学 位 論 文 の 要 旨

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学　位　論　文　名	Sublingual Immunotherapy Attenuates Nasal Symptoms Upon Allergen Exposure in Murine Allergic Rhinitis Model via an Induction of IL-10 producing T cells in Submandibular Lymph Node
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論 文 内 容 の 要 旨

INTRODUCTION

Allergic rhinitis is one of the commonest types of allergic inflammation affecting 10–25 % of the world's population, and its prevalence has increased over the last decade. Sublingual immunotherapy has been considered to be a painless and effective therapeutic treatment of patients with allergic rhinitis. Its mechanism of action has been elucidated, but there is still controversies among many reports between clinical efficacy and laboratory data.

More recently, Regulatory T (Treg) cells have been focused on as key players to downregulate excessive Th1 or Th2 pathologies such as autoimmune disease or allergy. Regulatory T cells also can control and regulate all the effector mechanisms activated during allergy and the Th2 responses through the production of IL-10/TGF- β and/or cell- cell contact. IL-10 and TGF- β acts directly or indirectly on the human airways to decrease both mucus production and airway hyperreactivity. Allergen-specific immunotherapy also modifies cellular and humoral responses to the allergen. Therefore, SLIT has previously been shown in many clinical studies to modulate allergen- specific Th2 responses with a decrease in the IgE to IgG4 ratio, its mechanism of action needs to be investigated further by using promising animal models such as rodents and monkeys.

However, upon the sublingual immunotherapy, dynamic immunological events in nasal

mucosae in relation with draining nearby submandibular lymph nodes (SMLNs) or distant mucosal sites of lymphoid organs of patients with allergic rhinitis has never been elucidated except allergen-specific serum antibody titers or peripheral blood T cell cytokine profiles. Therefore, in our present study, we constructed murine models of SLIT, that is, preventive SLIT model in which mice were sublingually treated with allergen before systemic and i.n. allergen challenge in allergic rhinitis.

MATERIALS AND METHODS

We successfully constructed an effective murine model for sublingual immunotherapy (SLIT) in allergic rhinitis in which mice were sublingually administered ovalbumin (OVA), and followed by intraperitoneal (i.p.) sensitization and intranasal (i.n.) challenge of OVA. Mice were intranasally challenged with OVA, After the final i.n. challenge with OVA, mice were placed into an observation cage for about 2 min. Clinical symptom was evaluated by counting the frequencies of sneezes and scratches. Mice were killed 12 hours after the last nasal challenge with OVA.

Coronal nasal sections were stained with hematoxylin and eosin, and the number of eosinophils in each side of the posterior edge of the nasal septum was counted microscopically in a high-power magnification field. Splenic lymphocytes and SMLN lymphocytes from individual tissues were isolated. Culture supernatants were collected and examined for the production of cytokines (IL-10, TGF-β). Real-time PCR was performed using commercial primer of mouse β-actin, Foxp3, IL-10, and TGF-β. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

In this study, we successfully constructed a murine model of allergic rhinitis, for a quantitative analysis in order to evaluate sublingual immunotherapy (SLIT) in murine allergic rhinitis. To summarize our experimental data, nasal symptoms such as sneezing and nasal rubbing of sublingually treated mice were significantly attenuated in accordance with lower specific IgE antibodies in sera. Histological analysis of eosinophil recruitment in nasal mucosae reveals less allergic inflammation in sublingually treated mice. It is clearly demonstrated that histological quantitation of eosinophil infiltration into nasal mucosa actually corresponds to the nasal symptom such as sneezing and scratching in this murine allergic rhinitis model. Therefore, we successfully focused on quantitative analysis of nasal symptom and histological analysis of nasal mucosa together with an induction profile of Tregs in draining SMLNs and spleen, a distant lymphoid tissue in periphery.

In addition, sublingual administration of OVA did not alter the mRNA expression pattern of Foxp3, IL-10, and TGF-β in cultured spleen cells obtained from allergic rhinitis group of mice.

Nevertheless, our data actually indicate that antigen-specific IL-10 producing T cells induced in SMLNs play an important role in suppressing allergic inflammation in nasal mucosa of sublingually treated mice.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that down-regulates cellular immunity and allergic inflammation. As results, IL-10 level in culture supernatants were significantly reduced with allergic rhinitis group, in comparison with control group of mice, which underwent only systemic sensitization but not i.n. challenge. IL-10 production and IL-10-specific mRNA gene expression of cultured submandibular lymph node (SMLN) cells with OVA, obtained from sublingually treated mice, were significantly higher than those of mice without sublingual treatment.

The mechanism of sublingual administration to attenuate allergic responses at nasal mucosa remained yet to be clearly determined, so we have been precisely performed our ongoing immunological experiments of SLIT to clarify the exact mechanism of it. Our present data in line with previous reports strongly suggests that inducible IL-10 producing T cells can be efficiently induced in an antigen-specific fashion at the nasal mucosa with antigen stimulation and also neighboring draining SMLNs, but not enough in distant lymphoid organs such as spleen.

CONCLUSION

These results demonstrate that sublingually introduced antigens can actually attenuate nasal symptoms in a murine allergic rhinitis model upon allergen exposures. Furthermore, our immunological data might indicate an important role of IL-10 producing T cells in SMLN to controlnasal allergic reaction.