ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 1999, 6, 91–97

THE IMMUNOLOGICAL AND CLINICAL EFFECTS OF IMMUNOTHERAPY IN PATIENTS SUFFERING FROM HOUSE DUST ALLERGY

Indrani Chowdhury[1], Bishnu P. Chatterjee[2]

[1]Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, Calcutta, India
[2]Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Calcutta, India

Chowdhury I, Chatterjee BP: The immunological and clinical effects of immunotherapy in patients suffering from house dust allergy. *Ann Agric Environ Med* 1999, **6**, 91–97.

Abstract: A group of 48 patients with asthma and rhinitis, sensitive to house dust allergen, underwent immunotherapy with an extract of crude house dust for a period of 6–12 months. The clinical results of the therapy, as evaluated by symptom medication scores, demonstrated the significant clinical improvement of the patients treated by crude dust extract (CDE) compared to those treated by placebo. A significant ($p < 0.001$) reduction of specific IgE and elevation ($p < 0.001$) of specific IgG in the post-therapeutic patient sera was demonstrated by serological tests. A good correlation was observed between the changes in specific IgE and IgG levels.

Address for correspondence: Dr. Indrani Chowdhury, Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, 93/1 Acharya P.C. Road, Calcutta - 700 009, India.

Key words: immunotherapy, house dust allergy, crude dust extract, IgE, IgG, ELISA, clinical status, pre-therapeutic, post-therapeutic.

INTRODUCTION

The beneficial effect of immunotherapy (IT) with crude extract or partially purified allergen had been demonstrated in certain IgE mediated disorders, such as seasonal allergic rhinitis, perennial allergic rhinitis and asthma [2, 3, 6, 11, 15, 25, 33]. Immunological changes that generally occur during IT include a rise in specific "blocking" IgG antibodies, decrease in IgE antibodies below pretreatment levels, and finally, the induction of suppressor T cells. For the evaluation of IT the clinical status of the patients is also considered [3]. However, the clinical relevance of these findings is not certain for individual patients since the beneficial effect of IT does not correlate precisely with the reduction of specific IgE level [6]. Gleich *et al.* [13] observed an abrupt increase in serum ragweed IgE in some patients receiving initial ragweed IT, parallel to a beneficial effect *in vivo*. An early rise in IgE antibodies was also demonstrated in Yellow Jacket Wasp Venom immunotherapy [34].

House dust is a well known inhalant allergen responsible for allergic rhinitis and asthma, and the extracts prepared from it have been employed for years in IT of house dust sensitive patients. House dust mite *Dermatophagoides* extracts appear to be more effective material where mites are the major allergen in dust. A number of studies have demonstrated the efficacy in controlling symptoms, a decrease in responsiveness to allergen on bronchial challenge [21], a significant decrease in skin and conjunctival sensitivity [9], a rise in specific IgG antibodies [14, 24, 30] and a decline in specific IgE antibodies [28, 31]. However, some other reports [4, 12] have not shown any significant improvement.

The aim of the present investigation is to study the changes in symptom-medication score and specific IgE and IgG responses in patients allergic to house dust before and after immunotherapy. The changes in antibody response to two allergenic fractions, Con A-sepharose column unbound and bound one, have been compared with those to crude dust extract (CDE).

Received: 31 March 1999
Accepted: 6 November 1999

MATERIALS AND METHODS

Patients. Sixty three patients ranging in age from 15 to 58 (mean ± SD = 32.2 ± 10.8 years) were included in the study. They were selected on the basis of the following criteria: (1) All patients had symptoms of asthma and/or rhinitis throughout the year. (2) The sensitivity of the patients to dust allergen had been established by skin prick test (SPT) to crude dust allergen extract (CDE) and the presence of dust allergen specific serum IgE detected by ELISA. (3) Immunotherapy was considered for those patients who did not show any satisfactory clinical improvement with medication.

Dust allergen. Dust samples were collected by scraping the dust from the flat surfaces of the unused book stocks on shelves in a library in Calcutta with the help of a piece of hard cardboard.

After washing with diethyl-ether, dust samples were extracted with 0.15 M phosphate buffered saline (PBS 1:10, pH 7.2) by stirring overnight at 4ºC. The extract was clarified by centrifugation at 12,000 × g for 40 min and filtered through Whatman No. 1 filter paper. The clear supernatant was then filtered through 0.22 µm Millipore filter (Millipore, Bedford, Mass., USA) and the filtrate stored at 4ºC in 5 ml aliquots in sterile vials. A portion of the extract was concentrated by lyophilization and stored at -20ºC until used. The crude dust extract (CDE) after gel filtration on columns of Sephadex G-25 and then G-50, was subjected to affinity chromatography on Con A Sepharose column and separated into two active fractions, Con A-bound and unbound fractions as described earlier [22].

For IT, CDE was standardized by its protein content [20] and 0.4% phenol was added. For placebo treatment, only carbol saline (0.15 M PBS containing 0.4% phenol) was used. Each solution was filtered through 0.22 µm Millipore filter and stored at 4ºC in 5 ml aliquots in sterile vials.

Skin prick test. CDE (0.5 mg protein/ml) and six other inhalant allergen extracts (kapok fibers, cotton fibers and pollen of *Cynodon dactylon, Lantana camara, Azadirachta indica* and *Cocos nucifera*) (1.5 mg protein/ml) were prepared individually in carbol saline (PBS containing 0.4% phenol) and sterilised by Millipore filtration (0.22 µm). The patients were advised to stop antihistamines, steroids and ephedrine 48 hrs before SPT. Prick test was performed by placing a drop (10 µl) of extract on the volar aspect of the forearm with a disposable No. 26 hypodermic needle. The wheal responses were measured after 20 min and graded according to Platts- Mills *et al.* [26] on the basis of wheal diameter: 1+ = 3-5 mm, 2+ = > 6 mm, 3+ = > 6 mm with pseudopodia and 4+ = any reaction more pronounced than 3+.

Human sera. Patients' sera were collected before starting IT and after 6–12 months of its progress. For comparative purposes, sera from nine normal individuals were also collected. All the sera were stored at -50ºC prior to use.

Enzyme-linked immunosorbent assay (ELISA). CDE and Con A bound and unbound specific serum IgE and IgG antibodies were measured by ELISA [10] before and after IT. 100 µl of each allergen (150 µg/ml in 10 mM PBS, pH 7.3) was added separately to coat each well of PVC microtiter plate (Flow Lab, UK) for 3 hrs at room temperature (RT). The plates after washing with PBS-T (10 mM PBS containing 0.05% Tween 20) were incubated with 1% BSA for 1 hr at RT and then overnight at 4ºC to block the non-specific sites. 100 µl aliquots of sera were added to each well after washing with PBS-T. Following incubation for 3 hrs at RT, the wells were again washed with PBS-T and incubated with 100 µl of horseradish peroxidase-antihuman IgE/IgG conjugate (1:100 for specific IgE and 1:250 for specific IgG) for 3 hrs at RT. The peroxidase-antibody conjugate was prepared according to Avrameas & Termynek [1]. Finally, after washing with PBS-T, the wells were incubated with 100 µl of o-phenylenediamine (1 mg/ml in 0.05 M citrate- phosphate buffer, pH 5.0, containing 0.01% H_2O_2) at RT for 30 min and the optical density (O.D.) was measured at 492 nm in a Titertek Multiskan automatic ELISA plate reader (Flow Lab, UK). The serum IgE was assayed from a standard curve obtained from serum pool of eight patients highly sensitive to dust allergen. The serum pool (dilution 1:5) was assigned to dust specific IgE concentration of 1000 arbitrary units per millilitre (Au/ml) [16]. The assay of serum IgG was made from a standard curve obtained from a serum pool of six highly desensitized (> 2 years) patients. The serum pool (dilution 1:50) was assigned to dust specific IgG concentration of 1000 Au/ml.

The dilution effects on serum IgE in pooled sera of 2 CDE treated, 2 placebo treated and 2 normal subjects were compared against CDE.

Immunotherapy schedules. For IT, a series of subcutaneous injections of graded doses of CDE in carbol saline were given to patients at varying intervals (Tab. 1) according to the gradation of SPT whereas the placebo patients received only carbol saline of the same volume. When a systemic reaction or a large local reaction (as wheal 73 mm in diameter) occurred, the dose increment was stopped and the second last dose given before this reaction was repeated. The next doses were followed according to Table 1.

Evaluation of the patients. IT was performed on the patients attending the clinic of the National Institute for Allergy and Asthma Research (NIFAAR). They were divided into two approximately equal groups: (1) < 25 years and (2) > 25 years. The patients were made aware of the possible symptoms that could appear and the type of medication which they should take (Tables 2 and 3). A chart presenting all possible treatment patterns that might be required was made available to the patients and doctors. Patients kept their daily records in the preceding month before starting IT and during the last half of every

Table 1. Dose schedule for immunotherapy.

Number of subcutaneous injections	Interval between two consecutive doses, N° of days	Doses (µl) × number of consecutive injections
1–16	16	50 × 4 100 × 4 150 × 2 200 × 2 250 × 2 300 × 2
17–26	15	350 × 2 400 × 2 450 × 2 500 × 2 550 × 2 600 × 2
29–36	20	650 × 2 700 × 2 750 × 2 800 × 2
37–Up to cure	30	850 × 2 900 × 2 950 × 2 1000 up to cure

The dose schedule was maintained the same for all patients irrespective of gender or age. For 1+ SPT sensitive patients the concntrations of CDE in vaccines were 0.05 µg/ml. For 2+, 3+ and 4+ patients the concentration 5 of CDE were 0.025 µg/ml, 0.0167 µg/ml and 0.0125 µg/ml respectively.

Table 2. Symptom medication score: Scoring system for medication.

No medication	0
Sodium cromoglycate (Inhaler or nasal spray)	1
Antihistamine (Terfenadine or Dexchlorophenaramine malate)	2
Salbutamol/Terbutaline (Inhaler/nasal spray)	3
Salbutamol/Terbutaline (Oral)	4
Steroid (beclomethasome or preduisolone)	5

Daily symptom scores were recorded.

Table 3. Diary charts given to the patients with asthma and rhinitis.

Asthma	
No symptoms	0
Mild occasional bronchospasm	1
Seasonal bronchospasm without any congestion	1 or 2
Perennial bronchospasm with or without emphysema	2 or 3
Rhinitis	
No symptoms	0
Episodes of sneezing (> 5)	1
Nasal blockade	1
Rhinorrhoea	1 or 2
Pruritus of the nose	1

Daily symptom scores were recorded.

third month after starting IT. The bronchial condition and the nasal symptoms were recorded along with all the possible medications. The symptom and medication scores were made according to Bosquet *et al.* [3]. The clinical status of each patient was graded as A, B or C depending upon the sum of symptom and medication scores as described in Tables 2 and 3: grade A - patients with score < 2; grade B - score between 2–8; and grade C - with score > 8.

Design of the study. For IT, patients were divided randomly into two groups, CDE treated and placebo-treated. Fifty patients were treated with CDE and 13 patients received placebo. The selection was performed blindly among the patients, independent of age, gender and levels of specific IgE or IgG. Ten patients discontinued IT (for personal reasons) over six months and five patients did not follow the treatment schedule properly and were considered as dropouts.

One physician was responsible for assigning the doses of allergen or placebo to be administered. Another physician, who was unaware of which treatment each patient was receiving, was responsible for the clinical management of the patient during the progress of IT.

CDE, Con A-bound and unbound dust allergens specific serum IgE and IgG of the patients were determined before and after 6–12 months of therapy.

Statistical analysis. The ELISA data were analysed for comparison of dust specific serum IgE and IgG levels before and after IT. Two tailed t-tests of significance for paired observation and Student's t-test for two different groups were used for the analyses, and $p < 0.05$ was considered significant. The correlation coefficient r, between the changes of IgE and IgG was computed from deviation of scores from respective means.

RESULTS

The characteristics of the 63 patients selected for the study and placed in the CDE treated or placebo treated groups are presented in Table 4.

Allergic response of the patients. A total of 200 consecutive individuals (112 males and 88 females) within an age range of 12–58 years, having symptoms of asthma and/or rhinitis, were subjected to SPT with CDE. Among them, 147 (73.5%) showed positive skin reactivity and out of this group 63 persons were selected arbitrarily for CDE treated and placebo treated IT. The CDE specific IgE of the active treated group was found higher than the placebo group (Tab. 4).

Table 4. Comparison of the patients groups.

	CDE treated	Placebo treated
No. of patients	50	13
Age (years)		
Range	15–38	16–44
Mean ± SD	32.2 ± 10.8	28.2 ± 8.9
<25	24	6
>25	26	7
Male : Female	29 : 21	8 : 5
SPT to CDE		
1+	9	9
2+	30	4
3+	9	
4+	2	
CDE specific IgE (Au/ml)	575 ± 60	483 ± 40
CDE specific IgG (Au/ml)	350 ± 43	324 ± 21
Asthma (%)	45(90)	9 (69.2)
Duration (years)		
Range	2–15	0.5–6
Mean	7.32 ± 1.85	4.56 ± 1.32
Severity score[a]	2.68 ± 0.92	2.92 ± 0.51
Rhinitis (%)	26 (52)	6 (46.1)
Duration (years)		
Range	0.5–10	0.5–7
Mean	6.53 ± 1.25	4.38 ± 1.40
Severity score[a]	2.36 ± 0.84	2.72 ± 0.46
Urticaria (%)	3 (6)	
Itiching of eyes (%)	5 (10)	2 (15.3)

[a]Asthma and rhinitis were graded 0–4 according to the severity of the symptoms and the medications required by the patients before starting IT.

Table 5. Clinical evaluation of the patients after desensitization with crude extract.

Age group (years)	Desensitization period					
	6 months			12 months		
	Grade[a]			Grade		
	A	B	C	A	B	C
<25	5	17	2	13	7	0
>25	2	20	4	7	7	1
Total	50 (male = 29, female = 21)			35 (male = 22, female = 13)		
	Placebo					
16–44	0	2	11	0	4	9
Total	13 (male=8, female=5)					

[a]Grade A: Symptom medication score: <2; Grade B: Symptom medication score: 2-8; Grade C: Symptom medication score: >8.

Symptom - medication scores. Before IT, the percentage of allergic disorders, such as asthma, was found to be higher in the active treated group than in the placebo treated group. In the case of rhinitis the figures were almost the same. The degrees of severity scores for asthma and rhinitis were comparable (Tab. 4).

After six months of therapy, among the patients who were < 25 years old, 20.83% (5/24) were in Grade A and this percentage increased to 65% (13/20) within one year of treatment. Among those in the age group of > 25 years, the percentage of Grade A patients was 7.7% after six months of therapy and raised to 46.6% after one year of therapy. The placebo treated patients did not show any significant improvement (Tab. 5).

Serological results. It is evident from Figure 1 that the binding of CDE to specific IgE in both pre-therapeutic (PrT) and post-therapeutic (PoT) patients sera had nearly the same dilution effect. Similar observation was made with Con A bound and unbound fractions. The dilution effects on placebo sera were nearly the same as that on patients' sera except the PoT IgE level was higher than that of PrT level. In normal sera, dilution effect was almost nil (Fig. 1).

The serum IgE and IgG antibody levels for two different age groups of patients, before and after immunotherapy, are presented in Table 6. Before IT, the specific IgE levels of the CDE treated groups were found to be significantly higher than the corresponding placebo treated groups ($p < 0.05$ for three groups, $p < 0.01$ for two groups and $p < 0.001$ for one group). The specific IgG levels in three CDE treated groups were not significantly different from the corresponding placebo groups.

After 12 months of therapy, in the CDE treated patients, CDE specific as well as Con A bound and unbound allergen specific IgE levels were found to be significantly lower compared to the respective PrT groups ($p < 0.001$ for all groups, except for one group of bound allergen where $p < 0.01$). When compared to the placebo groups, the specific IgE anti Con A unbound fraction was diminished significantly ($p < 0.01$ and $p < 0.001$ for one group each).

In contrast, the specific IgG levels anti-CDE and anti Con A bound and unbound fractions were found to be significantly ($p < 0.001$) increased as compared to the corresponding PrT or placebo groups. It has been observed that both IgE and IgG levels in the placebo-treated patients were increased either significantly or not significantly against CDE and the allergen fractions. The serum IgE and IgG levels against CDE and the allergen fractions in normal subjects were much lower ($p < 0.001$) than in all age groups of patients.

The correlation coefficients between the changes in specific IgE and IgG levels for CDE, bound dust allergen and unbound dust allergen were -0.716, -0.359, -0.188 and -0.332, -0.258, -0.468 respectively in the age groups of < 25 years and > 25 years.

DISCUSSION

The studies on house dust allergen reported so far were mainly carried out with the samples collected from bedding, mattresses or carpets [8, 17, 31]. The main object of the present study is to follow up immunotherapy

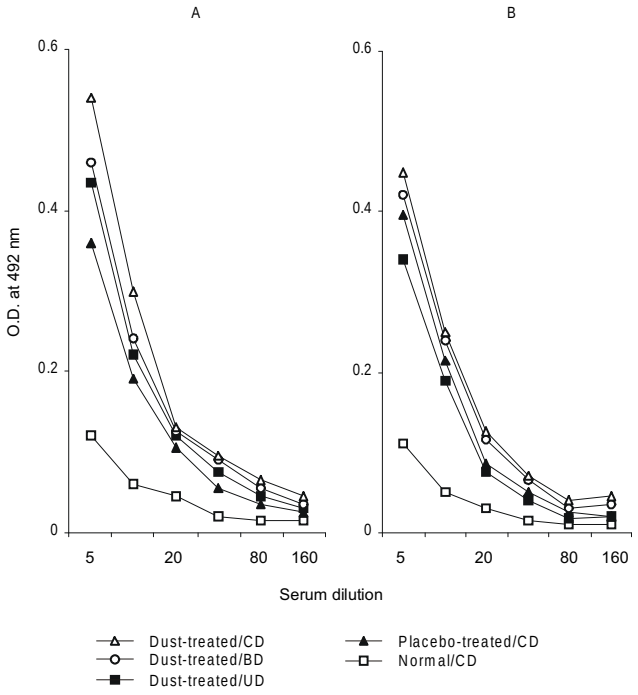

Figure 1. Immunoreactivity (mean O.D. + S.D. of each triplicate) of crude dust, bound dust and unbound dust allergens with specific serum IgE antibodies from two dust allergen treated patients, two placebo treated patients and two normal subjects.
A, the specific IgE before therapy and B, the same after therapy. In each section are shown: a) Reactivities of crude dust (CD), bound dust (BD) and unbound dust (UD) with specific IgE from dust-treated patients' sera. b) Reactivities of crude dust with specific IgE from normal and placebo-treated patients' sera.

conducted with crude dust extract (CDE) prepared from the dust collected from a different source (e.g., an unused book stock), including changes in specific IgE and IgG against CDE and two allergen fractions. The allergenic activity was characterized by skin prick test, by ELISA [22] and by chemical modification studies [23].

On progress of IT, the clinical responses (Tab. 5) of the patients increasingly attained Grade A, e.g. the best clinical condition. As much as 20.83% of patients under 25 years of age were within Grade A when immunized up to six months, whereas immunization up to 12 months scored a much better success, i.e., 65%. Among the older patients (> 25 yrs), only 7.7% scored Grade A after six months, but the success was significantly higher (46.6%) after 12 months of therapy. Thus, the symptom medication scores were significantly reduced in the CDE-treated group in comparison to placebo group. Similar observations were made by other workers, e.g. with ragweed pollen IT [7] and *Cocos nucifera* pollen IT [27]. The lack of efficacy in the placebo-treated patients might be due to the absence of CDE during the placebo treatment.

It is evident from Table 6 that both CDE specific IgE and IgG levels in the patients' sera were much higher than those of normals. Obviously, the higher IgE level is due to type I allergic reaction which enhances the specific IgE production on repeated exposure to the allergen, whereas the reason for higher IgG level is obscure. It might be partly explained by the fact that at the onset of inhalant allergy the patient usually takes some drugs which give temporarily relief, and thereby produce some amount of IgG_1 type of specific antibody [32].

Table 6. Specific IgE and IgG levels (mean ± SD of each triplicate) to CDE, Con A bound and unbound allergens in sera of CDE treated patients and normal subjects.

Allergen	Age group (years)	CDE treated		Placebo treated		Normal
		BT[a]	AT[b]	BT[c]	AT[d]	
		Specific IgE (Au/ml)				
CDE	< 25	567 ± 86*	467 ± 67***	482 ± 41**	516 ± 66[NS]	85 ± 20
	> 25	592 ± 72**	512 ± 55***	497 ± 38**	541 ± 52[NS]	105 ± 15
Con A bound allergen	< 25	580 ± 80*	495 ± 75***	502 ± 40*	538 ± 33[NS]	116 ± 25
	> 25	565 ± 62*	505 ± 45**	498 ± 54*	550 ± 28*	120 ± 12
Con A unbound allergen	< 25	564 ± 50***	406 ± 47***	436 ± 38*	475 ± 44**	90 ± 22
	> 25	531 ± 34**	421 ± 38***	465 ± 45[NS]	488 ± 34***	80 ± 16
		Specific IgG (Au/ml)				
CDE	< 25	320 ± 65[NS]	585 ± 33***	312 ± 20*	321 ± 32***	110 ± 18
	> 25	372 ± 32*	602 ± 41***	345 ± 18**	366 ± 21***	82 ± 28
Con A bound allergen	< 25	350 ± 52[NS]	662 ± 40***	336 ± 31[NS]	352 ± 27***	113 ± 20
	> 25	328 ± 46[NS]	602 ± 25***	312 ± 22[NS]	334 ± 18***	96 ± 22
Con A unbound allergen	< 25	395 ± 66*	675 ± 35***	337 ± 25*	360 ± 36***	122 ± 16
	> 25	346 ± 27*	601 ± 43***	322 ± 16*	340 ± 29***	94 ± 10

BT - Before therapy; AT - After therapy; [a] p values compared to BT placebo values; [b] p values compared to BT CDE treated values; [c] p values compared to AT placebo values; [d] p values compared to AT CDE treated values; *p < 0.05; **p < 0.01; ***p < 0.001; [NS] Not significant.

The number of patients in the placebo group was much smaller than in the active group. Their SPT reactivity was mostly 1+ whereas the active treated patients showed 2+ or more. This may explain the lower value of IgE levels in placebo group than in the actively treated group at the start of IT.

Most of the patients receiving IT with crude dust extract responded with significant lowering of specific IgE ($p < 0.001$ and $p < 0.01$) and significant elevation of specific IgG antibodies ($p < 0.001$). However, the IgE levels in the CDE treated patients were still high enough compared to normal levels even after 6–12 months of IT, while clinically the patients showed a good recovery. These findings suggest that an increase in specific IgG may play an important role in giving relief to the patients suffering from allergic illness and is likely to be related to the lowering of the specific IgE level. A number of studies of hyposensitization have demonstrated a selective rise in serum IgG [18, 29, 35]. Some of these showed quantitative increase of IgG, only weakly associated with clinical improvement [18, 29]. Decrease in allergen specific IgE level during IT was reported with many allergens [5, 19].

Although the CDE-IT resulted both in a decrease of IgE and increase in IgG levels, the rise of IgG level was more pronounced compared to lowering of IgE. The clinical efficacy was also found to be significant as determined by symptom-medication score.

The changes in Con A bound and unbound allergen specific serum IgE and IgG levels before and after CDE-IT were almost the same as those of CDE and thus do not suggest any greater differences in the response to both fractions.

It is evident that changes in specific IgE values by subsequent dilutions were of the same character in the presence of specific IgG in both PrT and PoT sera which suggests that the interfering factors, if any, for binding capacity of specific IgE levels were constant in both PrT and PoT patients sera.

CONCLUSION

From the results of the above study it may be concluded that the crude dust extract used for immunotherapy is a useful material, and that the IT schedule performed here is also effective one.

Acknowledgement

The authors are extremely grateful to Professor Sunirmal Chanda, Division of Palynology and Environmental Biology, Department of Botany, Bose Institute, without whose kind help and encouragement it would not have been possible to prepare the manuscript in its present form. We are also thankful to Dr. Swati Gupta-Bhattacharya, Lecturer, Department of Botany, and Ms. Pampa Chakraborty for their valuable co-operation. Thanks are also due to Dr. (Mrs.) S. Chatterjee, Allergy Unit, Institute of Child Health, Calcutta, for her assistance during clinical investigations.

REFERENCES

1. Avrameas S, Termynek T: Peroxidase labelled antibody and Fab conjugates with enhanced intracellular penetration (Coupling of glutaraldehyde treated peroxidase with antibody or Fab). *Immunochemistry* 1971, **8**, 1175-1179.

2. Berg T, Nordvall L, Lanner A: Clinical studies on a purified timothy pollen extract: Desensitization therapy with a purified timothy pollen preparation compared to a crude timothy pollen extract. *Int Arch Allergy Appl Immunol* 1980, **63**, 266-274.

3. Bosquet J, Becker WM, Hejjaoni A, Chanal I, Lebel B, Dhivert H, Michel FB: Differences in clinical and immunologic reactivities of patients allergic to grass pollen and multiple pollen species. II Efficacy of a double-blind placebo controlled specific immunotherapy with standardized extracts. *J Allergy Clin Immunol* 1991, **88**, 43-53.

4. British Tuberculosis Association: Treatment of house dust allergy. *Br Med J* 1968, 774-777.

5. Connell JT, Skerman WB: Changes in skin sensitizing antibody titer after injections of aqueous pollen extract. *J Allergy Clin Immunol* 1969, **43**, 22-30.

6. Creticos PS, VanMetre TE, Maradiney MT, 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.

7. Creticos PS, Marsh DG, Proud D: Responses to ragweed-pollen nasal challenge before and after immunotherapy. *J Allergy Clin Immunol* 1990, **85**, 934-940.

8. Dybendal T, Hellaud T, Vik H, Apold J, Elsayed S: Dust from carpeted and smooth floors. I. Comparative measurements of antigenic and allergenic proteins in dust vacuumed from carpeted and non-carpeted classrooms in Norwegian schools. *Clin Exp Allergy* 1989, **19**, 217-224.

9. Ebner H, Neuchrist C, Havlec L, Krait U: Comparative studies of the specific effectiveness of specific immunotherapy in house dust mite allergy. *Ambulatorium für Allergie und Klinische Immunologie* 1989, **101**, 504-511.

10. Engvall E, Pearlman P: Quantitation of specific antibodies by enzyme-linked antiimmunoglobulin in antisera coated tubes. *J Immunol* 1972, **109**, 129-135.

11. Foucard T, Johansson SGO: Allergen specific IgE and IgG in pollen-allergic children given immunotherapy for 2 to 6 years. *Clin Allergy* 1987, **8**, 249-252.

12. Gaddie J, Skinner C, Palmer KNV: Hyposensitisation with house dust mite vaccine in bronchial asthma. *Br Med J* 1976, 561-562.

13. 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**, 261-271.

14. Hasigawa M, Yasueda H, Maeda Y, Kaneko F, Hayakawa I, Akiyama K, Yanagihara Y, Shida I: Changes in antigen specific IgG, IgG_4 and IgE antibodies in patients receiving immunotherapy with house dust extract. *Arerugi* 1993, **42**, 809-816.

15. Heldin G, Graff-Lonnevig V, Heilborn H, Lilja G, Norriland K, Pegelow K, Sundin B, Lowenstein H: Immunotherapy with cat and dog dander extracts. Effects for three years of treatment. *J Allergy Clin Immunol* 1991, **87**, 955-964.

16. James TC, Yunginger JW, Reed CE, Jaffe HS, Nelson DR, Gleich GJ: Lack of suppression of IgE production by recombinant interferon gamma. A controlled trial in patients with allergic rhinitis. *J Allergy Clin Immunol* 1990, **85**, 934-940.

17. Kawai T, Marsh DG, Lichtenstein IN, Norman PS: The allergens responsible for house dust allergy. Comparison of *Dermatophagoides pteronyssinus* and house dust extracts by assay of histamine release from allergic human leukocytes. *J Allergy Clin Immunol* 1972, **50**, 117-122.

18. Levy DA, Lichtenstein LM, Goldstein EO, Ishizaka K: Immunologic and cellular changes accompanying the therapy of pollen allergy. *J Clin Invest* 1971, **50**, 360-369.

19. Lichtenstein LM, Norman PS, Winkenwerder WL: A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. *Ann Inter Med* 1971, **75**, 663-671.

20. Lowry OH, Rosenbrough NJ, Farr AL, Randal RJ: Protein measurement with Folin-phenol reagent. *J Biol Chem* 1951, **193**, 267-275.

21. Machiels JJ, Sonville MA, Lebrum PM, Lebeoque SJ, Jacquemin MG, Saint-Remy JM: Allergic bronchial asthma due to *Dermatophagoides pteronyssinus* hypersensitivity can be efficiently treated by inoculation of allergen antibody complex. *J Clin Invest* 1990, **85**, 1024-1035.

22. Mitra I, Chatterjee BP: Isolation of Concanavalin A-reactive low-molecular weight allergen from house dust. *Ind J Biochemistry & Biophysics* 1990, **27**, 28-32.

23. Mitra I, Sikdar S, Chatterjee BP: Effects of chemical reagents on the allergenicity of house dust. *Biochem International* 1992, **26**, 25-33.

24. Nagata M, Yamamoto H, Iabe K, Ianaka K, Kimora J, Sakamoto K, Sakamoto Y, Yamamoto K, Uohi Y: A clinical evaluation of rush immunotherapy in adult patients with severe bronchial asthma. *Arerugi* 1989, **38**, 1319-1326.

25. Negrana AC: Immunotherapy for respiratory allergic diseases: indications, efficacy and monitoring. *Allergy Immunol* 1993, **25**, 431-435.

26. Platts-Mills TAE, Chapman MD, Marsh DG: Human immunoglobulin E and immunoglobulin G antibody responses to minor ragweed allergen Ra3: Correlation with skin tests and comparison with other allergens. *J Allergy Clin Immunol* 1991, **67**, 129-136.

27. Roy Karmakar P, Das A, Chatterjee BP: Placebo-controlled immunotherapy with *Cocos nucifera* pollen extract. *Int Arch Allergy Immunol* 1994, **103**, 194-201.

28. Ruiz LLJ, Liuch PM, Valero SA, Zamorano CM, Huguet CJ, Malet CA, Garcia CPA: Evaluation of immune parameters during immunotherapy. *Allergol Immunopathol* 1989, **1**, 119-121.

29. Sadan N, Rhyne MB, Mellits DE, Goldstein EO, Levy DA, Lichtenstein LM: Immunotherapy of pollinosis in children. Investigation of immunologic basis of clinical improvement. *N Engl J Med* 1969, **280**, 623-627.

30. Sanchez PA, Schamann MF, Lamas RA, Hosch MC, Ramos SS, Garcia MJA: Comparative clinical-immunological study of oral and subcutaneous immunotherapy in children with extrinsic bronchial asthma. *Allergol Immunopathol* 1989, **1**, 323-329.

31. Satoh Y, Sugimoto K, Kuroki H, Fuzimoto N, Suzuki H: Evaluation of rapid injection immunotherapy by antigen specific IgE and IgG_4 antibodies. *Arerugi* 1989, **38**, 245-253.

32. Snapper CM, Finkelman FD, Paul WE: Differential regulation of IgG_1 and IgE synthesis by interleukin-4. *J Exp Med* 1988, **167**, 183-188.

33. Tarhini H, Kanani J, Machel FB, Bousquet J: Safety of venom immunotherapy administered by a cluster schedule. *J Allergy Clin Immunol* 1992, **89**, 1198-1200.

34. Yman L: Management of the allergen specific patient diagnosis and specific therapy. *Lab Clin Pract* 1985, **3**, 51-63.

35. Yunginger JW, Gleich GJ: Seasonal changes in IgE antibodies and their relationship to IgG antibodies during immunotherapy for ragweed hay fever. *J Clin Invest* 1973, **52**, 1268-1275.