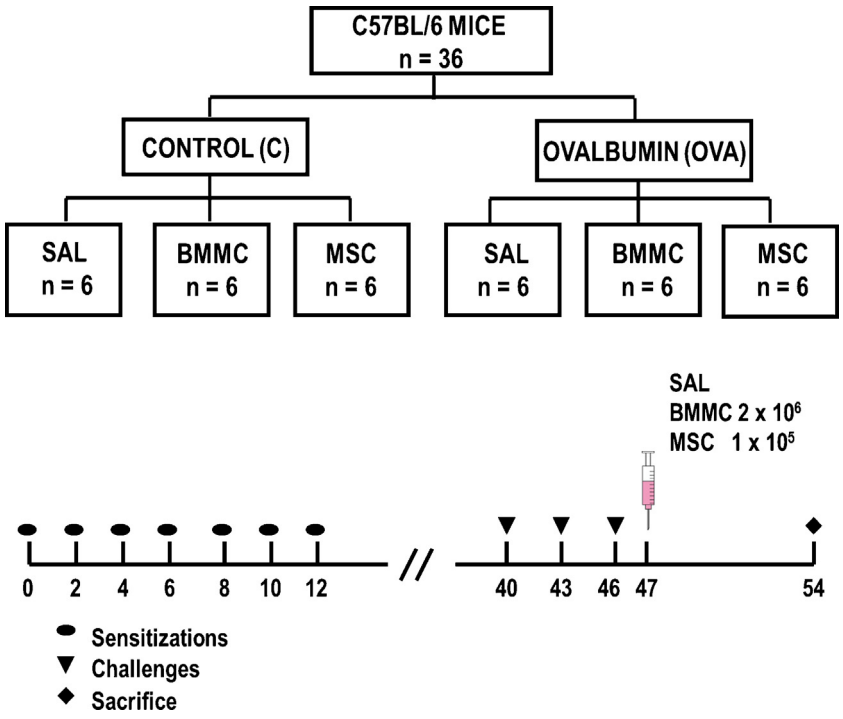

Fig. 1. Study design. (A) Schematic flow chart and (B) timeline of the study. Experimental groups: C, mice sensitized and challenged with saline; OVA, mice sensitized and challenged with ovalbumin; SAL, mice treated with saline; BMMC, mice treated with BMMC 24 h after the last challenge (2×10^6); MSC, mice treated with MSCs 24 h after the last challenge (1×10^5). All data were analyzed on day 54.

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, cells were centrifuged ($400 \times g$ for 10 min), plated in DMEM containing 20% fetal bovine serum (MSCs) or re-suspended in DMEM (BMMCs) and added to Ficoll-Hypaque (Histopaque 1083, Sigma Chemical Co., St. Louis, MO, USA), and again centrifuged and supplemented with phosphate-buffered saline (PBS). Cell characterization was performed by flow cytometry using antibodies against CD45 (leukocytes), CD34 (hematopoietic precursors), CD3, CD8, and CD4 (T lymphocytes), CD19 (B lymphocytes), CD14 (monocytes), and CD11b, CD29 and CD45 (non-hematopoietic precursors) (BD Biosciences, USA). The absence of CD34 and CD45 and the presence of CD14, CD29, and Sca-1 were used to identify MSCs. Furthermore, MSCs were identified by the capacity to differentiate into osteoblasts and chondroblasts.

2.2. Animal preparation and experimental protocol

Thirty-six female C57BL/6 mice (weight, 20–25 g) were randomly assigned to two groups. In the OVA group, mice were immunized using an adjuvant-free protocol by intraperitoneal injection of sterile ovalbumin (OVA, 10 μg OVA in 100 μl saline) on 7 alternate days. Forty days after the start of sensitization, 20 μg of OVA in 20 μl of saline were instilled intratracheally. This procedure was performed 3 times at 3-day intervals (Xisto et al., 2005). The control group (C) received saline using the same protocol. The C and OVA groups were further randomized to receive saline solution (0.9% NaCl, 50 μl, SAL), BMMCs (2×10^6 in 50 μl) or MSCs (1×10^5 in 50 μl) intratracheally, 24 h after the last challenge (Fig. 1). For the administration of saline, BMMCs or MSCs, mice were anesthetized with sevoflurane, the trachea of each mouse was dissected, and cells were slowly injected. Furthermore, small aliquots were used for immunophenotypic flow cytometry characterization of the injected cell populations and to evaluate the ability of MSCs to differentiate into osteoblasts and chondroblasts (Fig. 2).

2.3. Mechanical parameters

One week after cell therapy, the animals were sedated (diazepam 1 mg i.p.), anesthetized (thiopental sodium 20 mg/kg i.p.), tracheotomized, paralyzed (vecuronium bromide, 0.005 mg/kg i.v.), and ventilated with a constant flow ventilator (Samay VR15; Universidad de la Republica, Montevideo, Uruguay) set to the following parameters: frequency 100 breaths/min, tidal volume (V_T) 0.2 mL, and fraction of inspired oxygen (FiO_2) 0.21. The anterior chest wall was surgically removed and a positive end-expiratory pressure of 2 cm H_2O applied. Airflow and tracheal pressure (Ptr) were measured. Lung mechanics were analyzed by the end-inflation occlusion method. In an open chest preparation, Ptr reflects transpulmonary pressure (PL). Briefly, after end-inspiratory occlusion, there is a rapid initial decline in PL (ΔP_1,L) from the preocclusion value down to an inflection point (Pi), followed by a slow pressure decay (ΔP_2,L), until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lung (Pel). ΔP_1,L selectively reflects the pressure used to overcome airway resistance. ΔP_2,L reproduces the pressure spent by stress relaxation, or the viscoelastic properties of the lung, as well as a small contribution of pendelluft. Static lung elastance (Est,L) was determined by dividing Pel by V_T. Lung mechanics measurements were obtained 10 times in each animal. All data were analyzed using ANADAT software (RHT-InfoData, Inc., Montreal, Quebec, Canada). All experiments lasted less than 15 min.

2.4. Lung histology

Laparotomy was performed immediately after determination of lung mechanics. Heparin (1000 IU) was injected into the vena cava. The trachea was clamped at end-expiration, and the abdominal aorta and vena cava were sectioned, producing massive