Allergy 2002: 57: 785–790
Printed in UK. All rights reserved

ALLERGY
ISSN 0105-4538

Original article

Benefits of immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract in asthmatic children: a three-year prospective study

Background: Although widely practiced for over 80 years, the role of specific immunotherapy (SIT) in pediatric asthma treatment is still controversial. We assessed the effects of a 3-year period of subcutaneous administration of a standardized preparation of *Dermatophagoides pteronyssinus* (*D pt*) on the respiratory health in a group of asthmatic children monosensitized to house dust mite (HDM).
Methods: A randomized clinical trial was performed after 1-year run-in period. Fifteen children receiving SIT for HDM and 14 controls (four drop-outs), matched for age, allergen sensitization, asthma severity, lung function, and non-specific bronchial reactivity (BHR), were studied during the 3-year treatment period. During the whole trial, respiratory symptoms, pharmacological and respiratory function parameters were regularly evaluated. Skin prick tests and methacholine challenge were performed at the beginning and end of the study.
Results: In the SIT group significant improvement in asthmatic symptoms and marked reduction in drug intake was observed. The SIT group also showed a significant decrease in non-specific bronchial BHR. No new sensitivity occurred during the study period in the SIT group only. No major local or systemic side-effects were reported during the study.
Conclusions: Our results confirm that SIT is effective in asthmatic children sensitive to mites. It is associated with a decrease in BHR and it may prevent the development of new sensitizations in monosensitized subjects.

M. Pifferi[1], G. Baldini[1], G. Marrazzini[1], M. Baldini[1], V. Ragazzo[1], A. Pietrobelli[2], A. L. Boner[2]

[1]Departments of Pediatrics, University of Pisa, Pisa, Italy; [2]Department of Pediatrics, University of Verona and Istituto Pio XII, Misurina, Italy

Key words: allergy; bronchial reactivity; children; lung function; specific immunotherapy.

Massimo Pifferi, M.D.
Department of Pediatrics
University of Pisa
Via Roma 67
56100 Pisa
Italy

Accepted for publication 15 April 2002

Although widely practiced for over 80 years, the role of specific immunotherapy (SIT) in the treatment of pediatric bronchial asthma is still controversial. In fact, recommendations have ranged from cautious acceptance to outright dismissal (1, 2). The European Academy of Allergology and Clinical Immunology and the British Society for Allergy and Clinical Immunology advised against its utilization in patients less than 5 years of age, and raised some doubts in particular for the treatment of pediatric asthma (3, 4). Moreover, the NHLBI/WHO Working Group stressed that SIT should be considered only in cases where exposure to allergens cannot be avoided, or when a suitable pharmacological therapy has proved unable to control the disease (5). Abramson et al. (6) performed a meta-analysis of all randomized controlled trials using SIT in allergic asthma, and they found only two studies in pediatrics (7, 8). These two studies concluded that SIT in the pediatric population reduces significantly symptoms, drug intake and bronchial hyperreactivity.

Among the anti-asthma treatments available, SIT is still the only one that may modify the natural course of allergic asthma, because it interferes with the underlying immunological mechanism (9). Therefore, asthmatic children represent the age group most likely to benefit from SIT. In theory an intervention in early life may modify the development of the immune response to allergens, decreasing chronic inflammation and decline in lung function.

The current availability of a more standardized house dust mite (HDM) extract than those utilized in the studies analyzed by Abramson and co-workers (6) prompted us to undertake a prospective real-life study to verify the benefits of SIT in asthmatic children during a 3-year treatment period.

Material and methods

Study design

A randomized clinical trial aiming to evaluate the outcome of SIT with HDM standardized extract was performed. Twenty-nine asthmatic patients (16 boys and 13 girls), aged 6–14 years (mean ± SD: 10.61 ± 2.75), were enrolled in the trial.

Inclusion criteria were: the presence of at least five episodes of doctor-diagnosed asthma in the last 12 months, monosensitization

to HDM as evaluated by skin prick test, no previously treatment with SIT. Before the enrollment all children were monitored over a 1-year period (run-in) with a diary card to daily record respiratory symptoms and drug intake.

At the end of the run-in period, a skin prick test, lung function assessment and evaluation of non-specific bronchial hyper-responsiveness (BHR) by methacholine challenge (MC) were carried out. Fifteen children, seven of whom were also suffering from perennial allergic rhinitis, as defined by the EAACI position statement (10), were randomly assigned to the treatment group. Fourteen children (six of whom also had perennial allergic rhinitis) served as controls. Treatment and control groups were matched for age, asthma severity, respiratory function, and BHR.

The patients' characteristics at enrollment are reported in Table 1. During the study period, asthma exacerbations (defined as episodes of symptomatic asthma not responding to β_2 agonists and high dose Fluticasone – 500 μg/die – and requiring systemic corticosteriods) and anti-asthma therapy were evaluated. Flow volume curves were evaluated before and 30 min after SIT administration in the treatment group (11) and every 2 months in the control group.

Two weeks after the end of the study, a skin prick test and an MC were repeated. The tests were carried out at the same time of the year as the first ones, in order to have the same environmental exposure conditions.

The Local Hospital Ethical Committee approved the study and informed written consent was obtained by children's parents.

Specific immunotherapy

The SIT administration started in November 1996 using a purified, standardized allergenic extract of *Dermatophagoides pteronyssinus* (*D pt*) dialyzed and chemically conjugated to sodium alginate as a carrier (Dome/Hollister-Stier (DHS), Allergen Division, Bridgend, UK, Conjuvac, by Bayer Spa, Milan, Italy). The standardization performed by DHS consisted of clinical quantitation of the in-house reference (IHR) extract by skin prick test based on the Nordic Guidelines (12) and subsequent comparison of its biological potency with production batches by ELISA inhibition vs. the IHR. Biological activity of the IHR extract, expressed as a SARAH value (Skin Activity Reference Allergen vs. Histamine) (12), was defined as 200 Activity Units (AU). The biological activity of Conjuvac was 2500 AU/mL, which corresponds to 1000 label units.

Extract administration was conducted in accordance with the EAACI indication(11).

Extract doses were gradually increased (Table 2) up to the maximum tolerated dose and modulated during the treatment period according to clinical conditions and lung function results as suggested by the position paper (11). The SIT was continued for a period of 3 years. Side-effects were recorded.

Skin prick test

Skin prick tests were performed with a panel of standardized allergen extracts (Alphatests, concentration 400 AU/mL, DHS, Bridgend, UK) including *Dermatophagoides pteronyssinus*, *Cynodon dactylon*, *Lolium perenne*, *Parietaria officinalis*, *Olea europea*, *Platanus acerifolia*, *Pinus silvestris*, cat's dander, dog's dander, *Alternaria alternata*, histamine chlorhydrate (10 mg/mL) as positive control, and 50% glycerol solution as negative control.

After 15 min, the perimeter of the resulting wheal was drawn with a ballpoint pen, a procedure that allowed both the maximum diameter and the perpendicular to be measured. The mean diameter was then calculated. Results were evaluated according to the criteria recommended by EAACI (13).

Lung function test

Lung function tests were performed with a Multispiro-PC pneumotachograph (Burke & Burke, Wuerzburg, Germany). Subjects performed at least three forced expiratory maneuvers starting in the maximum inspiratory position, or at least until two comparable flow-volume curves (i.e., with FEV_1 and FVC values differing by no more than 5%) were obtained (14, 15).

Flow volume curves were performed monthly before SIT administration in all children, thereafter every 2 months in the control group and before injection in the treated group.

Table 1. Characteristics of SIT vs. control group at the beginning of the study. Symptoms and medications are expressed as the mean number of days during the run-in period. Lung function parameters are expressed as absolute values and percentage of theoretical values

Parameters	SIT group ($n = 15$)	Control group ($n = 10$)	*P*
Age (years)	10.7 ± 2.9	10.3 ± 2.5	NS
Symptoms:			
Asthma (exacerbations/year)	8.1 ± 1.8	8.5 ± 1.7	NS
Medications:			
Salbutamol (days/year)	40.7 ± 17.4	50.9 ± 19.9	NS
Systemic steroids (days/year)	22.4 ± 4.8	24.9 ± 3.7	NS
Lung function parameters:			
FVC (L)	2.7 ± 1.0	2.3 ± 0.7	NS
FVC (% pred)	100 ± 14.2	99.7 ± 12.3	
FEV1 (L)	2.2 ± 0.8	2.0 ± 0.6	NS
FEV1 (% pred)	93.5 ± 15	89.5 ± 13.6	
FEF25-75% (L/S)	2.0 ± 0.8	2.3 ± 1.0	NS
FEF25-75% (% pred)	72.5 ± 24%	84 ± 27.1	
Bronchial reactivity:			
Metacholine			
PD20 FEV1 (μg)	93.5 ± 56.3 ($n = 11$)	374.3 ± 505.5 ($n = 7$)	NS
Skin prick test:			
D far wheal (mm)	5.5 ± 3.7	6.0 ± 3.9	NS
D pt wheal (mm)	8.0 ± 5.7	7.4 ± 3.1	NS
Histamine wheal (mm)	4.6 ± 1.1	5.2 ± 2.0	NS

Table 2. SIT dosage schedule

	Injection no.	Concentration	Dose	Volume	Interval
Initial therapy					
Green	1	10 U/ml	1 U	0.1	1 week
	2	10 U/ml	2 U	0.2	1 week
	3	10 U/ml	4 U	0.4	1 week
	4	10 U/ml	8 U	0.8	1 week
Yellow	5	100 U/ml	10 U	0.1	1 week
	6	100 U/ml	20 U	0.2	1 week
	7	100 U/ml	40 U	0.4	1 week
	8	100 U/ml	80 U	0.8	1 week
Blue	9	500 U/ml	100 U	0.2	1 week
	10	500 U/ml	200 U	0.4	1 week
	11	500 U/ml	400 U	0.8	1 week
Red	12	1000 U/ml	800 U	0.8	1 week
Maintenance therapy					
Red	13	1000 U/ml	800 U	0.8	2 weeks
	14	1000 U/ml	800 U	0.8	3 weeks
	15	1000 U/ml	800 U	0.8	4 weeks
	16	1000 U/ml	800 U	0.8	4–6 weeks
	n	1000 U/ml	800 U	0.8	4–6 weeks

Methacholine challenge

Methacholine challenge was performed using the ME.FAR MB3 dosimeter (Markos, Monza, Italy) connected to a mouthpiece and activated by the patient's inspiratory maneuvers. After baseline lung function test, the children inhaled diluent control solution (phosphate-buffered saline). Lung function was repeated 1 min later. If there was no significant airflow limitation, the pharmacological challenge was performed (16). Twofold increasing doses of methacholine were given from 6.25 to 3.200 μg as cumulative doses by means of three increasingly concentrated solutions (0.125%, 1%, and 4%, respectively) (17, 18). Each inhalation was followed by lung function evaluation. The cumulative dose of methacholine that determines a 20% reduction in FEV_1 from the post-diluent value was then calculated (PD20 FEV1).

Diary card records

Occurrence and duration of the symptoms related to asthma, cough and rhinitis, and the drugs used for symptom control were reported in a daily clinical diary. Parents and children were asked to record symptoms defined as ''shortness of breath with wheezing'', ''cough apart from common colds'' and ''rhinitis with itch'', as well as to keep an accurate track of drug therapy. All the children were regularly receiving sodium cromoglycate (20 mg tid). Salbutamol (600 mg/day) and Fluticasone propionate (500 μg/day) were advised in case of symptoms. If no improvement was observed within 12 h the patients received prednisone (1 mg/kg/day) and the event was considered as an exacerbation.

Blinding of the study

The physicians (G.B and G.M) involved in the evaluation of skin prick tests, lung function and methacholine challenge were blinded of the child treatment.

Statistical analysis

Distribution of children's characteristics at enrollment was analyzed by the Kolmogorov–Smirnov Two-Sample test and the hypothesis of normal distribution of the two groups at the beginning by the Kolmogorov–Smirnov One-Sample test. Continuous (lung function and PD20 values, skin prick test wheals diameter) and discrete (age, days with symptoms and days on pharmacological treatment) numerical variables were analyzed by the unpaired Student's *t*-test. Furthermore, for each comparison, the homogeneity of the variance test on the two samples according to Levene was performed. In the case of a positive result the pooled variance *t*-test, and in the case of a negative result the separate variance *t*-test was applied. Results were expressed as the difference between the means of the two groups. A significance level of 0.05 was used. Categorical variables, such as severe, moderate, or mild BHR as expressed by PD20 $FEV_1 \leq 50$, 51–400, or 401–1600 γ, respectively, were analyzed by the chi-squared test (Pearson, Likelihood Ratio, and Mantel–Haenszel procedures). Relative risk of the occurrence of an event in SIT cases was compared with controls, with the significance level established at the 95% confidence interval.

The difference in lung function parameters, number of days with symptoms, and days on pharmacological treatment between run-in and the end of the trial was measured by comparing the difference between the first recorded value and the mean of the last two values (19,20).

Although lung growth determines an increase in the absolute values of lung function parameters, such increases occur in the whole pediatric population, and were thus unlikely to affect the comparisons between the two groups. Nonetheless, these data were verified by removing the growth effect, subtracting from the values recorded in the patients the normal values calculated on the basis of age and height (15). The occurrence of new sensitizations was compared by means of the chi-squared test.

Results

Four subjects in the control group failed to present at the scheduled visits and did not complete the study. All SIT subjects reached the suggested dose for the maintenance phase. However, the use of a flexible dosage schedule determined a cumulative mean extract dose of 24 758.33 U ± 1720.24 (range from 23 265 to 30 465; median: 24 465 U).

Lung function data are depicted in Fig. 1. Although SIT subjects showed a trend towards better performances throughout the whole 3-year period of study, the final comparison between final and initial values among SIT vs. controls did not yield significant differences. Such result was confirmed even after removing the growth effect (data not shown).

The number of asthma exacerbations significantly decreased among SIT patients compared to controls; the difference was observed after the first year ($P < 0.01$) and remained significant at the end of the 3-year period ($P < 0.01$) (Fig. 2).

Although drug use, expressed as number of days with treatment, diminished both in the treated and control groups, a significant reduction in Salbutamol and systemic steroids intake was observed between the SIT group compared to controls ($P < 0.01$) (Fig. 3).

At the end of the trial, PD20 methacholine values were 997.7 ± 974.0 μg in the SIT children, and 388.5 ± 516.4 μg in the control group.

Classification of bronchial reactivity severity was performed in all children at the beginning and at the end of the study. This allowed identifying the subjects moving from one severity class to another (21). The hypothesis of the independence of these values before and at the end of the study was verified by the chi-squared test. Unlike those of the control group (Pearson: 0.03; Likelihood Ratio: 0.02; Mantel–Haenszel: 0.02), the data of the SIT children did not allow this hypothesis to be rejected with the established level of significance, suggesting that an exogenous factor, very likely SIT, modified the theoretical distribution of bronchial reactivity.

The ratio of the incidence of ''non-improvement'' of bronchial reactivity in the SIT to the control group (Relative Risk: 0.3, and 95% CI between 0.11 and 0.87) indicated that the likelihood of non-improvement of the former was 1/3 of that of the latter (Fig. 4).

The comparisons between the differences in skin reactivity for *D pt* and for the positive control recorded at the end and at the beginning of the study were −3.9643 mm for *D pt* and 0.1333 mm for histamine.

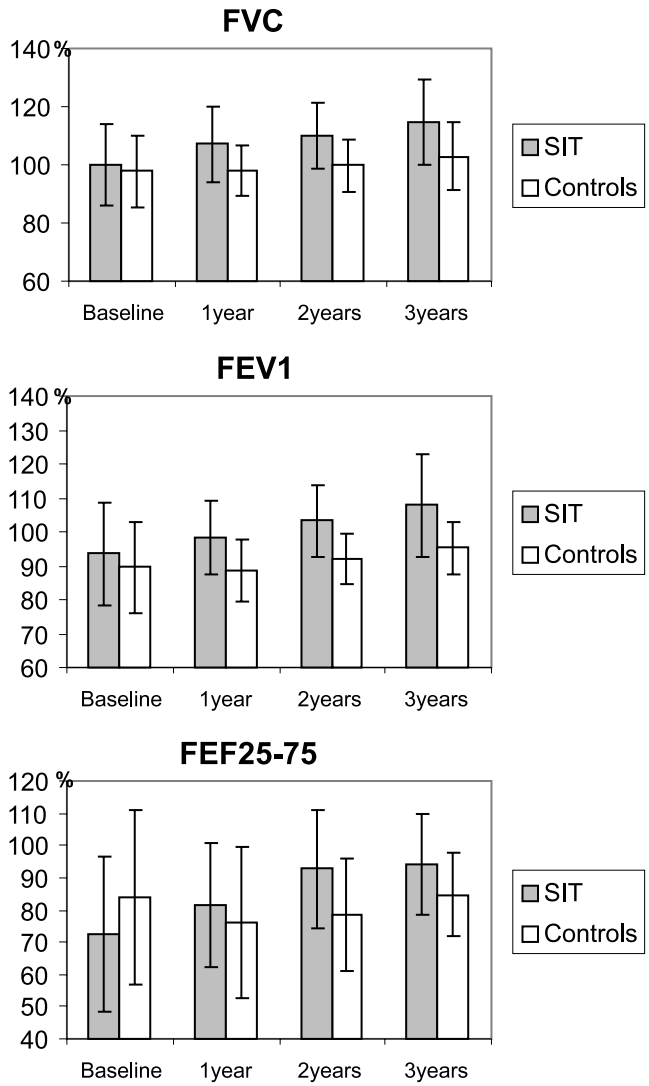

Figure 1. Lung function measurements (predicted values mean percent) in SIT subjects vs. controls.

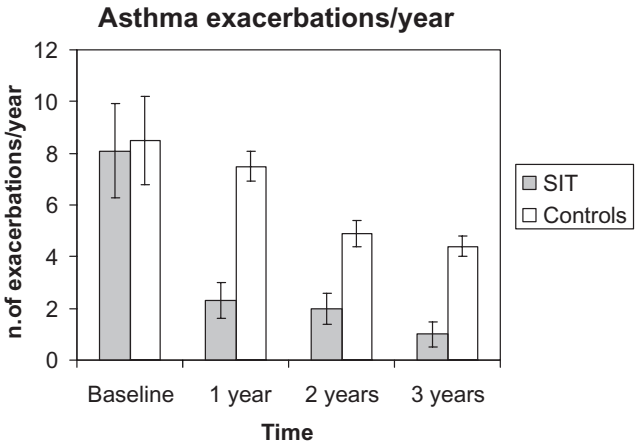

Figure 2. Numbers of asthma exacerbations over the year in SIT subjects vs. controls.

Although an evident reduction in the specific response was observed in the SIT group, this result just failed to reach statistical significance. The occurrence of new sensitizations was significantly lower in SIT treated children than in controls ($P = 0.01$). In the control group three children developed new sensitivities to pollen (*Lolium perenne*), and one patient became sensitive to cat and one to dog dander. None of the SIT group showed new sensitizations.

Finally, SIT was well tolerated and no major local or systemic side-effects were reported during the treatment period.

Discussion

Our results confirm that SIT is effective in the treatment of asthmatic children monosensitized to HDM (7). Our SIT group showed a more significant decrease in asthmatic symptoms and a more marked reduction in drug intake than those observed in the control group (Figs 2 and 3). In agreement with recent data, a significant reduction in non-specific BHR was observed in the SIT group (22,23). This was not caused by the corticosteroids treatment, since our patients were not receiving inhaled corticosteroids regularly. Furthermore, the use of corticosteroids was more frequent in the controls. Previous reports observed inconsistent reductions of BHR (23–27), while documenting the positive effect of SIT on allergen-specific provocation (7,25,27,29–32).

Our lung function results are in agreement with Abramson et al. (6). Significant improvement in respiratory function at the end of the trial in the SIT group was not observed, independently of the lung growth effect (Fig. 1). Although a trend towards better performance was observed in the SIT group, it did not reach statistical significance. Furthermore, although all participants underwent an identical 1-year run-in period, subjects belonging to the SIT group performed a larger number of lung function tests than the control group. Therefore, it cannot be ruled out that the SIT group may have benefited by an enhanced training effect, which may have contributed to its better performance.

In contrast, Adkinson et al. (33) report no detectable benefit of immunotherapy in children with perennial asthma in addition to correct medical treatment. This study, however, used therapeutic extracts containing mixtures of up to seven perennial and seasonal allergen sources, including pollen, molds and dust mites. The results therefore underline that to ensure efficacy, the use of allergen mixtures should be avoided. Dilution of multiple allergens may result in suboptimal doses of individual allergens, and the potency of individual allergens may be reduced more rapidly when diluted or mixed with other extracts (34).

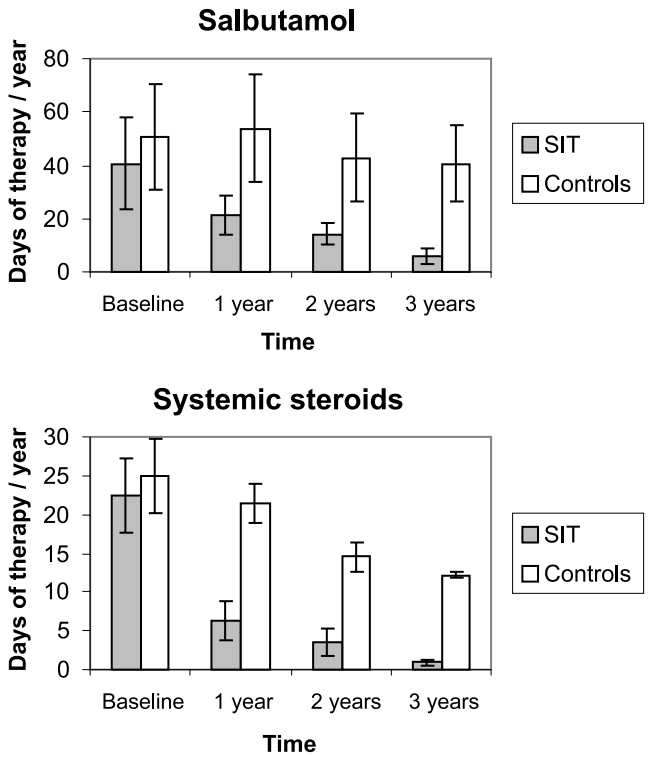

Figure 3. Self-reported drug intake (mean number of days of therapy over the year) in SIT subjects vs. controls.

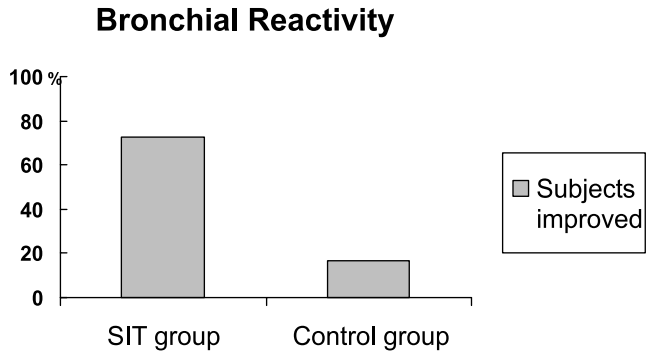

Figure 4. Improvement in bronchial responsiveness (percentage of subjects that entered a less severe class of PD20 bronchial reactivity class) in SIT subjects vs. controls.

There are now sufficient data to establish that SIT is effective as a curative treatment for perennial allergens as well as for pollen (35). The positive results with a perennial allergen such as *D pt* are particularly interesting since sensitization to indoor allergens has been demonstrated to induce chronic asthma and prolonged airways inflammation (36). These patients have been shown to undergo a remodeling of the airway (37) and eventually permanent abnormalities of the bronchial wall (38). These factors are thus likely to have a considerable role in lung function decline (39,40), and effectively bronchial obstruction has been demonstrated to be scarcely reversible with SIT, particularly when it is administered some decades after disease onset (41).

SIT "extinguishes" the allergic reaction by interrupting the chain of events which characterize the allergic disease. It induces a switch in the preferential differentiation of effector cells from Th2 to Th1, and thus modifies the cytokine response in an anti- inflammatory sense (42). A decreased release of the mediators of inflammation could thus underlie both the reductions in non-specific BHR and preventing of activity against the development of new sensitization in monosensitized subjects (43–45), as observed in our study. Furthermore, it has been suggested that an early administration of SIT may be able to reduce the inflammation process, modifying the natural course of the disease (46).

In conclusion, our data further support that SIT is effective. We confirm that an early adoption of SIT in asthmatic children monosensitized to HDM prevents sensitization to new allergens (43–45). This is particularly important since sensitivity to multiple allergens is usually associated with a more severe disease (47). Furthermore in our country children are subjected to a specific surveillance by family pediatricians. This assures a close monitoring of side-effects which may be reduced to an acceptable level by a flexible dosage schedule (48) and by full knowledge of its potential adverse effects (6).

References

1. Weeke B. The future for immunotherapy. Clin Exp Allergy 1991;**21**:86–92.
2. Henry RL, Landau L, Mellis C et al. Childhood asthma: application of the international view of management in Australia and New Zealand. J Paed Child Health 1990;**26**:72–74.
3. Malling H-J, Weeke B, eds. Position paper: Immunotherapy. Allergy 1993;48(S14):9–35.
4. Members of the Working Party BSACI. Position paper on allergen immunotherapy. Clin Exp Allergy 1993;**23**:1–38.
5. NHLBI/WHO. Workshop Report. Global strategy for asthma management and prevention. National Institutes of Health, Publication Number 95-3659, 1995.
6. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma?: a meta-analysis of randomized controlled trials. Am J Respir Crit Care Med 1995;**151**:969–974.
7. Warner JO, Price JF, Soothill JF, Hey EN. Controlled trial of hyposensitisation to *Dermatophagoides pteronyssinus* in children with asthma. Lancet 1978;**2**:912–915.
8. Dreborg S, Agrell B, Foucard T, Kjellman NI, Koivikko A, Nilsson S. A double-blind multicentre immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. Allergy 1986;**41**:131–140.
9. Bonifazi F, Bilò MB. Efficacy of specific immunotherapy in allergic asthma: myth or reality? Allergy 1997;**52**:698–710.
10. Johansson SGO, O'B Hourihane J, Bousquet J et al. A revised nomenclature for allergy. An EAACI position

statement from the EAACI nomenclature task force. Allergy 2001;**56**:813–824.
11. EAACI. Immunotherapy. Position paper. Allergy 1988;**43**:1–33.
12. Nordic Council on Medicines. Registration of allergenic preparations, Nordic guidelines, 2nd edn. Uppsala: NLN Publications no. 23, 1989.
13. Malling HJ. Methods of skin testing. Position paper. Allergy 1993;**48**:55–56.
14. American Thoracic Society. Standardization of spirometry–1987 update. Am Rev Respir Dis 1987;**136**:1285–1298.
15. Polgar G, Pramadhat V. Pulmonary Function Testing in Children: Techniques and Standards. Philadelphia: W.B. Saunders, 1971, 186–198.
16. SEPCR Working Group 'Bronchial Hyperreactivity'. Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents. Bull Eur Physiopathol Respir 1983;**19**:495–514.
17. Balzano G, Delli Carri I, Gallo C, Cocco G, Melillo G. Intrasubject between-day variability of PD20 methacholine assessed by the dosimeter inhalation test. Chest 1989;**95**:1239–1243.
18. Melillo G, Cocco G, Balzano G, Schiano M. Evaluation of non-specific bronchial hyperreactivity in different respiratory diseases. Eur J Respir Dis 1986;**69**:282–295.
19. Pocock SJ, ed. Clinical Trials. Chichester: John Wiley & Sons, 1994.
20. Kirkwood B, ed. Essentials of Medical Statistics. Oxford: Blackwell Scientific Publications, 1990.
21. Juniper EF, Cockcroft DW, Hargreave FE. Histamine and methacholine inhalation tests: tidal breathing method, laboratory procedure and standardization. Lund: AB Draco, 1991.
22. Pichler CE, Marquardsen A, Sparholt S et al. Specific immunotherapy with *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* results in decreased bronchial hyperreactivity. Allergy 1997;**52**:274–283.
23. Gruber W, Eder E, Mileder P, Modl M, Weinhaudl E, Zach MS. Effect of specific immunotherapy with house dust mite extract on the bronchial responsiveness of pediatric asthma patients. Clin Exp Allergy 1999;**29**:176–181.
24. Murray AB, Ferguson AC, Morrison BJ. Non-allergic bronchial hyperreactivity in asthmatic children decreases with age and increases with mite immunotherapy. Ann Allergy 1985;**54**:541–544.
25. Ohman JL Jr, Findlay SR, Leitermann KM. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses. J Allergy Clin Immunol 1984;**74**:230–239.
26. 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**:318–322.
27. Sundin B, Lilja G, Graff-Lonnevig V et al. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. J Allergy Clin Immunol 1986;**77**:478–487.
28. Machiels JJ, Lebrun PM, Jacquemin MG, Saint-Rémy 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**:1407–1412.
29. Frostad AB, Grimmer O, Sandvik L, Moxnes A, Aas K. Clinical effects of hyposensitization using a purified allergen preparation from Timothy pollen as compared to crude aqueous extracts from Timothy pollen and a four-Grass pollen mixture respectively. Clin Allergy 1983;**13**:337–357.
30. 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**:734–744.
31. 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;**15**:399–404.
32. Rohatgi N, Dunn K, Chai H. Cat- or dog-induced immediate and late asthmatic responses before and after immunotherapy. J Allergy Clin Immunol 1988;**82**:389–397.
33. Adkinson N Jr, Eggleston PA, Eney D et al. A controlled trial of immunotherapy for asthma in allergic children. N Engl J Med 1997;**336**:324–331.
34. Wahn U. Do asthmatic children benefit from specific immunotherapy? Clin Exp Allergy 1999;**29**:143.
35. Bousquet J, Demoly P, Michel FB. Specific immunotherapy in rhinitis and asthma. Ann Allergy Asthma Immunol 2001;**87**:38–42.
36. Djukanovic R, Roche WR, Wilson JW et al. Mucosal inflammation in asthma. Am Rev Respir Dis 1990;**142**:434–457.
37. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;**161**:1720–1745.
38. Paganin F, Séneterre E, Chanez P et al. Computed tomography of the lungs in asthma: influence of disease severity and etiology. Am J Respir Crit Care Med 1996;**153**:110–114.
39. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. Eur J Respir Dis 1987;**70**:171–179.
40. Ulrik CS, Backer V, Dirksen A. A 10 year follow up of 180 adults with bronchial asthma: factors important for the decline in lung function. Thorax 1992;**47**:14–18.
41. Bousquet J, Michel FB. Specific immunotherapy in asthma: is it effective? J Allergy Clin Immunol 1994;**94**:1–11.
42. Durham SR, Varney V, Gaga M, Frew AJ, Jacobson M, Kay AB. Immunotherapy and allergic inflammation. Clin Exp Allergy 1991;**21**: 206–210.
43. Des Roches A, Paradis L, Menardo J-L, Bouges S, Daurés J-P, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitization in children. J Allergy Clin Immunol 1997;**99**:450–453.
44. Yang K. Does allergen immunotherapy alter the natural course of allergic disorders? Drugs 2001;**61**:365–374.
45. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. Clin Exp Allergy 2001;**31**:1392–1397.
46. WHO. Position paper. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998;**53**:1–42.
47. Burrows B, Sears MR, Flannery EM, Herbison GP, Holdaway MD. Relations of bronchial responsiveness to allergy skin test reactivity, lung function, respiratory symptoms, and diagnoses in thirteen-year-old New Zealand children. J Allergy Clin Immunol 1995;**95**:548–556.
48. Olaguibel JM, Tabar AI, Garcia Figueroa BE, Cortés C. Immunotherapy with standardized extract of *Dermatophagoides pteronyssinus* in bronchial asthma: a dose-titration study. Allergy 1997;**52**:168–178.