Sphingosine-1-Phosphate Aggravates Antigen-Induced Airway Inflammation in Mice

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Abstract: Recent investigations suggest an involvement of sphingosine-1-phosphate (S1P) in the pathogenesis of allergic bronchial asthma. However, the role of S1P in the development of asthma is still controversial. Our aim was to investigate the effects of intranasal application of S1P on antigen-induced airway inflammation in a mouse model of allergic bronchial asthma.

Methodology: Male BALB/c mice were actively sensitized with ovalbumin antigen, and were repeatedly challenged with aerosolized antigen. Animals also received an intranasal administration of S1P (10^{-5} M, 20 \muL) or its vehicle (1% methanol in sterile PBS, 20 \muL) 30 min prior to each antigen challenge. Histological examinations of the lungs and determination of cell number in the bronchoalveolar lavage fluids (BALFs) were studied.

Results: The airway inflammation induced by antigen exposure was significantly augmented by the intranasal administration of S1P: the cell number in BALFs of the S1P-treated, antigen-challenged mice (S1P-Challenged, 48.9±4.8 x 10^4/mL BALF) was significantly increased as compared with those of the vehicle-treated, antigen-challenged ones (Vehicle-Challenged, 26.3±5.7 x 10^4/mL BALF, P<0.01).

Conclusion: In mice, the intranasal administration of S1P might aggravate the antigen-induced airway inflammation.

Keywords: Sphingosine-1-phosphate (S1P), intranasal administration, allergic bronchial asthma, airway inflammation, mouse.

INTRODUCTION

The dramatic increase in the number of asthma cases over the last decades is of great concern for public health in the world [1]. Allergic bronchial asthma is characterized by structural and functional abnormalities of the bronchial epithelium, accumulation of inflammatory cells in the bronchial mucosa, remodeling of the airway tissue structure, and airway hyperresponsiveness. Many mediators have been identified that play significant roles in the initiation and progression of the disease, but the exact mechanism of the pathogenesis of asthma is still unclear.

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that mediates diverse biological responses [2-6]. Recently, an involvement of S1P in allergic bronchial asthma has been suggested [7-13]. Ammit and colleagues [7] firstly demonstrated that S1P levels are elevated in the airways of individuals with asthma after segmental allergen challenge. The finding that S1P can act as a chemotactic agent for eosinophils further suggests an involvement of S1P in pathophysiology of asthma [8]. Indeed, inhalation of inhibitors for sphingosine kinase (SphK), which produces S1P directly from sphingosine, attenuated antigen-induced airway inflammation in mice [9]. In addition, S1P might have an ability to cause airway hyperresponsiveness [10-13], one of the characteristic features of allergic bronchial asthma. Contrary to these observations, an intratracheal instillation of FTY720, an S1P receptor agonist, prevented antigen-induced airway inflammation and hyperresponsiveness in mice [14]. Thus, the role of S1P in the development of asthma is still controversial.

In the present study, the effects of pretreatment with S1P by intranasal administration on antigen-induced inflammatory signs of the airways were determined in a murine model of allergic bronchial asthma.

METHODS

Animals and Treatments

Male BALB/c mice were purchased from the Charles River Japan, Inc. (Kanagawa, Japan) and housed in a pathogen-free facility. All animal experiments were approved by the Animal Care Committee of the Hoshi University (Tokyo, Japan).

Preparation of a murine model of allergic bronchial asthma, which has an in vivo AHR [15], was performed as described previously [16-20]. In brief, BALB/c mice (8 weeks of age) were actively sensitized by intraperitoneal injections of 8 \mug ovalbumin (OA; Seikagaku Co., Tokyo, Japan) with 2 mg Imject Alum (Pierce Biotechnology, Inc., Rockfold, IL, USA) on Day 0 and Day 5. The sensitized mice were challenged with aerosolized OA-saline solution (5 mg/mL) for 30 min on Days 12, 16 and 20. The OA aerosol was generated with an ultrasonic nebulizer (Nihon Kohden, Tokyo, Japan) and introduced to a Plexiglas chamber box...
(130 x 200 mm, 100 mm height) in which the mice were placed. The animals also received intranasal administration of S1P (10^{-5} M, 20 µL) or its vehicle (1% methanol in sterile PBS, 20 µL) 30 min prior to each antigen challenge by the method previously described [18]. The dose of S1P was decided based on the previous report [7]. Twenty-four hours after the last OA challenge, mice were sacrificed by exsanguination from abdominal aorta under urethane (1.6 g/kg, i.p.; Sigma and Aldrich, St. Louis, MO) anesthesia. Then histologic examination and cell count in bronchoalveolar lavage (BAL) fluid were carried out by the methods previously described [18].

**Statistical Analyses**

The cell counts data were expressed as the mean with S.E. Statistical significance of difference was determined by unpaired Student's t-test or two-way analysis of variance (ANOVA) with post hoc Bonferroni/Dunn (StatView for Macintosh ver. 5.0, SAS Institute, Inc., NC). A value of p<0.05 was considered significant.

**RESULTS AND DISCUSSION**

In the present study, we used our well-established murine model of allergic bronchial asthma [16-20]. Histochemical examination using hematoxylin and eosin staining revealed a marked lung inflammation in the repeatedly antigen-challenged mice (Fig. 1C) as compared with the control animals (Fig. 1A): a marked infiltration of inflammatory cells, mainly eosinophils, was observed in the lungs of the antigen-challenged mice. The inflammation score determined as previously described [19] was significantly increased in lungs of the antigen-challenged mice (2.4±0.4) than that of the control animals (0.9±0.3, P<0.05). To further quantify the airway inflammation, cell counts in bronchoalveolar lavage (BAL) fluids were carried out. As shown in Fig. (2), the cell counts in BAL fluids of the repeatedly antigen-challenged mice were significantly increased compared to the control mice.
challenged mice were significantly increased as compared with those of the control group (Fig. 2, Vehicle-Control vs Vehicle-Challenged groups; \( P < 0.05 \)). As previously reported [19, 20], most of the increased cells were eosinophils. The vehicle used had no effect on baseline lung histology and BAL cell counts and the airway inflammation induced by antigen exposure (data not shown).

Contrary to our results, Izdzo and colleagues [14] reported that an intratracheal instillation of FTY720, an S1P receptor agonist, or S1P itself prior to each antigen inhalation abolished inflammatory cells infiltration into the airways in the BALB/c mice. Although the reason for the discrepancy is not known for certain, differences in the experimental conditions, such as difference in the protocols for antigen sensitization and inhalation, difference in the concentration and volume of S1P solution (20 \( \mu L \) of \( 10^{-5} \) M currently vs 80 \( \mu L \) of \( 10^{-6} \) M in their study), and/or difference in the administration route of S1P (intranasally vs intratracheally, respectively), might be involved in it. While S1P and/or S1P receptor agonists, such as FTY720, have been suggested as a novel therapeutic strategy for airway inflammation [14, 21], further studies are required for making clear the exact role of S1P in the pathogenesis of allergic bronchial asthma.

In summary, the intranasal application of S1P caused an aggravation of airway inflammation induced by antigen exposure in a mouse model of allergic bronchial asthma. The results might raise a caution for clinical use of S1P receptor agonists, such as FTY720, in asthma treatment.

**CONFLICT OF INTEREST STATEMENT**

None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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