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Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract

I. In vivo and in vitro parameters after a short course of treatment

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*Dermatophagoides pteronyssinus (Dp) is the major allergen in allergic asthma in France. Standardized and lyophilized Dp extracts are available, and their effectiveness after a short course of rush immunotherapy was examined in a placebo-controlled, double-blind study. Twenty patients received the Dp standardized extract, and 10 other patients received a placebo extract. Before and 7 weeks after rush immunotherapy, in vivo and in vitro parameters were examined. Bronchial provocation tests performed in a standardized manner demonstrated that a provocative dose causing a 20% fall in FEV₁, a 25% fall in maximum mild expiratory flow rate, a 25% fall in maximum flow when 50% of the forced vital capacity has been expired, and a 35% fall in specific airway conductance were significantly ($p < 0.005$ to $p < 0.01$, Wilcoxon W test) improved in the treated group and remained unchanged in the placebo group. Skin test titration demonstrated that patients placed in the treated group had a significant ($p < 0.001$, Wilcoxon W test) decrease of both end point titer and size of the largest wheal. No significant difference was observed in the placebo group. Serum Dp-IgE did not vary significantly in either group. Serum Dp antigen P1-IgG was significantly ($p < 0.001$, Wilcoxon W test) increased in the treated group and slightly increased in the placebo-treated group. This study demonstrated that a Dp standardized extract administered by a rush protocol elicits a rapid and significant immune response and leads to a significant protection of the patients. (*J ALLERGY CLIN IMMUNOL* 76:734-44, 1985.)*

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Abbreviations used

BPT:	Bronchial provocation tests
BU:	Biologic units
CIE:	Crossed immunoelectrophoresis
Dp:	<i>Dermatophagoides pteronyssinus</i>
Df:	<i>Dermatophagoides farinae</i>
Dp-IgE:	Serum <i>Dermatophagoides pteronyssinus</i> -specific IgE
Dp-IgG:	Serum <i>Dermatophagoides pteronyssinus</i> -specific IgG
HSA:	Human serum albumin
MMEF:	Maximum mild expiratory flow rate
P1-IgG:	Serum <i>Dermatophagoides pteronyssinus</i> antigen P1-specific IgG
PD:	Provocative dose
RI:	Rush immunotherapy
SG _{aw} :	Specific airway conductance
V _{max50} :	Maximum flow when 50% of the forced vital capacity has been expired
PD ₂₀ , PD ₂₅ , PD ₃₅ :	Provocative dose causing a 20% fall, 25% fall, and 35% fall
IEF:	Isoelectric focusing
WHO IRP:	World Health Organization International Reference Preparation

Dp is the most common house dust mite in Western Europe and certainly one of the most common allergen around the world. Trials with Dp or Df have been performed since 1970. Many conclusive trials have been produced in children or adult subjects¹⁻¹⁰ to treat asthma and/or rhinitis by use of either aqueous or tyrosine-adsorbed extracts, but most had to be prolonged during long periods of time to demonstrate efficacy. In most trials the differences between the placebo-treated and the extracts-treated groups were not very large. By contrast, other trials did not report any benefit from house dust-mite immunotherapy.¹¹⁻¹⁴ In fact, the evaluation of the clinical benefits of mite immunotherapy appears to be very difficult, since the amount of mite allergens inhaled by the patients varies widely according to their home environment¹⁵ and since none of the previous studies were performed with characterized and standardized extracts.

The mechanisms by which immunotherapy with Dp extracts operates are still unclear. Dp-IgG usually increases,^{3, 4, 16, 17} but it was not possible to correlate

Dp-IgG with clinical benefits. Skin tests did not vary in the studies that examined this parameter,^{1, 3, 11} and Dp-IgE varies widely from patient to patient.^{1, 4, 11, 17}

Many protocols of immunotherapy have been proposed. In 1930 Freeman¹⁸ described three different protocols according to the frequency of allergen injections and the increment of doses: weekly, daily or "intensive," and rush requiring five to six injections per day. In this study Freeman¹⁸ pointed out the rapid effectiveness of the treatment. RI has never been performed in Dp allergy, since some severe side systemic reactions might occur with unstandardized allergens. In 1980 in France, standardized and lyophilized allergens were made available, and it was therefore possible to start an RI program with Dp allergens.

Thirty patients with asthma only sensitive to Dp had RI with either placebo or a standardized Dp extract. Patients were assessed before RI and 7 weeks after RI by means of in vivo (BPT and quantitative skin tests) and in vitro (Dp-IgE and Dp-IgG) parameters in order to determine the early effects of RI with Dp.

MATERIAL AND METHODS

Patients

Thirty patients (21 men) ranging in age from 18 to 42 years (mean $\pm$ SD, 28.8 $\pm$ 5.7 years) were recruited from the Clinic of Respiratory Diseases of Montpellier. They had to fulfill the following criteria: (1) a definite history of perennial allergy characterized by asthma and eventually rhinitis (Asthma was defined by either a reversible bronchoconstriction after the inhalation of β -agonists or a significant sensitivity to metacholine. All patients also had a BPT with Dp to confirm their sensitivity.), (2) a positive prick test with a Dp standardized extract according to the criteria used in our clinic,¹⁹ (3) a Phadebas RAST (Pharmacia AB, Uppsala, Sweden) of class 3 to 4 in the Pharmacia scoring system, and (4) no previous mite or house dust immunotherapy.

After informed consent was obtained, the patients were allocated at random to one of the two treatment groups. Twenty patients were treated with the Dp extract, and 10 were treated with the placebo in a double-blind fashion.

Extract

The same lyophilized and standardized Dp extract was used for both immunotherapy and tests. It was prepared by the Laboratoire des Stallergènes (Fresnes, France) according to the proposals of the allergen subcommittee of the International Unions of Immunological Societies. Dp were grown on medium containing equal parts of *Saccharomyces cerevisiae* and human dander. After a 3-week culture mites were carefully isolated from the culture medium, and somatic and metabolic allergens were extracted in ammonium bicarbonate (0.125 mol/L, pH 8.0). Extraction at +4° C

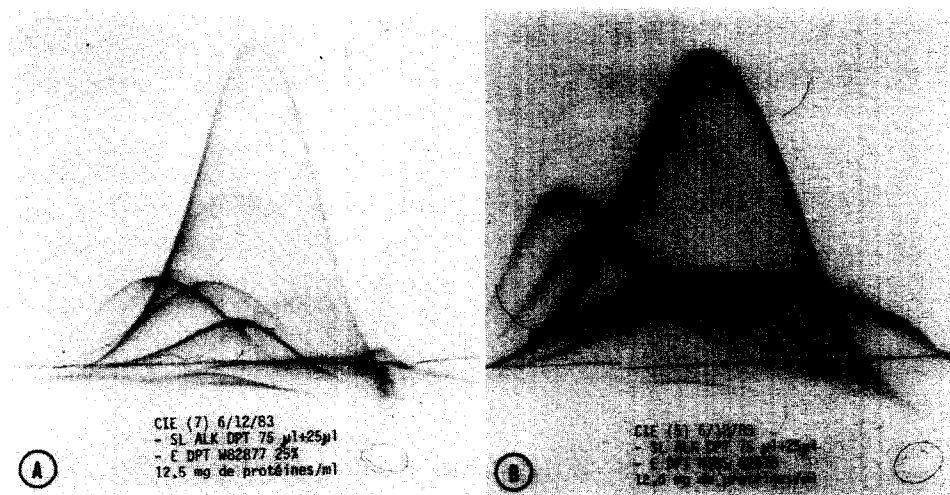


FIG. 1. CIE. **A**, The internal standard W82877. **B**, WHO IRP NIBS 82518. The same ALK (Copenhagen, Denmark) rabbit antiserum was used for both CIE. The same amount of proteins (12.5 mg/ml) was used for both CIE.

was continued for 24 hours with continuous stirring, and the extracting liquid was subsequently passed through a sterilizing grade filter. An aliquot was then removed for control testing, and the bulk of the extracting solution was frozen. A control of the potency of the removed aliquot was performed by RAST inhibition and IEF and compared with the internal standard that had been controlled by RAST inhibition, IEF, CIE, and skin test titration. The internal standard was compared with the forthcoming WHO IRP by means of CIE and RAST inhibition and was demonstrated to contain all antigens present in the IRP (Fig. 1). The extracts contain the major Dp antigen (antigen P1). A given amount of the extracting solution was then distributed into vials that were subsequently lyophilized. A control of the final product by RAST inhibition and IEF was then performed. The freeze-dried extracts were stored at $+4^{\circ}\text{C}$.

The strength of the Dp extract was expressed in biologic units that were defined by comparing skin tests induced by a 9% codeine phosphate positive control with serial dilutions of the Dp extract. An arbitrary potency of 1000 BU/ml was added to the strength of allergen extract that elicited a median wheal size identical to that provoked by the positive control solution when both solutions were tested in parallel in 30 highly allergic subjects.

The shelf life of DP extracts freshly reconstituted either in saline containing 0.02% of HSA or in 50% glycerol was assayed.²⁰ It was observed that extracts preserved in 50% glycerol had lost more than a quarter of their total activity within 2 months. Extracts preserved in HSA had an even shorter half life. For these reasons extracts that were used for skin test titration were discarded 2 weeks after reconstitution. Extracts used for immunotherapy were reconstituted in saline containing 0.02% of HSA and discarded 1 week after their reconstitution. When the maintenance dose was reached, single dose vials were used.

Treatment

The RI consisted in a rapid increase of allergen doses that made it possible to reach the maintenance dose in 3 to 6 days. The maintenance dose was estimated from preliminary experiments that demonstrated that (1) skin test reactivity was decreased in almost all patients for a single dose of 2000 BU, and (2) more than 50% of the subjects had a systemic reaction when a dose of more than 4000 BU was administered in a single shot.²¹ A 3-day protocol was proposed (Table I). After the maintenance dose was reached, the dose was administered every week.

When a local reaction occurred, the protocol was unchanged. By contrast, when a systemic reaction was observed, the next dose was reduced by half.

BPT

BPT were only performed when factors that may affect the tests were absent.²²⁻²⁴ Thus BPT were not performed in subjects who had suffered from an asthma attack within the preceding 2 weeks or from any bronchial infection in the previous month. Beta₂-agonists, theophylline, and antihistamine were withheld from each patient for 24 hours before testing. Beclomethasone dipropionate, atropine derivatives, and cromoglycate were stopped for 3 days before testing; ketotifen or oral imipramine was stopped 2 weeks before the tests. Patients receiving oral or parenteral corticosteroids were not included in the study. Patients who developed spontaneous wheezing when medications were withheld were also excluded from the study. Finally, the baseline pulmonary function test of the patient (FEV₁) was required to be within 20% of predicted values.

Bronchial challenges were performed by use of the standardized and lyophilized extracts that were freshly reconstituted with phosphate-buffered saline on the day of each test. Saline or allergen extracts were delivered by use of the

TABLE I. RI protocol

	Hours	BU	Weight by volume	
Day 1	9	30	0.1 ml	1/10,000
	9½	150	0.5 ml	1/10,000
	10	300	0.1 ml	1/1,000
	11	600	0.2 ml	1/1,000
	14	1200	0.4 ml	1/1,000
Day 2	9	1800	0.6 ml	1/1,000
	14	2400	0.8 ml	1/1,000
Day 3	9	3000	0.1 ml	1/100
Weekly injection		3000	0.1 ml	1/100

TABLE II. Comparison between allergen and placebo-treated groups

	Group I (allergen)	Group II (placebo)	Statistical difference*
Age			
Limits (yr)	18 to 41	19 to 42	NS
Mean ± SD	29.1 ± 4.8	27.1 ± 5.9	
Sex			
M	13	7	NS
F	7	3	
Asthma			
Duration of symptoms (yr)	6.3 ± 4.6	9.1 ± 3.3	<0.05
Severity†	2.9 ± 1.2	3.3 ± 1.2	NS
Skin test end point‡	4.7 ± 1.2	4.7 ± 0.9	NS
RAST (% binding)	15.9 ± 9.6	20.1 ± 8.2	<0.05
FEV ₁ §			
Limits	-18 to 22%	-17 to 18%	NS
Mean ± SD	-4.7 ± 13.1	0.1 ± 11.2	
PD ₂₀ in FEV ₁			
Mean ± SD (µg)	96.3 ± 82.1	79.1 ± 93.6	NS

NS = not significant.

*Statistical analysis by Mann-Whitney U test.

†According to Charpin et al.¹⁶‡Log₃ allergen dose.

§Percent from predicted values.

Pigłowski's apparatus.²⁵ Aerosols were delivered by a modified Tiffeneau nebulizer that delivers a reproducible output of 0.22 L/min with a particle size of 2 to 4 µm in aerodynamic mass median diameter. The reproducibility of the generation of aerosols is controlled by a constant aerosolization pressure that is determined at ±0.5%. The nose was clipped, and aerosols were inhaled via a mouth piece by tidal breathing. The Pigłowski's apparatus recorded the amount of air inhaled by the subject. After the concentration of aerosol is known, it is therefore easy to calculate the amount of extract inhaled. The bronchial response to aerosol challenge was assessed by measuring in a total body plethysmograph SG_{aw}, FEV₁, MMEF, and V_{max50} with SG_{aw}

being first recorded when the subjects were breathing at tidal volume.

All BPT were started between 8 and 9 A.M. Inhalation tests were performed as follows: Baseline tests of the pulmonary function were first recorded. If the FEV₁ was within 20% of the predicted values, BPT were done. The diluent for the allergen solutions (phosphate-buffered saline) was delivered for 3 minutes. SG_{aw}, FEV₁, MMEF, and V_{max50} were serially measured before inhalation and 10, 15, 20, 30, and 45 minutes after the control or the allergen inhalation. Allergen solutions were administered in doubling concentration from 10 to 1280 µg. Inhalations were continued until a PD₂₀ in FEV₁ and a PD₃₅ in SG_{aw} were obtained

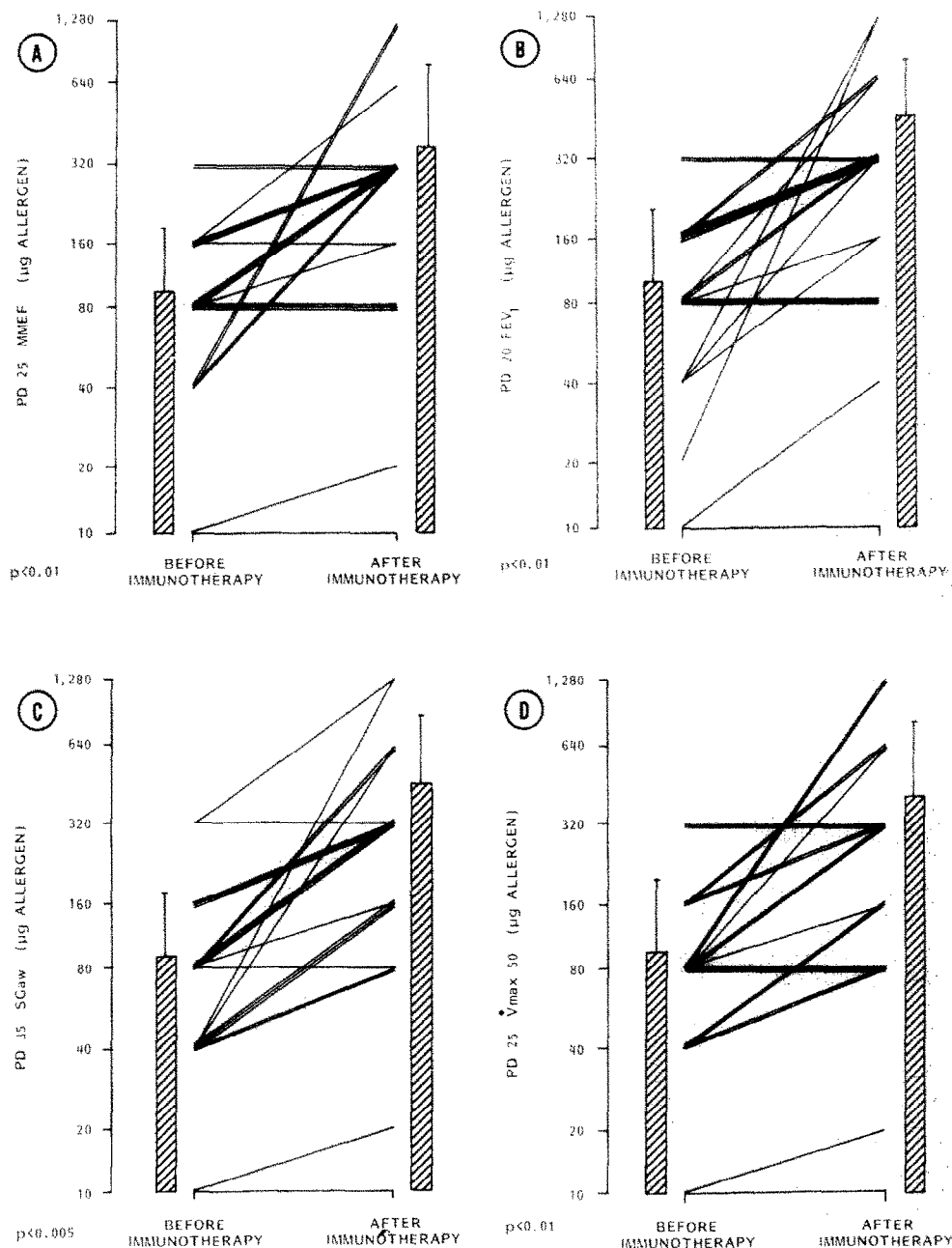


FIG. 2. Evolution of the PD eliciting a specific bronchial response before and after immunotherapy in 20 patients who had a rush protocol with a standardized Dp extract. Statistical analysis by Wilcoxon W test.

or until the maximal concentration had been administered. The late-phase reaction induced by allergen was also sought, and after a positive early phase reaction, the patient was monitored every hour for the following 8 hours.

Skin test titration

Prick tests were performed by the Stallerpointe²⁰ (Stallergènes Laboratories) derived from the Morrow Brown standardized needle. This device makes it possible to stan-

dardize the penetration of allergens into the epidermis and is very reproducible, at least in subjects more than 3 years of age. All patients were tested with three positive control solutions (histamine hydrochloride, 2 mg/ml and 10 mg/ml, and codeine phosphate, 9%). Patients allergic to Dp were tested with Dp extract. Patients allergic to both pollens and Dp were tested with Dp and the relevant allergen. Three-fold dilutions for allergen extracts were tested from 30,000 BU to 1.5 BU. The highly diluted extracts were chosen in

order to investigate possible anomalous effects of allergen concentration.²⁶ To avoid possible circadian differences in skin reactivity, skin tests were done between 9:00 A.M. and 11:00 A.M. The same investigator conducted all the studies. None of the subjects had received any antihistamine, systemic corticosteroids, or any form of theophylline for at least 72 hours before the tests, or any ketotifen or imipramine for at least 2 weeks because these drugs can affect the production of the wheal-and-flare reaction. The wheals induced by the allergen extracts were recorded 15 minutes after the tests, and wheals elicited by the positive control solutions were recorded 10 minutes after the tests by the Scotch tape technique. The end point skin titration was calculated from the strength of an extract that produced a wheal size of >75% of the positive control solution. The size of the largest wheal was also recorded.

Dp-IgE

Dp-IgE was measured by the RAST. RAST discs were prepared according to the method of Ceska et al.²⁷ with Dp extracts of the same origin as those used for both immunotherapy and skin testing. All serum samples from a given patient were diluted 1/5 and tested in duplicate at the same time to avoid possible intertest variation. Mean results from the duplicates were used to calculate percentage binding by dividing the counts per minute bound to the test disc by the total activity added.

Dp-IgG

Dp-IgG was initially titrated with a solid-phase radioimmunoassay with *Staphylococcus* protein A to reveal IgG, but this assay was demonstrated to be less efficient than Dp antigen P1-IgG so that only the latter was used in this study. P1-IgG was titrated by Dr. B. Mitchell (Clinical Research Center, Division of Immunological Medicine, Harrow, Great Britain) according to the technique of Chapman and Platts-Mills.²⁸ This technique is based on a double antibody radioimmunoassay with ¹²⁵I-radiolabeled antigen P1. The results are expressed as binding activity unit per milliliter against antigen P1.

Protocol

All patients were submitted to a first BPT and then randomly assigned to RI with either allergen (group 1) or placebo-treated (group 2). The same day blood was drawn for the titration of Dp-IgE and Dp-IgG, but the titration of these parameters was only performed at the end of the protocol to analyze all samples at the same time. RI was started 1 week after the BPT, and skin tests were performed just before starting RI. Seven weeks after the first BPT, a second identical BPT was performed; skin tests and Dp-IgE and Dp-IgG were also studied.

Statistical analysis of the data

Data were analyzed by use of nonparametric statistical methods for group comparison. The Wilcoxon W, Mann-Whitney U tests, and Spearman's rank correlation coefficient test were used with a Snedecor computed program.

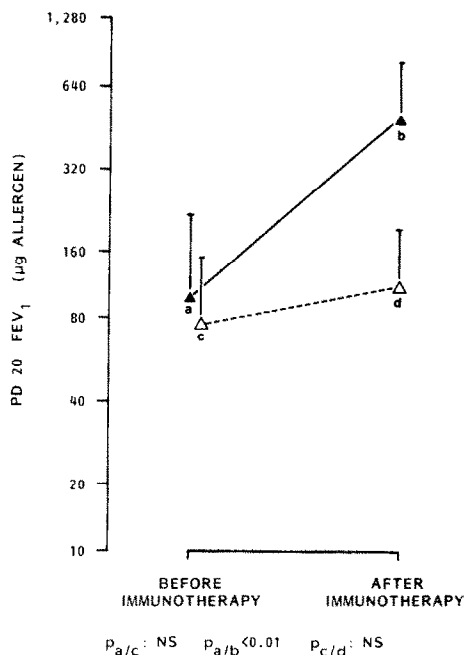


FIG. 3. Evolution of PD₂₀ in FEV₁ before and 7 weeks after RI with Dp (▲) or placebo (△). Statistical analysis by Wilcoxon W test (paired data) and Mann-Whitney U test (nonpaired data).

RESULTS

Homogeneity of RI and placebo-treated groups

The groups were compared on the basis of the age of the patients, symptoms, allergenic sensitivities, skin test reactivity, and Dp-IgE. Table II illustrates that the two groups did not differ significantly except for Dp-IgE that were significantly ($p < 0.05$) increased in the placebo-treated group.

Duration of the protocol and side reactions

RI with a standardized mite extract was performed in 20 patients in whom the maintenance dose of 3000 BU could be reached in 3 to 4 days. Four patients experienced a systemic reaction during the RI protocol, and adrenalin was required in three occasions. No systemic reaction occurred when patients were receiving maintenance immunotherapy. No systemic reaction occurred in patients placed in the placebo-treated group.

Pulmonary function before BPT

Deep inspiration did not elicit any change in pulmonary function tests performed before BPT.

Before and 6 weeks after RI, FEV₁ was measured and found to be similar in all but one of these selected patients. One of the patients placed in the placebo-treated group had an FEV₁ sharply decreased (65% of

TABLE III. Evolution of PD of mite extract before and after immunotherapy in patients treated with the same mite extract

	Improved	Equal	Worsened	Statistical difference
PD ₂₀ in FEV ₁	15	5	0	$p < 0.01$
PD ₃₅ in SG _{aw}	18	2	0	$p < 0.005$
PD ₂₅ in V _{max50}	15	5	0	$p < 0.01$
PD ₂₅ in MMEF	16	4	0	$p < 0.01$

Statistical analysis by Wilcoxon W test.

TABLE IV. Evolution of PD of mite extract before and after immunotherapy in patients treated with placebo

	Improved	Equal	Worsened	Statistical difference
PD ₂₀ in FEV ₁	2	5	2	NS
PD ₃₅ in SG _{aw}	3	4	2	NS
PD ₂₅ in V _{max50}	2	5	2	NS
PD ₂₅ in MMEF	2	5	2	NS

Statistical analysis by Wilcoxon W test; NS = not significant.

TABLE V. Nonimmediate reactions occurring in patients before and after immunotherapy

Nonimmediate reaction		Mite extract	Placebo
Before RI	After RI		
+	+	4	4
+	—	4	0
—	+	1	1
—	—	11	2

predicted values) after RI, and this patient could not have a subsequent BPT.

Evolution of BPT

The results of BPT before and after treatment are presented in Figs. 2 and 3 and Tables III and IV. It is obvious that the PD increased significantly ($p < 0.01$ to $p < 0.005$ by Wilcoxon W test) in patients who had RI with Dp extract but did not change in those treated with a placebo. The difference between placebo-treated and Dp-treated groups is also significant ($p < 0.01$ by Mann-Whitney U test) when PD₂₀ in FEV₁ after 7 weeks are compared.

A nonimmediate allergic reaction was observed in some patients (Table V), but there was no significant difference in the incidence of late reactions before and after treatment. A recurrent reaction was noticed in five patients before RI and in two subjects after RI with placebo.

Evolution of skin test reactivity

A decrease in Dp skin test reactivity was observed in 19 of 20 patients who had RI with Dp extract. Both the size of the largest wheal and the end point were significantly ($p < 0.001$ by Wilcoxon W test) reduced (Fig. 4). By contrast, codeine phosphate and both histamine positive control solutions did not vary. Four subjects who were also allergic to grass pollens had unchanged grass pollen skin tests.

There was no significant difference between quantitative prick tests performed before and after RI with placebo, although some patients' skin tests were not identical (Table VI). The positive control solutions elicited similar skin tests.

Evolution of Dp-IgE

Some variability was observed when individual results were compared, and it was found that most patients who had RI with Dp extract had a slight but not significant increase in Dp-IgE.

In the placebo-treated group, levels of Dp-IgE were very similar, and no significant mean variation occurred (Table VI).

Evolution of Dp-IgG

Dp-IgG increased in the treatment group in all patients, and mean results are significantly ($p < 0.001$ by Wilcoxon W test) increased after RI with Dp extract (Fig. 5). By contrast, patients placed in the placebo-treated group did not have any mean increase of

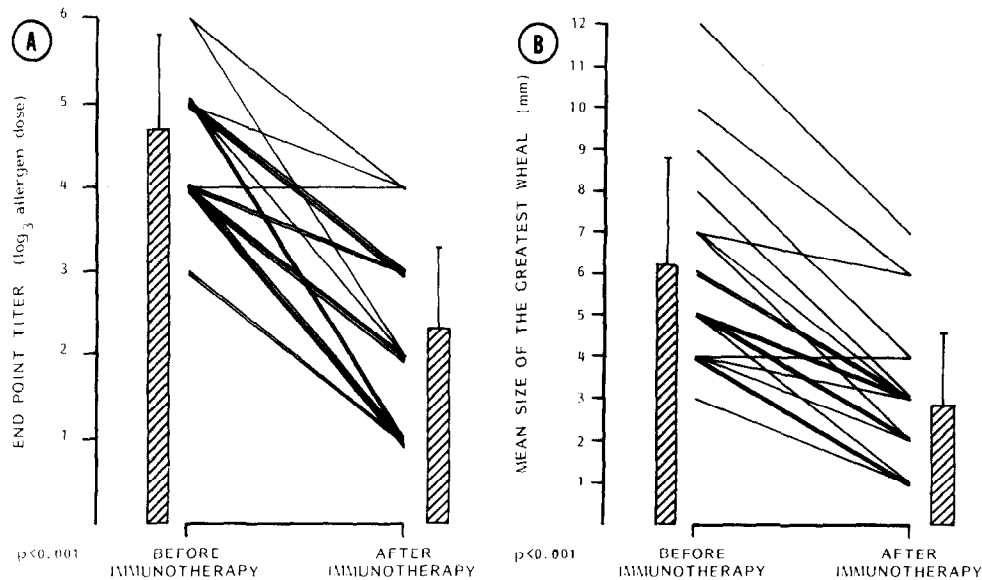


FIG. 4. Evolution of the skin test reactivity in 20 patients who had a rush protocol with a standardized Dp extract. Statistical analysis by Wilcoxon W test.

TABLE VI. Evolution of in vivo and in vitro parameters after immunotherapy

	Group I (allergen)	Group II (placebo)	P/groups I and II
Prick test end point			
Before immunotherapy	4.7 ± 1.2	4.7 ± 0.9	NS
After immunotherapy	2.3 ± 1.1	4.8 ± 1.3	p < 0.001
	p < 0.001	NS	
PD ₂₀ in FEV ₁			
Before immunotherapy	96.3 ± 82.1	79.1 ± 93.6	NS
After immunotherapy	432 ± 171	95.0 ± 99.8	p < 0.01
	p < 0.01	NS	
Dp-IgE (RAST binding)			
Before immunotherapy	15.9 ± 9.6	20.1 ± 8.2	p < 0.05
After immunotherapy	17.2 ± 10.4	19.7 ± 7.7	NS
	NS	NS	
Dp-IgG (binding units)			
Before immunotherapy	277 ± 476	343 ± 708	NS
After immunotherapy	3181 ± 1441	354 ± 632	p < 0.005
	p < 0.001	NS	

NS = not significant.

Dp-IgG, although two of them had a slight increase (Table VI).

DISCUSSION

The data presented in this study demonstrate that RI with a standardized Dp extract can be performed easily and elicits a rapid immune response and a protection of the patient within weeks after its initiation.

RI has been used for many years,¹⁸ but this method

became widely applicable only when standardized extracts became available. Pollen extracts^{29,31} and cat and dog danders have been used, and it was found that this type of immunotherapy elicited a rapid onset of protection. Standardized and lyophilized extracts represent a significant improvement in the diagnosis and treatment of allergic disorders, and, as stated by Aas,³² it is not immunotherapy as a therapeutic principle that should be put on trial but the quality of

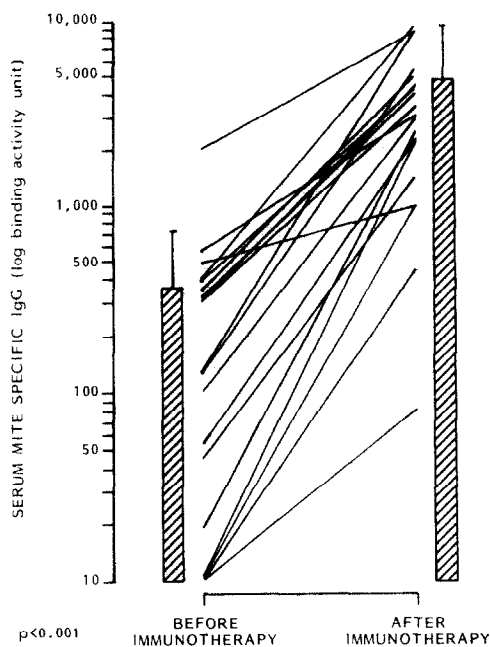


FIG. 5. Evolution of Dp-IgG in 20 patients who had a rush protocol with a standardized Dp extract. Statistical analysis by Wilcoxon W test.

allergen extracts used. In the present study the extract used is comparable to the forthcoming WHO IRP (Fig. 1).

The technique used to perform BPT did not vary from standardized procedures already reported.^{33, 34} Only asymptomatic patients who had normal or minimally subnormal baseline pulmonary function were included in the study; moreover, they were not receiving treatment that may affect the BPT. The delivery system is widely used in Europe and compares favorably with other devices.³⁵ The amount of aerosol was checked each month and was found to be constant throughout the study. Since the same batch of lyophilized material was reconstituted for every test, it can be assumed that the amount of allergen inhaled by the patients at tidal volume was comparable each time BPT was performed. The assessment of the bronchial response was first done by measuring SG_{aw} , since forced expiratory maneuvers may alter airway caliber,³⁶ although this was not the case in the patients presented herein.

Only carefully characterized patients were selected for this study, and this mode of selection may have introduced a bias in the study, since many patients with asthma and allergic to mites have permanently altered pulmonary function. Other biases may also be found in the study: (1) Immunotherapy and provocative challenges were performed with the same aller-

gen extract, as it is usually done,^{29, 31, 37-39} but, if one or several specific antigens are missing in the extract, some patients will not improve clinically, although the PD may be increased after immunotherapy. (2) BPT are very different from natural exposure, since allergens are in particular form and likely to be borne on 20 μ m fecal particles during natural exposure, whereas they are on 2 to 5 μ m droplets in BPT. It has also been calculated that the amount of allergen entering into the lung may be 2 ng/hr with natural exposure and 100 ng/min with BPT.⁴⁰ These considerations may not be critical for this study, since it is not the pathophysiologic mechanisms of BPT that were investigated but the difference in specific bronchial sensitivity after immunotherapy. The main issue for this study was the reproducibility of the test and not its similarity to the conditions of natural exposure.

Bronchial challenges performed before and after specific immunotherapy have been used to evaluate indirectly the clinical efficacy of allergen injections in house dust,⁴¹ house dust mite,^{10, 42} pollen,^{43, 44} and cat-induced asthma.³⁹ In all these studies bronchial challenges were performed after long-term immunotherapy. In the present study, after a few weeks of treatment, the PD eliciting a specific bronchial response was significantly greater in subjects undergoing specific immunotherapy with a Dp extract, whereas it is unchanged in subjects treated with a placebo. Although the improvement in PD may be related to clinical benefits, this may not be the case in all patients because of the quality of the allergen extract and nonspecific bronchial hyperreactivity.⁴⁵ Moreover, Warner et al.¹⁰ have postulated that the clinical improvement of Dp-allergic children, after specific immunotherapy, was more related to the disappearance of the nonimmediate response rather than a greater PD after bronchial challenges; however, in their study they used tenfold dilutions of allergen extracts instead of twofold dilutions used in the present one. This difference may account for a far greater number of late reactions, since they are often related to the severity of the immediate type I reaction.

The decrease in skin test reactivity was clear in the Dp group and was demonstrated to be allergen specific since skin tests induced by either positive control challenges or nonrelevant allergens did not change. Both the size of the largest wheal and the end point titer decreased significantly. In the study skin tests were performed in a standardized fashion with standardized extracts freshly reconstituted throughout the study so that they were absolutely comparable, as demonstrated in a preliminary study. These findings accord with previous studies in pollen immunother-

apy^{29,31, 46-50} and are in favor of a decrease in mast cell release of mediators similar to that observed after nasal provocation tests with allergens in patients undergoing immunotherapy.^{51, 52} The findings are also in agreement with decreased histamine release by leukocytes of allergic subjects after immunotherapy.⁵³⁻⁵⁵

Dp-IgE levels did not change significantly either in the Dp-treated group or in the placebo-treated group, as it is usually found in all immunotherapy procedures.

Dp-IgG levels significantly increased in the Dp-IgG-treated group, and this study contrasts with others performed with tyrosine-adsorbed Dp extracts^{2, 17} but not with the study of Chapman et al.¹⁶ The use of a very potent extract demonstrated that mites are highly immunogenic and can mount a significant IgG immune response in all patients. However, from this study it cannot be stated whether Dp-IgG may play a protective role in mite immunotherapy.

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