

A three-year double-blind placebo-controlled study with specific oral immunotherapy to *Dermatophagoides*: evidence of safety and efficacy in paediatric patients

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Summary

Very few double-blind trials of oral immunotherapy have been reported. The majority of these have been performed with pollen extracts and the results have often been equivocal. The major weaknesses of these studies have been the short periods of the trials, the low doses of allergen employed and inadequate evaluation of efficacy. The present study has involved a placebo-controlled double-blind trial of oral immunotherapy for three years with *Dermatophagoides pteronyssinus* at relatively high doses in 18 paediatric patients. Throughout the trial clinical parameters (symptom and medication scores) and immunological parameters (specific IgE, IgG1 and IgG4 levels) were monitored in order to assess the safety and efficacy of the treatment. The treatment was well tolerated by all patients and no side-effects were experienced. Clinical improvement was evident after the second year of therapy and this was confirmed by a significant reduction in conjunctival reactivity assessed by a specific conjunctival provocation test. In addition, there were significant changes in the immunological parameters with a reduction in specific IgE and increased levels of IgG4 and IgG1, results in keeping with previous studies of oral and subcutaneous immunotherapy. Although the results do not provide an explanation of the basis of successful oral immunotherapy, they clearly demonstrate the efficacy and safety of the treatment and suggest that it may be a useful and more acceptable alternative for patients than the traditional subcutaneous immunotherapy.

Clinical and Experimental Allergy, Vol. 24, pp. 53–59. Submitted 14 April 1993; revised 18 August 1993; accepted 6 September 1993.

Introduction

Oral immunotherapy (OIT) for the treatment of allergic disease was suggested as early as 1920 as an alternative to the traditional subcutaneous immunotherapy (SIT), and several clinical investigations have been performed since 1939 [1]. The aim of this treatment is to reduce or avoid the possible side-effects of conventional treatment [2], yet still evoke the immunological response observed with SIT. The oral route for immunotherapy appears more

acceptable, particularly for children whom the compliance for SIT may sometimes be unsatisfactory.

A limited number of double-blind controlled trials have been reported and the results of these have often been equivocal. The majority of these studies have been conducted with pollen extracts, and only those with birch pollen [3] and *Artemisia* pollen [4] have shown clearly demonstrable effects, whilst double-blind trials with grass pollen [5–7] and *Dermatophagoides* [8] have failed to demonstrate efficacy. The birch pollen was administered using gastric resistant capsules [3] and the *Artemisia* preparation in aqueous solution. Recently, Creticos *et al.* [9] provided evidence of the effectiveness of the oral route

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for pollen immunotherapy in a double-blind study with high doses of ragweed in aqueous solution.

In the birch pollen study [3] very high doses were used, approximately 200 times higher than the total cumulative dose in subcutaneous immunotherapy. High doses were also used in the *Artemisia* study [4]. It should be borne in mind that the effectiveness of subcutaneous IT has been found to be dose-related.

Three double-blind studies, employing administration of allergen preparations by the sublingual route alone or the sublingual plus oral routes, have provided evidence of clinical effectiveness of the method with *Der* and grass pollen [10–12]. All these investigations apparently involved the use of low doses of the allergen extract.

Another interesting observation from the literature is the short time period considered for the evaluation of the effectiveness of the active treatment compared with placebo in OIT studies. OIT has been shown to induce immunological changes in the birch [3] and *Artemisia* [4] studies, and also in an open study with grass pollen [13] in which increased levels of specific IgG1 and IgG4 were detected.

In the light of all these observations, and in accordance with the position statements of the *EAACI*, *IUIS* and *SIAIC*, we designed a double-blind placebo-controlled study with relatively high doses of *D. pteronyssinus* extract in aqueous solution, to be administered by the oral route to a paediatric subjects suffering from rhinitis and/or asthma. The study has been running for 3 years.

Materials and methods

Study design

The present study involved a randomized, double-blind, placebo-controlled trial of a standardized preparation of *D. pteronyssinus*. Before beginning the treatment a run-in period (September–December 1987) was observed. Patients were assigned numbered treatment sets that were randomized by computer and coded prior to the study (Rancode IDV, Munich). All patients commenced treatment in January 1988 and continued until January 1991, when the double-blind key was revealed. During the whole trial several clinical and immunological parameters were evaluated as reported below.

Patients

Eighteen patients (15 boys and 3 girls) aged between 3 and 13 years (mean age: 6 years), were enrolled for the trial, with parental consent. They were selected by the trial physician from a population of 600 asthmatic children identified amongst 3000 children attending an out-patients clinic in Milan. Asthma was the principal inclusion criterion. All the patients had experienced

symptoms for at least 1 year, and 13 were affected by asthma and rhinitis, and five by asthma and rhinoconjunctivitis. At the time of enrolment all the patients showed single sensitization to *D. pteronyssinus*, assessed by clinical history, skin-prick test and detection of specific IgE antibodies. The severity of their symptoms was similar and therefore no attempt was made to stratify patients for disease severity. None of the patients had previously received specific immunotherapy. At the end of the run-in period, the patients were randomly assigned to the two groups: the active treatment group was comprised of 10 patients and the placebo group of eight patients.

OIT treatment

The treatment started in January 1988 using an aqueous extract of *D. pteronyssinus* (Allergopharma, Reinbek, Germany) prepared especially for the trial in three 10-fold different concentrations in accordance with the commercially available treatments, and standardized in therapeutic units (TU). Placebo and active treatment preparations were indistinguishable with regard to colour, flavour, density and consistency. Each patient received the therapy as oral drops administered each morning. Treatment commenced with 1 drop of the lowest concentration of 10 TU/ml and the dosage was increased by one drop per day to a maximum of 20 drops. Treatment continued with 2 drops of 100 TU/ml, increasing to 20 drops per day by increments of one drop, and then progressed from 2 to 30 drops of the highest concentration of 1000 TU/ml. Maintenance therapy was continued with 30 drops of the highest concentration three times per week. On this basis the annual dose administered (Table 2) was approximately 5 times greater than by subcutaneous injection of an alum-adsorbed depot-allergen preparation.

Administration of active and placebo preparations was conducted over a 3 year period, 1988 until January 1991, when the double-blind code was broken. Each patient was assessed at the Paediatric Clinic every 2 months during the first 20 months of the study and every 3 months thereafter. On these occasions an allergist collected the symptom-medication score diaries and assessed the clinical status.

Allergen avoidance measures

Parents were asked to exercise the following measures in order to reduce the level of house dust mite exposure. The home had to be adequately ventilated to avoid excessive humidity and condensation. Wherever possible, carpets, upholstered furniture, feather and wool cushions and

Table 1. Patients: basal parameters

	Active	Placebo
Number	10	8
Sex (M/F)	7/3	7/1
Age (years)		
Mean $\pm$ s.d.	6.56 $\pm$ 1.97	6.08 $\pm$ 2.39
Range	4.7–11.0	3.0–9.5
Asthma (number of attacks in previous year)	5–10	5–75
Confidence interval	3.7–6.5	4.1–7.4
Drug Therapy		
β -agonists	10	8
DSCG	6	5
Ketotifen	3	1
*Oral corticosteroids	0	2
Environmental prevention measures	10	8

* Treatment of one exacerbation.

pillows, cuddly toys etc. were to be removed from the home. Cleaning of floors, furniture and walls was to be undertaken on a daily basis, in the absence of the patient, using a vacuum cleaner and a damp cloth. The mattress and all laundry had to be dried thoroughly, preferably by placing it in the sun. The mattress was to be covered with a thin plastic sheet that was wiped with a damp cloth at regular intervals, and pillows were to be covered with four or five cotton pillow cases. No pets were to be allowed in the home.

Symptom-medication score

The patients' parents were requested to keep a daily record of the presence and grade (from 0 to 3) of the following symptoms: asthma (wheezing, dyspnoea, reduction of physical activity), cough and rhinitis (rhinorrhoea, sneezing, nasal blockage), conjunctivitis (itching, burning, palpebral oedema). In addition, the drug intake (sodium chromoglycate, antihistamines, salbutamol or beclomethasone) was recorded on the same diary.

Statistical analysis was performed on global scores (symptoms + medications), and a corrective factor was considered for the medication score, in relation to the class of drugs used, as described by Norman *et al.* [14] and Grammer *et al.* [15]. Non-parametric analysis of the data involved the use of the Wilcoxon test for intra-group comparisons and Mann-Whitney test for inter-group comparisons. $P < 0.05$ was considered significant.

Specific conjunctival provocation test (SCPT)

SCPT was performed before treatment and after 12, 24 and 36 months of therapy, using an aqueous extract (Pharmacia, Uppsala, Sweden) at graded allergen concentrations ranging between 100 and 100 000 BU. The procedure employed has been described previously by our group [16] according to Bonini *et al.* [17] and Schoenheit & Pecoud [18].

Briefly, one drop of the lowest allergen concentration was instilled into the conjunctival sac of the right eye, whilst a drop of diluent (negative control) was instilled into the left eye. Patients were then rechallenged every 15 min with one drop of allergen at a time, making threefold increases in the allergen concentration, until allergic symptoms occurred, and so a threshold dose was established. Median threshold doses were compared at different times, intra-group by the Wilcoxon test and inter-group by the Mann-Whitney test. $P < 0.05$ was considered significant.

Skin-prick test

Cutaneous reactivity was evaluated before treatment and after 2 years of therapy, by the end-point technique, employing a *D. pteronyssinus* extract at increasing concentrations (1,100; 3500; 10 000 and 35 000 SBE/ml). In order to perform a statistical analysis the surface area of the weal induced with 10 000 SBE/ml was expressed as a percentage of the 0.1% histamine weal. Stochastic probabilities < 0.01 were considered significant.

Immunological parameters

Allergen-specific IgE, IgG1 and IgG4 were evaluated in serum of all patients before the treatment and after 6, 12, 24 and 36 months of therapy. Results were analysed statistically by Wilcoxon test for paired samples and Friedman's ANOVA. $P < 0.05$ was considered significant.

Specific IgE An immunofluorimetric assay was employed for evaluation of serum IgE (IgE FAST PLUS, Eurospital Pharma, Trieste, Italy). Fifteen microliters of serum was incubated for 2 h in microvials coated with the allergen; after washing, a specific anti-IgE-alkaline phosphatase conjugate was added and incubated for 2 h. After standardized rinsing the fluorescent substrate (4-methylumbelliferin) was added, and the samples were read with a specific fluorometer (Fluorofast Reader). Results are expressed as fluorescence units (FSU).

Specific IgG4 This was measured by a fluorimetric ELISA method (IgG4 FAST, Eurospital Pharma, Trieste, Italy).

analogous to that for specific IgE. In this procedure serum samples were diluted 1:20 and an anti-IgG4-alkaline phosphatase conjugate was employed. Results are expressed as fluorescence units (FSU).

Specific IgG1 A colorimetric ELISA method (ALLER-BLOCK EIA, Medical Systems, Genova, Italy), was employed. One hundred microliters of serum, diluted 2:1 were incubated overnight in allergen coated microtiter plates. After standardized washing an anti-human IgG1 was added and incubated for 2 h. The microtiter plate was then washed and an anti-mouse horse radish peroxidase conjugate was added and incubated for 2 h. Finally, a chromogenic substrate (ortho-phenylenediamine) was added, together with hydrogen peroxide, and microtiter plates were read in a spectrometer after about 30 min. Results were expressed as optical density (OD) or as ELISA Index (EI = sample OD - negative control OD / negative control OD).

Results

Patients

All the patients completed the trial. The therapy was well tolerated and no local or systemic side-effects were reported during the treatment. Only one patient was unable to achieve the maximum dose of OIT during the first year because of exacerbations of his asthma, but during the second year he reached the maintenance dose without problems.

Clinical parameters

Basal homogeneity This was verified during the run-in period: no significant difference was found in clinical

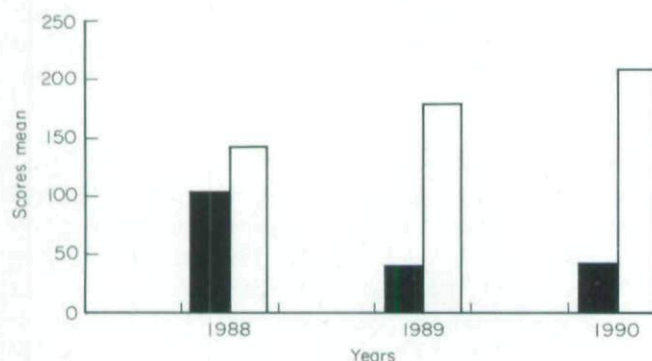


Fig. 1. Symptom-medication scores for rhinitis in the active treatment ($n=10$) and placebo ($n=8$) groups for each year of the study. Wilcoxon test: active treatment 88 vs 89: $P<0.05$; 89 vs 90: $P<0.05$; placebo: $P=n.s.$ Mann-Whitney: treatment vs placebo 1989: $P<0.05$; 1990: $P<0.05$. ■. Active treatment. □. Placebo.

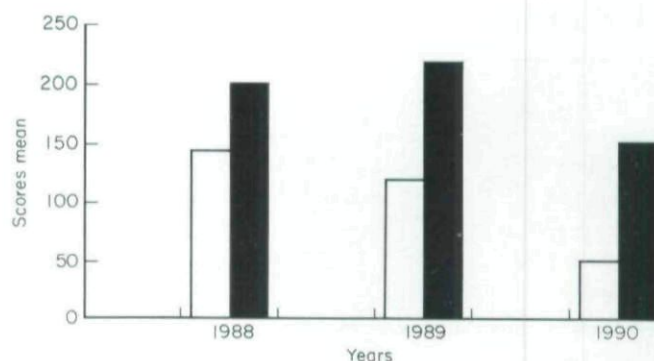


Fig. 2. Symptom-medication scores for asthma in the active treatment ($n=10$) and placebo ($n=8$) groups for each year of the study. Wilcoxon test: active treatment 88 vs 90: $P<0.01$; 89 vs 90: $P<0.01$, 88 vs 89: $P=n.s.$ Placebo: $P=n.s.$ Mann-Whitney: treatment vs placebo 1990: $P<0.05$. □. Active treatment. ■. Placebo.

scores (Mann-Whitney test: asthma, $P>0.1$, rhinitis, $P>0.4$, cough, $P>0.1$; skin-prick test and SCPT ($P>0.1$) between active treatment and placebo groups. Very low symptom scores were recorded for conjunctivitis and consequently no statistical analysis could be performed.

Clinical scores These showed a significant improvement after the second year of therapy especially for rhinitis (Fig. 1) and asthma (Fig. 2). The symptom 'cough' did not show a statistically significant change, nevertheless an improvement was evident in the actively treated group.

SCPT An increase in the threshold dose was observed in the actively treated group after the first year of therapy; the increase became highly significant at the end of the

Table 2. Cumulative allergen dose administered (TU/patient/year)

Patient no	1988	1989	1990
2	210 000	240 000	255 000
6	210 000	240 000	255 000
8	210 000	210 000	255 000
10	210 000	240 000	255 000
12	240 000	270 000	300 000
14	60 000	180 000	250 000
16	210 000	260 000	285 000
18	210 000	300 000	315 000
20	210 000	180 000	255 000
21	120 000	180 000	255 000

Table 3. Specific conjunctival provocation test (SCPT) (Median threshold doses and statistical analysis [doses expressed in BU/ml])

	Placebo (<i>n</i> =8)	Active (<i>n</i> =10)	Wilcoxon-Mann-Whitney <i>U</i> -test
Run-in			
1987	5500	1000	<i>P</i> =1
1988	1000	10 000	<i>P</i> =0.056
	vs 1987 <i>P</i> =n.s.*	vs 1987 <i>P</i> =n.s.*	
1989	2000	10 000	<i>P</i> =0.029
	vs 1987 <i>P</i> =n.s.*	vs 1987 <i>P</i> =n.s.*	
1990	10 000	100 000	<i>P</i> =0.011
	vs 1987 <i>P</i> =n.s.*	vs 1987 <i>P</i> <0.01*	

* Wilcoxon-(Pratt) test.

third year ($P < 0.01$), whilst no significant change was detectable in the placebo group during the whole trial.

In addition, a significant difference in threshold dose between actively treated and placebo groups was evident after the second year of therapy ($P = 0.029$) and this increased by the end of the third year ($P = 0.011$) (Table 3).

Skin-prick test Three patients on active treatment and one on placebo were excluded from the within-group analysis of skin-prick test data because of inadequate testing before therapy. Cutaneous reactivity was defined as the surface area of 10 000 SBE/ml weal expressed as a percentage of histamine weal. No significance in the end-

point test was detected in either the actively treated or placebo groups, and there was no significant difference between the groups before and after therapy. In the active treatment group medians of histamine related responses decreased from 319.9% to 57.2% ($P < 0.05$), but this change was not statistically significant by the set criteria. There was no significant change for the placebo group.

Immunological parameters

The 36 month samples from two of the placebo patients were not available, and therefore all results for these subjects were excluded from the statistical evaluation. Basal homogeneity was verified by Mann-Whitney test. No statistical differences were revealed in the IgE, IgG1, and IgG4 levels between the two groups ($P > 0.1$).

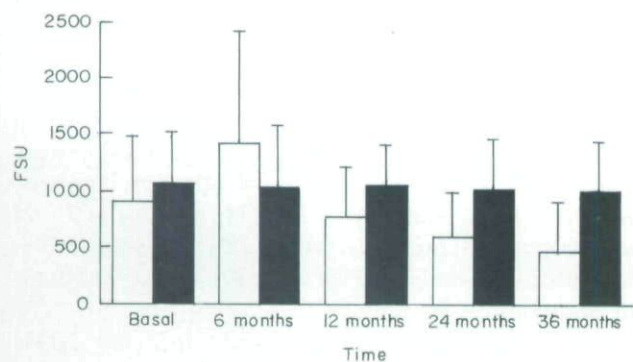


Fig. 3. *D. pteronyssinus* specific IgE measured by RAST in the sera from active treatment and placebo patients. Statistical analysis performed by Wilcoxon test: Active-treated group: Basal vs 6 months $P = 0.02$; Basal vs 12 months $P = \text{n.s.}$; Basal vs 24 months $P = 0.03$; Basal vs 36 months $P < 0.01$. Placebo-treated group: All times $P = \text{n.s.}$ Friedman's ANOVA by ranks: Active-treated group $P = 0.01$; Placebo-treated group $P = \text{n.s.}$ □. active-treated patients. ■. Placebo-treated patients.

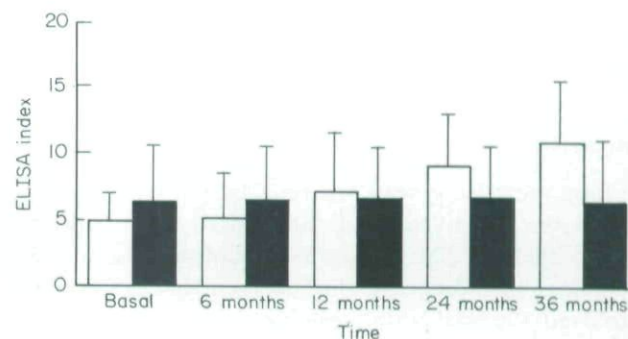


Fig. 4. *D. pteronyssinus* specific IgG1, measured by ELISA in the sera from active treatment and placebo patients. Statistical analysis performed by Wilcoxon test: Active-treated group: Basal vs 6 months $P = \text{n.s.}$; Basal vs 12 months $P = \text{n.s.}$; Basal vs 24 months $P = 0.02$; Basal vs 36 months $P = 0.02$. Placebo-treated group: All times $P = \text{n.s.}$ Friedman's ANOVA by ranks: Active-treated group: $P = 0.017$; Placebo-treated group: $P = \text{n.s.}$ □. Active-treated patients. ■. Placebo-treated patients.

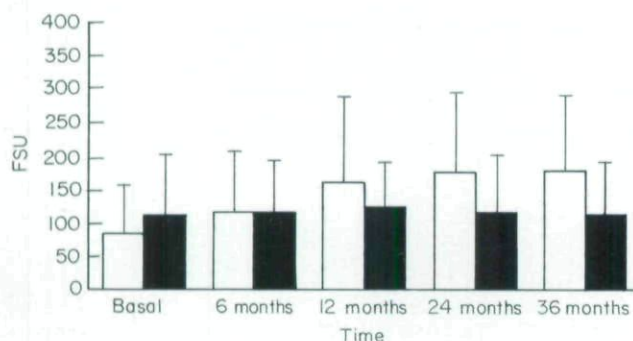


Fig. 5. *D. pteronyssinus* specific IgG4 measured by ELISA in the sera from active treatment and placebo patients. Statistical analysis performed by Wilcoxon test: Active-treated group: Basal vs 6 months $P = \text{n.s.}$; Basal vs 12 months $P = 0.01$; Basal vs 24 months $P = 0.002$; Basal vs 36 months $P = 0.002$. Placebo-treated group: All times $P = \text{n.s.}$ Friedman's ANOVA by ranks: Active-treated group $P = 0.006$; Placebo-treated group $P = \text{n.s.}$ □. Active-treated patients. ■. Placebo-treated patients.

Allergen-specific IgE There was a significant increase in specific IgE after 6 months of treatment, as described for conventional SIT, and subsequently a significant decrease became evident after the second and third years of treatment (Fig. 3).

Allergen-specific IgG1 Specific IgG1 levels showed a continuous increase that was statistically significant from the first year of treatment, and achieved greater significance after the second and third years of therapy as shown in Fig. 4.

Allergen-specific IgG4 Specific IgG4 levels increased during the first year of treatment and increases continued and achieved statistical significance after the second year (Fig. 5). The increase was maintained after the third year, apparently in accordance with clinical improvement.

Discussion

To date there have been few reports that have demonstrated the effectiveness of the oral route for specific immunotherapy. This may be a consequence of a number of factors including the low allergen dosages usually employed; the brief periods of observation for evaluating the clinical effectiveness; the kind of extract used, some of which may be particularly susceptible to the actions of gastric and intestinal secretions; the side-effects observed with high dosages, in particular gastrointestinal disturbances, nausea, vomiting, diarrhoea; the weak relationships between the allergist and the patient; and inadequate clinical and laboratory parameters for monitoring the effects of treatment.

The design of the present investigation was made to obviate at least some of these weak points. The study incorporated a long period of observation (3 years in a double-blind fashion), relatively high dosages, complete and constant monitoring of clinical and immunological effects, specific allergen challenges, and accurate recording of any possible side-effects.

Unfortunately the present study does not provide any information about *in vivo* processing of the allergen extract introduced by the oral route, nor about the mechanisms involved in the immunological responses to allergen administration. It is worth noting that a previous report showed 'normal' intestinal processing of the allergen, even when it was enclosed in gastroprotective capsules [19].

The effectiveness of OIT has been clearly demonstrated for birth [3], ragweed [9] and *Artemisia* pollen [4]. In addition, *Der* and grass pollen extracts have been shown to be effective using the sublingual route [10–12] although little information about the amounts of allergen extracts actually administered in these two studies was provided.

In the present study, statistically significant data about the clinical effectiveness of OIT on both asthma and rhinitis have been obtained. Effects were only clearly manifest after one year and this suggests that many of the previous studies on OIT probably failed to demonstrate effectiveness of this treatment because the study designs involved too short a period of observation, in fact few investigations have reached a 12 or 18 month follow-up.

In the present study, a clearcut decrease in conjunctival responsiveness to specific allergen has been detected in the treatment group. As recently pointed out [20], SCPT represents a very useful tool for investigating the inflammatory processes involved in allergy, contributing to a more detailed diagnosis and more critical monitoring and evaluation of the treatment of allergic diseases.

No side-effects, either local or systemic, were observed. This is obviously an important point, since a possible general reaction has to be anticipated in OIT treated subjects just as in other forms of allergen immunotherapy, a point made in *SIAIC* [21] and *EAACI* [22] position papers. Our study provides further support for the safety of the oral route for immunotherapy, although our observations are limited.

Immunological parameters, namely IgE and IgG4, showed a trend similar to the one generally observed in subcutaneous IT treatment [23]. On the other hand, IgG1 showed a different trend compared with subcutaneous IT. In the case of the latter IgG1 levels show mainly an initial increase followed by a slow decrease, whilst in the present study we observed a continuous increase, significant by the end of the first year and thereafter. This effect has been observed previously with OIT in an open study with grass

pollen in children [12], and represents an interesting difference between OIT and SIT, although it is not possible to say if the persistence of IgG1 is due to the oral route of administration or to the high dosages employed. Little information is available on the association of IgG1 levels with OIT and further studies are needed to investigate this point in more detail.

In conclusion, the present investigation over a 3-year-period provides evidence for the clinical efficacy and safety of OIT, and of the induction of an immunological response to the allergen.

Acknowledgments

The present study was partially supported by A.R.M.I.A. (Associazione Ricerca sulle Malattie Immunologiche e Allergiche); P.F. CNR FATMA SP2, grant to Professor G. W. Canonica, n. 5710; CNR Target Project 'Ingegneria Genetica', P.F. 99 n. 91.0018, grant to Professor M. Bagnasco. We are grateful to Dr O. Cromwell and Dr E. Frank for their help in the preparation of this manuscript.

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