

# Effect of allergen immunotherapy on nasal responses in guinea-pigs with allergic rhinitis

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## Summary

**Methods** We have investigated the effects of allergen immunotherapy on the nasal responses in the guinea-pigs with allergic rhinitis. Thirty-three male Hartley guinea-pigs with allergic rhinitis were divided into three groups; those receiving intradermal injection of saline (Group 1) or 0.1% ovalbumin (Group 2) 6 days after the last intranasal sensitization, and those injected with 0.1% ovalbumin intradermally once daily for 6 consecutive days from the next day after the last intranasal sensitization (Group 3).

**Results** The dye leakage and histamine content into the nasal lavage significantly decreased at 30 min after antigen challenge in Group 3, compared with Group 1 or 2. We also observed the change of mast cell numbers in superficial nasal mucosa, lamina propria and injected dorsal skin. The number of mast cells in superficial nasal mucosa significantly decreased in Group 3 compared with Group 1 or 2, but not those in nasal lamina propria or dorsal skin.

**Conclusions** These results suggest that the improvements of nasal responses such as dye leakage and histamine content may be caused by the decrease of mast cell numbers in the superficial mucosal layer after the specific immunotherapy, which may be developing tolerance and one of the mechanisms underlying the beneficial effect of immunotherapy.

**Keywords:** guinea-pigs, allergen immunotherapy, allergic rhinitis, ovalbumin, dye leakage, mast cell

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## Introduction

Since specific immunotherapy was introduced in 1911 for the treatment of pollinosis by Noon and Freeman [1], this treatment has been used as one of the common treatments on the IgE-mediated allergic disease in many parts of the world [2–5]. However, immunotherapy is still controversial because protocols have been devised empirically, many allergens are still poorly defined, the mechanisms of action are not yet clear, the duration is poorly characterized, and the efficacy index of immunotherapy has been contested for many years.

It is impossible to compare the efficacy of specific immunotherapy strictly in human although the double-blind studies have been reported [6–8]. In this study, we investigated the pathophysiological responses to the antigen in the nose of animal model with allergic rhinitis, and

compared the effect of antigen-specific immunotherapy with the control.

## Materials and methods

### Immunization

Thirty-three male Hartley guinea-pigs, weighing about 300 g at the beginning of the experiment, were actively sensitized by intraperitoneal injection of 20 µg ovalbumin containing 10 mg Al(OH)<sub>3</sub> and boosted intraperitoneally by the same antigen-Al(OH)<sub>3</sub> mixture every 2 weeks three times after the initial sensitization. Furthermore, 2 weeks after the last sensitization, the animals were boosted intranasally by 3 mL of 0.01% ovalbumin (OA) for 5 consecutive days [9–11].

### Antigen-specific immunotherapy

Sensitized animals were divided into three groups; those

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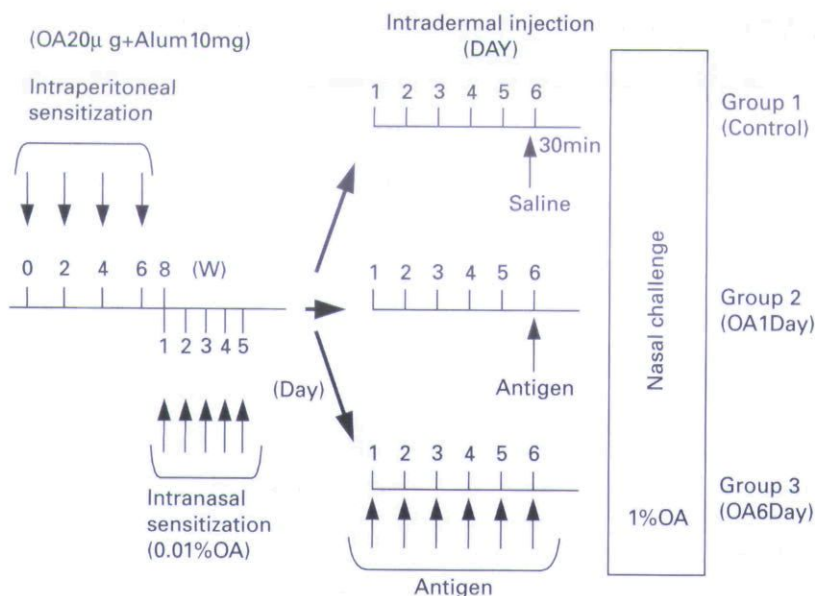


Fig. 1. Experimental resume on three groups.

receiving intradermal injection of saline (Group 1) or 0.1% OA (Group 2) 6 days after the last intranasal sensitization, and those treated with 0.1% OA intradermally once daily for 6 consecutive days from the next day after the last intranasal sensitization (Group 3). All groups were provoked intranasally by 1% OA 30 min after the last intradermal injection (Fig. 1). Intradermal treatment was performed by the intracutaneous injection of 0.1 mL saline or OA at different dorsal areas which has been previously shaved.

#### Antigen challenge

Sensitized guinea-pigs were anaesthetized with intraperitoneal injection of sodium pentobarbital (30 mg/kg) and tracheostomy was performed. A polyethylene tube was inserted into the nasopharyngeal space through the tracheal stoma. Oral cavity was sealed by glycerin-soaked cotton. One per cent OA which is submaximal concentration was perfused for 3 min through the nasal cavity at a rate of 0.2 mL/min.

#### Experimental protocol

The dye of 4% pontamine sky blue (0.2 mL/100 gr body-weight) was injected into the middle internal jugular vein, and the perfusion pump was connected to the polyethylene tube inserted into the nasopharyngeal space. After the physiological saline was intranasally perfused via the same route and cannula as antigen challenge for 5 min at a rate of 0.2 mL/min, the perfusate effused from the nostrils was collected four times before (Period 1) and until 30 min after antigen challenge (Periods 2, 3, 4) at intervals of 10 min. The dye

concentration in the intranasal perfusate was determined by measuring the absorbance (620 nm) of the supernatant obtained after centrifugation of the perfusate (3000 rpm for 10 min), and the histamine concentration was determined by radioimmunoassay kit (IMMUNOTHECH, France).

After collection of the perfusate at each Period, nasal airway resistance (NAR) were measured as follow; compressed air was aerated to the nasal cavity through the polyethylene tube, which was connected with the pressure transducer, after air flow was adjusted to 200 mL/min. NAR was measured in terms of recording on the X-Y recorder after the signal was amplified [12]. Per cent change of NAR was calculated by the formula of  $B-A/A \times 100$  (A: value before challenge, B10, B20, B30: value of 10, 20, 30 min after challenge, respectively).

#### Titration of antibodies

The titre of IgE and IgG antibody to OA of sera from each group were determined by PCA reactions. PCA was performed as previously described [13]; that is, to determine IgE titre, 0.01 mL of serial dilutions of unheated antisera were injected intradermally on the shaved dorsal skin of guinea-pigs. To determine IgG titre, sera heated at 56°C for 1 h was injected by the same as the determination of IgE. The animals were challenged intravenously by 1 mL saline containing 0.5% Evans blue and 500 mg OA 8 days for IgE and 6 h for IgG after the injection of antisera. Blue spots larger than 5 mm in diameter were judged as positive PCA reaction. The antibody titer were taken as the last dilution which produced a positive reaction.

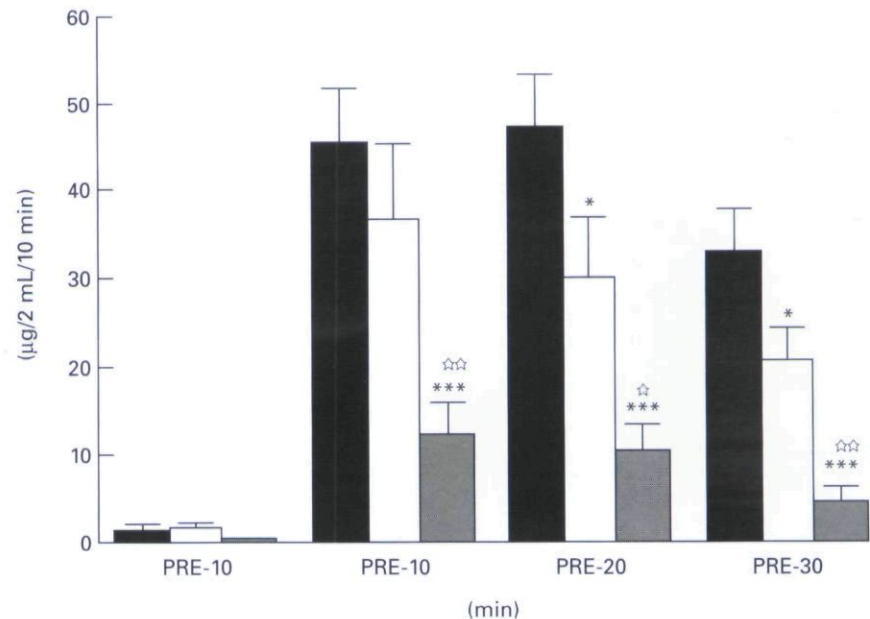


**Fig. 2.** The dye leakage into the nasal cavity. Values represent mean  $\pm$  SE. ■, Group 1 ( $n = 11$ ); ▨, Group 2 ( $n = 11$ ), ■, Group 3 ( $n = 11$ ). Statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ) compared with Group 1 (\* $P < 0.05$ , \*\* $P < 0.01$ ) compared with Group 2.

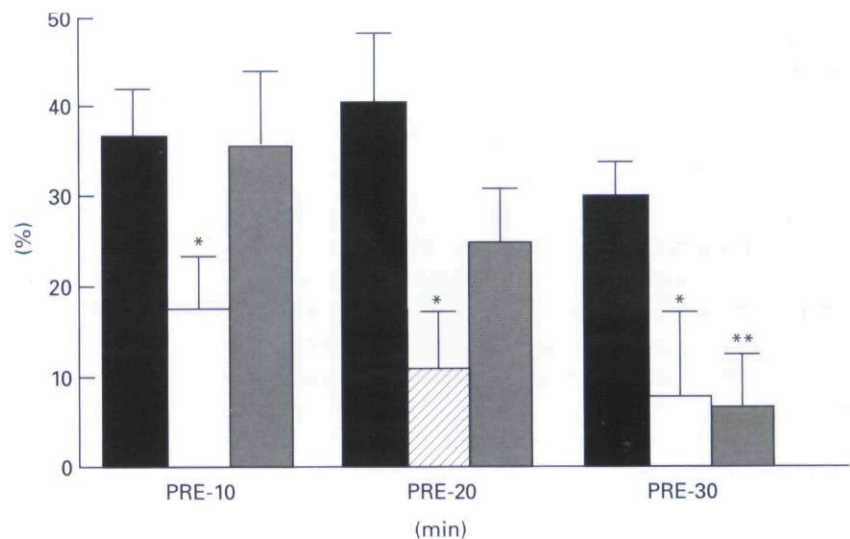


**Fig. 3.** Per cent changes of nasal resistance. Values represent mean  $\pm$  SE. ■, Group 1 ( $n = 11$ ); ▨, Group 2 ( $n = 11$ ); ■, Group 3 ( $n = 11$ ). Statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ ) compared with Group 1.

#### Preparation of specimens

The nasal mucosa of  $\approx 25\text{mm}^2$  were excised at the 1 cm from the anterior part of collumela. The skin of about  $30\text{mm}^2$  in size were obtained from the most caudal of dorsal skin injected.

#### Light microscopic observation

The excised nasal mucosa and dorsal skin were immediately placed in Carnoy's fixative containing 60 mL of absolute alcohol, 30 mL of chloroform and 10 mL of glacial acetic acid for 3 h. They were embedded in paraffin, sectioned at  $4\mu\text{m}$  and stained with 1% toluidine blue at pH 1.0 for nasal mucosa and at pH 4.2 for skin. The number of mast cells in

the mucosal epithelium, lamina propria and shallow corium which was defined between dermoepidermal interface and  $8\mu\text{m}$  deeper area were counted at 1000 folds. The area of the tissue section was measured by an image analyser (SP-500, Olympus, Tokyo, Japan) in combination with a light microscope. The number of mast cells per  $\text{mm}^3$  was calculated by Lange's method [14].

#### Statistical analysis

Data were shown as the mean value  $\pm$  standard error (SE). Statistical evaluation was performed using Wilcoxon's signed rank test. A  $P$ -value of less than 0.05 was recognized as significant.

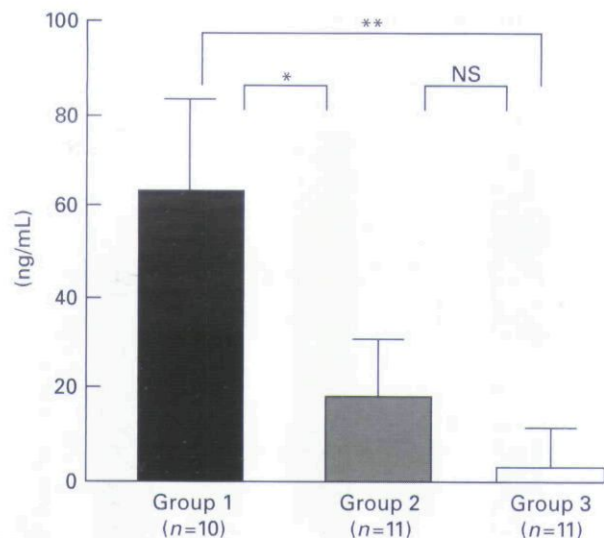


**Fig. 4.** Histamine content into the nasal secretion. Values represent mean + SE. Statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ ) compared with 10 min before challenge.

## Results

### *Effect of specific immunotherapy on nasal responses to the antigen*

The dye leakage into intranasal perfusates was shown in Fig. 2. The dye concentration of Group 3, which was intradermally treated with antigen most frequently in the three groups, was the significant low level compared with other two groups at each period after nasal challenge to antigen. The dye leakage at Periods 3 and 4 in Group 2, which was intradermally treated once before nasal challenge, was significant lower than that of Group 1, which was not intradermally treated antigen, and also significant higher than that of Group 3.

The percentage change of NAR was shown in Fig. 3. Group 2 at each period was significantly lower than those of Group 1, and Group 3 at 30 min after challenge was significantly lower than Group 1. There was no statistical difference between Groups 2 and 3 at each period.

The histamine content in the intranasal perfusates was shown in Fig. 4. The histamine content was indicated as the increased value of Period 2 to Period 1. Histamine content in Groups 2 and 3 was significantly lower than that in Group 1, especially Group 3 was lowest in three groups. There was no statistical difference between Groups 2 and 3.

### *Effect of specific immunotherapy on the number of mast cells in nasal mucosa and dorsal skin*

The effect of immunotherapy on the number of mast cells in nasal mucosal epithelium was shown in Fig. 5. The number

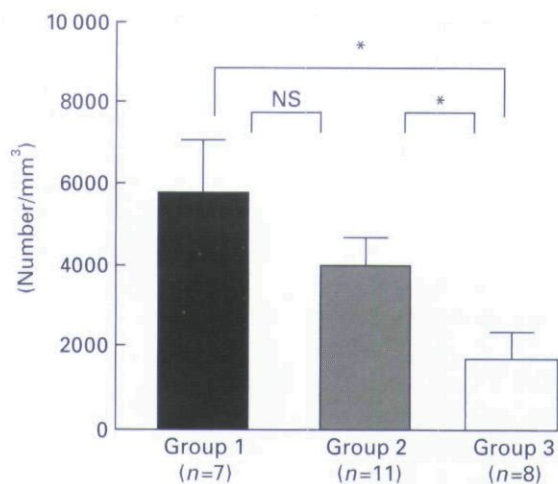


**Fig. 5.** The number of mast cells in the nasal mucosal epithelium. Values represent mean + SE. Statistically significant (\* $P < 0.05$ ) compared with Group 3.

of mast cells in Groups 1, 2, and 3 was  $5751 \text{ cells/mm}^3 \pm 1354$ ,  $3930 \pm 761$ , and  $1659 \pm 694$ , respectively. The number in Group 3 was significantly lower than those in other two groups. The number of mast cells in the lamina propria of Groups 1, 2 and 3 was, respectively,  $4441 \text{ cells/mm}^3 \pm 1111$ ,  $5583 \pm 1092$  and  $4342 \pm 1199$ . There was no significant difference between the three groups. The number of mast cells in the shallow corium was  $441 \text{ cells/mm}^3 \pm 115$  in Group 1,  $304 \pm 126$  in Group 2 and  $359 \pm 97$  in Group 3, respectively. There was no significant difference between the three groups.

### *Effect of immunotherapy on specific IgE and IgG antibody titre to OA*

The PCA titre of IgE was  $70.4 \pm 20.4$  in Group 1,  $136.3 \pm 23.4$  in Group 2 and  $111.3 \pm 23.1$  in Group 3, and also the titre of IgG was  $454.5 \pm 85.6$  in Group 1,  $333.3 \pm 66.6$  in Group 2 and  $444.4 \pm 92.9$  in Group 3. There was no statistical difference between the three groups on IgE and IgG antibody titre.

## Discussion

The mechanisms of action of specific immunotherapy (SIT) are still unclear. For decades it has been postulated that the changes of allergen-specific immunoglobulin such as IgE and IgG or IgG4 may be the principal causes of the clinical improvements after SIT. The production of antigen specific IgG and/or IgG4 antibody in serum have been reported in human study [15–17], but there is no definite conclusion since in some studies IgG and IgG4 was associated with the efficacy of clinical symptoms after SIT whereas in others the contrary was observed [18,19].

It has recently been shown that the quantity of IL-4 produced by allergen specific memory CD4<sup>+</sup>T cells from allergic individuals could be considerably reduced by *in vivo* treatment with allergen [20]. The induction of specific IgE antibody in serum may be suppressed by the decreased IL-4 production [21]. In this study, IgE and IgG antibody in sera did not show the significant difference between immunotherapy and non-immunotherapy. The changes of allergen-specific IgE and IgG may appear later than the improvement of local responsiveness in nasal cavity [22,23].

It has been shown in our allergic model that the number of mast cells in nasal mucosa increase after intranasal sensitization [11], and the histamine, dye leakage into the nasal irrigation and nasal resistance increase at 30 min after intranasal antigen challenge [9,10,24]. Since nasal resistance and dye leakage into the nasal cavity in nonsensitized guinea-pigs dose-dependently increase by histamine intranasal administration and there is a significant correlation between these two parameters [12], it is supposed that increase of nasal resistance and dye leakage into the nasal cavity at acute phase after antigen challenge may be mainly induced by the histamine released from mast cells in the nasal mucosal epithelium.

In this experiment, the sensitized guinea-pigs injected with a sublethal dose of antigen intradermally once daily for six consecutive days (Group 3), showed the significant improvements of dye leakage and histamine content in nasal lavage, and the number of mast cells in nasal mucosal epithelium, compared with saline group (Group 1) or one shot-antigen group (Group 2). Presumably those animals receiving the repeated injection of antigen were developing tolerance, since the final responses except NAR were generally reduced. These results may be interpreted by the reduced number of mast cells and/or the decrease of histamine content released from each mast cell in nasal mucosa after SIT. The reason why the contradictory result of NAR produced in Groups 2 and 3, is unclear.

Changes in nasal metachromatic cells during allergen immunotherapy has been described, that is, Otsuka *et al.* [25] demonstrated the mean number of metachromatic cells in nasal epithelial scrapings was significantly reduced at both 3 and 6 months after immunotherapy compared with the pretreatment values. The reduced sensitivity of metachromatic cells and their release of chemical mediators by immunotherapy have been shown by Pruzansky and Patterson [26]. These results in human are consistent with our present data in animal model.

The reason why the mast cells accumulated into the superficial layer of nasal epithelium by sensitization decrease after SIT have not been resolved yet. However, recently it has been focused to the chemokines like the stem cell factor [27] and RANTES [28] which stimulate the

growth, differentiation and migration of mast cells, and which has been released from nasal epithelial cells. It is speculated that the function of epithelial cell may be responsible and inhibited for the increased mast cells in superficial layer of nasal mucosal epithelium after SIT.

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