Abstract

Asthmatic mice were injected with a PGE2 inhibitor or TGF-β neutralizing antibodies at the same time as adipose derived stem cell (ASCs) injection. In asthmatic mice, ASCs significantly reduced airway hyperresponsiveness, the number of total inflammatory cells and eosinophils in the bronchoalveolar lavage fluid (BALF), eosinophilic inflammation and serum total and specific IgE and IgG1. ASCs significantly inhibited Th2 cytokines and enhanced the Th1 and regulatory cytokines in the BALF and lung draining lymph nodes (LLNs). However, blocking PGE2 or TGF-β eliminated the immunosuppressive effect of ASCs in allergic airway inflammation. ASCs are capable of secreting PGE2 and TGF-β, which may play a role in inducing regulatory T cell (Treg) expansion. Furthermore, treatment with a PGE2 inhibitor or TGF-β neutralizing antibodies eliminated the beneficial effect of ASCs treatment in asthmatic mice, suggesting that PGE2 and TGF-β are the major soluble factors responsible for suppressing allergic airway inflammation.

Methods and Materials

Adipose tissue was obtained from the abdominal fat of C57BL/6 mice, washed and digested with 0.075% collagenase type I. Enzyme activity was neutralized followed by centrifugation. The pellet was filtered and incubated and then washed cell population was maintained. One week later, once the monolayer of adherent cells had reached confluence, cells were trypsinized, resuspended and subcultured. Third- or fourth-passage ASCs was used. Flow cytometric analysis was used to characterize the phenotype of ASCs. ASCs were analyzed for their capacity to differentiate into adipogenic, osteogenic, and chondrogenic lineages.

Mice were divided into 5 groups with 5 mice per group (Fig 1). These experiments were repeated four times. Twenty-four hours after the last challenge, AHR was assessed in conscious, unrestrained mice using non-invasive whole-body plethysmography. The tracheas of mice were exposed and cut just below the larynx to obtain BALF and LLNs were obtained between trachea and both lung lobes.

Introduction

Asthma is a chronic inflammatory airway disease and is characterized by Th2-mediated eosinophilic inflammation, mucus hypersecretion, and airway hyperresponsiveness (AHR). There is mounting evidence that insufficient suppression of Tregs is responsible for the excessive Th2 response in allergic airway disease. The immunomodulatory effects of mesenchymal stem cell (MSCs) in allergic airway diseases may be mediated by the upregulation of Tregs and increases in several soluble factors such as indoleamine 2, 3-dioxygenase (IDO), PGE2, TGF-β, and IL-10. However, the role of these soluble factors in the suppression of allergic airway inflammation by MSCs remains to be elucidated, and the major soluble factors responsible for the immunomodulatory effects of MSCs in allergic airway diseases have not been well documented. The purpose of this study was to determine whether PGE2 or TGF-β contributes to the immunomodulatory effects of ASCs in asthmatic mice by evaluating the effects of a PGE2 inhibitor or TGF-β-specific neutralizing antibody (Ab) on allergic inflammation.

Results

In asthmatic mice, ASCs significantly reduced airway hyperresponsiveness, the number of total inflammatory cells and eosinophils in the BALF, eosinophilic inflammation, goblet cell hyperplasia, and serum total and specific IgE and IgG1 (Fig 2 and 3). ASCs significantly inhibited Th2 cytokines, such as IL-4, IL-5, and IL-13, and enhanced the Th1 cytokines (Interferon-γ) and regulatory cytokines (IL-10 and TGF-β) in the BALF and LLNs (Fig 4 and 5). ASCs engraftment caused significant increases in the Treg and IL-10+ T cell populations in LLNs. However, blocking PGE2 or TGF-β eliminated immunosuppressive effect of ASCs in allergic airway inflammation.

Conclusions

Inhibition of PGE2 or TGF-β-specific neutralizing Abs eliminated the beneficial effect of ASC treatment in asthmatic mice, suggesting that PGE2 and TGF-β are the major soluble factors involved in suppressing allergic airway inflammation.

References