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Effects of bone marrow-derived mononuclear cells on airway and lung parenchyma remodeling in a murine model of chronic allergic inflammation

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ABSTRACT

We hypothesized that bone marrow-derived mononuclear cells (BMDMC) would attenuate the remodeling process in a chronic allergic inflammation model. C57BL/6 mice were assigned to two groups. In OVA, mice were sensitized and repeatedly challenged with ovalbumin. Control mice (C) received saline under the same protocol. C and OVA were further randomized to receive BMDMC (2×10^6) or saline intravenously 24 h before the first challenge. BMDMC therapy reduced eosinophil infiltration, smooth muscle-specific actin expression, subepithelial fibrosis, and myocyte hypertrophy and hyperplasia, thus causing a decrease in airway hyperresponsiveness and lung mechanical parameters. BMDMC from green fluorescent protein (GFP)-transgenic mice transplanted into GFP-negative mice yielded lower engraftment in OVA. BMDMC increased insulin-like growth factor expression, but reduced interleukin-5, transforming growth factor-β, platelet-derived growth factor, and vascular endothelial growth factor mRNA expression. In conclusion, in the present chronic allergic inflammation model, BMDMC therapy was an effective pre-treatment protocol that potentiated airway epithelial cell repair and prevented inflammatory and remodeling processes.

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1. Introduction

Recently, new attention has been directed to the long-term changes in asthmatic airways as indicated by the accelerated rate of lung function decline despite therapy with inhaled corticosteroids. These structural changes in the airway wall, termed airway remodeling, are now thought to be a key component of the pathophysiology of asthma. Airway remodeling is characterized by: subepithelial fibrosis, mucous metaplasia, wall thickening, smooth muscle cell hypertrophy and hyperplasia, myofibroblast hyperplasia, vascular proliferation, and changes in the extracellular matrix, such as deposition of collagen fiber and elastic fiber fragmentation (Holgate et al., 2004; Xisto et al., 2005). Therefore, a therapy that hastens the repair process and attenuates both inflammatory and remodeling responses is thought to be important to improve asthma management.

Bone marrow-derived stem cells are potent modulators of immune responses, promoting the proliferation and re-epithelization of lung cells. Several studies have shown beneficial effects of cell-based therapy in respiratory diseases (Rojas et al., 2005; Gupta et al., 2007; Zhao et al., 2008; Zhen et al., 2008) resulting from cell plasticity (Krause, 2002; Herzog et al., 2003) and/or the release of paracrine factors (Rojas et al., 2005; Ortiz et al., 2007). So far, however, no study has analyzed the effects of bone marrow-derived stem cells in experimental allergic asthma.

We tested the hypothesis that pre-treatment with bone marrow-derived mononuclear cells (BMDMC) may prevent inflammatory and remodeling processes while improving lung function in a murine model of chronic allergic inflammation. For this purpose, lung mechanics and histology, elastic and collagen fiber content in airways and alveolar septa, the amount of smooth muscle-specific actin present in distal airways and alveolar duct walls, and the expression of inflammatory cytokines and growth factors were analyzed.

2. Material and methods

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

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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, USA.

2.1. Extraction and characterization of bone marrow-derived mononuclear cells

Bone marrow cells were extracted from male C57BL/6 (20–25 g, $n = 13$) mice and administered on the day of collection. BMDMC from GFP + male mice (20–22 g, $n = 3$) were administered to 16 C57BL/6 female mice to evaluate the degree of pulmonary GFP + cell engraftment. Briefly, under anesthesia with ketamine (25 mg/kg) and xylazine (2 mg/kg) *iv*, bone marrow cells 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), re-suspended in DMEM and added to Ficoll-Hypaque (Histopaque 1083, Sigma Chemical Co., St. Louis, MO, USA), and again centrifuged and supplemented with phosphate-buffered saline (PBS). Cells were counted in a Neubauer chamber with Trypan Blue for evaluation of viability. For the administration of saline or BMDMC, mice were anesthetized with sevoflurane, the jugular vein of each mouse was dissected, and cells were slowly injected. A small aliquot of the mononuclear cells was used for immunophenotipic characterization of the injected cell population. Cell characterization was performed by flow cytometry using antibodies CD45 (leukocyte), CD34 (hematopoietic precursors), CD3, CD8, and CD4 (T lymphocyte), CD14 (monocytes and macrophages), CD11b, CD29 and CD45- (non-hematopoietic precursors), all from BD Biosciences, USA.

2.2. Animal preparation and experimental protocol

Eighty female C57BL/6 mice (20–25 g) were used. Lung mechanics and histology as well as molecular biology were analyzed in 24 female C57BL/6 mice ($n = 6$/group). Of the remaining 56 female C57BL/6 mice, 20 ($n = 5$/group) were used to compute airway hyperresponsiveness, 20 to evaluate total and differential cell count in bronchoalveolar lavage fluid (BALF, $n = 5$/group) and 16 to evaluate the degree of pulmonary GFP + cell engraftment ($n = 4$/group). All females 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 of OVA in 100 μl) on 7 alternate days. Forty days after the beginning of sensitization, 20 μg of OVA in 20 μl saline was intratracheally instilled. This procedure was performed 3 times with 3-day intervals between applications (Xisto et al., 2005). The control group (C) received saline using the same protocol. C and OVA groups were further randomized to receive saline solution (0.9% NaCl, 50 μl, SAL) or BMDMC (2×10^6 in 50 μl, CELL) administration through the left jugular vein 24 h before the first challenge (Fig. 1).

2.3. Mechanical parameters

Twenty-four hours after the last intratracheal challenge with saline or OVA, animals were sedated (diazepam 1 mg ip), anesthetized (thiopental sodium 20 mg/kg ip), tracheotomized, paralyzed (vecuronium bromide, 0.005 mg kg iv), and ventilated with a constant flow ventilator (Samay VR15; Universidad de la Republica, Montevideo, Uruguay) with the following parameters: frequency of 100 breaths/min, 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. Airflow and tracheal pressure (Ptr) were measured (Burburan et al., 2007). Lung mechanics was analyzed by the end-inflation occlusion method (Bates et al., 1988). In an open chest preparation, Ptr reflects transpulmonary pressure (PL). Briefly, after end-inspiratory occlusion, there is an initial fast drop in PL ($\Delta P1$) from the preocclusion value down to an inflection point (Pi), followed by a slow pressure decay ($\Delta P2$), until a plateau is reached. This plateau corresponds to the elastic recoil pressure of the lung (Pel). $\Delta P1$ selectively reflects the pressure used to overcome the airway resistance. $\Delta P2$ reproduces the pressure spent by stress relaxation, or viscoelastic properties of the lung, together with a small contribution of *pendelluft*. Static lung elastance (Est) was determined by dividing Pel by V_T. Lung mechanics measurements

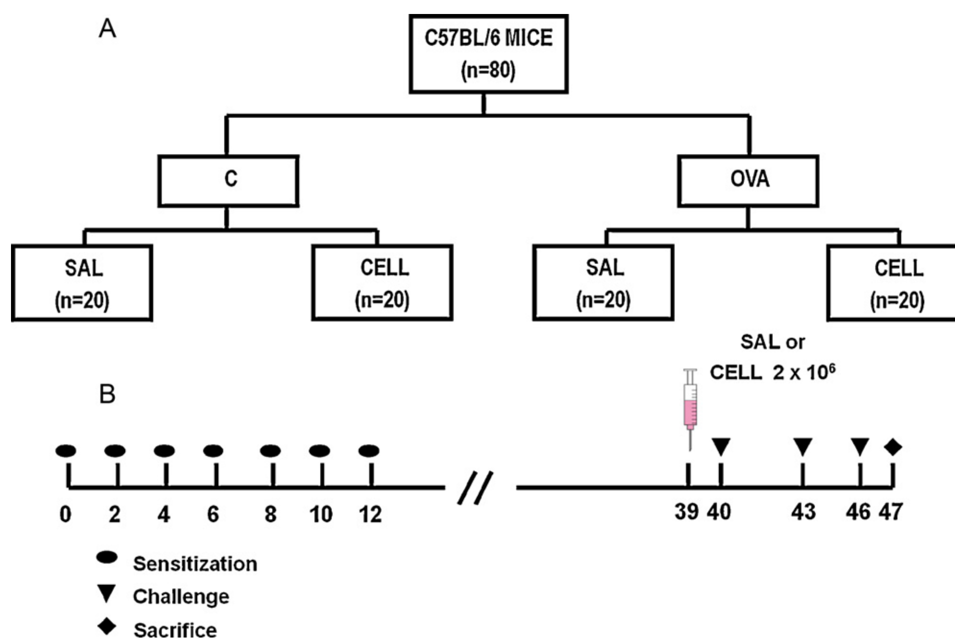

Fig. 1. Schematic flow chart (A) and timeline (B) of the study design. C: mice sensitized and challenged with saline; OVA: mice sensitized and challenged with ovalbumin; SAL: mice treated with intravenous injection of saline; CELL: mice treated with BMDMC (2×10^6) 24 h before the first challenge. All data were analyzed at day 47.

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