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TSLP receptor is not essential for house dust mite-induced allergic rhinitis in mice

Wakako Nakanishi [a,b], Yoshihisa Hiraishi [a,c], Sachiko Yamaguchi [a], Ayako Takamori [a], Hideaki Morita [d], Kenji Matsumoto [d], Hirohisa Saito [d], Katsuko Sudo [e], Tatsuya Yamasoba [b], Susumu Nakae [a,f,*]

[a] Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[b] Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[c] Department of Respiratory Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[d] Department of Allergy and Clinical Immunology, National Research Institute for Child Health and Development, Tokyo, Japan
[e] Animal Research Center, Tokyo Medical University, Tokyo, Japan
[f] Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Saitama, Japan

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ABSTRACT

TSLP induces Th2 cytokine production by Th2 cells and various other types of cells, thereby contributing to Th2-type immune responses and development of allergic disorders. We found that house dust mite (HDM) extract induced TSLP production by nasal epithelial cells, suggesting that TSLP may be involved in development of HDM-induced allergic rhinitis (AR). To investigate that possibility in greater detail, wild-type and TSLP receptor-deficient ($TSLPR^{-/-}$) mice on the C57BL/6J background were repeatedly treated intranasally with HDM extract. The frequency of sneezing, numbers of eosinophils and goblet cells, thickness of submucosal layers, serum levels of total IgE and HDM-specific IgG1, and levels of IL-4, IL-5 and IL-13 in the culture supernatants of HDM-stimulated LN cells were comparable in the two mouse strains. Those findings indicate that, in mice, TSLPR is not crucial for development of HDM-induced AR.

1.. Introduction

The number of patients with allergic rhinitis (AR) has been increasing since around 1965. Its incidence is estimated at 10–20% in advanced countries, and it has become a health problem worldwide. In particular, patients with allergic reactions to house dust mites (HDM) and pollens are increasing. AR is accompanied by three major symptoms, i.e., sneezing, rhinorrhea and nasal congestion, and it is roughly divided into intermittent/seasonal AR (pollinosis) induced by pollens, and persistent/perennial rhinitis induced by HDM-derived antigens [1]. In general, patients with AR show an increased serum concentration of antigen-specific IgE and increased numbers of eosinophils in rhinorrhea. Crosslinking of high-affinity IgE receptors (FcεRI) by antigens and antigen-specific IgE complexes induces degranulation and activation of mast cells and basophils. Those cells then release a variety of proinflammatory mediators, such as histamine, leukotrienes, prostaglandins, cytokines and chemokines, which induce immediate-phase and/or delayed-phase reactions. In immediate-phase reactions, mast cell- and/or basophil-derived chemical mediators cause sneezing by stimulating the sensory nerves, secretion of serous nasal discharge from serous gland cells and nasal congestion associated with interstitial edema and increased vascular volume due to smooth muscle relaxation.

Accumulation of such leukocytes as eosinophils, neutrophils, macrophages and Th2 cells can be observed in the nasal mucosa as a delayed reaction [2]. Th2-cytokines such as IL-4, IL-5 and IL-13 are crucial for development of AR; IL-4, IL-5 and IL-13 are involved in induction of IgE, eosinophilia and mucus secretion, respectively. In support of this, Th2 cells are increased in the blood of patients with AR [3].

Thymic stromal lymphopoietin (TSLP) was identified as an IL-7 family cytokine expressed in thymic stromal cells [4–6]. Airway and intestinal epithelial cells, mast cells and basophils also express TSLP [4–6]. TSLP receptors (TSLPR), which consist of the IL-7Rα chain and the TSLPR chain, are expressed on dendritic cells (DCs) and $CD4^+$ T cells [4–6]. TSLP is considered to be involved in Th2

Abbreviations: AR, allergic rhinitis; HDM, house dust mites; TSLP, thymic stromal lymphopoietin; TSLPR, thymic stromal lymphopoietin receptor

* Corresponding author at: Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.
E-mail address: snakae@ims.u-tokyo.ac.jp (S. Nakae).

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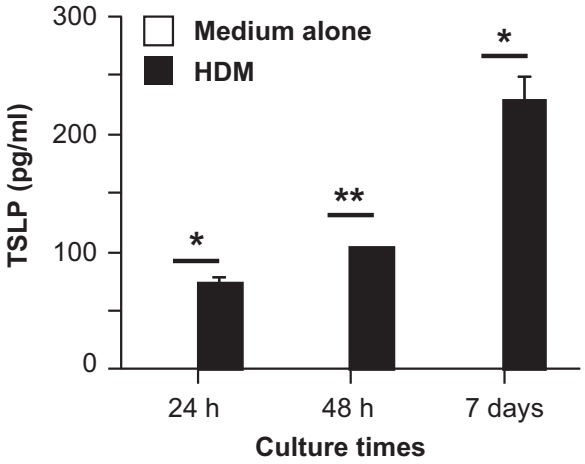

Fig. 1. TSLP induction by nasal epithelial cells in response to HDM extract. Nasal epithelial cells from wild-type mice were cultured in the presence and absence of HDM extract for 24 h, 48 h and 7 days. The levels of TSLP in the culture supernatants were determined by ELISA. Data show the mean ± SEM (n=3). $^{*}p < 0.05$ and $^{**}p < 0.01$. The data show representative results from 3 independent experiments.

cell differentiation by acting directly on naïve $CD4^+$ T cells [7] and by promoting DC functions such as increased expression of OX40 ligands on their cell surface [5]. TSLP contributes to host defenses against parasites by augmenting Th2-type immune responses [8].

On the other hand, inappropriate/excessive expression of TSLP is thought to be involved in development of Th2-type allergic disorders [9]. In support of that, TSLP levels were increased in the nasal secretion and nasal epithelial cells of patients with AR induced by HDM [10–12]. However, the precise roles of TSLP in the development of HDM-induced AR remain poorly understood. Therefore, in the present study, we investigated TSLP using TSLP receptor-deficient ($TSLPR^{-/-}$) mice as a model of AR induced by HDM.

2.. Materials and methods

2.1. Animals

C57BL/6J wild-type mice were purchased from Japan SLC Inc. (Shizuoka, Japan). $TSLPR^{-/-}$ mice on the C57BL/6J background were generated as described previously [13]. Eight- to 10-week-old female mice were used in the experiments. All animals were housed under specific-pathogen-free conditions in an environmentally controlled clean room at The Institute of Medical Science, The University of Tokyo. All animal experiments were approved by the institution's Ethics Committee and conducted in accordance with its ethical safety guidelines (A11-28).

2.2. HDM-induced allergic rhinitis

AR was induced in mice by treatment with HDM extract from *Dermatophagoides farinae* (Greer Laboratories, Lenoir, NC, USA) as described previously [14].

2.3. Epithelial cell culture

Nasal epithelial cells were harvested from wild-type mice. Red blood cells were removed using a red blood cell removal solution (Sigma-Aldrich, St. Louis, MO, USA). The cells were then suspended in RPMI1640 medium (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS (Invitrogen, Grand Island, NY, USA), 50 μg/ml streptomycin (Invitrogen), 50 U/ml penicillin (Invitrogen), 5 mg/ml Transferrin (Sigma-Aldrich), 50 pM hydrocortisone (Sigma-Aldrich), 50 pM β-estradiol (Sigma-Aldrich), 10 mM HEPES (Invitrogen), and Insulin Transferrin Selenium (Invitrogen). The cells were cultured in a ϕ10-cm dish at 37 °C for 4 days in a 5% CO_2 incubator. Cells were passaged two to four times, and the culture medium was changed every four days. Epithelial cells (2×10^5 cells/well in a 96-well flat-bottom plate) were cultured in the presence and absence of 50 μg/ml HDM extracts at 37 °C for 24 h, 48 h and 7 days in a 5% CO_2 incubator.

2.4. Lymph node cell culture

At 48 h after the last inhalation of HDM or PBS, cervical lymph nodes (LNs) were collected, and LN cells were suspended in RPMI1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated FBS (Invitrogen), 50 μM 2-mercaptoethanol (Invitrogen), 50 μg/ml streptomycin and 50 U/ml penicillin (Invitrogen). LN cells (5×10^5 cells/well in 0.2 ml in a 96-well flat-bottom plate)

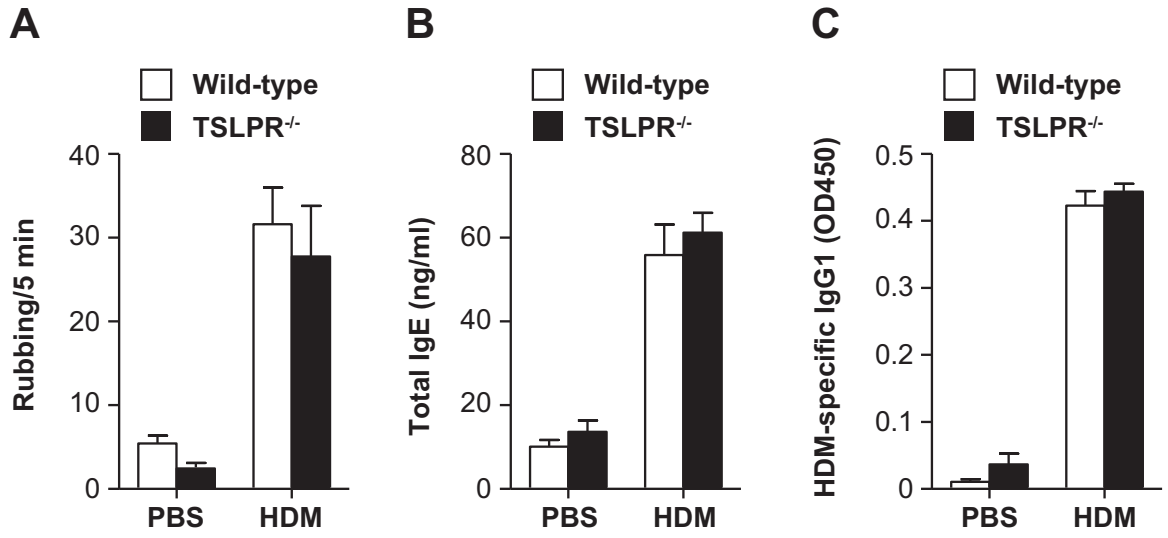

Fig. 2. TSLPR is not essential for immediate reaction during HDM-induced AR. Wild-type and $TSLPR^{-/-}$ mice were treated intranasally with HDM extract or PBS. Sera were collected 48 h after the last inhalation. (A) The frequency of sneezing was counted for 5 min after the last HDM or PBS treatment. (B)The serum levels of total IgE and (C) HDM-specific IgG1 were determined by ELISA. Data show the mean ± SEM (wild-type mice, n=4 [PBS] and n=6–8 [HDM]; and $TSLPR^{-/-}$ mice, n=4 [PBS] and n=6-8 [HDM]).