

Allergen-specific conventional immunotherapy decreases immunoglobulin E-mediated basophil histamine releasability

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Summary

Background Allergen-specific immunotherapy has proven to be clinically effective in the treatment of patients with atopic asthma; however, the mechanisms are still unclear. Several noted immunological changes include an increase of the allergen-specific IgG antibody, a reduction in the allergen-specific IgE antibody subsequent to transient increase, an allergen-specific T cell shift in cytokine production from Th2 to Th1, and a decrease in quantity and activity of basophils and mast cells.

Objective To analyse the changes of basophil histamine release in response to IgE-mediated and non-IgE-mediated stimuli before and after conventional house-dust mite immunotherapy in children who suffer from atopic asthma.

Methods Fourteen *Dermatophagoides farinae* (Df) sensitive asthmatic children with conventional immunotherapy were examined. Basophil histamine releasability was measured 0 months (just before immunotherapy), 4 months and 9 months after immunotherapy. Basophils were stimulated with Df and goat anti-human IgE antibody as IgE-mediated stimuli; and formyl-Met-Leu-Phe (fMLP) and calcium ionophore A23187 as non-IgE-mediated stimuli. Accordingly, the asthma symptom score was used to assess clinical outcome and the skin test reactivity to Df was measured.

Results In contrast to pre-immunotherapy activity, 4 and 9 months after immunotherapy there were significant decreases in histamine release by Df and by anti-IgE antibody. The histamine release by fMLP and by calcium ionophore showed no significant changes after immunotherapy. Histamine release by Df demonstrated significant correlation to that by anti-IgE antibody and by fMLP, yet there was no observable correlation between histamine release by Df and by calcium ionophore. The asthma symptom score decreased significantly 4 and 9 months after immunotherapy and showed significant correlation with histamine release by Df. The skin test reactivity (allergen/histamine ratio) remained constant 4 months after immunotherapy, but decreased significantly 9 months after immunotherapy.

Conclusion Basophils have the potential to play an important role in the early clinical improvement of conventional immunotherapy in children with atopic asthma, which may be a result of the decreased IgE-mediated histamine releasability during immunotherapy.

Keywords atopic asthma, IgE-mediated basophil histamine releasability, immunotherapy, non-IgE-mediated basophil histamine releasability

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Introduction

Bronchial asthma is a chronic inflammatory airway disease characterized by bronchial hyper-responsiveness and airway inflammation. This airway inflammation may be significantly affected by eosinophils, lymphocytes, mast cells and basophils. The cross-linking of membrane-bound IgE on basophils and mast cells by allergens triggers a release of mediators such as histamine and leukotriene. In addition, this cross-linking also triggers the release of IL-4, which regulates adhesion molecules

on vascular endothelial cells, promotes infiltration of inflammatory cells to the tissue site and plays an essential role in the switching of B cells to produce IgE and T cell differentiation into Th2 cells. Therefore, this cross-linking of membrane-bound IgE on basophils and mast cells by allergens plays an important role during the initial phase of allergic reaction as well as during the late phase reaction, which has been known to be a model of chronic inflammatory reaction [1, 2]. Also, basophils are increased in bronchoalveolar lavage (BAL) fluid after antigen challenge and basophil count is correlated with the changes in airway responsiveness [3].

Although the precise mechanism had not been fully recognized until recently, immunotherapy is a well-known treatment modality in atopic asthma. This treatment is associated with a decrease of allergen-specific IgE antibody after transient early

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increase [4, 5], an increase of allergen-specific IgG₁ and IgG₄ antibody [6, 7], a decrease in numbers and activities of mast cells in the target tissue [8], anergy of T cells [9], an increase of Th1 cells and decrease of Th2 cells [10], and a decrease of basophil releasability [11]. The changes in IgG, IgE antibodies and cytokine profile of T cells develop late after initiation of immunotherapy [5], thus these findings do not fully explain early clinical tolerance after immunotherapy. Subsequently, some changes may be induced in the effector cells, such as basophils or mast cells, during the initial phase of immunotherapy rather than in immunoregulatory lymphocytes. Furthermore, it has been reported that basophil sensitivity and clinical symptoms are well correlated in patients with pollen allergy and that clinical tolerance within several hours after rush immunotherapy in venom allergy is associated with changes of effector cells rather than of lymphocytes [10].

In our previous study, we found that spontaneous and IgE-mediated histamine release was linked to the presence of both atopy and asthma, whereas non-IgE-mediated releasability was heavily dependent on the stimuli [12]. There have been conflicting reports on the variation of basophil releasability after immunotherapy [13–15]. Most notably, after bee venom immunotherapy, total histamine content decreased, yet IgE-mediated and non-IgE-mediated releasability showed no significant changes [10]. Although pollen immunotherapy did not reduce IgE-mediated releasability, total histamine release was substantially decreased [16].

In this study, we analysed the changes of basophil histamine release in response to different stimuli mediated by IgE and non-IgE along with clinical response in atopic asthmatics before and after conventional house-dust mite immunotherapy. The focal point was to evaluate the early effects of immunotherapy on basophils.

Materials and methods

Subjects

This study included 14 children with asthma (11 males, 3 females, mean age 9 years, age range 6 to 13 years) who showed positive skin test to *Df* and *Dp*. All 14 patients were treated with conventional house-dust immunotherapy. Immunotherapy was performed routinely with a purified extract of *Df* and *Dp* (Novo-Helisen, Allergopharma, Reinbek, Germany). Injections had been given for 9 months, weekly during the induction period and monthly during the maintenance period. The concentrations of serum total IgE and *Df*-specific IgE were 909 ± 344 IU/mL (mean $\pm$ SEM) and 3.53 ± 0.23 class, respectively.

This study was approved by the ethics committee of the Ulsan University Asan Medical Center Institutional Review Board, and written consent was obtained from all parents after they were fully informed of the details and the potential risks of the study.

Measurement of serum total IgE, specific IgE, and skin test reactivity

Concentrations of serum total IgE and *Df*-specific IgE were measured with COAT-A-COUNT Total IgE IRMB kit (Diagnostic Products Corporation, Los Angeles, USA) and AlaSTAT RIA Allergen-specific IgE kit (Diagnostic Products Corporation), respectively.

The clinical respiratory symptom scores were measured based on the modified asthma symptom score [17]. Skin tests were performed by the same physician on the backs of the patients with *Df* allergen (50 000 BU/mL, Allergopharma), histamine 1 mg/mL, and saline. The mean weal size was measured and skin test reactivity was analysed to the weal-size ratio of allergen to histamine.

Histamine release from washed human leucocytes

Basophil histamine releasability was measured 0 months (just before immunotherapy), 4 months (finishing point of vial 1) and 9 months (finishing point of initial therapy) after immunotherapy.

The techniques for leucocytes isolation from peripheral blood for histamine release, staging the histamine release reaction and automated fluorometric assay of histamine were as previously described [18–20]. Six millilitres of venous blood was obtained from patients using a plastic syringe and mixed with 3 mL of 6% dextran/3% dextrose/0.9% normal saline and 1.2 mL of 0.1 M EDTA. Erythrocytes were allowed to sediment at room temperature for 90 min. The leucocyte-rich plasma layer was removed, transferred to a clean tube, and then centrifuged for 8 min at 110 g at 4 °C. After removing the upper plasma layer, the leucocyte pellet was resuspended in 40 mL of PIPES (piperazine-N,N'-bis[2-ethanesulphonic acid])-albumin-glucose (PAG) buffer and centrifuged as described above. The wash step was repeated once and the cell pellet was finally re-suspended in 6 mL PAGCM buffer. Cell density was adjusted to 4×10^3 to 3×10^4 /mL. PIPES buffer is composed of PIPES 250 mmol/L, NaCl 1100 mmol/L and KCl 50 mmol/L. PAG buffer is composed of 10 mL of PIPES buffer diluted to 100 mL, and 0.03% human serum albumin, 1% glucose. PAGCM buffer is made with PAG buffer containing 1 mM Ca⁺⁺ and 1 mM Mg⁺⁺.

Cells in PAGCM buffer were warmed up for 5 min in a shaking water bath at 37 °C. For stimulated histamine release, 1 mL of cells was incubated with 1 mL of various stimulating agents for 45 min in a water bath at 37 °C. We used 20 BU/mL of *D. farinae* (Allergopharma) and 2 µg/mL of goat anti-human IgE antibody (Sigma, St Louis, MO, USA) as IgE-mediated stimulation agents and 2×10^{-5} M/L fMLP (formyl-Met-Leu-Phe, Sigma) and 2×10^{-2} µM calcium ionophore A23187 (Sigma) as non-IgE-mediated stimulating agents. We selected the appropriate concentration of stimulating agents with regard to the dose–response curve. Tubes containing only cells and buffer were used to measure the spontaneous release of histamine. Total histamine content was obtained by lysing the cells using 55% TCA. All tests were performed twice. After incubation, the test tubes were transferred into an ice-water bath and centrifuged (700 g, 15 min, 4 °C). The supernatants were stabilized with 0.2 mL of 55% trichloroacetic acid (TCA) and this was followed by freezing at –20 °C. Histamine was measured by the automated fluorometric technique. Stimulated histamine release is expressed as percentage histamine release (HR, stimulated HR–spontaneous HR/total HR–spontaneous HR $\times$ 100).

Statistical analysis

The data were analysed through a Wilcoxon signed rank test and Spearman correlation test. Only those tests with *P*-values less than 0.05 were considered as significant.

Results

Asthma symptom score and its relationship with histamine release by various stimuli

The asthma symptoms of all patients who received immunotherapy significantly improved at both 4 and 9 months compared with those before immunotherapy (median values before, and at 4 and 9 months = 9.5, 4 and 2.1, respectively) (Fig. 1).

There was a significant correlation between asthma symptom score and histamine release by *Df* ($r_s = 0.76$, $P < 0.05$), but no correlations were found between asthma symptom score and histamine release by the other stimuli (data not shown).

Skin test reactivity

The skin test reactivity (weal size ratio of allergen to histamine) to *Df* remained constant 4 months after immunotherapy, but decreased significantly 9 months after immunotherapy (median values = 2, 2.3, 1.6, respectively) (Fig. 2).

Basophil histamine release before and after immunotherapy

Basophil histamine release was compared before, and 4 and 9 months after immunotherapy. There were significant decreases

of histamine release by *Df* (median values = 64%, 46%, 40%, respectively) (Fig. 3a) and by anti-IgE antibody (median values = 54%, 46%, 26%, respectively) (Fig. 3b) at 4 and 9 months after immunotherapy compared with those before immunotherapy.

The histamine release by fMLP (median values = 30%, 23%, 20%, respectively) (Fig. 4a) and by calcium ionophore (median values = 76%, 77%, 67%, respectively) (Fig. 4b) showed no significant changes after immunotherapy.

There was no significant change in total histamine content before and after immunotherapy (median values = 41 ng/mL, 37 ng/mL, 50 ng/mL, respectively) (data not shown).

There was no observable difference in spontaneous histamine release before and after immunotherapy (median values = 10%, 14%, 9%, respectively) (data not shown).

Relationship of histamine release between various stimuli before and during immunotherapy

Histamine release by *Df* before immunotherapy showed significant correlation with that by anti-IgE antibody ($r_s = 0.869$, $P < 0.001$) (Fig. 5a). Also, there was significant correlation between histamine release by *Df* and by fMLP ($r_s = 0.799$,

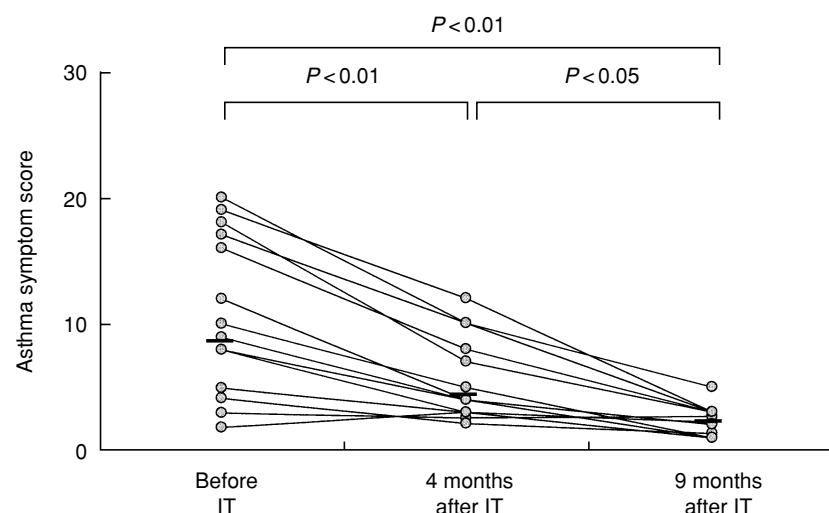


Fig. 1. Asthma symptom scores before and after immunotherapy. They decreased 4 months and 9 months after immunotherapy compared with those before immunotherapy.

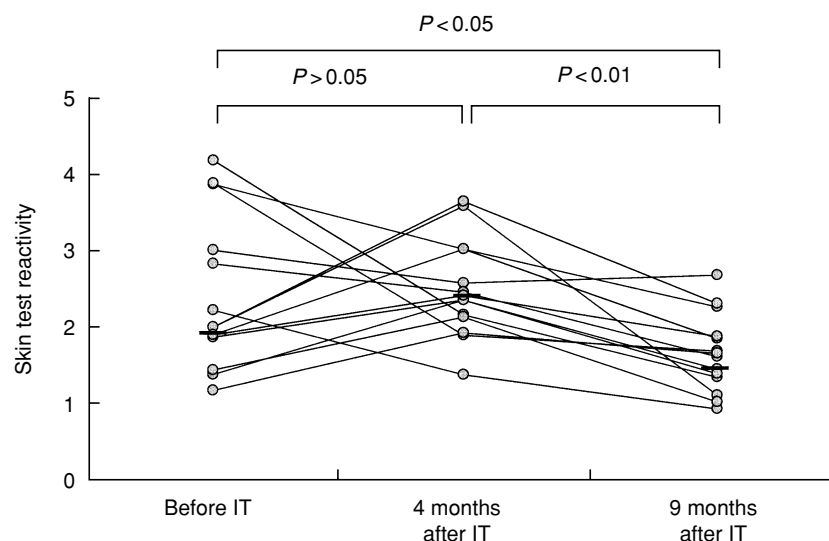


Fig. 2. The skin reactivity (weal size ratio of allergen to histamine) to *D. farinae* before and after immunotherapy. It did not decrease 4 months after immunotherapy, but decreased significantly 9 months after immunotherapy.

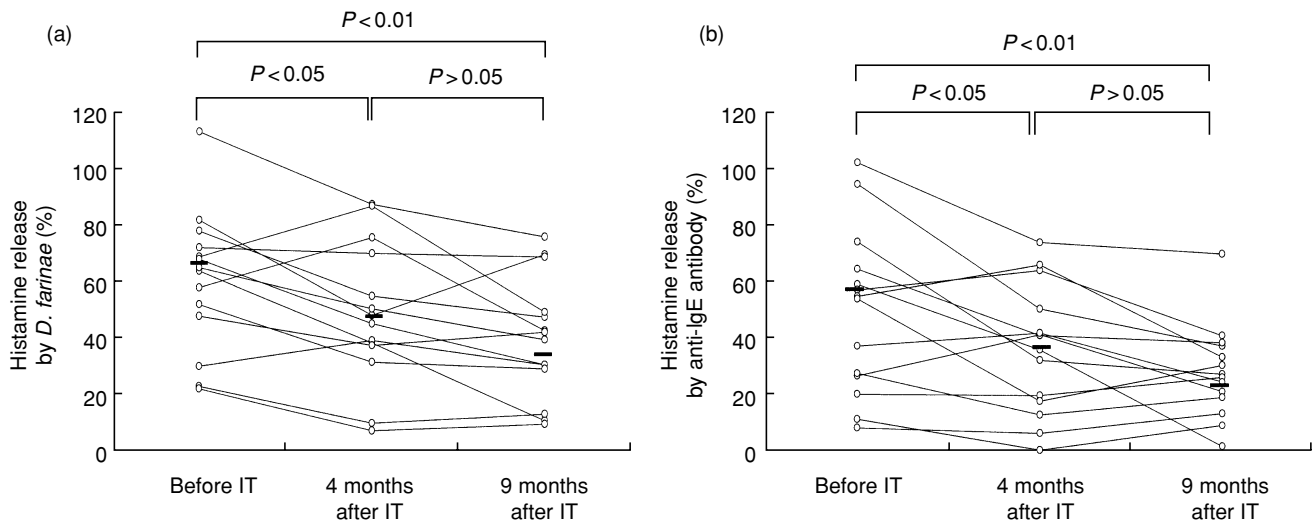


Fig. 3. Basophil histamine release by *D. farinae* (a) and by anti-IgE antibody (b) before and after immunotherapy. It decreased 4 months and 9 months after immunotherapy.

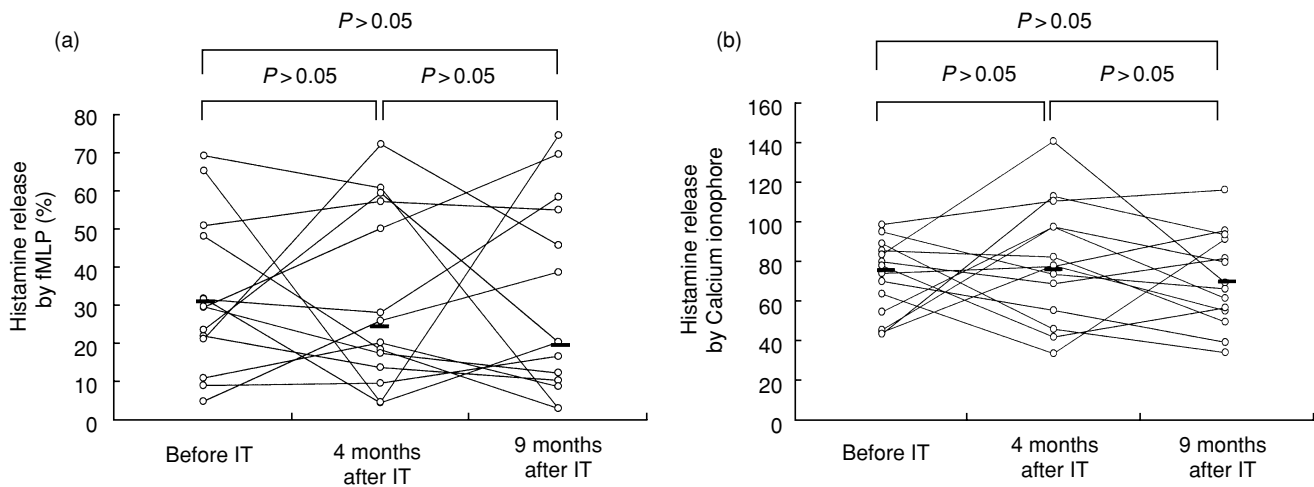


Fig. 4. Basophil histamine release by fMLP (a) and by calcium ionophore (b) before and after immunotherapy. It did not decrease 4 months and 9 months after immunotherapy compared with that before immunotherapy.

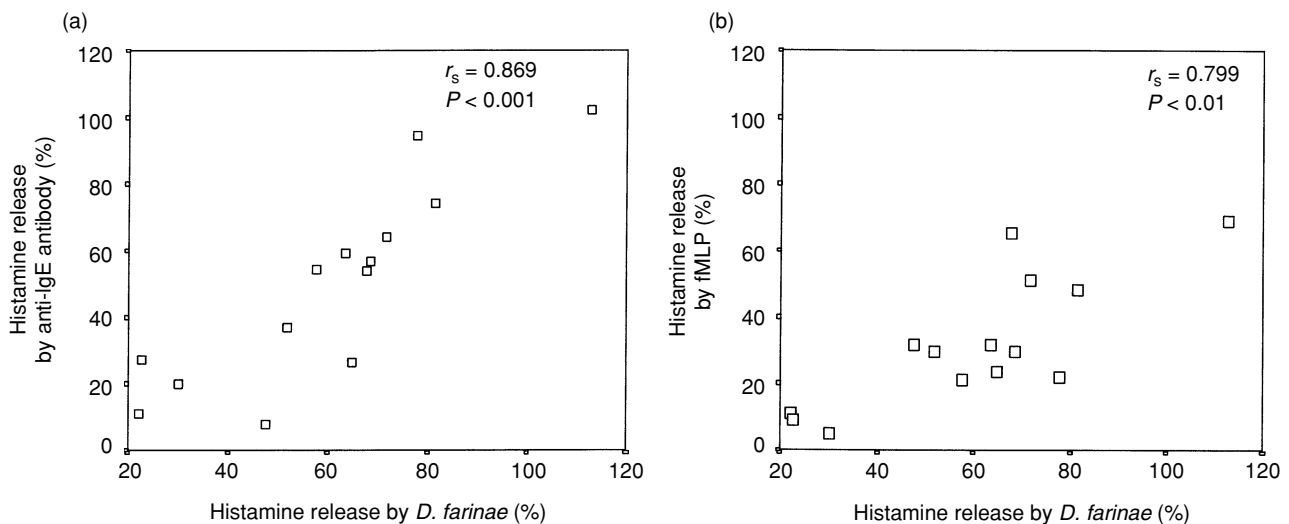


Fig. 5. Correlations of basophil histamine release by *D. farinae* with that by anti-IgE antibody (a) and by fMLP (b) before immunotherapy. Before immunotherapy, basophil histamine release by *D. farinae* was well correlated with histamine release by anti-IgE antibody and that by fMLP.

$P < 0.01$, Fig. 5b), but no correlation between histamine release by *Df* and by calcium ionophore ($r_s = -0.002$, $P > 0.05$). During immunotherapy, there was a correlation between histamine release by *Df* and by fMLP at 4 months of immunotherapy ($r_s = 0.618$, $P < 0.05$), but not at 9 months ($r_s = -0.049$, $P > 0.05$) (data not shown). Spontaneous histamine release showed no correlation with histamine release by all of the stimuli (data not shown) before and during immunotherapy.

Changes in serum total IgE levels and Df-specific IgE levels after immunotherapy

Serum total IgE was decreased 4 months after immunotherapy, followed by an increase 9 months afterwards (median values = 556 IU/mL, 435 IU/mL, 579 IU/mL, respectively). In contrast, *Df*-specific IgE antibody was increased 4 months after immunotherapy, and then decreased after 9 months (median values = 3.5 class, 3.6 class, 3.5 class, respectively). However, there were no statistical changes in serum total IgE levels and *Df*-specific IgE levels after immunotherapy compared with those before immunotherapy.

Discussion

Df and anti-IgE antibody mediate histamine release through IgE receptors, while fMLP mediates it through non-IgE receptors, and calcium ionophore does not interact with surface receptors, but induces it by transmembrane Ca^{++} influx. Accordingly, we can term the former as IgE-mediated and the latter as non-IgE-mediated histamine release.

In this study, IgE-mediated histamine release was decreased 4 and 9 months after initiation of conventional immunotherapy with house dust mite allergen. Conversely, non-IgE-mediated histamine release remains unaffected. Thus, it is significant that IgE-mediated histamine-releasing pathways may be changed in the initial phase of immunotherapy, including the number of free or surface IgE molecules, IgE receptor densities on basophils, and intracellular signal transduction pathway. There were no consistent findings between the numbers of surface IgE molecules on basophils and histamine release by anti-IgE antibody [3]. Although there was a report that the concentration of serum IgE was correlated with the number of IgE molecules per basophil [21], there was no relationship between the serum IgE levels and the concentration of anti-IgE antibody required for 30% histamine release [3]. In our study, there was the early decrease of IgE-mediated histamine release along with the early clinical improvement at 4 months of immunotherapy, whereas *Df*-specific IgE levels and skin test reactivity to *Df* increased. Despite a decrease in total IgE levels at 4 months of immunotherapy, it increased up to the concentrations before immunotherapy at 9 months. Hence, these results suggest that the early decrease of IgE-mediated histamine release may not be caused by changes in the number of total or specific IgE molecules.

Desensitization, which inhibits the release of inflammatory mediators, is also associated with down-regulation of the cell surface receptor numbers in most receptor-mediated cellular systems [22, 23]. MacGlashan et al. [24] demonstrated that treatment with anti-IgE antibody lowered circulating free IgE levels, which might in turn down-regulate $\text{Fc}\epsilon\text{RI}$ on basophils

and histamine release to dust mite antigen. However, there has been no study demonstrating that a decrease of IgE-mediated releasability after immunotherapy is associated with a decrease in the number of surface IgE receptors. Moreover, the levels of serum-specific IgE have been known to be increased in the early phase of immunotherapy [25], and the changes of surface IgE receptors on basophils may not be associated with a decrease of IgE-mediated releasability.

Remarkably, it is possible that the functional modification of IgE receptors or intracellular signal transduction pathways may be responsible for the decline of IgE-mediated releasability. Through the impact of immunotherapy, the function of IgE receptors can be altered directly and/or indirectly through T cell immunoregulations. However, there have been no proven data to support the theory. Further studies on the changes of IgE receptor as well as intracellular signal transduction as one of the mechanisms in decreased IgE-mediated basophil releasability after immunotherapy are required.

Despite the fact that *Df*-mediated basophil releasability was decreased 4 months after immunotherapy, skin test reactivity to *Df* was not significantly decreased after the same 4-month interval. This suggests that basophils are more influenced in earlier stages of immunotherapy than are skin mast cells. Nish et al. [25] demonstrated that skin test reactivity was increased 3 months after immunotherapy. This was followed by a decline 6 months after immunotherapy, resulting in levels less than those of pre-immunotherapy, whereas histamine release from basophils was decreased at 3 months. These results support our data.

In this study, total histamine content failed to demonstrate any changes during immunotherapy. Immunotherapy did not influence the formation of histamine in each basophil, but only influenced the IgE-mediated histamine-releasing pathways. In a recent report of venom immunotherapy [10], total histamine release was decreased after rush immunotherapy, whereas stimulated histamine release was unaffected. This finding is to some extent different from ours in as much as the type of allergen, the method of immunotherapy and timing of sampling applied are dissimilar.

From our data, there was a significant correlation between asthma symptom score and histamine release by *Df*. Therefore, early clinical improvement during immunotherapy was strongly related to the decrease in IgE-mediated basophil releasability. Future studies focusing on IgE-mediated histamine-releasing pathways are required.

References

- MacGlashan D Jr, Shite JM, Huang SK, Ono SJ, Schroeder J, Lichtenstein LM. Secretion of interleukin-4 from human basophils: the relationship between IL-4 mRNA and protein in resting and stimulated basophils. *J Immunol* 1994; 152:3006–16.
- Guo CB, Liu MC, Galli SJ, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Identification of IgE-bearing cells in the late-phase response to antigen in the lung as basophils. *Am J Respir Cell Mol Biol* 1994; 10:384–90.
- Marone G, Spadaro G, Patella V, Genovese A. The clinical relevance of basophil releasability. *J Allergy Clin Immunol* 1994; 94:1293–303.
- Lichtenstein LM, Ishizaka K, Norman PS, Sobotka AK, Hill BM. IgE antibody measurements in ragweed hay fever, relationship to clinical severity and the results of immunotherapy. *J Clin Invest* 1973; 52:472–82.

- 5 Mastrandrea F, Serio G, Minardi A et al. IgE responses to *Dermatophagoides pteronyssinus* native major allergens Der p 1 and Der p 2 during long-term specific immunotherapy. *Allergy* 1997; 52:1115–9.
- 6 Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J Allergy Clin Immunol* 1982; 70:261–71.
- 7 Djurup R, Malling HJ. High IgG4 antibody level is associated with failure of immunotherapy with inhalant allergens. *Clin Allergy* 1989; 17:459–68.
- 8 Otsuka H, Mezawa A, Ohnishi M, Okubo K, Seki H, Okuda M. Changes in nasal metachromatic cells during allergen immunotherapy. *Clin Exp Allergy* 1991; 21:115–9.
- 9 O'Hehir RE, Yssel H, Verma S, de Vries JE, Spits H, Lamb JR. Clonal analysis of differential lymphokine production in peptide and superantigen induced T cell anergy. *Int Immunol* 1991; 3:819–26.
- 10 Jutel M, Muller UR, Fricker M, Rihs S, Pichler WJ, Dahinden C. Influence of bee venom immunotherapy on degranulation and leukotriene generation in human blood basophils. *Clin Exp Allergy* 1996; 26:1112–8.
- 11 Kimura I, Tanizaki Y, Goda Y, Komagoe H, Kitani H. Decrease in reactivity of basophils by immunotherapy with housedust extract. *Clin Allergy* 1985; 15:1–7.
- 12 Hong SJ, Shim JY, Byun WC, Hong JS, Choi SW, Moon HB. Basophil histamine releasability in children with atopy and asthma [abstract]. *J Allergy Clin Immunol* 1997; 99(1):S88.
- 13 Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol* 1998; 102:157–64.
- 14 Pierkes M, Bellinghausen I, Hultsch T, Metz G, Knop J, Saloga J. Decreased release of histamine and sulfidoleukotrienes by human peripheral blood leukocytes after wasp venom immunotherapy is partially due to induction of IL-10 and IFN-gamma production of T cells. *J Allergy Clin Immunol* 1999; 103:326–32.
- 15 Brunet C, Bedard PM, Lavoie A, Jobin M, Hebert J. Allergic rhinitis to ragweed pollen. I. Reassessment of the effects of immunotherapy on cellular and humoral responses. *J Allergy Clin Immunol* 1992; 89:76–86.
- 16 Creticos PS, van Metre TE, Mardiney MR, Rosenberg GL, Norman PS, Adkinson NF Jr. Dose-response of IgE and IgG antibodies during ragweed immunotherapy. *J Allergy Clin Immunol* 1984; 73:94–104.
- 17 Wasserfallen J-B, God K, Schulman KA, Baraniuk JN. Development and validation of a rhinoconjunctivitis and asthma symptom score for use as an outcome measure in clinical trials. *J Allergy Clin Immunol* 1997; 100:16–22.
- 18 Kim YK, Oh SY, Oh HB et al. Coding single nucleotide polymorphism in the high-affinity immunoglobulin E receptor b chain (FcεRI-b) gene is associated with immunoglobulin E receptor-mediated histamine release from basophils. *Clin Exp Allergy* 2002; 32:751–5.
- 19 Park HS, Jung KS, Kang KR, Nahm DH, Cho SH, Kim YY. Enhanced basophil histamine release and neutrophil chemotactic activity predispose grain dust-induced airway obstruction. *Clin Exp Allergy* 1999; 29:543–9.
- 20 Kim YK, Cho SH, Koh YY et al. Linkage between IgE receptor-mediated histamine releasability from basophils and gene marker of chromosome 11q13. *J Allergy Clin Immunol* 1999; 104:618–22.
- 21 Conroy MC, Adkinson NF Jr, Lichtenstein LM. Measurement of IgE on human basophils: relation to serum IgE and anti-IgE-induced histamine release. *J Immunol* 1977; 118:1317–21.
- 22 MacGlashan DW Jr. Desensitization of IgE-mediated IL-4 release from human basophils. *J Leukoc Biol* 1998; 63:59–67.
- 23 MacGlashan DW Jr. Single cell analysis of free cytosolic calcium changes in human lung mast cells. II. The relationship between desensitization and the cellular regulation of calcium changes. *Mol Immunol* 1991; 28:585–97.
- 24 MacGlashan DW, Bochner BS, Adelman DC et al. Down-regulation of FcεRI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 1997; 158:1438–45.
- 25 Nish WA, Charlesworth EN, Davis TL et al. The effect of immunotherapy on the cutaneous late phase response to antigen. *J Allergy Clin Immunol* 1994; 93:484–93.