

Production of Salivary Immunoglobulin A and Suppression of *Dermatophagoides pteronyssinus*-Induced Airway Inflammation by Local Nasal Immunotherapy

Yi-Hsia Liu Jaw-Ji Tsai^a

Section of Allergy and Immunology, Taichung Veterans General Hospital, Taichung, Taiwan, ROC

Key Words

Local nasal immunotherapy · *Dermatophagoides pteronyssinus* · Fungal immunomodulatory peptide

Abstract

Background: Local nasal immunotherapy (LNIT) is an effective immunotherapeutic modality, especially when targeting a single immunodominant peptide from an allergen. However, the working mechanisms of LNIT are poorly understood. We hypothesized that LNIT with a mixture of group 2 allergens of *Dermatophagoides pteronyssinus* (Der p 2) protein and fungal immunomodulatory peptide (FIP) would generate suppression of Der p-induced airway inflammation through immunoglobulin (Ig) A secretion in the airways. **Method:** Mice were sensitized with recombinant Der p 2 (rDer p 2) and Der p followed by LNIT with rDer p 2 in conjunction with FIP. After intratracheal challenge with rDer p 2 and Der p, the airway inflammation was determined by analyzing the cell subpopulation and cytokine concentration in the bronchoalveolar lavage fluid. The allergen-specific IgE, IgG2a and IgG in the sera and IgA in the saliva were measured by ELISA. **Results:** LNIT with rDer p 2 in conjunction with FIP could downregulate the lymphocyte infiltration in both rDer p 2- and Der p-induced airway inflammation. Both total and specific IgA in the saliva were

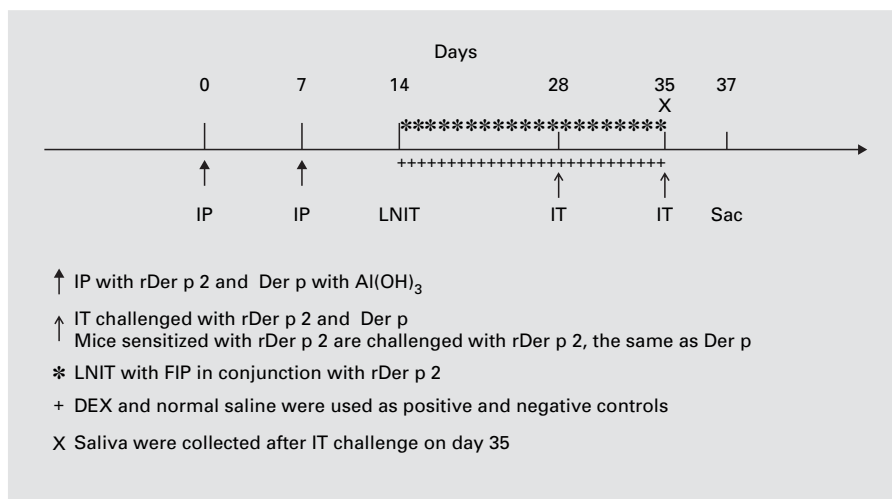
increased after LNIT. Serum levels of IL-4, IL-10 and specific IgE were reduced and the specific IgG2a and IgG increased after LNIT. After LNIT, there was a reduction of airway hypersensitivity at 30 min after allergen challenge in the rDer p 2- and Der p-sensitized mice, with a significant decrease only in rDer p 2-sensitized mice. **Conclusion:** LNIT with rDer p 2 in conjunction with FIP was not only able to suppress rDer p 2-induced airway inflammation but also generate suppression of Der p-induced airway inflammation. The simultaneous reduction of IL-4 and IL-10 indicated that IL-10-producing cells were not activated by LNIT. The increment of IgA in the airway might play a role in the prevention of airway inflammation.

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Introduction

Epidemiological studies suggest that between 10 and 20% of the world's population exhibit some form of immunoglobulin (Ig) E-mediated hypersensitivity, which is manifested as asthma, atopic dermatitis or allergic rhinitis [1]. House dust mites are the most important risk factor for asthma; more than 10 mite allergens have been defined, and the 14-kDa group 2 allergens of *Dermatophagoides pteronyssinus* (Der p 2) and *Dermatophagoi-*

Fig. 1. Experimental schedule for IP sensitization and the therapeutic procedure. Allergen sensitization, LNIT with rDer p 2 in conjunction with FIP and IT allergen challenges. DEX and NS were used as positive and negative controls. Sac = Sacrifice after the second pulmonary function measurement.



des farinae (Der f 2) are considered major allergens because of the fact that 80–90% of patients have specific IgE antibodies to these allergens [2–4]. Therapy for allergic disease includes allergen avoidance, pharmacotherapy and allergen-specific immunotherapy [5].

There has been a long-standing interest in the use of local nasal immunotherapy (LNIT) for the treatment of allergic rhinitis and asthma. LNIT is capable of controlling the symptoms of seasonal allergic rhinitis in a dose-dependent manner [6, 7]. Several animal and human studies have already shown that LNIT attenuates airway hypersensitivities [8–11]. There is also evidence that with high allergen doses, LNIT sometimes causes local adverse reactions [12–15]. The studies which used a low-dose administration schedule did not show clinical effectiveness, although tolerance was found to be good [7, 16, 17]. Therefore, the working mechanism of LNIT remains unclear.

Currently, LNIT, with a single immunodominant peptide from an allergen, can induce tolerance to the whole protein in a process called ‘linked suppression’ [18]. Moreover, LNIT with ovalbumin in conjunction with an immunostimulatory sequence induced airway IgA production and was more effective in protecting allergic mice from allergen-induced airway hypersensitivity [19].

FIP-fve is a fungal immunomodulatory protein (FIP) isolated from the edible mushroom *Flammulina velutipes*, a common oriental food. It is a simple protein consisting of 114 amino acid residues with a molecular mass of 13 kDa, and its cDNA has been cloned [20, 21]. A recent report has showed that oral administration of FIP-fve has a Th1-skewing effect on the development of the allergen-specific immune response and may serve the purpose of immunoprophylaxis for food allergy and other allergic diseases [22].

FIP appears to be a lectin with a specificity for complex cell surface carbohydrates. It is a noncovalently linked homodimer, and it is the first fibronectin III domain containing protein of fungal origin [23]. We have previously demonstrated that LNIT with FIP targeting the Der p 2 peptide and mixtures of both were able to inhibit Der p 2-induced airway inflammation [24]. In this study, we hypothesized that LNIT with a mixture of Der p 2 and FIP would not only downregulate Der p 2-induced airway inflammation, but would also cause suppression of Der p-induced airway inflammation through increased concentrations of secretory IgA in the airways.

Materials and Methods

Animals

Female Balb/c mice were obtained from the National Laboratory Breeding Research Center in Taiwan and were raised in a specific pathogen-free environment. These mice were used at 6–8 weeks of age. Groups of 6 mice were caged separately according to their treatment. Recombinant Der p 2 (rDer p 2) and Der p were prepared as previously described [4]. The immunotherapy agent with rDer p 2 was kindly donated by Dr. Kaw-Yan Chua of the Department of Pediatrics, National University of Singapore, Singapore. FIP was a gift from Dr. Jiunn-Liang Ko at the Institute of Toxicology, Chung Shan Medical University Hospital, Taichung, Taiwan [20].

Allergen Sensitization and LNIT with rDer p 2 in Conjunction with FIP

Mice were immunized by intraperitoneal (IP) injection of 1 µg/0.1 ml of rDer p 2 or 4.0 µg/0.1 ml of Der p adsorbed in

4.0 mg/0.062 ml aluminum hydroxide $\text{Al}(\text{OH})_3$ (Whitehall Lab Ltd., Punchbowl, Australia) on day 0 and day 7 (fig. 1). Seven days after the second immunization, mice received LNIT with FIP mixed with rDer p 2 daily from days 14 to 35 (FIP 50 $\mu\text{g}/12 \mu\text{l}$ /mouse/day, rDer p 2 2.5 $\mu\text{g}/2.5 \mu\text{l}$ /mouse/day). The positive control groups of mice were fed with dexamethasone (DEX) 10 $\mu\text{g}/100 \mu\text{l}$ /mouse/day and negative control groups of mice received LNIT with normal saline (NS). On days 28 and 35, mice were lightly anesthetized with an IP injection of 60 mg/kg of sodium pentobarbital (Sigma Chemical Co., St. Louis, Mo., USA) and intratracheally (IT) inoculated with 1 $\mu\text{g}/50 \mu\text{l}$ of different antigens. Saliva collection from mice was done using carbachol (10 $\mu\text{g}/\text{mouse}$; Sigma), by IP injection [25] before the first pulmonary function measurement. Two days after the second IT inoculation, the mice were sacrificed after the second pulmonary function measurement and bronchoalveolar lavage.

Sample Collection and Preparation

Groups of 4 mice had received bronchoalveolar lavage, and the fluid (BALF) was collected as previously described [24]. One separate dose of 1 ml of sterile endotoxin-free saline was injected into the lungs via the trachea of each mouse. Approximately 0.85 ml of the washing solution was recovered constantly. The BALF was aspirated and stored at -70°C until assayed. After total leukocyte counting, cytospin preparations using 100 μl of BALF were stained with Liu stain (Tonyar Diagnostic Inc., Taipei and Hien, Taiwan), and differential counts were performed on 200 cells. Blood samples were obtained via the orbital sinus, and sera were collected and stored at -70°C until assayed.

Determination of Antigen-Specific Igs in Sera and Saliva

Serum IgG and non-IgG were separated from the pooled sera by means of protein G affinity chromatography (HiTrap affinity columns; Pharmacia, Uppsala, Sweden) [19].

Specific IgG and IgE antibodies against rDer p 2 and Der p 1 in the sera were then determined by ELISA. Briefly, each sample was diluted 1:100 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin. One hundred microliters of the sample solution were added to ELISA plates (Nunc Lab, Ill., USA) coated with rDer p 2 and Der p 1 (1 $\mu\text{g}/\text{ml}$) and incubated overnight at 4°C . The plates were washed with PBS three times. For detection of antibodies, horseradish peroxidase-conjugated goat antimouse IgG2a and IgE antibodies diluted 1:800 and IgG antibodies diluted 1:2,000 (Southern Biotech Assoc., Inc., Birmingham, Ala., USA) were added separately for each assay, and the bound enzyme substrate was detected using 3,3',5,5'-tetramethylbenzidine (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md., USA). The reaction was stopped after 30 min and the optical density was measured at 405 nm in a multiscan spectrophotometer (model A-5682; SLT Lab Instruments, Salzburg, Austria). Results were expressed as ELISA units. The total IgA and antigen-specific IgA in the saliva were measured using an ELISA method as described above. Plates were coated with 50 μl of goat antimouse IgA antibody (Sigma) or coated with 100 μl of rDer p 2 and Der p 1 overnight at a concentration of 5 $\mu\text{g}/\text{ml}$ in a refrigerator at 4°C . Detection antibodies using horseradish peroxidase-conjugated goat antimouse IgA antibodies diluted 1:4,000 (Southern Biotech Assoc.) were added separately for each assay, and the bound enzyme substrate was detected using 3,3',5,5'-tetramethylbenzidine. The lowest detectable quantity was 0.078 ng/ml. Results were expressed as ng/ml in the saliva.

Cytokine Measurement and Tracheal Tissue Staining

IL-4, IL-10 and IFN- γ were measured using commercially available ELISA kits (R&D Systems, Minneapolis, Minn., USA) containing mouse monoclonal antibodies recognizing different epitopes of the cytokine molecules. The lowest detectable quantity was 10 pg/ml. The lung and tracheal tissue of 2 mice from the experimental and control groups were removed and rinsed with PBS. Tissue sections were fixed with 10% formalin and stained with hematoxylin-eosin, and the slides with these sections were mounted with cover slides.

Pulmonary Function Determination

Each mouse was placed inside a barometric plethysmograph (Buxco Electronics, Troy, N.Y., USA). The plethysmograph has two chambers: one is the main or animal chamber (internal diameter 7.5 and 5.5 cm), and the other is the reference chamber (internal diameter 7.5 and 3.5 cm). A differential pressure transducer was employed to detect the pressure difference between the two chambers [24]. The pressure signal was amplified, digitized via an A/D convert card and sent to a computer with a BioSystem XA program (Buxco Electronics), which sampled and calculated the desired respiratory parameters. These parameters were enhanced pause (Penh), tidal volume, breathing frequency, peak inspiratory flow, peak expiratory flow, end-inspiratory pause and end-expiratory pause. Aerosol was generated by placing 5 ml of saline or methacholine (1.56–25.0 mg/ml; Sigma) solution in the cup of an ultrasonic nebulizer (DeVilbiss, Somerset, Pa., USA). It was delivered via a connecting tube and a three-way connector to the animal chamber of the plethysmograph [24]. The median size of the aerosol was approximately 3 μm (range 1–5 μm), according to the manufacturer's information. The aerosol usually filled the chamber within 15–20 s. At first, each mouse inhaled saline aerosol for 3 min; saline aerosol was then replaced with aerosols of methacholine solution for 3 min. In both cases, the aerosol in the chamber was cleared immediately after the exposure. Respiratory parameters were then measured for 4 min following inhalation of methacholine. A dose-response curve for methacholine was calculated starting from a low dose of 0 mg/dl to a higher dose of 25.0 mg/dl. There was a 15-min interval between exposures. The percentage changes of the Penh obtained from each dose of methacholine were represented as means $\pm$ SEMs. Differences in parameters among groups were analyzed using analysis of variance.

Statistical Analysis

Results were expressed using the arithmetic mean $\pm$ SEM. Differences among the groups were assessed using Student's *t* test. A *p* value of less than 0.05 was considered statistically significant.

Results

Effects of LNIT on the Inflammatory Cell Infiltration in the Airway

The inflammatory cell infiltration in the airway was compared between the two groups of mice by analyzing the leukocytes in the BALF after LNIT. The results showed that LNIT had an anti-inflammatory effect on

Fig. 2. LNIT on systemic immune responses. Serum concentration of antigen-specific IgG, IgE and IgG2a were measured by ELISA. Values are expressed as means $\pm$ SEM of optical density (OD) of 6 mice in each group. Serum cytokine levels were measured by ELISA. Data are represented as means $\pm$ SEM (pg/ml) of 6 mice. * $p < 0.05$ as compared with the NS group; + $p < 0.05$ as compared with the naive group.

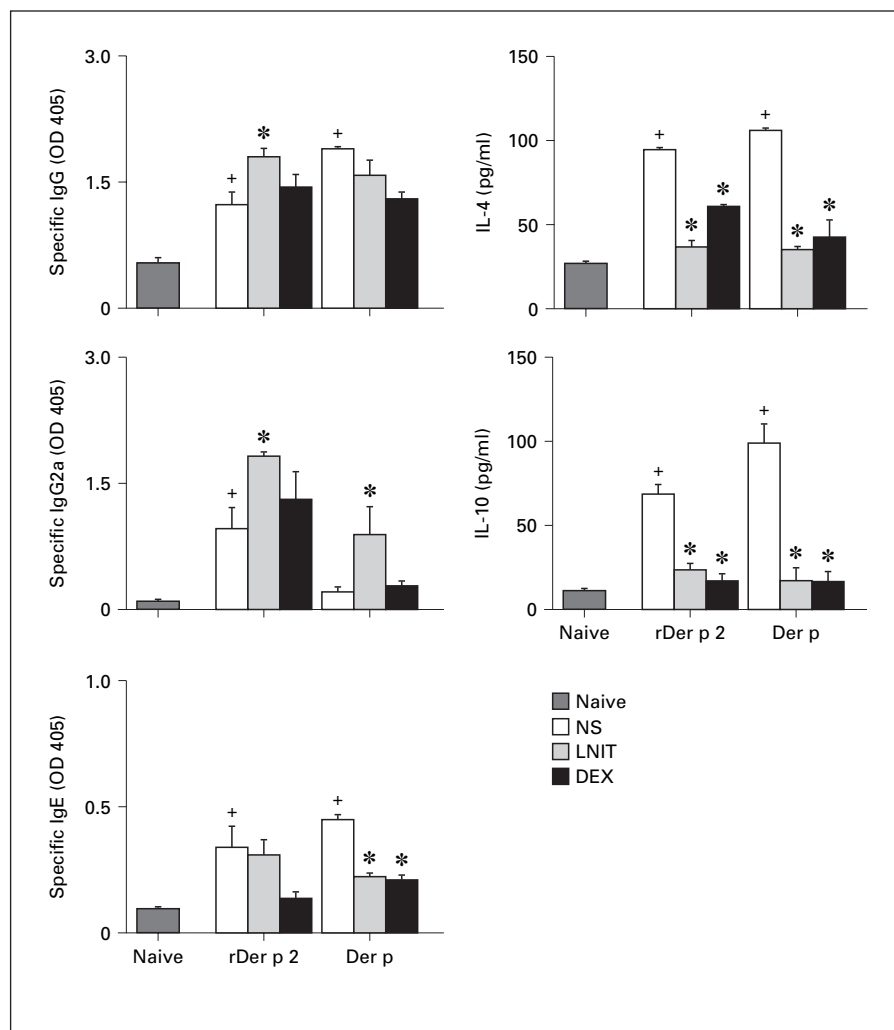


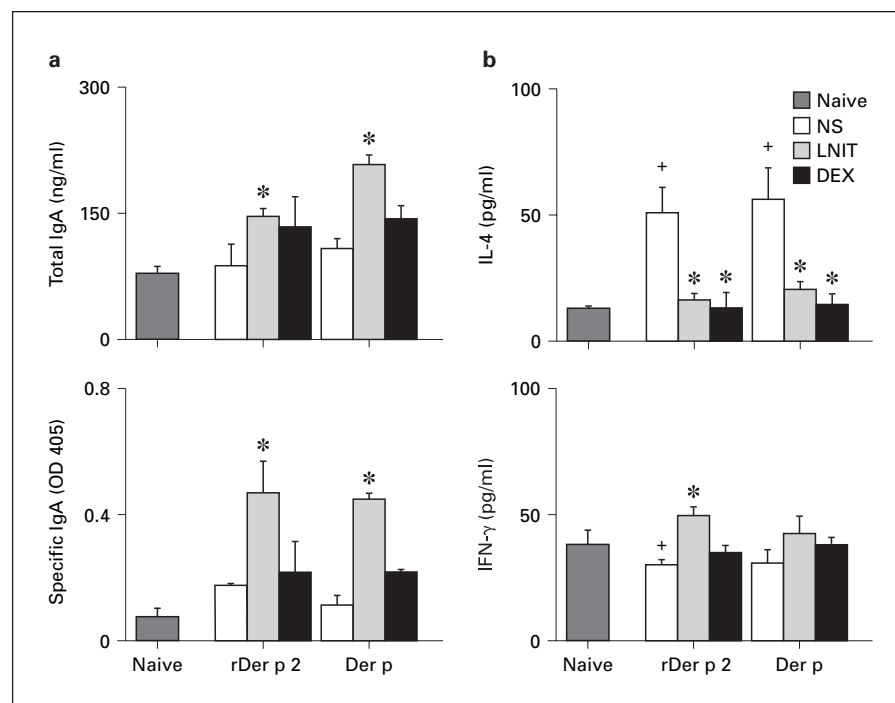
Table 1. Leukocyte subpopulation in the BALF derived from all groups of mice after immunotherapy

	Total cells	Macrophages	Lymphocytes	Neutrophils	Eosinophils
Naive	13.5 $\pm$ 1.5	12.5 $\pm$ 1.5	1.0 $\pm$ 0	0	0
rDer p 2-sensitized mice					
NS	146.0 $\pm$ 6.0	26.5 $\pm$ 2.5	38.5 $\pm$ 3.5	65.5 $\pm$ 12.5	16.0 $\pm$ 1.0
LNIT	59.7 $\pm$ 2.5*	14.3 $\pm$ 1.9*	14.0 $\pm$ 0.9*	24.3 $\pm$ 2.5*	5.0 $\pm$ 1.1*
DEX	70.0 $\pm$ 10.0*	25.5 $\pm$ 3.5*	10.0 $\pm$ 0*	9.0 $\pm$ 2.0*	1.5 $\pm$ 0.5*
Der p-sensitized mice					
NS	134.0 $\pm$ 2.0	23.5 $\pm$ 3.5	39.5 $\pm$ 2.5	54.0 $\pm$ 0	17.5 $\pm$ 1.5
LNIT	108.0 $\pm$ 12.0	25.3 $\pm$ 3.4	24.7 $\pm$ 3.0*	39.3 $\pm$ 4.6*	18.0 $\pm$ 3.2
DEX	70.0 $\pm$ 14.0*	29.0 $\pm$ 7.0	26.0 $\pm$ 4.0*	14.0 $\pm$ 4.0*	1.0 $\pm$ 1.0*

Data are represented as means $\pm$ SEM of total cell number obtained from 4 animals ($\times 10^4$).

* $p < 0.05$ when compared with the NS group.

Fig. 3. Effects of LNIT on airway immune responses. The concentration of total and specific IgA and cytokines in the saliva (a) and BALF (b) were measured by ELISA. Data are represented as means $\pm$ SEM of 6 mice. * $p < 0.05$ as compared with the NS group; + $p < 0.05$ as compared with the naive group.



both rDer p 2- and Der p-sensitized mice. The number of both lymphocytes and neutrophils was significantly decreased after LNIT compared with the NS buffer control. In the rDer p 2-sensitized mice, total leukocyte counts, as well as their subpopulations, including macrophages, lymphocytes and neutrophils, were all decreased. In the DEX-treated mice, both total leukocyte counts and all the cell subpopulations were also decreased when compared with the NS-treated mice in both rDer p 2- and Der p-sensitized mice (table 1).

Effect of LNIT on Systemic Immune Responses

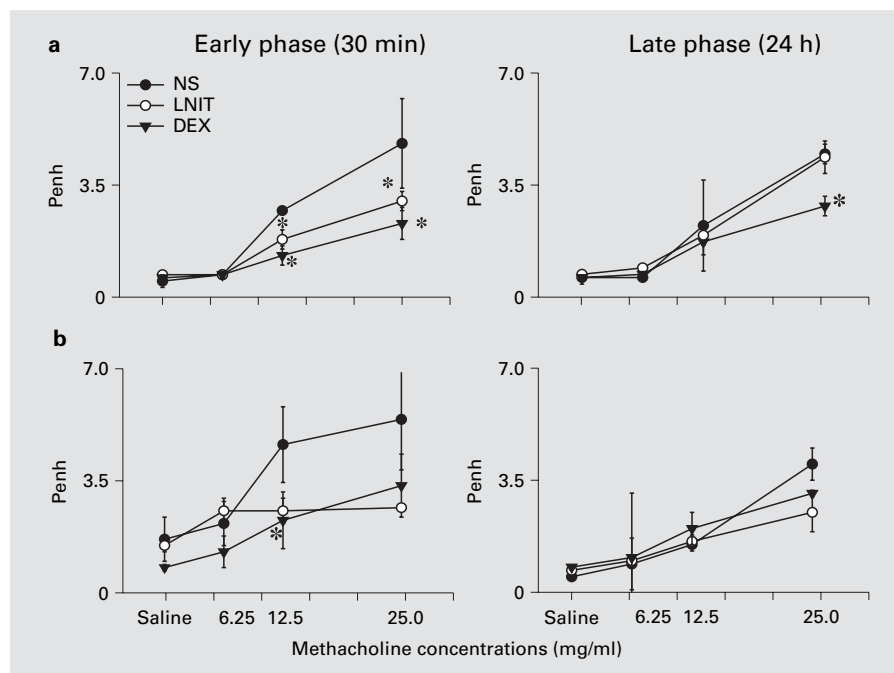
In both rDer p 2- and Der p-sensitized mice, allergen-specific IgG, IgG2a and IgE were significantly increased in sera when compared with those obtained from the naive group of mice ($p < 0.05$; fig. 2). LNIT significantly increased allergen-specific IgG and IgG2a production in both groups of mice (fig. 2). However, specific IgE was reduced after LNIT in both rDer p 2- and Der p-sensitized mice, but only Der p-sensitized mice reached a significant level ($p < 0.05$; fig. 2). When the serum concentrations of cytokines were analyzed, both IL-4 and IL-10 were increased in both rDer p 2- and Der p-sensitized mice in comparison with those obtained from naive controls ($p < 0.05$; fig. 2). After LNIT, both IL-4 and IL-10 were sig-

nificantly decreased when compared with the NS-treated mice in all allergen-sensitized mice ($p < 0.05$; fig. 2). Decreased IL-4 and IL-10 were also found in the DEX-treated mice. The serum concentrations of IFN- γ in rDer p 2- and Der p-sensitized mice were 90.6 ± 16 and 90.5 ± 35 pg/ml, which were not significantly different from NS-treated mice (77.3 ± 2.0 and 79.8 ± 7.4 pg/ml).

Effect of LNIT on Airway Immune Response

In both rDer p 2- and Der p-sensitized mice, allergen-specific IgA and total IgA in the saliva were significantly increased after LNIT when compared with NS buffer control ($p < 0.05$; fig. 3a). In the NS-treated mice, both total and specific IgA in saliva were not changed (fig. 3a). The total and specific IgA were not changed in the DEX-treated mice either. When the cytokine concentrations in the BALF were measured, the IL-4 concentrations were significantly decreased after LNIT in both rDer p 2- and Der p-sensitized mice, in comparison with those of the NS buffer control ($p < 0.05$; fig. 3b). LNIT could increase IFN- γ secretion in both rDer p 2- and Der p-treated mice; however, the increase was only significant in the rDer p 2-treated mice (fig. 3b).

Fig. 4. Effects of LNIT on the pulmonary function after antigen IT challenge on rDer p 2-sensitized mice (**a**) and Der p-sensitized mice (**b**). The airway hypersensitivity to methacholine was measured 30 min (early phase) and 24 h (late phase) after antigen IT challenge. * $p < 0.05$ as compared with the NS control group.



Effects of LNIT on the Pulmonary Function

Airway hypersensitivity to methacholine was increased at both 30 min and 24 h after allergen challenge. Penh was increased after methacholine inhalation in all groups of mice. After LNIT, there was a reduction of airway hypersensitivity at 30 min after allergen challenge in the rDer p 2- and Der p-sensitized mice, but only significantly decreased in rDer p 2-sensitized mice. The significant decrease of the Penh was not observed in Der p-sensitized mice at 24 h after allergen challenge; only the DEX-treated group in rDer p 2-sensitized mice reached a significant level (fig. 4). For mice treated with DEX, the airway hypersensitivity to methacholine was diminished in all allergen-sensitized mice, with a decreased Penh when compared with NS-treated mice (fig. 4).

Pathology of Different Groups of Mice after LNIT

The inflammatory cell infiltration in and around the epithelium of the airway was compared between rDer p 2- and Der p-sensitized mice after LNIT (fig. 5). The results revealed that there were reductions of airway inflammatory cell infiltration in both rDer p 2- and Der p-sensitized mice after LNIT compared with NS buffer controls (fig. 5).

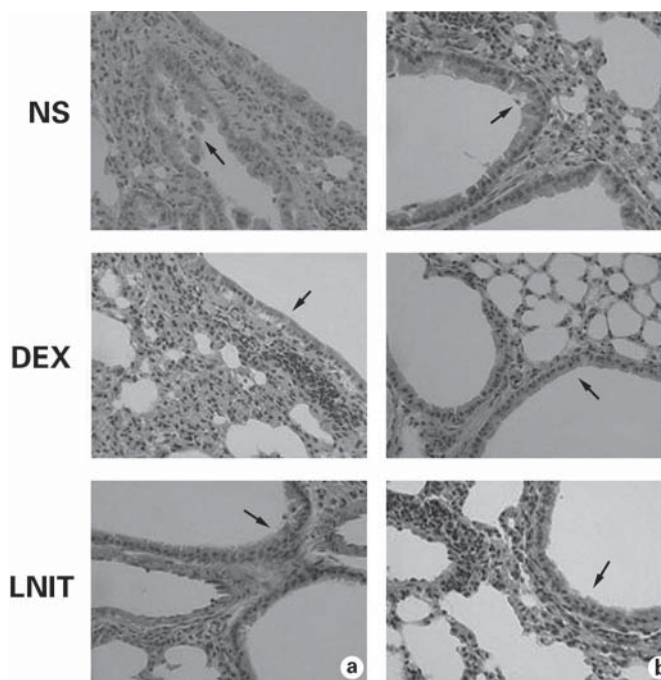


Fig. 5. Lung pathology after LNIT. The lung tissues were obtained 2 days after IT exposure to rDer p 2 in rDer p 2-sensitized mice (**a**) and Der p in Der p-sensitized mice (**b**). Data in the upper row represent different groups of mice treated with NS buffer control, whereas the middle row and the lower row show data obtained from mice treated with DEX and rDer p 2 in conjunction with FIP, respectively. Arrows indicate airway mucosa damage. HE. $\times 400$.

Discussion

In this study, we demonstrated that LNIT with rDer p 2 in conjunction with FIP could downregulate the airway inflammation in both rDer p 2- and Der p-sensitized mice. The significantly increased concentration of total IgA and specific IgA in the saliva of both rDer p 2- and Der p-sensitized mice indicated that LNIT might have some immunomodulatory effect on the airway. It has been previously reported that airway delivery of allergen-specific IgA monoclonal antibodies is able to attenuate pulmonary hypersensitivity responses in Th2-sensitized mice [19, 26]. Therefore, the local increment of IgA in response to LNIT may contribute to its improved effectiveness in protecting Th2-sensitized mice from airway allergen challenge. With respect to the use of recombinant allergens for mucosal tolerance induction as a treatment strategy against allergic sensitization, it has been shown in a murine model of birch pollen allergy that intranasal application of recombinant Bet v 1 (the major birch pollen allergen) led to significant responses in naive and in sensitized mice [27]; more importantly, the immunosuppression effect of LNIT with rBet v 1 was able to be directed against rBet v 1 itself, as well as towards the whole pollen [28]. In our study, similar results were found for LNIT with rDer p 2 in conjunction with FIP which did not only downregulate rDer p 2-sensitized mice but also those mice sensitized by Der p.

We previously demonstrated that LNIT using Der p 2 in conjunction with FIP enhanced Th1-biased immunogenicity and reduced Th2-biased allergenicity; TGF- β decreased after LNIT as well [24]. It has been reported that Th2 cells can be downregulated by IL-10, which is the major cytokine generated by regulatory T cells [29]. However, in this study, the simultaneous reduction of IL-4 and IL-10 after LNIT indicated that the downregulation of IL-4 was not due to the activation of IL-10-producing cells. Similarly, it has been confirmed that IL-10 can be reduced in the sera of mice after LNIT with an immunodominant peptide, which indicates that intranasal delivery of a single immunodominant peptide from an allergen can induce tolerance to the whole protein [18]. Based on this result and our previous studies, both IL-10 and TGF- β were decreased after LNIT. Since regulatory T cells secreted both cytokines, it is unlikely that LNIT activated the regulatory T cells. Whether, LNIT had an influence on regulatory T cells remains to be further clarified.

LNIT with rDer p 2 could enhance allergen-specific IgG and IgG2a production in both Der p- and rDer-p 2-sensitized mice, but the enhancement of IgG and IgG2a

in rDer p 2-sensitized mice was higher than that of Der p-sensitized mice. These results indicated that Der p 2 was the major allergen in Der p and LNIT could play a role in the activation of IgG-producing cells in Der p-sensitized mice. On the other hand, the reduction of specific IgE was found in both rDer p 2- and Der p-sensitized mice, and LNIT could decrease antigen-specific IgE and IL-4 production in the sera. For the airway hypersensitivity study, our results revealed that LNIT was effective for the prevention of an early-phase reaction. Similar results have been reported for FIP, namely that FIP abolishes the immediate anaphylactic reaction [20]. LNIT with FIP was not effective for the prevention of late-phase airway hyperreactivity despite the fact that the inflammatory cells in the BALF were decreased. These results suggested that late-phase airway hyperreactivity is only partially correlated with airway inflammatory cell infiltration. Although it is now widely accepted that airway inflammation is the basis of airway hyperreactivity, there are at least three independent studies of airway hyperreactivity in mice [30]. The first pathway is dependent on IL-4 and mast cells, the second is dependent on IL-5 and eosinophils and the third is mediated by IL-13. Whether IL-4 and IL-13 can be downregulated by LNIT remains to be clarified.

In conclusion, LNIT with Der p 2 in conjunction with FIP could suppress both rDer p 2- and Der p-induced airway inflammation and cause tolerance to these antigens. The enhancement of IgG2a in the sera and IgA in the saliva indicated that LNIT might exert both local and systemic immunomodulation on both rDer p 2- and Der p-induced allergic reactions.

Acknowledgements

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References

- Platts-Mills TA, Vervloet D, Thomas WR, Aalberse RC, Chapman MD: Indoor allergens and asthma: report of the Third International Workshop. *J Allergy Clin Immunol* 1997;100: S2–S24.
- Sporik R, Chapman M, Platts-Mills TA: House dust mite exposure as a cause of asthma. *Clin Exp Allergy* 1992;22:897–906.
- Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TA: Antigenic and structural analysis of group II allergens (Der f II and Der p II) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989;83: 1055–1067.
- Tsai JJ, Shen HD, Chua KY: Purification of group 2 *Dermatophagoides pteronyssinus* allergen and prevalence of its specific IgE in asthmatics. *Int Arch Allergy Immunol* 2000;121: 205–210.
- Smith AM, Chapman MD: Allergen-specific immunotherapy: new strategies using recombinant allergens; in Bousquet J, Yssel H (eds): *Immunotherapy in Asthma*. New York, Marcel Dekker, 1999, pp 99–118.
- Fox PC, Siraganian RP: IgE antibody suppression following aerosol exposure to antigens. *Immunology* 1981;43:227–234.
- Georgitis JW, Clayton WF, Wypych JI, Barde SH, Reisman RE: Further evaluation of local intranasal immunotherapy with aqueous and allergoid grass extracts. *J Allergy Clin Immunol* 1984;74:694–700.
- Wiedermann U, Herz U, Vrtala S, Neuhaus-Steinmetz U, Renz H, Ebner C, et al: Mucosal tolerance induction with hypoallergenic molecules in a murine model of allergic asthma. *Int Arch Allergy Immunol* 2001;124:391–394.
- Zambetti G, Moresi M, Romeo R, Luce M, Filiaci F: Non-specific nasal provocation test with histamine. Analysis of the dose-response curve. *Rhinology* 1999;37:168–174.
- Pocobelli D, Del Bono A, Venuti L, Falagiani P, Venuti A: Nasal immunotherapy at constant dosage: a double-blind, placebo-controlled study in grass-allergic rhinoconjunctivitis. *J Invest Allergol Clin Immunol* 2001;11:79–88.
- Olivieri M, Mohaddes Zadeh MR, Talamini G, Lampronti G, Lo Cascio V: Local nasal immunotherapy and bronchial hyperreactivity in seasonal allergic rhinitis: an observational pilot study. *J Invest Allergol Clin Immunol* 2000; 10:300–304.
- Nickelsen JA, Goldstein S, Mueller UR, Wypych J, Reisman RE, Arbesman CE: Local intranasal immunotherapy for ragweed allergic rhinitis. 2. Immunologic response. *J Allergy Clin Immunol* 1981;68:41–45.
- Nickelsen JA, Georgitis JW, Mueller UR, Kane J, Wypych J, Goldstein S, et al: Local intranasal immunotherapy for ragweed allergic rhinitis. 3. A second year of treatment. *Clin Allergy* 1983;13:509–519.
- Welsh PW, Butterfield JH, Yunginger JW: Allergen-controlled study of intranasal immunotherapy for ragweed hay fever. *J Allergy Clin Immunol* 1983;71:454–460.
- Bjorksten B: Local immunotherapy is not documented for clinical use. *Allergy* 1994;49:299–301.
- Georgitis JW, Nickelsen JA, Wypych JI: Local nasal immunotherapy: efficacy of low-dose aqueous extract. *J Allergy Clin Immunol* 1985; 75:496–500.
- Bertoni M, Cosmi F, Bianchi I, Berardino L: Clinical efficacy and tolerability of a steady dosage schedule of local nasal immunotherapy. Results of preseasonal treatment in grass pollen rhinitis. *Ann Allergy Asthma Immunol* 1999;82:47–51.
- Hoyne GF, Askonas BA, Hetzel C, Thomas WR, Lamb JR: Regulation of house dust mite responses by intranasally administered peptide: transient activation of CD4+ T cells precedes the development of tolerance in vivo. *Int Immunol* 1996;8:335–342.
- Takabayashi K, Libet L, Chisholm D, Zubeldia J, Horner AA: Intranasal immunotherapy is more effective than intradermal immunotherapy for the induction of airway allergen tolerance in Th2-sensitized mice. *J Immunol* 2003;170:3898–3905.
- Ko JL, Hsu CI, Lin RH, Kao CL, Lin JY: A new fungal immunomodulatory protein, FIP-fve isolated from the edible mushroom, *Flammulina velutipes* and its complete amino acid sequence. *Eur J Biochem* 1995;228:244–249.
- Ko JL, Lin SJ, Hsu CI, Kao CL, Lin JY: Molecular cloning and expression of a fungal immunomodulatory protein, FIP-fve, from *Flammulina velutipes*. *J Formos Med Assoc* 1997; 96:517–524.
- Hsieh KY, Hsu CI, Lin JY, Tsai CC, Lin RH: Oral administration of an edible-mushroom-derived protein inhibits the development of food-allergic reactions in mice. *Clin Exp Allergy* 2003;33:1595–1602.
- Seow SV, Kuo IC, Paaventhann P, Kolatkar PR, Chua KY: Crystallization and preliminary X-ray crystallographic studies on the fungal immunomodulatory protein Fve from the golden needle mushroom (*Flammulina velutipes*). *Acta Crystallogr D Biol Crystallogr* 2003;59: 1487–1489.
- Liu YH, Kao MC, Lai YL, Tsai JJ: Efficacy of local nasal immunotherapy for Dp2-induced airway inflammation in mice: using Dp2 peptide and fungal immunomodulatory peptide. *J Allergy Clin Immunol* 2003;112:301–310.
- Deslauriers N, Oudghiri M, Seguin J, Trudel L: The oral immune system: dynamics of salivary immunoglobulin production in the inbred mouse. *Immunol Invest* 1986;15:339–349.
- Schwarze J, Cieslewicz G, Joetham A, Sun LK, Sun WN, Chang TW, et al: Antigen-specific immunoglobulin-A prevents increased airway responsiveness and lung eosinophilia after airway challenge in sensitized mice. *Am J Respir Crit Care Med* 1998;158:519–525.
- Wiedermann U, Jahn-Schmid B, Bohle B, Repa A, Renz H, Kraft D, Ebner C: Suppression of antigen-specific T- and B-cell responses by intranasal or oral administration of recombinant Bet v 1, the major birch pollen allergen, in a murine model of type I allergy. *J Allergy Clin Immunol* 1999;103:1202–1210.
- Winkler B, Baier K, Wagner S, Repa A, Eichler HG, Scheiner O, Kraft D, Wiedermann U: Mucosal tolerance as therapy of type I allergy: intranasal application of recombinant Bet v 1, the major birch pollen allergen, leads to the suppression of allergic immune responses and airway inflammation in sensitized mice. *Clin Exp Allergy* 2002;32:30–36.
- Ling EM, Smith T, Nguyen XD, Pridgeon C, Dallman M, Arbery J, Carr VA, Robinson DS: Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet* 2004;363:608–615.
- Leong KP, Huston DP: Understanding the pathogenesis of allergic asthma using mouse models. *Ann Allergy Asthma Immunol* 2000; 87:96–110.