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Original article

Effects of sublingual immunotherapy in a murine asthma model sensitized by intranasal administration of house dust mite extracts

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Abbreviations:
AHR, airway hyperresponsiveness; AIT, allergen immunotherapy; BALF, bronchoalveolar lavage fluid Der f, *Dermatophagoides farinae*; ELISA, enzyme-linked immunosorbent assay; HDM, house dust mite; ICS, inhaled corticosteroid; IL, interleukin; IFN-γ, interferon-γ; MNC, mononuclear cell; PAS, periodic acid-Schiff; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; TGFβ, transforming growth factor β; Treg, regulatory T cell

ABSTRACT

Background: Sublingual immunotherapy (SLIT) has received attention as a method for allergen immunotherapy. However, the mechanism of SLIT has not yet been fully investigated. Therefore, we evaluated the effects of SLIT in a murine asthma model, sensitized by intranasal administration of house dust mite (HDM) extracts.

Methods: Female BALB/c mice were intranasally exposed to HDM for either 3 or 5 weeks (5 consecutive days per week). Mice were administered either low-dose (0.5 mg/day) or high-dose (5 mg/day) sublingual HDM extracts for 2 weeks, followed by an additional week of intranasal exposure. Airway hyperresponsiveness (AHR), bronchoalveolar lavage fluid (BALF) cell count, cytokine levels in the BALF and lymph node cell culture supernatants, and allergen-specific antibodies were measured. Lung histology was also investigated.

Results: In mice sensitized for 5 weeks, high-dose SLIT ameliorated AHR, airway eosinophilia and goblet cell metaplasia. In mice sensitized for 3 weeks, even low dose SLIT ameliorated AHR and airway eosinophilia. Th2 cytokine levels in culture supernatants of submandibular lymph node cells in high-dose SLIT mice decreased, whereas IL-10 levels increased. Total IgA in BALF increased in mice sensitized for 3 or 5 weeks, and high-dose SLIT also increased allergen-specific IgG2a in mice sensitized for 5 weeks.

Conclusions: These data suggest that earlier induction of SLIT in HDM-sensitized mice provides superior suppression of AHR and goblet cell metaplasia. The modulation of allergen specific IgG2a and local IgA might play a role in the amelioration of AHR and airway inflammation.

Introduction

Bronchial asthma is characterized by chronic allergic airway inflammation, variable airway obstruction, and airway hyperresponsiveness (AHR) to various stimuli. In addition, prolonged airway inflammation induces structural changes in the airway that are difficult to treat.[1] To date, the initial therapy for bronchial asthma is inhaled corticosteroids (ICS), which are powerful enough to control the inflammation, and can alleviate symptoms and restore respiratory functions. However, ICS only treats the symptoms of inflammation and cannot modify the natural history of disease.[2,3]

Allergen immunotherapy (AIT) involves the administration of specific allergens to patients with IgE-mediated conditions. In this

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context, the major objectives of AIT are to reduce responses to allergic triggers, decrease the inflammatory response and prevent the development of persistent disease. Furthermore, AIT is the only curative treatment for allergic diseases, and subcutaneous immunotherapy (SCIT) has been demonstrated to be clinically effective in treating asthma and rhinitis caused by allergic sensitization[4]; however, SCIT requires inconvenient injections and is associated with potentially severe systemic reactions. Thus, sublingual immunotherapy (SLIT) has been introduced to avoid systemic reactions, and has recently received increased attention regarding its potential clinical application in AIT.[5]

House dust mite (HDM) is the major allergen source in cases of human asthma and perennial allergic rhinitis. The randomized controlled trials have demonstrated that SLIT with HDM (using SLIT-droplets or tablets) is effective in treating allergic rhinitis and allergic bronchial asthma.[6,7] Another large clinical trial of HDM SLIT tablets in both adult and adolescent patients with bronchial asthma is under way. Interestingly, although a large number of animal models of HDM-induced allergic asthma have been developed,[8,9] the efficacy and mechanism(s) of action for SLIT with HDM are not fully understood. Therefore, this study aimed to develop an animal model of allergic asthma induced by HDM, and to investigate the effects of SLIT in mice intranasally sensitized with HDM.

Methods

Animals

Eight-week-old female BALB/c mice, free of murine-specific pathogens, were used in this study (CLEA Japan, Tokyo, Japan). Animals were housed under specific pathogen-free conditions and a 12:12 h light:dark cycle. All experiments were conducted under a protocol approved by the Niigata University ethics committee for animal experiments.

Allergen

The species of HDM used was *Dermatophagoides farinae* (Der f), which was collected for preparing the dried extract (Torii Pharmaceutical, Tokyo, Japan). To characterize the extract, the major allergens (Der f 1; 27 kDa and Der f 2; 15 kDa) were detected by SDS-PAGE. The BCA assay was used to determine the 4protein concentration by absorption spectrophotometry with bovine serum albumin as the internal standard (0.31 $mg^{BSA}/mg^{extract}$). The major allergens' concentrations were measured using enzyme-linked immunosorbent assays (ELISA) (Der f 1; 4.8 $\mu g/mg^{extract}$, Der f 2; 4.0 $\mu g/mg^{extract}$).

HDM-induced asthma model and treatment protocol

To induce AHR with HDM, mice were intranasally exposed to the HDM extract (25 μg in 10 μL of saline) for 3, 5 or 7 weeks (5 consecutive days/week), without immunization (Fig. 1A). SLIT was performed for 2 weeks (5 consecutive days/week) after intranasal exposure, followed by an additional week of intranasal exposure (Fig. 1B, C). Saline was sublingually administered to mice as the control for SLIT. Twenty-four hours after the last HDM challenge, AHR was assessed, and bronchoalveolar lavage fluid (BALF), serum and lungs were obtained for further analyses.

Sublingual administration of allergen extract

SLIT was performed by grasping the mouse by the scruff of the neck, and carefully applying 10 μL of the allergen extract under the tongue. The mouse was maintained in this position for 20–30 s after administration, to prevent the animal from immediately

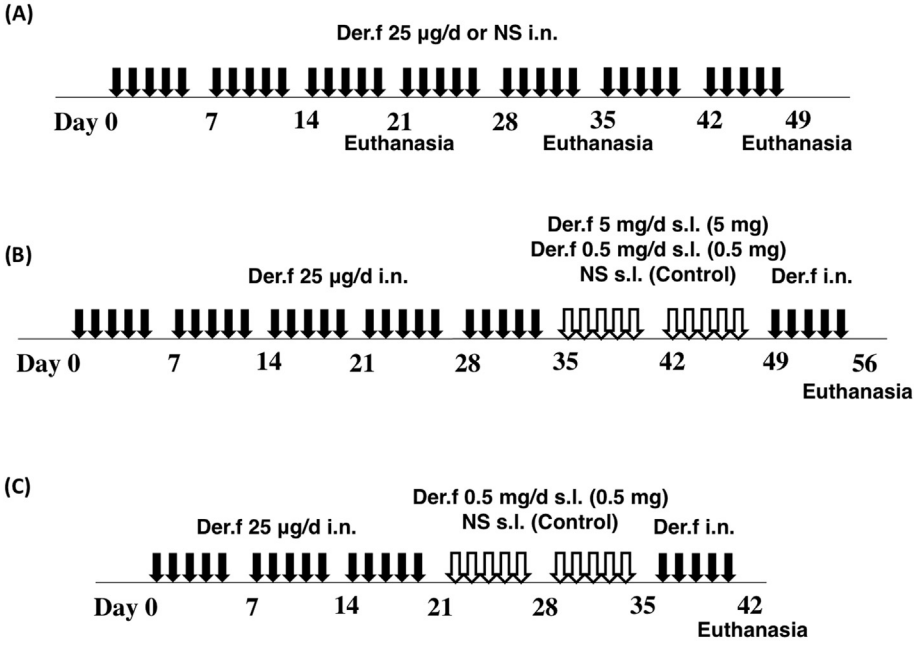

Fig. 1. Experimental protocols. **(A)** Mice were intranasally exposed to purified house dust mite (HDM) extract (black arrows, 25 μg Der. f in 10 μL saline) for 5 consecutive days/week for 3–7 weeks without immunization. Sublingual immunotherapy (white arrows) was administered for 5 consecutive days/week for 2 weeks after intranasal exposure for 5 weeks **(B)** or 3 weeks **(C)**, as either high dose (5 mg/day) or low dose (0.5 mg/day), followed by an additional week of intranasal exposure. Instead of HDM extract, control mice received saline (NS) for SLIT. At 24 h after the last HDM challenge, airway hyperresponsiveness was assessed, and bronchoalveolar lavage fluid (BALF), serum and lungs were obtained for further analyses. i.n., intranasal; s.l., sublingual.