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Original article

Beneficial effects of Galectin-9 on allergen-specific sublingual immunotherapy in a *Dermatophagoides farinae*-induced mouse model of chronic asthma

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Abbreviations:
AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; Df, *Dermatophagoides farinae*; EAR, early asthmatic response; Gal-9, galectin-9; SLIT, sublingual immunotherapy; Treg, regulatory T cells

ABSTRACT

Background: Allergen-specific sublingual immunotherapy is a potential disease-modifying treatment for allergic asthma. Galectin-9 (Gal-9), a β-galactoside-binding protein with various biologic effects, acts as an immunomodulator in excessive immunologic reactions by expanding regulatory T cells (Treg) and enhancing transforming growth factor (TGF)-β signaling. We investigated the efficacy of sublingually administered Gal-9 as an adjuvant to a specific allergen in a *Dermatophagoides farinae* (Df)-induced mouse model of chronic asthma.

Methods: BALB/c mice were intranasally sensitized with Df extract 5 days/week for 5 weeks, and then sublingual Df-allergen extract for 2 weeks (5 days/week). Three days after the final sublingual treatment, mice were intranasally challenged with Df extract. The early asthmatic response (EAR) was evaluated 5 min after the last Df challenge. Airway hyperresponsiveness (AHR) was assayed and bronchoalveolar lavage (BAL) was performed 24 h after the last allergen challenge. Serum IgE and cytokine levels, and number of inflammatory cells in the BAL fluid (BALF) were analyzed.

Results: Sublingual Df treatment in the presence of Gal-9, but not alone, significantly reduced AHR; EAR; number of eosinophils and interleukin-13 in the BALF; and serum IgE levels. BALF TGF-β1 levels were significantly increased in the presence of Gal-9 compared with Df alone. Treg depletion blocked the inhibitory effects of Gal-9 on the EAR, AHR, eosinophilic airway inflammation, and Df-specific serum IgE levels, and suppressed BALF TGF-β1 levels.

Conclusions: Gal-9 exhibited beneficial effects of sublingual Df allergen-specific immunotherapy in a Df-induced mouse model of chronic asthma, possibly by Gal-9-induced TGF-β1 production in the lung.

Introduction

Asthma is a common respiratory disease characterized by reversible airway obstruction, airway hyperresponsiveness (AHR), and chronic airway inflammation with eosinophils. The majority of patients with asthma are well controlled by combined treatment with inhaled corticosteroids, long-acting β-agonists, and leukotriene receptor antagonists.[1–3] These drugs have anti-inflammatory effects on chronic airway inflammation, but do not cure asthma. While allergen-specific immunotherapy might cure asthma, some problems, such as the administration route of the allergen, must be overcome.[4] Recently, patients with allergic rhinitis and asthma induced by mite-allergen were treated with an allergen-specific sublingual immunotherapy (SLIT). The SLIT route could be beneficial toward curing these allergic airway diseases, but some difficulties remain.[5,6]

Galectin-9 (Gal-9) is a β-galactoside binding animal lectin that induces various biologic reactions, such as cell chemoattraction, activation, and apoptosis.[7] Gal-9 functions as an immunomodulator in excessive immunologic reactions by expanding regulatory T cells (Treg) and immunosuppressive macrophages.[8–10] Furthermore, Gal-9 and CD44 interactions enhance the stability and function of adaptive Treg through smad3-dependent mechanisms.[11] The role of Gal-9 in allergic respiratory diseases, however, remains unclear.

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In the present study, we developed a SLIT model of mite allergen-induced chronic asthma using the *Dermatophagoides farinae* (Df)-induced murine model of chronic asthma that we previously developed.[12] We also used recombinant stable human Gal-9[13] as an adjuvant to the mite-allergen. The additive effects of Gal-9 in this SLIT model were evaluated by physiological examination, eosinophilic airway inflammation, and serum allergen-specific immunoglobulin levels, especially immunoglobulin IgE.

Methods

Animal model and sublingual immunotherapy

BALB/c mice were obtained from Charles River Laboratory (Yokohama, Japan). Female mice (8–12 weeks old) were intranasally sensitized with Df extract (40 μl of 1 mg/ml, LSL, Tokyo, Japan) 5 days/week for 5 weeks (Fig. 1). Negative control animals were intranasally exposed to phosphate-buffered saline (PBS) in a similar manner. Following intranasal administration of the Df extract for 5 weeks, SLIT was performed 5 days/week, twice daily, for 2 weeks (Fig. 1). The SLIT agents were PBS, Df-allergen extract (250 μg/day, Torii, Tokyo, Japan), and Df-allergen extract with recombinant human stable Gal-9 (0.1, 0.3, or 1.0 μg [3 μM], GalPharma, Takamatsu, Japan).[13] For SLIT, the mouth of an anesthetized mouse was kept open by placing forceps under the tongue. We administered 10 μl of SLIT agent under the tongue with a micropipette that was kept in place for 1 min. In preliminary experiments, we used trypan blue to confirm that this method does not deliver the SLIT agent to the stomach or lung. Endotoxin contamination in the Df and Gal-9 solution was minimal, as previously described (<0.25 EU/mg).[13,14] In this SLIT model, intranasal Df exposure is stopped during the SLIT because it is important for the SLIT to prevent allergen contact in patients with atopic asthma, as previously described.[15,16] All experiments in the present study were performed under a protocol approved by the Institutional Animal Care and Use Committee of the Kawasaki Medical School.

Early asthmatic response (EAR) and airway hyperresponsiveness (AHR)

The early asthmatic response (EAR) was evaluated as the rate of increase (ΔsRaw) in airway resistance (sRaw) after the last Df challenge, compared with PBS exposure just before the last Df challenge in each mouse using a two-chambered, double-flow

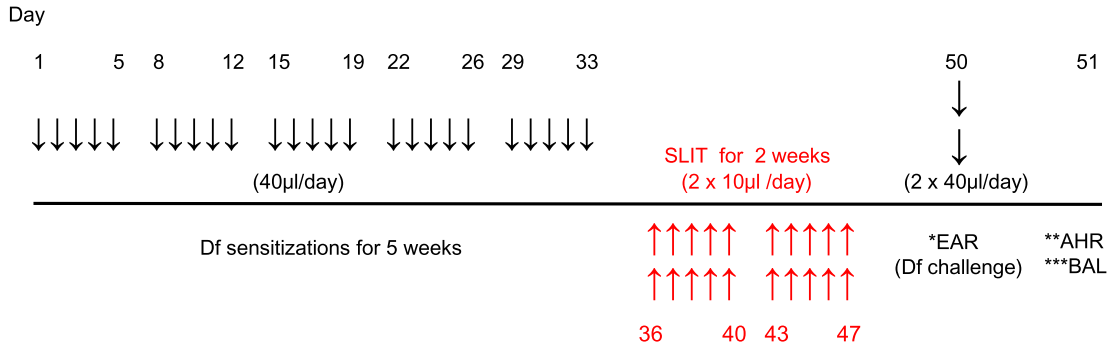

Fig. 1. Animal model and sublingual immunotherapy. BALB/c mice were intranasally sensitized with *Dermatophagoides farinae* (Df) extract (40 μl of 1 mg/ml) 5 days/week for 5 weeks. Negative control animals were intranasally exposed to PBS in a similar manner. Following intranasal administration of Df extract for 5 weeks, sublingual immunotherapy (SLIT) was performed 5 days/week, twice daily, for 2 weeks. Early asthmatic response (EAR) was evaluated on day 50 just after the last Df challenge. Airway hyperresponsiveness (AHR) and bronchoalveolar lavage (BAL) were evaluated on day 51, 24 h after the last Df (80 μl: 40 μl twice of 1 mg/ml) challenge.

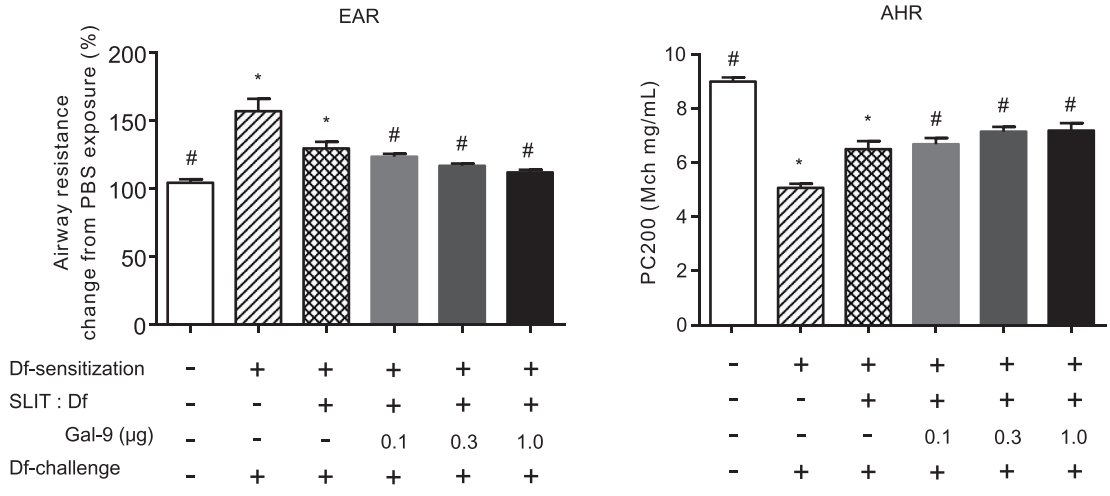

Fig. 2. Effects of Gal-9 on early asthmatic response (EAR) and airway hyperresponsiveness (AHR) in mite allergen-specific SLIT. EAR was evaluated and expressed as airway resistance change from PBS exposure (%) as described in the Methods. Airway hyperresponsiveness (AHR) was measured and expressed as PC200 sRaw (mg/ml). Data represent means ± SEM. Values shown are means from 12 mice per group. These results are typical of those obtained in three independent experiments. The Kruskal–Wallis test was used to compare values of different groups followed by Dunn's multiple comparisons test. *$p < 0.05$ compared with Df-sensitization (−), SLIT: Df (−) Gal-9 (−), and Df-challenge (−) group. #$p < 0.05$ compared with Df-sensitization (+), SLIT: Df (−) Gal-9 (−), and Df-challenge (+) group.