

Double-blind trial of house-dust mite immunotherapy in asthmatic children resident at high altitude

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Twenty-three *Dermatophagoides pteronyssinus* (Dpt)-sensitive asthmatic children aged 7–14 years entered a double-blind, placebo-controlled trial of standardized immunotherapy (IT) (Alpare) while resident at high altitude. Dpt sensitivity was evaluated by skin prick tests at different allergen concentrations at the enrollment and after 6 and 12 months of treatment. Bronchial hyperreactivity was evaluated at the same time points, and on each occasion, histamine challenge and, the following day, Dpt bronchial challenge were performed. All patients, irrespective of active treatment, improved clinically and in lung function with increased PC_{20} and Dpt-PD₂₀. Alpare-treated patients had a significantly decreased sensitivity on Dpt skin testing ($P < 0.009$) and felt that their asthma had improved ($P < 0.001$) compared with placebo-treated subjects, but there was no difference between the treatment groups in lung function or bronchial challenge response. IT neither increased nor decreased bronchial histamine sensitivity. Our results indicate that Dpt IT benefits asthmatic children, but improvement by allergen avoidance at high altitude is even greater.

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Evidence supports the efficacy of parenteral immunotherapy (IT) in ameliorating the symptoms of hay fever during the pollen season (1, 2). In double-blind, placebo-controlled trials, the use of IT with extracts of grass pollen was associated with a significant reduction in symptoms, use of medications, and mediators secreted after nasal allergen challenge (1, 3). The clinical improvement was accompanied by a reduction of immediate nasal hypersensitivity and a decrease in cutaneous late reactions (1). There has been general agreement about the efficacy of IT in the treatment of seasonal allergic rhinitis, but doubt remains about the use of IT in house-dust mite (HDM)-sensitive asthma and in perennial rhinitis. In addition to increased concern about the side-effects of IT and incomplete knowledge of the mechanisms of action of IT, it is still debated whether this treatment can be considered to affect the natural history of allergic disorders in childhood. According to the literature, IT has greater beneficial effects in children than in adults. This was dramatically indicated in some studies which evaluated the effects of IT for Dpt in HDM-sensitive asthmatic children (4).

Price et al. (5) showed the effects of IT on the Dpt-induced bronchial early- and late-phase reaction (LPR) and reported that the clinical improvement was significantly associated with a preferential reduction of LPR.

The aim of the present study was to verify the effects of IT with Dpt allergen on skin prick test (SPT) sensitivity, bronchial hyperresponsiveness (BHR), and symptoms in a cohort of asthmatic children resident for at least 12 months at high altitude in an environment practically allergen free (6).

Material and methods

Patients

Twenty-three Dpt-sensitive children, 19 boys and four girls, aged 7–14 years (mean $\pm$ SD, 10.8 ± 2.0 years) with a diagnosis of moderate to severe asthma, according to the definition of the American Thoracic Society (7), were recruited to the study during two consecutive years. All the children presented immediate hypersensitivity to Dpt, evaluated by SPT, a positive (score ≥ 3) RAST for Dpt (Pharmacia,

Phadebas RAST), and a positive history of clinical sensitivity to HDM. They lived for 12 consecutive months in the residential house for asthmatic children, Istituto Pio XII, at Misurina in the Italian Alps, 1756 m above sea level, where the mean level of HDM antigens is class 0 by the guanine test (8). Furthermore, because of the Alpine weather conditions, the ground in Misurina is usually covered with snow from November to May, reducing to a short summer period the presence of pollens in the atmosphere (unpublished data). No contact with pets was allowed during the study period.

At admission to the study, all of the children were receiving either beclomethasone dipropionate 200 µg t.i.d. or sodium cromoglycate in addition to p.r.n. inhaled salbutamol. β₂-sympathomimetic drugs and sodium cromoglycate were stopped 24 h before challenge. Inhaled steroids were gradually withdrawn during a few weeks after arrival in the Alpine environment. No patient received oral steroids during the study period.

Study design

The patients were treated with parenteral IT (12/23) using a standardized Alpare Dpt extract or placebo (11/23) in a double-blind, placebo-controlled trial. The Alpare treatment solutions were at concentrations of 10, 100, and 1000 units/ml according to the manufacturer's indications (Table 1). To evaluate the effects of the IT, we performed SPT for Dpt, histamine bronchial challenge, and Dpt allergen challenge at admission to the study, after 6 months, and after 12 months from the beginning of the treatment. During the study period, the patients kept diaries in which they recorded their chest symptoms

(coughing or wheezing attacks) during the night and the day and the medication requirement.

Prick tests

The patients were first tested with a range of common allergens (Dpt, grass pollen, *Parietaria judaica*, birch, dog, cat) to verify the degree of their atopic sensitivity. All the patients were allergic to Dpt, four had a positive response to cat, three to dog, 12 to grass, three to *Parietaria*, and two to birch, several patients reacting to more than one allergen. Seven patients reported having allergies to pets, but none had contact with animals during the trial. The children participating in the study were tested in duplicate, one in each arm, with 20, 40, 100, 200, 400, and 1000 activity units by RAST (AUR)/ml of Alpha-base for Dpt and positive and negative control on entry, after 6 months, and at the end of the study. All tests were done at the same time in each patient in the afternoon by the same investigator. Fresh solutions of prick extracts were made each day of testing, starting from the same batch, by dissolving lyophilized Alphabase mite 2000 AUR/vial in Coca's glycerol solution to give the required final concentrations. All SPT were done with DHS-standardized lancets. After exactly 15 min, wheals were outlined with a ballpoint pen, and the outlines were transferred to report forms with adhesive tape in order to calculate the wheal surface.

BHR evaluations

BHR was evaluated by histamine and Dpt bronchial challenge within a few days of admission to the residential house, after 6 and 12 months, and at the end of the study. Each set of tests consisted of histamine challenge followed the day after by specific Dpt bronchial provocation. All patients used the same nebulizer for all the tests.

Histamine bronchial challenge

The histamine bronchial challenge was performed by the method of Cockcroft et al. (9). In brief, the patients inhaled for 2 min, from a Wright nebulizer with an airflow of 8 l/min, histamine acid phosphate at 5-min intervals in doubling concentrations, from 0.03 to 32 mg/ml. FEV₁ was evaluated 30 s, 90 s, and 3 min after each inhalation, and the best of three determinations on each occasion was used. Inhalations continued until the FEV₁ fell by 20% or more below the postsaline value. The histamine concentration required to produce a 20% fall in FEV₁ was recorded (PD₂₀-FEV₁) by extrapolation between the last two points on the dose-response curve above and below 20% fall in FEV₁ (10).

Table 1. Immunotherapy dosage schedule

Dose number	Vial concentration	Dose in units	Volume injected (ml)	Interval between injections (weeks)
1	10 units/ml	1	0.1	
2	10 units/ml	2	0.2	1
3	10 units/ml	4	0.4	1
4	10 units/ml	8	0.8	1
5	100 units/ml	10	0.1	1
6	100 units/ml	20	0.2	1
7	100 units/ml	40	0.4	1
8	100 units/ml	80	0.8	1
9	1000 units/ml	100	0.1	1
10	1000 units/ml	200	0.2	1
11	1000 units/ml	400	0.4	1
12	1000 units/ml	800	0.8	1
13 and following	1000 units/ml	800	0.8	4

HDM bronchial challenge

The Dpt specific bronchial challenge was performed as previously described (11), by administering a standardized lyophilized Dpt extract (Alphabase, Dome Hollister-Stier, Slough, UK). The children inhaled with a Wright nebulizer (airflow of 8 l/m) normal saline followed at 5-min intervals by Dpt in fivefold concentrations from 8 to 1000 AUR/ml. The inhalations were stopped when the FEV₁ fell by 20% or more below the postsaline value. The allergen concentration (AUR/ml) required to produce PD₂₀-FEV₁ was recorded. After the mite challenge, to evaluate the late asthmatic response, we measured the pulmonary function values every hour for 10 h. The lowest FEV₁ and PEFR between 4 and 10 h after the challenge was recorded and expressed as a percentage of the prechallenge result.

Statistical analysis

The data were transformed as necessary to normalize them, usually by logarithmic transformation. The computer package SAS was used for all the analyses. The data were analyzed by ANOVA analysis of variance and by paired *t*-test.

The study was approved by the local hospital committee, and all the children's parents gave written informed consent.

Results

Only two children did not complete the trial, one on placebo and the other on active treatment. There was no evidence that these withdrawals were related to the treatment.

Side-effects

All but two patients undergoing Alpare treatment were maintained at the recommended dose: one patient at 0.6 ml of the top-strength solution (600 units) and the other at 0.4 ml at top strength (400 units). No systemic reactions were observed. There were six large delayed local reactions (swelling > 5 cm), all from four Alpare patients, and 19 small delayed local reactions, 16 in the actively treated group and three in the placebo group. The percentages of large and small delayed reactions in comparison to the total number of injections during the treatment were 3% and 7%, respectively.

Skin prick tests

The SPT results, expressed as the mean $\pm$ SD of log wheal area + 1, are shown in Fig. 1. At admission to the study, there was no evidence of a difference

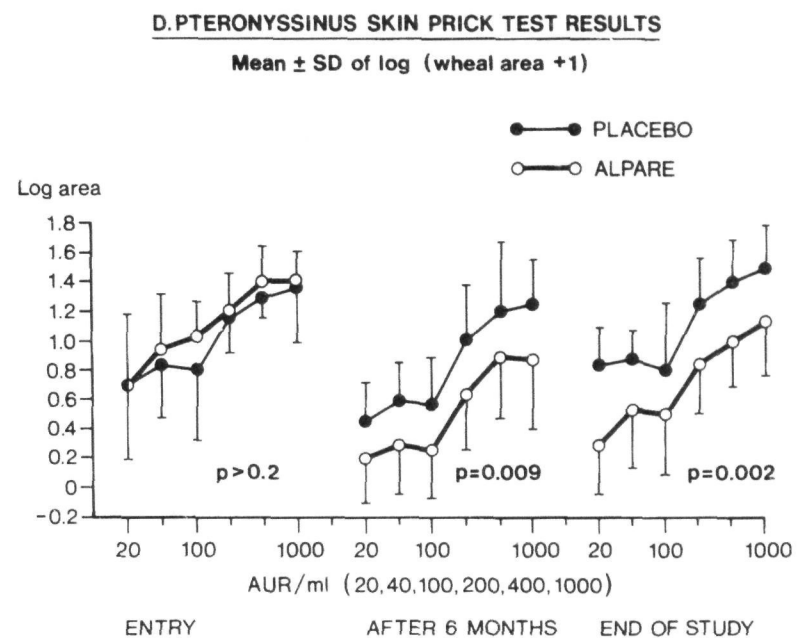


Fig. 1. Results of SPT for Dpt expressed as mean $\pm$ SD of log wheal area + 1.

between the treatment groups and there was a constant increase in log area with log dose ($P < 0.001$). After 6 months of treatment, the Alpare group had smaller wheals than the placebo group in all the concentrations tested ($P = 0.009$).

At the final assessment, the difference between the treatment groups in the wheal measurements for all the concentrations was significant ($P = 0.002$), and a straight line relationship between concentration and wheal area ($P = 0.001$) was observed.

Lung function measurement

The pulmonary function levels obtained before the challenges showed a significant difference between the treatment groups from the first observation (Table 2). This difference, in which the placebo group

Table 2. Prechallenge lung function tests

		FEV ₁		PEFR	
	Day	Mean	SD	Mean	SD
Alpare					
On entry	1	90.9	11.4	107.1	14.3
	2	94.8	10.9	107.1	10.8
6 months	1	93.8	9.9	108.7	16.7
	2	94.2	10.8	105.3	12.7
12 months	1	90.9	9.8	103.4	15.7
	2	90.5	9.3	107.4	14.6
Placebo					
On entry	1	84.5	7.4	98.8	14.9
	2	83.4	8.1	93.2	15.7
6 months	1	87.9	7.0	103.7	11.5
	2	85.5	9.0	95.6	14.2
12 months	1	85.1	86.6	102.2	10.0
	2	83.5	8.0	99.1	9.6

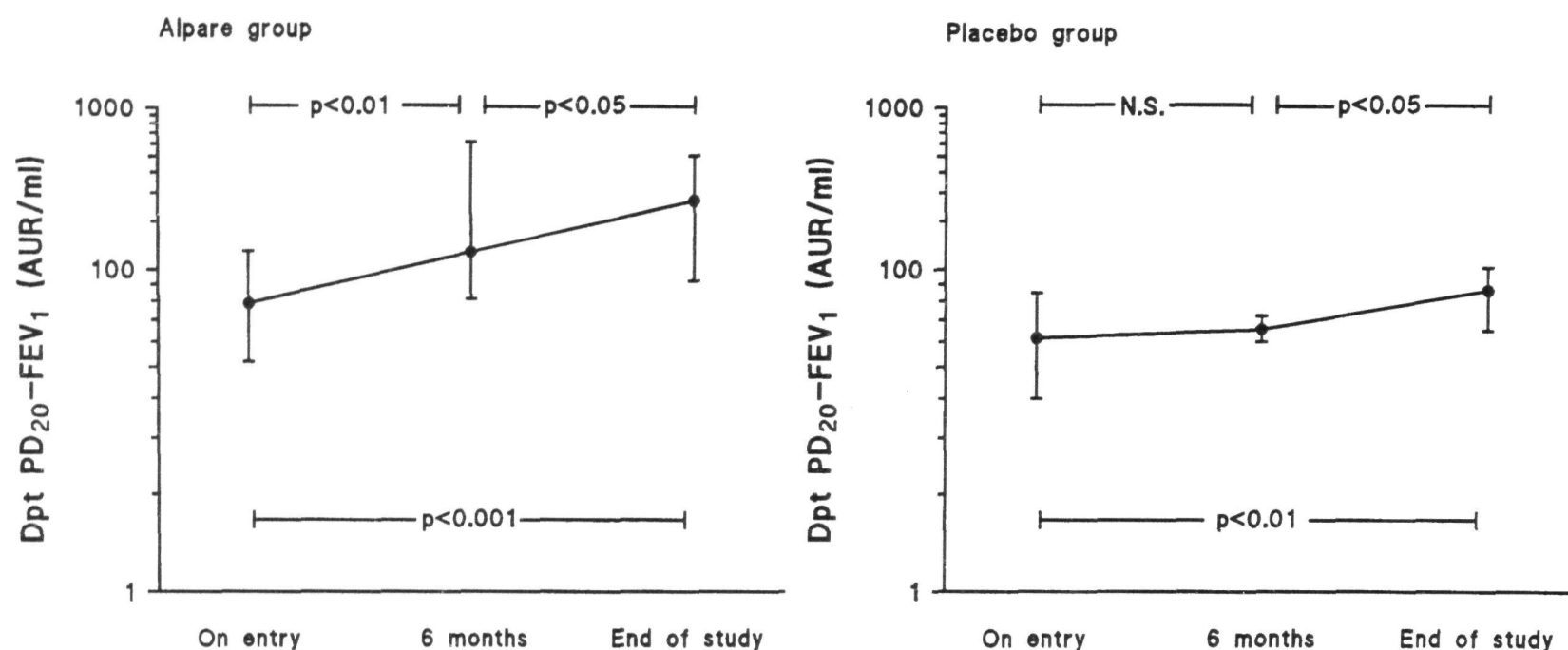


Fig. 2. Values of Dpt PD₂₀-FEV₁ in activity units by RAST (AUR)/ml expressed as geometric means, with range in bars in logarithmic scale.

had a consistently lower FEV₁ ($P = 0.043$), but not PEFR, was sustained to the end of the study. However, there was no change in lung function within each group during the trial.

Bronchial provocation tests

The results of the Dpt challenge are shown in Fig. 2. Analysis of the results showed a clear improvement in Dpt-PD₂₀ during the trial both in the active ($P < 0.001$) and in the placebo treatment groups ($P < 0.01$). The Alpare-treated patients could tolerate higher concentrations of mite on entry, and this difference was sustained throughout the trial. When the data at 6 and 12 months were adjusted for the

entry PD₂₀, the difference between the treatments disappeared. No difference was observed between the 6- and 12-month results. These results imply that the two treatment groups improved in parallel during the trial. The results of the late responses after the Dpt challenge are shown in Fig. 3. There was no difference between treatments in either FEV₁ or PEFR. There was a slight improvement in FEV₁, though it was not apparent in PEFR, which was maintained to the end of the study ($P < 0.01$). No difference was observed between the groups.

The results of the histamine BPT are summarized in Table 3. Analysis of the data showed no difference between the treatment groups.

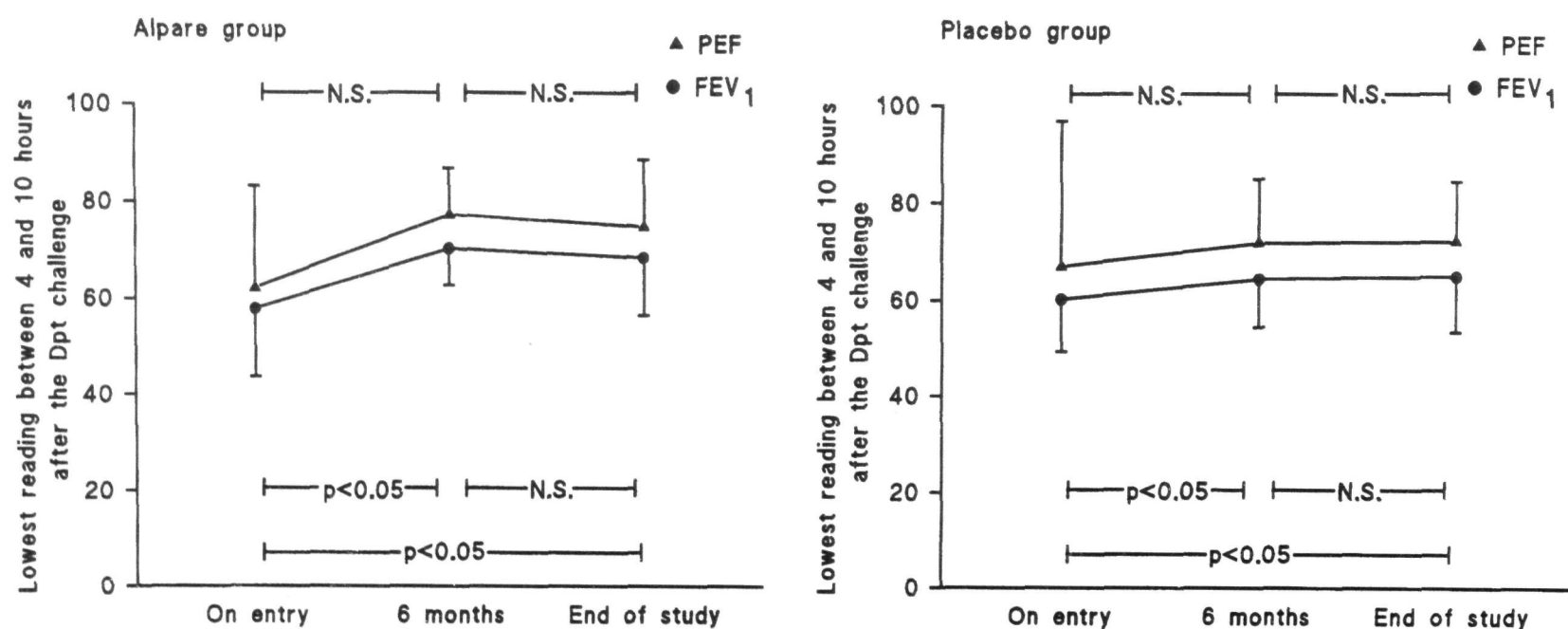


Fig. 3. Lowest readings (mean $\pm$ SD) between 4 and 10 h, evaluated by FEV₁ (circles) and PEF (triangles) after Dpt bronchial challenge.

Table 3. Histamine bronchial challenge PD₂₀-FEV₁ (mg/ml)

	Median	Interquartile range
Alpare		
On entry	1.87	1.29–4.76
6 months	3.80	1.43–13.6
12 months	3.79	2.16–8.44
Placebo		
On entry	1.62	0.55–2.45
6 months	2.59	2.15–4.28
12 months	2.63	1.35–4.8

Diaries

In the diaries that the patients kept from their admission into the study until their last assessment, very few symptoms were recorded. Out of all the morning and evening observations, the recorded chest symptoms were less than 2%, and there were more at the start than at the end of the trial. Few patients had symptoms or used medications, and no difference was observed between the groups. No symptom exacerbation was noted during the pollen season in the patients sensitized to pollens. At the end of the study, none of the subjects was receiving regular treatment other than specific IT.

At the beginning and at the end of the study, the patients were asked to evaluate their asthma. All the patients in the Alpare group felt that their asthma had improved, but only a minority of placebo patients did so (three patients out of 12).

Discussion

From our study population, we can come to no conclusion about the clinical benefits of IT in asthmatic children. In fact, after a 12-month treatment with Dpt IT, we could not observe any improvement in the BHR in comparison to the placebo controls. The prolonged stay at high altitude, as previously described (6), decreased the BHR in both groups; the beneficial effect was observed when the children were tested with either histamine or HDM extract.

However, in the actively treated group, we were able to demonstrate a significant improvement in HDM immediate hypersensitivity as evaluated by SPT with all the concentrations tested. This effect was apparent after 6 months and was sustained to the end of the study. Despite the few chest symptoms recorded by the patients throughout the study period, the children treated with Alpare felt better at the end of the trial, as compared to only 30% of the placebo-treated subjects.

In this study, we have also demonstrated the safety of Alpare HDM IT. Indeed, we observed only 3%

of large (> 5 cm) local reactions and 7% of small (< 5 cm) local reactions. No anaphylactic reactions occurred during the trial. In only two of the patients in active treatment was it necessary to reduce the maintenance dose because of persistence of local reactions with the highest dose.

We have previously demonstrated (6) that a prolonged stay at high altitude leads to a decrease in asthma symptoms and medical requirements as the consequence of reduced exposure to aeroallergens, especially HDM. Repeated dust collections from the mattresses of the children living in the residential house Istituto Pio XII always failed to show detectable levels of mite antigen (8) because of climatic conditions such as the low temperature and the low humidity of the Alpine environment, which are unfavorable to mite growth. At a high altitude, other factors can modulate the BHR: the levels of pollution and cigarette smoking are low, and the colder climate is associated with reduced incidence of airway infections. The results of this study confirm that in both groups there was an improvement in BHR while they were resident at the high-altitude institute, and this improvement was even greater than that obtained from the HDM IT. However, the late reaction incidence was only slightly reduced by the active treatment and by the stay at high altitude, probably because a longer period of IT and/or stay at high altitude would be necessary to achieve better results.

In a previous study, Price et al. were able to demonstrate that IT attenuated the late asthmatic reaction to allergen inhalation in children (5), but no change was observed in the immediate reactions in lung and skin (5). In the present study, we obtained a significant reduction in the skin immediate hypersensitivity tested by SPT, as a general measure of mast cell reactivity to the offending allergen. There is strong evidence that this parameter improves with successful IT (12, 13), although it might be considered less important to the clinical state. In a position paper, the European Academy of Allergology and Clinical Immunology suggested that SPT should be monitored during IT (14, 15). Furthermore, the other papers mentioned provide evidence of good correlation between changes in sensitivity in the target organs and the skin (12, 13). We were unable to demonstrate an effect of IT on the BHR, probably as a result of the short period of the treatment and the beneficial effect of the stay at high altitude. However, we observed no increase by IT of the bronchial histamine sensitivity, as shown by previous studies (4, 16) and in contrast to an observation by Murray et al. (17).

The reports of fatal reactions due to IT (18) and the relatively high incidence of systemic reactions observed in other studies (19), ranging from 5% to

35%, suggest that this therapy may present some risks. Careful supervision of the dose and procedure and facilities for resuscitation are essential. Therefore, the disease must be severe enough to justify this possible risk. However, in the present study, conducted with the dosage schedule suggested by the manufacturer's indications, the incidence of serious reactions was extremely low, with the occurrence of a minimal percentage of minor local reactions.

We conclude that IT for Dpt could alleviate allergic asthma in childhood by reducing immediate hypersensitivity to the antigen. No conclusion can be drawn about the clinical benefits, probably because a longer period of treatment may be required to obtain a significant positive effect on the BHR. In our population, the improvement by allergen avoidance at high altitude was positively greater, but, because this beneficial effect is lost after only 3 months of residence at sea level in the presence of the offending allergen (20), we suggest that the role of IT should be to support and enhance the positive effect of effective allergen avoidance.

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