

Effect of specific immunotherapy with house dust mite extract on the bronchial responsiveness of paediatric asthma patients

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Summary

Background and objective Allergic asthma is common in children, and house dust mite (HDM) is an important source of perennial allergens. Bronchial hyperresponsiveness is a functional hallmark of asthma. Specific immunotherapy (SIT) with HDM extracts were shown to decrease symptoms, but its effect on bronchial responsiveness, as measured by non-pharmacological challenges, has not been evaluated.

Methods Twenty-six paediatric asthma patients allergic to HDM participated in this study. Fourteen patients received SIT with a HDM extract (Alavac, Bencard) for 2 years, and 12 served as controls. Bronchial responsiveness was assessed non-pharmacologically by cold dry air challenge (CACH) before and 3, 6, 12 and 24 months after SIT, and 12 months after termination of SIT.

Results After 24 months, the SIT group showed a statistically significant reduction of the mean CACH-induced changes of both forced expiratory volume in one second ($-21.8 \pm 2.7\%$ vs $-13.7 \pm 2.4\%$; $P = 0.03$) and maximal expiratory flow at 25% remaining vital capacity ($-48.9 \pm 4.9\%$ vs $-27.9 \pm 6.2\%$; $P = 0.01$). In contrast, no significant changes of bronchial responsiveness were observed in the control group. In the SIT group more patients lost their bronchial hyperresponsiveness than in the control group (6/14 vs 1/12; $P < 0.05$). One year after terminating SIT, the treatment group showed a tendency towards returning bronchial hyperresponsiveness.

Conclusion These results demonstrate that during 2 years of SIT there was a reduction of bronchial hyperresponsiveness in HDM-allergic paediatric asthma patients.

Keywords: asthma, bronchial responsiveness, house dust mite, specific immunotherapy, children

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Introduction

House dust mite (HDM) of the species *Dermatophagoides* represents the most common source of perennial allergens [1]. In allergic asthma patients chronic airway inflammation was found both in biopsy and in bronchoalveolar lavage studies [2,3]. Bronchial hyperresponsiveness (BHR) is a consequence of this bronchial inflammation; thus, it can be used as a marker of inflammatory activity [4].

Many controlled studies of specific immunotherapy (SIT)

with HDM-extracts have shown a beneficial effect on symptoms and on allergen-induced bronchial responsiveness, especially in childhood [5–12]. So far, however, there are only few investigations that examined the effect of HDM-SIT on non-specific bronchial responsiveness (BR), and their results have remained contradictory [10–17]. In all of these studies, bronchial challenges were performed with pharmacologically active substances, such as histamine or methacholine. In contrast with these pharmacological provocations, non-pharmacological challenges use naturally occurring physical stimuli to provoke bronchoconstriction. By evoking intermediate events like the release of mediators from reactive cell systems in the mucosa, non-pharmacological challenges might be of greater clinical relevance than

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pharmacological ones [18–20]. Thus, it should be of special interest to evaluate non-specific BR during SIT with HDM-extracts by a non-pharmacological challenge.

In the present study, we therefore investigated the influence of HDM-SIT on BR as measured by cold dry air challenge (CACH) in paediatric asthma patients.

Materials and methods

Subjects

Subjects were recruited from the asthma patients of the local paediatric respiratory centre. Inclusion criteria were as follows: a clinical diagnosis of bronchial asthma as based on standard criteria [21], a positive skin-prick test (mean weal ≥ 5 mm at 15 min) to *Dermatophagoides pteronyssinus* (DP) and/or *Dermatophagoides farinae* (DF), a specific IgE (Phadebas RAST, Pharmacia, Sweden) to DP and/or DF $>$ RAST-class 2, a positive bronchial challenge test with DP and/or DF (threshold-HDM concentration of 1:9 v/v dilution producing a fall in FEV₁ of 20% or more), a positive bronchial reaction, i.e. a decrease in forced expiratory volume in one second (FEV₁) of 9% or more, to a single-step CACH [22], and age ≥ 7 years.

Patients with one or more of the following conditions were excluded: severe instable asthma, a positive skin-prick test to moulds, a clinically relevant sensitization to animal dander (i.e. reproducible symptoms after exposure) and occasional contact with the animal, prior SIT with HDM-extracts, and other immunological or systemic diseases.

Thirty-one patients were recruited for the study. Allocation to the groups (SIT and control group) was done by randomization of every two or four subsequent patients. All subjects were on treatment with different combinations of antiasthmatic drugs (disodium cromoglycate, beclomethasone dipropionate, budesonide, salbutamol on demand).

Lung function and bronchial responsiveness

Lung function testing was performed in accordance with standardized guidelines [23]. A forced expiratory vital capacity manoeuvre was recorded in form of a volume–time and a flow–volume curve on a pneumotachygraph spirometer (Body-Test; Jaeger, Würzburg, Germany). FEV₁ and maximal expiratory flow at 25% remaining vital capacity (MEF₂₅) were measured from these curves. Results were expressed as percentage of predicted normal, based on relevant reference standards [24].

BR was assessed non-pharmacologically by a standardized single-step CACH [22,25]. Prior to the CACH, medication with disodium cromoglycate and salbutamol was withheld for 12 h; medication with topical steroids was

continued. After the measurement of prechallenge FEV₁ and MEF₂₅, CACH consisted of a 4 min isocapnic hyperventilation of absolutely dry, minus 10 °C air at 75% of maximal voluntary ventilation. Cold dry air was produced by a commercially available heat exchanger (RHES; Jaeger). Three minutes after termination of the challenge, FEV₁ and MEF₂₅ were measured again. The changes in FEV₁ (Δ FEV₁) and in MEF₂₅ (Δ MEF₂₅) from the pre- to the post-CACH measurements were expressed in percentage baseline. For this method, a Δ FEV₁ of minus 9% or more defines BHR [22].

Study protocol

The parents of all patients gave their written informed consent, and the study was approved by the ethics committee of the University Hospital Graz. Antiasthmatic medication remained unchanged for the entire study period in both the SIT and the control group.

Lung function measurements and CACH were done in the SIT group before and after 3, 6, 12 and 24 months of SIT. In addition, these measurements were repeated 12 months after SIT was stopped. The control group was evaluated at the same time points.

Specific immunotherapy

SIT was performed with an alum-adsorbed HDM (50% DP/50% DF) extract (Alavac, Bencard, Germany). In the first 3 months subcutaneous injections were given in weekly incremental allergen doses up to the highest dose of 4 QAU (Quality Assurance Units) of DP (= 0.56 μ g/mL content of Der p 1) and 30 QAU of DF. This maintenance dose was then given in intervals of 4–6 weeks for 2 years.

At each visit patients were interviewed about their asthma status, and local and/or systemic reactions after the previous injection. After allergen injection, patients had to stay at the outpatient clinic for at least 30 min, and signs and symptoms of systemic reactions as well as the diameter of the local weal-and-flare were recorded.

Statistical methods

Data are given as means $\pm$ SEM. Differences between groups and within groups were analysed by one-way analysis of variance (ANOVA). Data in contingency tables were analysed by the chi square test. A *P*-value of 0.05 or less was taken as indicating statistical significance.

Results

Initially, 16 patients were assigned to the SIT group and 15

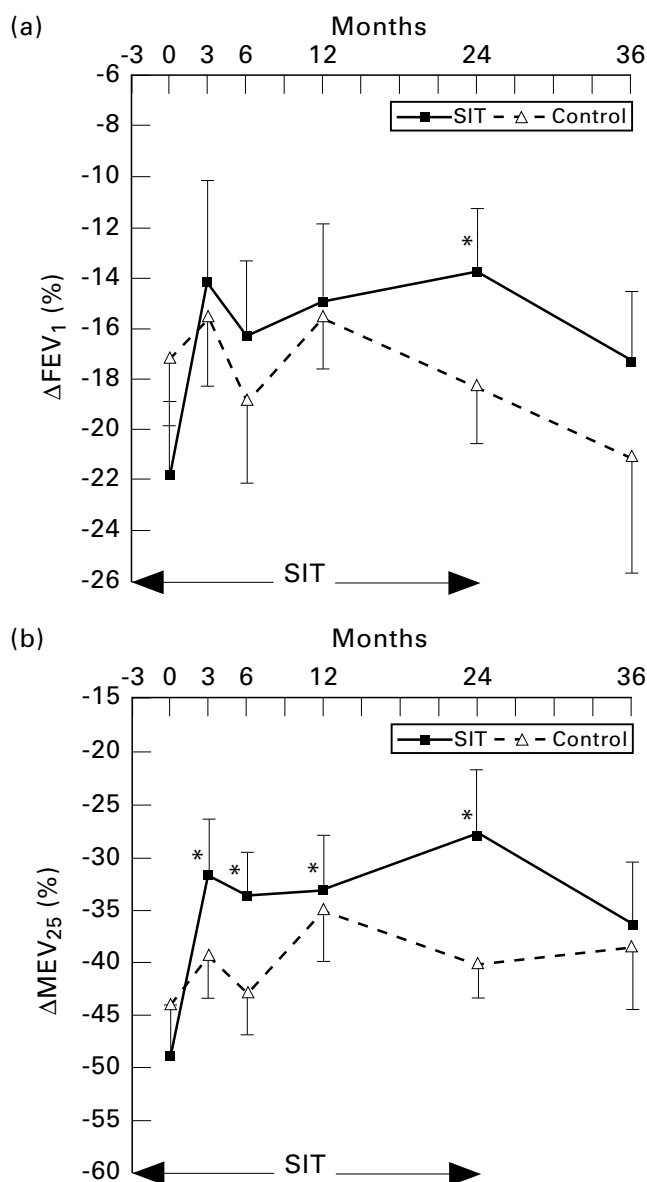


Fig. 1. Provocation-induced changes in FEV_1 (a) and in MEF_{25} (b) before and after 3, 6, 12, and 24 months of specific immunotherapy (SIT), and after stopping SIT for 12 months. Values are mean $\pm$ SEM. * $P < 0.05$; difference from pretreatment values.

to the control group. Five subjects were withdrawn from the study: two in the SIT group (one patient objected to the injections and one stopped regular antiasthmatic treatment) and three in the control group (two patients missed appointments and one stopped his antiasthmatic treatment).

Demographic data at inclusion for those patients completing the study are shown in Table 1. The SIT group and the control group were comparable in age, gender

Table 1. Demographic data

	SIT group	Control group	P-value
<i>Age (years)</i>			
Mean $\pm$ SEM	11.4 $\pm$ 0.7	12.1 $\pm$ 0.7	NS
Range	7.7–15.3	8.8–15.6	
<i>Gender</i>			
Male/Female	10/4	10/2	NS
<i>Medication</i>			
DSCG/inhaled steroids/no.	7/5/2	6/3/3	NS
<i>Baseline lung function</i>			
FEV_1 (% predicted)	90.0 $\pm$ 2.9	98.8 $\pm$ 3.6	NS
MEF_{25} (% predicted)	77.3 $\pm$ 5.8	90.4 $\pm$ 8.2	NS
<i>Cold dry air challenge</i>			
ΔFEV_1 (% baseline)	-21.8 $\pm$ 2.7	-17.2 $\pm$ 2.7	NS
ΔMEF_{25} (% baseline)	-48.9 $\pm$ 4.9	-43.9 $\pm$ 4.9	NS

NS, not significant; DSCG, disodium cromoglycate.

and medication. When comparing the results of prechallenge lung function testing at the beginning of the study, the values for FEV_1 and MEF_{25} tended to be lower in the SIT group than in the control group. In addition, CACH-induced lung function changes tended to be higher in the SIT group. These differences, however, remained statistically insignificant. Two out of 14 patients of the SIT group were grass pollen allergic. In these subjects bronchial provocation was performed prior to the pollen season.

After the first year of the study, six patients in the SIT group and one patient in the control group had lost their BHR to CACH ($P < 0.05$). This result remained unchanged after two years of SIT. One year after SIT was stopped, five patients of the SIT group and three of the control group showed a normalized BR; this difference was no longer statistically significant.

The CACH-induced changes in FEV_1 and MEF_{25} over the whole study period are presented in Fig. 1. A significant reduction in ΔFEV_1 ($-21.8 \pm 2.7\%$ at the beginning of the study vs $-13.7 \pm 2.4\%$ after the second year; $P = 0.03$) was observed after 24 months in the SIT group, whereas ΔFEV_1 remained essentially unchanged in the control group ($-17.2 \pm 2.7\%$ vs $-18.3 \pm 2.3\%$; ns). A significant decrease in ΔMEF_{25} ($-48.9 \pm 4.9\%$ at the beginning of the study vs $-27.9 \pm 6.2\%$ after the second year; $P = 0.01$) could be demonstrated for the SIT group after 24 months. In the control group no significant change in ΔMEF_{25} ($-43.9 \pm 4.9\%$ at the beginning of the study vs $-40.2 \pm 3.3\%$ after the second year) occurred. Twelve

months after SIT was terminated, there was a tendency towards a higher ΔFEV_1 ($-13.7 \pm 2.4\%$ after the second year of SIT vs $-17.7 \pm 3.7\%$ one year after stopping SIT) and ΔMEF_{25} ($-27.9 \pm 6.2\%$ vs $-36.8 \pm 5.4\%$) in the SIT-group, but these changes remained insignificant.

Differences between the groups did not reach statistical significance at any time of the study.

Large local reactions, defined as a local weal of 5 cm or greater in diameter after an injection of HDM-extract, were noted after 13 of the 495 injections. One patient complained of a mild allergic reaction consisting of wheezing and rhinitis 1 h after an injection. No generalized cutaneous or anaphylactic reactions were observed during SIT.

Discussion

The findings of this study demonstrate that 2 years of SIT cause a significant reduction of BHR in HDM-allergic paediatric asthma patients. When BHR is a marker of bronchial inflammation in asthma [4], this result suggests a beneficial effect of SIT on the basic pathophysiology of the disease.

Only a few studies have evaluated non-specific BR in asthma patients under SIT with HDM-extracts [10–17]. All these studies used pharmacological challenges for assessing BR, and the majority of them were performed in adult asthma patients. Results have remained somewhat contradictory. Mosbech *et al.* found a significant reduction of BR to histamine in the first year of SIT; after the second year, however, this positive effect disappeared [15]. Haugaard *et al.*, using standardized HDM-extracts, observed a significant reduction of BR to histamine after two years of SIT; however, this was also found for the control group [17]. Other authors failed to show a SIT-effected decrease of BR to methacholine or histamine [13,16]. In a placebo-controlled study, Machiels *et al.* could not find a significant reduction of BR to acetylcholine in asthma patients treated with DP-IgG complexes for 2 years [10]. In a follow-up study, however, SIT was continued for another year, and the authors then observed a significantly decreased BR in the SIT group [12].

The biggest improvement in asthma symptoms and baseline lung function after HDM-SIT was demonstrated in children and in patients with mild asthma [7]. As yet, however, there are only two paediatric studies evaluating the effect of HDM-SIT on non-specific BR. In a placebo-controlled study, Van Bever *et al.* found no significant change of BR to histamine after one year of SIT with a standardized HDM-extract [11]. Murray *et al.* observed an insignificant increase of BR to histamine after HDM-SIT, while BR decreased in subjects without SIT [14].

The results of the present study differ from the findings of these two other paediatric investigations. This

difference in outcome might be explained by differences in methodology. One such difference between the present and the above-quoted investigations was the use of a non-pharmacological challenge for assessing BR. Different challenges apply different stimuli and activate different bronchoconstrictor mechanisms; thus, they should measure different types of bronchial responsiveness [20,26]. Non-pharmacological challenges, such as CACh, do not act directly on bronchial smooth muscle, but instead evoke intermediate events, such as the release of bronchoactive mediators from cells in the airway lumen and submucosa [27]. Consequently, they might provide measurements of greater clinical relevance than pharmacological provocations [19].

In addition, the present study differs from the two other paediatric studies in two further aspects. In our study, the number of subjects was higher, and HDM-SIT was performed for 2 years as compared with 1 year in the two others. The difference in outcome could suggest that a SIT course of one year might be too short for effecting a significant therapeutic effect on BR.

In the present study, BR improved significantly in the SIT group while showing no significant changes in the control group. It is highly unlikely that this improvement in BR in the SIT-group is due to the concurrent asthma medication, as pharmacotherapy was similar in the two groups. Changes in ΔFEV_1 and ΔMEF_{25} , however, did not reach a big enough dimension for resulting in a significant difference between SIT and control group towards the end of the study. One factor that might have contributed to this outcome was a tendency towards more BHR in the SIT group at the beginning of the study. Starting from a lower baseline, the SIT group would have needed a still bigger improvement to end up significantly better than the controls. However, there was a significant difference between both groups in the number of patients who normalized their BR during the course of the study; thus, these beneficial changes in BR can hardly be attributed to spontaneous variations of asthma severity alone.

One year after termination of SIT, BR showed a tendency to increase again in the SIT group. The duration of SIT with HDM-extract, necessary for a longer-lasting therapeutic effect, is still under discussion. Price *et al.* showed that paediatric asthma patients had more asthma symptoms and needed more antiasthmatic treatment within one year after termination of SIT than during SIT [28]. This was confirmed by the findings of Van Bever and Stevens [29]. In their study, a suppression of the late phase asthmatic reaction under SIT was observed, but 1 year after SIT was stopped, this positive effect was no longer present. From these findings and our results, it seems that a 1–2-year course of HDM-SIT may be too short for providing a beneficial long-term effect on BR. Two studies in adult asthma patients investigated the effects of longer HDM-SIT. In

the study of Des Roches *et al.* patients received a standardized HDM-extract for 12–96 months [30]. After cessation of SIT, the subjects were followed for up to 3 years. These authors found a relapse rate of asthma and/or rhinitis in 48% of patients who had SIT for more than 35 months, and in 62% of those who had SIT for under 35 months. Machiels *et al.* showed that most adult asthma patients treated with immune complexes (DP-IgG) for 3 years did not experience a relapse after cessation of SIT [12]. Furthermore, a study of asthma patients treated with cat or dog dander extracts for three years could demonstrate that 5 years after stopping immunotherapy asthma symptoms and bronchial responsiveness to histamine had remained improved, whereas BR to these allergens had increased again [31]. One can speculate from these results that SIT should be performed for at least 3 years to provide a beneficial long-term effect on BR. This speculation, however, needs to be evaluated by further studies.

In conclusion, during a 2-year course of SIT with HDM-extract bronchial responsiveness to a non-pharmacological stimulus decreased in HDM-allergic paediatric asthma patients. A tendency towards relapsing hyperresponsiveness after termination of SIT suggests that longer treatment periods might be necessary for providing a longer lasting therapeutic effect.

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References

- Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma – a world wide problem. *J Allergy Clin Immunol* 1989; 83:416–27.
- Bousquet J, Chané P, Lacoste JY et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990; 323:1033–9.
- Djukanovic R, Roche WR, Wilson JH et al. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142:434–57.
- Kerrebijn KF. (ed.) Similarities and discrepancies between asthma and chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1991; 143:1149–96 and 1419–72.
- Warner JO, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978; II:912–5.
- Bousquet J, Calvayrac P, Guérin B et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. *J Allergy Clin Immunol* 1985; 76:734–44.
- Bousquet J, Hejjaoui A, Clauzel AM et al. Specific immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. II Prediction efficacy immunotherapy. *J Allergy Clin Immunol* 1988; 82:971–7.
- Mosbech H, Dreborg S, Frolund L et al. Hyposensitization in asthmatics with mPEG modified and unmodified house dust mite extract. I. Clinical effect evaluated by diary cards and a retrospective assessment. *Allergy* 1989; 44:487–98.
- Van Bever HP, Stevens WJ. Suppression of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*). *Clin Exp Allergy* 1989; 19:399–404.
- Machiels JJ, Somville MA, Lebrun PM et al. Allergic bronchial asthma due to *Dermatophagoides pteronyssinus* hypersensitivity can be efficiently treated by inoculation of allergen-antibody complexes. *J Clin Invest* 1990; 85:1024–35.
- Van Bever HP, Stevens WJ. Effect of hyposensitization upon the immediate and late asthmatic reaction and upon histamine reactivity in patients allergic to house dust mite (*Dermatophagoides pteronyssinus*). *Eur Respir J* 1992; 5:318–22.
- Machiels JJ, Lebrun PM, Jacquemin MG, Saint-Remy JMR. Significant reduction of nonspecific bronchial reactivity in patients with *Dermatophagoides pteronyssinus*-sensitive allergic asthma under therapy with allergen-antibody complexes. *Am Rev Respir Dis* 1993; 147:1407–12.
- Formgren H, Lanner A, Lindholm N, Löwhagen O, Dreborg S. Effects of immunotherapy on specific and non-specific sensitivity of the airways. *J Allergy Clin Immunol* 1984; 73:140.
- Murray AB, Ferguson AC, Morrison BJ. Non-allergic bronchial hyperreactivity in asthmatic children decreases with age and increases with immunotherapy. *Ann Allergy* 1985; 54:541–4.
- Mosbech H, Dreborg S, Frolund L et al. Hyposensitization in asthmatics with mPEG modified and unmodified house dust mite extract. II. Effect evaluated by challenges with allergen and histamine. *Allergy* 1989; 44:499–509.
- Garcia-Ortega P, Merelo A, Marrugat J, Richart C. Decrease of skin and bronchial sensitization following short-intensive scheduled immunotherapy in mite-allergic asthma. *Chest* 1993; 103:183–7.
- Haugaard L, Dahl R, Jacobsen L. A controlled dose–response study of immunotherapy with standardized, partially purified extract of house dust mite: clinical efficacy and side effects. *J Allergy Clin Immunol* 1993; 91:709–22.
- Galdes-Sebaldo M, McLaughlin FJ, Levison H. Comparison of cold air, ultrasonic mist, and methacholine inhalations as test of bronchial reactivity in normal and asthmatic children. *J Pediatr* 1985; 107:525–30.
- Anderson SD, Smith CM. Osmotic challenges in the assessment of bronchial responsiveness. *Am Rev Respir Dis* 1991; 143:S43–6.
- Zach M. Cold dry air challenge for measuring bronchial responsiveness – Where do we stand? *Pediatr Pulmonol* 1995; 19:323–5.
- National Heart, Lung, Blood Institute National Institutes of Health Bethesda Maryland 20892. International consensus report on diagnosis and treatment of asthma. *Eur Respir J* 1992; 5:601–41.
- Zach M, Polgar G, Kump H, Kroisel P. Cold air challenge of airway hyperreactivity in children: practical application and theoretical aspects. *Pediatr Res* 1984; 18:469–78.
- Quanjer PH, Tammeling GJ, Cotes JE et al. Lung volumes and

- forced ventilatory flows. Report working party standardization of lung function tests, European Community for Steel and Coal. Official statement of the European Respiratory Society. *Eur Respir J* 1993; 6 (Suppl. 16):5–40.
- 24 Hibbert ME, Lannigan A, Landau LI, Phelan PD. Lung function values from a longitudinal study of healthy children and adolescents. *Pediatr Pulmonol* 1989; 7:101–9.
- 25 Zach M, Polgar G. Cold air challenge of airway hyperreactivity in children: dose–response interrelation with a reaction plateau. *J Allergy Clin Immunol* 1987; 80:9–17.
- 26 Modl M, Eber E, Steinbrugger B, Weinhandl E, Zach MS. Comparing methods for assessing bronchial responsiveness in children: single step cold air challenge, multiple step cold air challenge, and histamine provocation. *Eur Respir J* 1995; 1742–7.
- 27 Pliss LB, Ingenito EP, Ingram RH, Pichurko B. Assessment of bronchoalveolar cell and mediator response to isocapnic hyperpnea in asthma. *Am Rev Respir Dis* 1990; 142:73–8.
- 28 Price JF, Warner JO, Hey EN, Turner MW, Soothill JF. A controlled trial of hyposensitization with adsorbed tyrosine Dermatophagoides pteronyssinus antigen in childhood asthma: in vivo aspects. *Clin Allergy* 1984; 14:209–19.
- 29 Van Bever HP, Stevens WJ. Evolution of the late asthmatic reaction during immunotherapy and after stopping immunotherapy. *J Allergy Clin Immunol* 1990; 86:141–6.
- 30 Des Roches A, Paradis L, Knani J et al. Immunotherapy with standardized Dermatophagoides pteronyssinus extract. V. Duration of the efficacy of immunotherapy after its cessation. *Allergy* 1996; 51:430–3.
- 31 Hedlin G, Heilborn H, Lilja G et al. Long-term follow-up of patients treated with a three-year course of cat or dog immunotherapy. *J Allergy Clin Immunol* 1995; 96:879–85.