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Mesenchymal stem cells ameliorate the histopathological changes in a murine model of chronic asthma

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ABSTRACT

Asthma therapies are effective in reducing inflammation but airway remodeling is poorly responsive to these agents. New therapeutic options that have fewer side effects and reverse chronic changes in the lungs are essential. Mesenchymal stem cells (MSCs) are promising for the development of novel therapies in regenerative medicine. This study aimed to examine the efficacy of MSCs on lung histopathology in a murine model of chronic asthma. BALB/c mice were divided into four groups: Group 1 (control group, n = 6), Group 2 (ovalbumin induced asthma only, n = 10), Group 3 (ovalbumin induced asthma + MSCs, n = 10), and Group 4 (MSCs only, n = 10). Histological findings (basement membrane, epithelium, subepithelial smooth muscle thickness, numbers of goblet and mast cells) of the airways and MSC migration were evaluated by light, electron, and confocal microscopes. In Group 3, all early histopathological changes except epithelial thickness and all of the chronic changes were significantly ameliorated when compared with Group 2. Evaluation with confocal microscopy showed that no noteworthy amount of MSCs were present in the lung tissues of Group 4 while significant amount of MSCs was detected in Group 3. Serum NO levels in Group 3, were significantly lower than Group 2. The results of this study revealed that MSCs migrated to lung tissue and ameliorated bronchial asthma in murine model. Further studies are needed to evaluate the efficacy of MSCs for the treatment of asthma.

1. Introduction

Asthma is a chronic disease characterized by reversible airway obstruction, airway inflammation and remodeling [1]. Current strategies for the management of asthma focus on suppressing airway inflammation [2]. Inhaled glucocorticoids are currently the mainstay of asthma therapy although several side effects may arise when they are used at high doses or for a prolonged time [3]. Airway remodeling consists of progressive structural changes in the composition, content, and organization of the cellular and molecular constituents of the airway wall [4]. Although current asthma therapies are effective in reducing inflammation, airway remodeling is poorly responsive to current therapies, such as inhaled corticosteroids, antileukotrienes, and theophylline [4,5]. For this reason, new therapeutic options are required.

Mesenchymal stem cells (MSCs) are emerging as a therapeutic modality in various inflammatory diseases. MSCs have the potential to differentiate into various connective tissue lineages including adipose tissue, marrow stroma, cartilage, tendon and bone [6]. Additionally, studies during the last decade have suggested that MSCs may have more plasticity and are able to differentiate into bronchial and alveolar epithelium, vascular endothelium, and interstitial cell types [7]. MSCs can be isolated from multiple tissues including adipose tissue, skeletal muscle, synovium, spleen, thymus, blood, lung, fetal blood, and amniotic fluid [8]. The most accessible and the best characterized source of MSCs is the bone marrow although they are found in relatively small numbers with an estimate of about 10 MSCs for 1 million total bone marrow cells [9].

However, to the best of our knowledge, there is no report in the medical literature regarding the efficacy of MSCs in the treatment of asthma. The aim of the present study was to investigate the efficacy of MSCs on lung histopathology in a murine model of asthma.

2. Materials and methods

2.1. Experimental animals

Specific pathogen-free, 6- to 8-week-old, female BALB/c mice, weighing 18 to 20 g, were maintained in the animal laboratory of

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Dokuz Eylul University. They were kept in hygienic macrolene cages in air-conditioned rooms and allowed ad libitum with food and water on a 12-hour light/12-hour dark cycle. All experimental procedures were prepared according to the requirements of the Animal Care and Ethics Committee of the Dokuz Eylul University. Thirty-six BALB/c mice were divided into four groups; Group 1 (control group, n = 6), Group 2 (ovalbumin induced asthma only, n = 10), Group 3 (ovalbumin induced asthma + MSC, n = 10), and Group 4 (MSC only, n = 10).

2.2. Sensitization and inhalational exposure

BALB/c mice are high responders to ovalbumin [10]. Mice in control group were not exposed to any intervention. The mice in study Groups 2 and 3 were sensitized via two intraperitoneal injections, on days 0 and 14 of the experiment, of 10 μg/0.1 ml chicken egg albumin (ovalbumin, grade V, ≥98% pure; Sigma, St. Louis, MO, USA) with alum as an adjuvant. After the sensitization, the mice in study Groups 2 and 3 were exposed to aerosolized ovalbumin for 30 min per day on three days a week for eight weeks, beginning from the 21st day of the study. The mice in control group were administered normal saline with alum intraperitoneally on days 0 and 14 of the experiment and exposed to aerosolized saline for 30 min per day on three days a week for eight weeks, beginning from the 21st day of the study [11]. Exposures were carried out in a whole body inhalation exposure system. Temperature and relative humidity were maintained between 20–25 °C and 40–60%, respectively. A solution of 2.5% ovalbumin in normal saline was delivered by aerosolization via compressed air to a sidestream jet nebulizer injected into a chamber. The aerosol generated by this nebulizer comprised >80% particles with a diameter of <4 μm. Particle concentration was maintained in the range of 10 to 20 mg/mm^3 [11].

2.3. Generation and administration of mesenchymal stem cells

2.3.1. Bone marrow harvest

In order to isolate mesenchymal stem cells, after sacrificing 6- to 8-week-old BALB/c mice obtained from the Dokuz Eylul University, femurs and tibias were dissected away from attached muscle and connective tissue; the ends of the bones were removed. Bone marrow was extruded by inserting a 21-gauge needle into the shaft of the bone with cuts on both ends and flushing it with 1 ml of Dulbecco's modified Eagle's medium (DMEM; Biological Industries, Israel) supplemented with 10% fetal bovine serum (FBS). The marrow plugs were dispersed by passage through a 16-gauge needle and the marrow was filtered through a 75-μm filter. Cells were plated in tissue culture flask.

2.3.2. Isolation and culture of mesenchymal stem cells (MSCs)

Between 10 and 15×10^6 whole marrow cells were placed in a 75 cm^2 tissue culture flask in DMEM containing 15% fetal bovine serum (FBS), 2 mmol/l L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin (Biological Industries, Israel) at 37 °C in a humidified atmosphere of 5% carbon dioxide. After 72 h, the nonadherent cells were removed by changing the medium. The medium was completely replaced every 3 days and nonadherent cells were discarded. Cultured MSCs were observed under inverted microscope to assess the level of expansion and to verify the morphology at each culture medium change. In order to prevent the MSCs from differentiating or slowing their rate of division, each primary culture was replaced (first passage) to 3 new flasks when the cell density within colonies became 80–90% confluent, approximately 2 weeks after seeding. The adherent cells were released from flasks with 0.25% trypsin in 1 mmol/l sodium ethylenediaminetetraacetic acid (EDTA, Sigma, St. Louis, MO, USA). After the twice-passaged cells became nearly confluent, they were harvested and used for the experiments [12].

2.3.3. Transfection of MSCs by GFP

In order to track stem cells in vivo, cells were transfected with pEGFP-N1 Vector including GFP encoding gene (ClonTech, USA). Lipid based transfection was performed with PolyFect Transfection Reagent according to the kit instructions (Qiagen, USA). Briefly, 5×10^5 cells were plated and grown when they reach 60% confluency. The day before transfection, MSCs grown in 75 cm^2 flasks were detached by trypsin-EDTA treatment and replated into a new sterile 75 cm^2 flask at a density of 1.6×10^6 cells in 8 ml of complete media. The cells were incubated at 37 °C and 5% CO_2. The next day, transfection mixture including 10 μg of plasmid DNA and total volume was transferred drop wise onto the cells in the flasks. The flask was gently swirled in an attempt to ensure uniform distribution of the complexes. Finally, cells were incubated overnight with the complexes and then were visualized under the fluorescent microscope [12].

2.3.4. Flow cytometry

The cells were incubated with antibodies against CD45 PC5 (Beckman Coulter, Marsillia, France), NG2 PE (Beckman Coulter), CD73 PE (Becton-Dickinson, Bioscience Pharmingen, San Diego, CA, USA), and CD105 FITC (Serotec, Oxford, UK). Fluorescence histograms were obtained by recording 20,000 cells/sample at a flow rate of approximately 200 cell events/s. Experiments were conducted using Coulter Epics XL-MCL and flow cytometric data were analyzed using EXPO 32 ADC software (Beckman Coulter Inc, Miami, FL, USA) [13]. Flow cytometry analysis demonstrated that there were significant expressions of MSC specific antigens (CD105, CD73, and NG2) and absence of hematopoietic marker antigen (CD45).

2.4. MSC administration

Flow cytometry demonstrated that the cells were MSCs. Subsequently, 10^6 MSCs were administered via an insulin syringe from the tail vein following OVA nebulization under superficial sedation to each of the 10 mice in both Groups 3 and 4 (Fig. 1).

2.5. Histopathological analysis

Animals were sacrificed after two different time periods by an overdose of ketamine after last MSC administration. First, half of the animals were sacrificed at the end of one week in order to analyze the histological findings of the lung tissue and whether MSCs migrated to the inflamed lung tissue or not. Second, remaining mice were sacrificed at the end of the second week in order to evaluate the histopathological features of inflammation in the lungs. Two investigators who were blinded to the treatment groups interpreted the histopathology. Tissue specimens were obtained from the mid zone of the left lung of mice. Samples were fixed in 10% formalin for light microscopic evaluation. Some tissue samples of 1 to 2 mm^3 obtained from adjacent regions were stocked in 2.5% glutaraldehyde for electron microscopic evaluation. After fixation, samples were embedded in paraffin for light microscopic evaluation and serial sections of 5-μm thickness were prepared. After choosing the first section randomly, 10 sections in each mouse were selected by skipping over 10 sections and proceeded to staining process. For light microscopic evaluation, 3 different staining processes were used. The first 10 samples were stained with hematoxylin and eosin (H&E). In these samples general tissue features were examined and thicknesses of epithelium and subepithelial smooth muscle layers of the medium and small airways were measured. In order to evaluate the thicknesses of epithelium and subepithelial smooth muscle layers, the measurements were performed from 4 points of each airway