

Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract

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Specific immunotherapy is ineffective with unstandardized mold extracts. A double-blind, placebo-controlled study was performed in 24 patients (5 to 56 years of age) only allergic to Alternaria. The extract was standardized by isoelectric focusing, crossed immunoelectrophoresis, crossed radioimmuno-electrophoresis, RAST inhibition, and skin tests and contained allergen Alternaria major allergen a I and antigen B. Thirteen patients received the active treatment, and 11 received the placebo. Immunotherapy was started by a 2-day rush protocol; maintenance injections were administered for 1 year. The patient's self-evaluation of the treatment was significantly ($p < 0.001$) lower in the placebo-treated group. Global symptom-medication scores, including asthma and rhinoconjunctivitis, were significantly ($p < 0.005$) lower in the actively treated group. Nasal challenges with Alternaria extract were performed before immunotherapy and after 1 year of treatment. There was no difference in the placebo-treated group and a significantly ($p < 0.01$) increased mean provocative dose in the actively treated group. Skin tests were significantly reduced in the actively treated group. Specific IgG increased significantly in the actively treated group and were stable in the placebo-treated group. There was a significant correlation between nasal challenges and nasal symptom-medication scores ($p < 0.03$) or the patient's self-evaluation of efficacy ($p < 0.05$). This study demonstrated that patients only sensitized to Alternaria benefit from specific immunotherapy with a standardized Alternaria extract. (J ALLERGY CLIN IMMUNOL 1990;85:460-72.)

Specific immunotherapy has been demonstrated to be effective with extracts of ragweed and grass pollens, Hymenoptera venoms, house dust mites, or animal danders.¹⁻⁵ However, not all immunotherapy trials have resulted in efficacy of the extracts. Low doses of allergen extracts are ineffective⁶; therefore, it has been proposed to administer the "highest tolerated dose,"⁷ but this administration inevitably leads to a high incidence of side reactions. We have suggested methods to find an optimal dose that would result in clinical efficacy with an acceptable number of systemic reactions.⁸

Although immunotherapy with mold extracts has been performed for many years,⁹⁻¹² the final demonstration of its clinical efficacy is still missing. Because

Abbreviations used

Alt:	<i>Alternaria</i>
Alt a I:	<i>Alternaria</i> major allergen a I
BU:	Biological units
CBS:	Central Bureau Voor Schimmel Culture, Holland
Dpt:	<i>Dermatophagoides pteronyssinus</i>
IUIS:	International Union of Immunological Societies
RI:	Rush immunotherapy
PRU:	Phadebas RAST unit

of the variability and complexity of fungal organisms, mold allergy is particularly difficult to study.^{13,14} Commercial extracts differ considerably in their composition and allergenic potency.¹⁵⁻¹⁷ However, now that major allergenic fractions of two of the most important mold species in respiratory allergy,¹⁸⁻²⁰ *Alt* and *Cladosporium*, have been identified,²¹⁻²⁵ Standardized extracts of these fungi are becoming available.²⁶⁻²⁸ Recent studies have indicated that patients allergic to several mold species had an improved allergen-

From Clinique des Maladies Respiratoires, Hôpital l'Aiguelongue, Montpellier, France.

Received for publication Oct. 4, 1988.

Revised Aug. 24, 1989.

Accepted for publication Aug. 24, 1989.

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induced nasal or ocular challenge after treatment with standardized *Cladosporium* extracts, but on the whole, symptom-medication scores were not significantly improved.^{11, 29, 30} This lack of clinical efficacy might have been related to the polysensitization of patients, since they were allergic to at least several mold species. Moreover, Goldstein and Chai³¹ have demonstrated a decrease in the specific bronchial reactivity of *Alt*-allergic patients after immunotherapy.

A. alternata is considered one of the most important aeroallergens in the United States and in Europe.³²⁻³⁴ Although it is usually present in atmospheric air in much smaller numbers than *Cladosporium*,³⁵ it appears to be at least equally relevant in clinical practice.^{36, 37} *Alt* shares with *Stemphylium* some major allergenic fractions, among them the major allergen *Alt a* I.³⁸

In the present study, 24 patients exclusively sensitized to *Alt* and *Stemphylium* were enrolled in a 1-year double-blind, placebo-controlled study with a lyophilized and standardized *Alt* extract. Efficacy of immunotherapy was assessed by means of patient self-evaluation and symptom-medication scores, as well as nasal challenge, skin prick tests, and serum-specific IgG and IgE done before and after immunotherapy.

MATERIAL AND METHODS

Patients

Twenty-four patients ranging in age from 5 to 56 years were included in the study on the basis of the following criteria: (1) A clinical history of rhinitis with or without asthma; symptoms of the perennial type with possible seasonal exacerbations in summer and autumn. Asthma was defined according to the criteria of the American Thoracic Society,³⁹ and a reversible airway obstruction was documented by pulmonary function tests in all subjects older than 5 years who suffered from asthma. Bronchial hyper-reactivity to carbachol was demonstrated in patients with normal respiratory function. (2) A positive skin prick test with standardized *Alt* extract (Stallergènes Laboratories, Fresnes, France). The prick test was considered positive if the wheal size induced by the allergen extract was at least 70% the size of the wheal size elicited by a 9% codeine-phosphate positive control according to previous studies.⁴⁰ (3) A positive Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden) to *Alt* with the classic RAST disk of the company. (4) No other perennial allergy, above all, negative skin tests and Phadebas RAST to *Dpt*, *D. farinae*, and animal danders, as well as an exclusive mold sensitivity to *Alt* and *Stemphylium* assessed by the negativity of skin tests to other molds (*Aspergillus fumigatus*, *Candida albicans*, *C. herbarum*, *Epicoccum purpurescens*, *Fusarium moniliforme*, *Mucor racemosus*, *Neurospora sitophila*, *Penicillium notatum*, and *Rhizopus nigricans*, extracts prepared by Stallergènes Laboratories) and negativity of Phadebas RAST

TABLE I. Crossed immunoelectrophoresis patterns of *Alt* extracts and quantitative determination of two purified *Alt* allergens by rocket immunoelectrophoresis

	IUIS No. 4	Stallergènes Laboratories
No. of precipitates in CIE (150 µg against 150 µl antiserum)	20	20
<i>Alt a</i> I content	3.8	0.3
Ag B content	0.3	0.7

CIE, Crossed immunoelectrophoresis; Ag B, antigen B.
Alt a content and antigen B content are expressed in micrograms.

(Pharmacia Diagnostics AB) to the 14 other mold species of the Pharmacia RAST panel.³² Since most patients with *Alt* allergy are also sensitized to other perennial allergens, we had to screen up to 6000 allergic individuals to find 50 patients that fulfilled these inclusion criteria. (5) No previous or ongoing immunotherapy of any form.

The study was approved by the ethical committee of the clinic. Patients or their parents gave informed consent for the study.

Extracts

Alt extract. The same lyophilized and standardized *Alt* extract was used for both immunotherapy and tests. It was prepared by the Stallergènes Laboratoires according to the proposals of the allergen subcommittee of the IUIS for the preparation of allergenic extracts. Five different cultures (CBS 10333, CBS 10524, CBS 11341, and American Type Culture Collection 11680 and 16086) were grown on a synthetic, stationary medium. After a 3-week culture, fungal mycelium and spores were filtrated, isolated from the culture medium, and subsequently lyophilized at -80°C . The resulting dry material was ground and weighed, and the five batches were mixed together in equal weight proportions. Extraction in a 4 gm/L ammonium bicarbonate solution of pH 8 and 0.05 mol/L molarity was performed at 4°C with continuous stirring overnight. The extraction liquid was then passed through a sterilizing grade filter. An aliquot was removed for potency control, and comparison was made with the internal standard by means of RAST inhibition; the rest of the liquid was distributed in vials before undergoing a final lyophilization and being stored at $+4^{\circ}\text{C}$. The internal standard had been evaluated by isoelectric focusing,⁴¹ RAST inhibition,⁴² skin test titration,⁴³ and crossed immunoelectrophoresis,⁴⁴ and its allergen content was compared by crossed radioimmunoelectrophoresis⁴⁵ to two other extracts, an ALK (ALK Laboratories, Copenhagen, Denmark) standard extract and the candidate extract for the future IUIS standard.²⁷ It contained the antigens present in the IUIS extract, above all, *Alt a* I (former Ag I and Alt I) and antigen B (Table I).

The strength of the *Alt* extract was expressed in BUs,

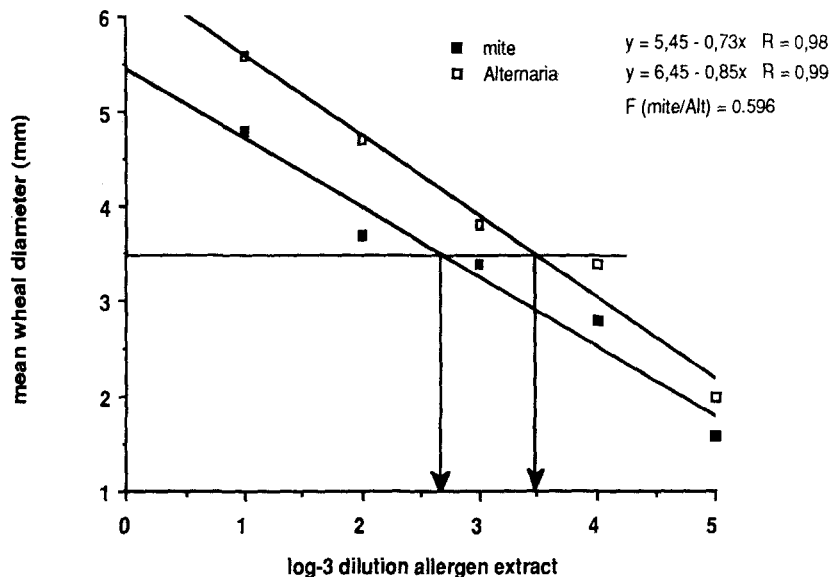


FIG. 1. Parallel-line bioassay for the determination of the maintenance dose.

defined by comparing skin test reactivity induced by a 9% codeine-phosphate positive control with serial dilutions of the extract according to the guidelines on allergen standardization of the nordic countries.⁴⁶ An arbitrary potency of 1000 BU/ml was attributed to the allergen extract that elicited an average wheal size equal to the wheal size provoked by the positive control solution when both were tested in 20 *Alt*-allergic individuals. The extract contained 0.8 µg of *Alt a 1* per 1000 BU. The shelflife of *Alt* extracts freshly reconstituted either in saline containing 0.02% of human serum albumin or in 50% glycerol was assayed according to the method of Bousquet et al.⁴⁰ The potency of the extract was maintained for more than 6 months in both solutions. Moreover, we tested the shelf life of the lyophilized material by means of skin test titration and RAST-inhibition assay and found that there was a decrease in activity of about 20% after a storage of 1 year.

Extracts for skin testing were reconstituted in 50% glycerol every 2 months; extracts for immunotherapy were prepared on the first day of the rush protocol. As soon as maintenance dose was reached, single-dose vials were used. The extracts for nasal challenge were newly reconstituted in normal saline for each test.

Placebo. A preparation containing 0.9% NaCl, 0.4% phenol as a preservative, and 0.5 to 0.05 mg of histamine dihydrochloride served as placebo and had a color similar to the color of the *Alt* extract.

Specific immunotherapy

Determination of the maintenance dose. The maintenance dose was characterized according to our experience with mite immunotherapy.⁴⁷⁻⁵⁰ Before starting any injection, we attempted to define the optimal dose that is effective and at the same time provokes a low rate of systemic reactions. We had previously determined that a dose of 3000 BU of a standardized *Dpt* extract was able (1) to decrease the skin

prick test reactivity of >80% of treated patients,^{47, 50} (2) to reduce significantly the provocative dose causing a 20% fall in FEV₁ during bronchial challenge with a *Dpt* extract,⁴⁷ and (3) to protect significantly patients treated for 1 year.⁴⁸

We first compared skin prick tests induced by serial dilutions of *Dpt* or *Alt* extracts in allergic patients. Each extract was tested in 20 patients (12 to 25 years of age); six of these patients were allergic to both *Alt* and *Dpt*. Five threefold dilutions of the most concentrated *Alt* and *Dpt* lyophilized and standardized extracts (Stallergènes Laboratories) were prepared in 50% glycerol. Skin prick tests were performed on the volar surface of the forearm with uncoated Phazet (Pharmacia Diagnostics)⁵¹ according to a technique previously published in detail.⁴⁷ In particular, all drugs that might have modified the results were carefully avoided. Wheal sizes were recorded 15 minutes after the prick test. Dose-response curves were constructed with mean results of the five threefold increasing concentrations of both extracts (Fig. 1). Statistical analysis of the dose-response curves was done according to parallel-line bioassay. We examined linearity by linear regression analysis and parallelism by F test.⁵² Dose-response lines induced by *Dpt* and *Alt* in the 20 tested subjects were found to be linear and parallel. We then determined the effective concentration for 50% of the group, and since the optimal maintenance dose for *Dpt* was 3000 BU,⁴⁷⁻⁵⁰ we calculated the effective concentration for 50% of the group for *Alt*, which was approximately 2000 BU.

RI. RI was administered according to our previous experience with mite RI.⁴⁷⁻⁵⁰ The RI consisted of a rapid increase of allergen doses that made it possible to reach the optimal maintenance dose under hospital supervision in 2 days (Table II). Both placebo and *Alt* extract were administered in an identical fashion. Patients were carefully monitored to assess the possible occurrence of a systemic reaction.

TABLE II. RI protocol

	Time (AM)	BU	Weight (ml)	Weight by volume
Day 1	9	40	0.1	1/10,000
	10	200	0.5	1/10,000
	11	400	0.1	1/1,000
	2 PM	800	0.2	1/1,000
Day 2	9	1400	0.35	1/1,000
	11	2000	0.5	1/1,000
Maintenance injections		2000	0.5	1/1,000

TABLE III. Symptom-medication scores

Asthma	
Symptom free	0
Isolated cough	1
Asthma attack	3
Sympathomimetics	
Inhaled	1
Oral	2
Theophylline	2
Bronchial DSCG	2
Ketotifen	2
Corticosteroids	
Inhaled	2
Systemic	3
Rhinoconjunctivitis	
Symptom free	0
Bouts of sneezing	1
Rhinorrhea	1
Obstruction	1
Conjunctivitis	1
Nasal DSCG	1
Ocular DSCG	1
Beclomethasone	1
Terfenadine	2
Ketotifen	2
Oral steroids	3

DSCG, Disodium cromoglycate.

Maintenance injections. Maintenance injections were administered every week for the first 6 weeks after the RI protocol and then every 2 weeks for 1 year. Systemic reactions were carefully checked.

Evaluation of patients

Patient's evaluation of efficacy. Each patient was asked at the end of the first year to give a percentage evaluation on a visual analogue scale for the efficacy of the treatment he or she was receiving; 100% corresponded thus to a total success and 0% to a complete failure.

Symptom-medication scores. Patients were taught on their first day of treatment how to mark their symptoms into a standardized chart and which medication they had to take

TABLE IV. Nasal challenge symptom score

Sneezing	
<5	1
>5	3
Rhinorrhea	
Mild (anterior)	1
Moderate (anterior and posterior)	2
Abundant (anterior and posterior)	3
Obstruction	
One nostril	2
Both nostrils	3
Nasal itching	1
Palatal or auricular pruritus	1
Conjunctivitis	2

to counteract those symptoms. These charts were provided for the patients or their parents and were subsequently brought to our clinic every 2 months. These forms reported the nasal, ocular, and bronchial symptoms, as well as the possible necessary medications, for every day. We defined both a scoring system for rhinitis, since all patients had nasal symptoms, and a global scoring system, including nasal, conjunctival, and bronchial symptoms (Table III). We did not calculate bronchial symptoms alone, since the number of patients presenting with asthma or at entry was too low. We calculated each patient's average daily score after 1 year of treatment.

Skin testing. Prick tests were performed with uncoated Phazet. All patients were tested with a 9% codeine-phosphate control solution in 50% glycerol. *Alt* extract was applied in threefold dilutions ranging from 400,000 to 2 BU/ml. All skin tests were performed by the same investigator between 9 AM and 11 AM, according to a technique previously described in detail, and in particular, drugs that might modify the performance of the tests were carefully checked.^{40, 47, 48, 50} The wheals induced by the extracts were recorded 15 minutes after the test by a transparent adhesive tape technique. The skin test end point was defined as the highest dilution of the extract that produced a wheal size of 75% of the wheal size elicited by the control solution.

Nasal challenge. The nasal provocation test was regularly performed between 9 AM and 11 AM to avoid possible cir-

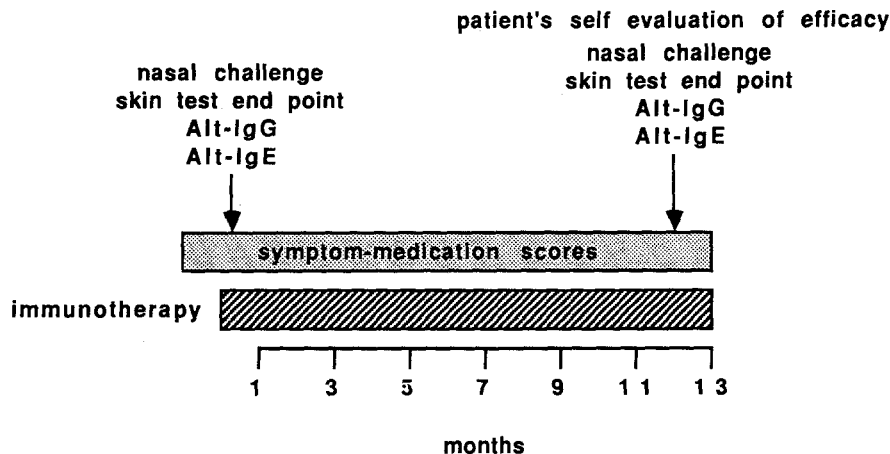


FIG. 2. Design of the study.

cadian variations.⁵³ All patients were tested by the same investigator. All drugs that might affect the nasal response to allergic challenge were carefully avoided. Nasal cromoglycate was stopped for 2 days. H₁ histamine-receptor antagonists, nasal beclomethasone, and oral corticosteroids were stopped for 1 week, and ketotifen, astemizole, or imipramines were stopped for 1 month before the test. Subjects with a history of infectious rhinitis up to 2 weeks before the scheduled challenge or having an obstructed nose on the day of testing were scheduled on another test date. Current asthma was also considered prohibitive for the test.

We used the same standardized and lyophilized *Alt* extract for immunotherapy and nasal challenge. A new vial was reconstituted each test day with normal saline as diluent. Four tenfold dilutions (400 to 400,000 BU/ml) were prepared.

The positivity of the challenge was assessed by a symptom score including three major and one minor symptoms of allergic rhinitis (Table IV) that had previously been validated as being characteristic of nasal allergy in grass pollen-allergic patients.⁵⁴

The test was performed by means of a pump-dose spray⁵⁵ discharging 100 µl of diluent or *Alt* extract in one application. We began with normal saline as the negative control. When positivity indicated an irritant reaction, the test was stopped. The lowest concentration of *Alt* extract was then applied. The test was stopped and considered positive at a given concentration if the patient's symptom score attained or exceeded 5 points according to Table IV. The test was considered negative if, after the application of 100 µl of the strongest solution (400,000 BU/ml), the patient's symptom score remained at <5 points.

Titration of serum *Alt*-specific IgE. Specific IgE to *Alt* was measured by the RAST. RAST disks were prepared according to the method of Ceska et al.⁵⁶ with a standardized *Alt* extract prepared by Allergopharma Joachim Ganzer (Reinbeck, West Germany). This extract was standardized by comparison to the IUIS standardized extract. RAST was performed with Phadebas RAST reagents and technique

(Pharmacia Diagnostics AB). All serum samples were diluted one fifth and tested in duplicate. Mean results from the duplicates were expressed in PRU per milliliter.

Titration of serum *Alt*-specific IgG. Serum *Alt*-specific IgG was measured according to the method of Putonen and Maasch.⁵⁷ RAST disks were prepared according to the method of Ceska et al.⁵⁶ with a standardized *Alt* extract prepared by Allergopharma Joachim Ganzer. IgG coupled on the RAST disk was detected by ¹²⁵I-labeled *Staphylococcus* protein A. Serum samples of a given patient were diluted one tenth and analyzed in duplicate. The mean results were expressed as percent of allergen binding compared to binding of a positive control.

***Alternaria* spore counts**

Alternaria spore counts were done by means of a Hirst spore trap (Burkard Manufacturing Co., Ltd., Rickmansworth, England) placed in Montpellier.⁵⁸

Study design

Since very few patients were eligible for the study, we included patients directly in a double-blind, placebo-controlled study. We estimated the maintenance dose as described above to obtain efficacy and safety. Two to three patients started the RI protocol every week to modify eventually the protocol if side reactions had been observed. This did not occur, and we used the same RI protocol for all patients. Thus, we performed RI between Oct. 1 and Dec. 15, 1986. Patients were randomly assigned to one of the treatment groups on the first day of the RI protocol and had follow-up for more than 12 to 15 months with symptom-medication diaries. Each patient was observed 1 day before immunotherapy, and at 2, 4, 8, and 12 months after the start of immunotherapy. Complete evaluation with skin testing, blood sampling, and nasal challenge was carried out only the day before and 1 year after the start of immunotherapy (Fig. 2). The other visits served, above all, as a supervision of patient compliance and possible problems that might arise in the course of treatment.

TABLE V. Comparison between groups before treatment

	Actively treated group	Placebo-treated group	Statistical difference
No. of patients	13	11	
Age			
Limits (yr)	7-23	5-56	
Mean $\pm$ SD	12.15 $\pm$ 4.69	13.27 $\pm$ 14.61	NS*
Sex			
M	9	8	NS†
F	4	3	NS†
Asthma (%)	38.5	45.5	NS†
Rhinitis (%)	100.0	100.0	NS†
Conjunctivitis (%)	69.2	54.5	NS†
Skin test end point‡	4.7 $\pm$ 2.2	3.8 $\pm$ 2.3	NS*
Greatest wheal (mm)	5.0 $\pm$ 1.1	5.3 $\pm$ 2.0	NS*
Nasal challenge§	2.8 $\pm$ 0.6	2.8 $\pm$ 1.0	NS*
RAST Alt (PRU/ml)	5.0 $\pm$ 3.3	5.3 $\pm$ 2.0	NS*
Specific IgG (% binding)	18.95 $\pm$ 9.35	23.37 $\pm$ 21.19	NS*
Pollen (%)	30.8	36.7	NS†

NS, Not significant.

*Mann-Whitney U test.

†Corrected chi-square (Yates).

‡Log₃ allergen dose.§Log₁₀ allergen dose.

||Positive skin prick test.

Statistical analysis of data

Data were analyzed by nonparametric statistical methods for group comparison. The Wilcoxon W test was used for intragroup, and the Mann-Whitney U test was used for intergroup comparisons. The chi-square, corrected according to Yates, was used to compare sex and symptom distribution in the two groups. Correlations were established with Spearman's rank-correlation test.

RESULTS

Homogeneity of patient groups

The actively treated group is compared to the placebo-treated group in Table V. The two treated groups did not differ significantly before treatment was instituted. We did not use baseline values measured before treatment for symptom-medication scores because RI was started during a period of 10 weeks and seasonal variations in symptoms might have occurred.

RI protocol and side reactions

All the actively treated patients reached the maintenance dose of 2000 BU in 2 days. Two patients experienced asthmatic reactions on the first day of treatment, but reactions were easily controlled by inhaled β -mimetics. Subsequently, the maintenance-dose injections were well tolerated by all the patients.

No systemic reactions were observed in the placebo-treated group.

Two of the placebo-treated patients dropped out of the protocol after 6 and 8 months, respectively: one patient for social problems not related to treatment and the other patient for inefficacy of the treatment. Both patients had a nasal challenge at the end point visit and were included in the final analysis, with a comparison of their evaluation before and at the end of treatment.

Patient's self-evaluation of efficacy

The patients receiving active treatment rated its efficacy significantly higher than patients receiving placebo (actively treated group: mean $\pm$ SD, 76.5 $\pm$ 27.9%; placebo-treated group, mean $\pm$ SD, 39.5% $\pm$ 30.4%; $p < 0.001$, Mann-Whitney U test) (Fig. 3).

Symptom-medication scores

Four patients (three patients in the actively treated group and one patient in the placebo-treated group) did not fill in the symptom-medication forms correctly for reasons obviously not related to the treatment. We therefore believed that these patients could be excluded from this analysis. Patients receiving *Alt* ex-

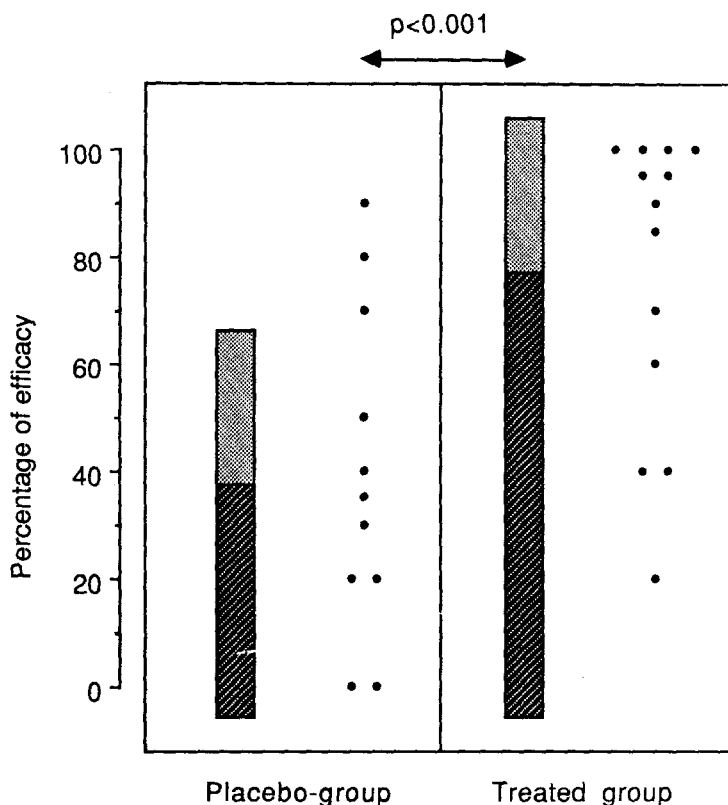


FIG. 3. Patient's self-evaluation of efficacy; mean (▨) and standard deviation (▩) (statistical analysis by Mann-Whitney U test).

tract almost immediately improved. The average daily symptom-medication scores of each patient are illustrated in Fig. 4.

When nasal and bronchial symptoms were pooled, there was a highly significant difference between the two treated groups ($p < 0.005$, Mann-Whitney U test). There was also a significant difference for nasal symptoms alone ($p < 0.005$, Mann-Whitney U test) (Table VI).

Average daily total symptom-medication scores for rhinitis, asthma, and conjunctivitis in the two groups for each month of the year after 1 to 3 months of treatment and *Alt* spore counts are illustrated in Fig. 5. An allergy of the perennial type, accentuated in summer and fall months, is easily recognizable in the placebo-treated group, whereas patients receiving the *Alt* extract did not demonstrate such a seasonal increase.

Evolution of skin test reactivity

The skin test reactivity decreased significantly in the actively treated group (Table VI). Both end point titer and size of the largest wheal were reduced significantly ($p < 0.005$ and $p < 0.001$, respectively, Wilcoxon W test). The mean wheal size elicited by the positive control solution did not vary.

Nasal challenge

Twenty patients could be tested by nasal challenge before the start of immunotherapy. All patients reacted positively, and there was no significant difference in the threshold dose inducing a positive challenge between the two treated groups. At the end of the first year of treatment, five of these patients could not be tested; three patients in the placebo-treated group and one patient in the actively treated group had prohibitive symptoms (obstructed nose and asthma attack), and one patient of the actively treated group refused to undergo the test again. Two of the actively treated patients demonstrated no retained reactivity. The threshold dose necessary for a positive nasal reaction had increased significantly in the actively treated group ($p < 0.01$, Wilcoxon W test), whereas the dose stayed at the same level in the placebo-treated group (Table VI; Fig. 6).

Alt-specific IgE

Serum *Alt*-IgE titers did not change significantly in either treated group. Titers, however, increased, but not significantly, in the actively treated group (6.9 ± 3.6 PRU/ml compared to 5.0 ± 3.3 PRU/ml before treatment).

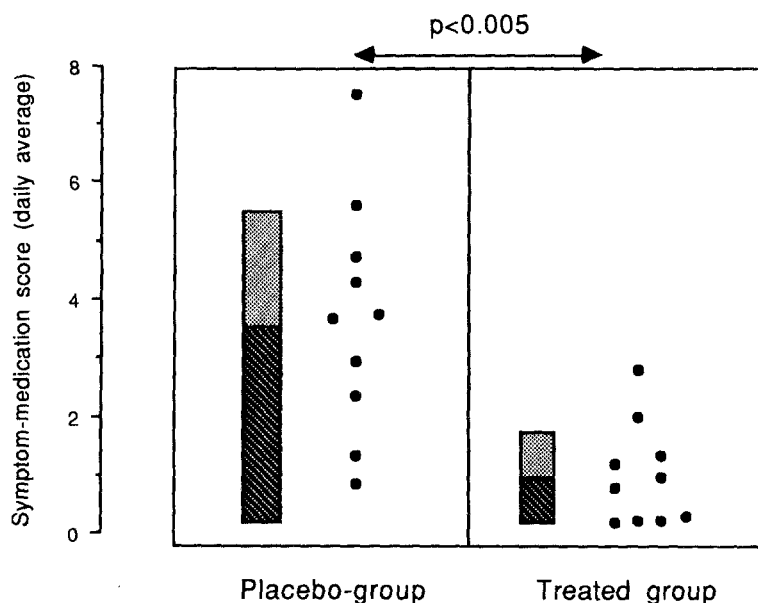


FIG. 4. Average daily symptom-medication score for each patient; mean (▨) and standard deviation (▩) (statistical analysis by Mann-Whitney U test).

TABLE VI. Parameters after 1 year of *Alternaria* immunotherapy

	Group I (<i>Alternaria</i>)	Group II (placebo-treated)	Statistical difference
Patients' self-evaluation of efficacy	76.5% ± 27.9%	39.5% ± 30.4%	$p < 0.001$
Sympt-med scores			
Global	0.84 ± 0.93	3.55 ± 2.00	$p < 0.005$
Rhinitis	0.64 ± 0.83	2.65 ± 1.89	$p < 0.005$
Prick test end point*			
Before treatment	4.7 ± 2.2	3.8 ± 2.3	NS
After treatment	1.7 ± 1.4	3.8 ± 1.8	$p < 0.001$
	$p < 0.005$	NS	
Greatest wheal (mm)			
Before treatment	5.0 ± 1.1	5.3 ± 2.0	NS
After treatment	3.5 ± 0.8	4.8 ± 1.5	$p < 0.05$
	$p < 0.001$	NS	
Nasal challenge†			
Before treatment	2.8 ± 0.6	2.8 ± 1.0	NS
After treatment	1.6 ± 1.2	2.5 ± 1.1	$p < 0.05$
	$p < 0.01$	NS	
RAST Alt (PRU/ml)			
Before treatment	5.0 ± 3.3	5.3 ± 2.0	NS
After treatment	6.9 ± 3.6	5.7 ± 2.7	NS
	NS	NS	
Specific IgG (% binding)			
Before treatment	19.0 ± 9.4	23.4 ± 21.2	NS
After treatment	28.6 ± 11.0	20.9 ± 12.6	$p < 0.05$
	$p < 0.05$	NS	

NS, Not significant.

Wilcoxon W test used for intragroup comparison; Mann-Whitney U test used for intergroup comparison.

*Log₃ allergen dose.

†Log₁₀ allergen dose.

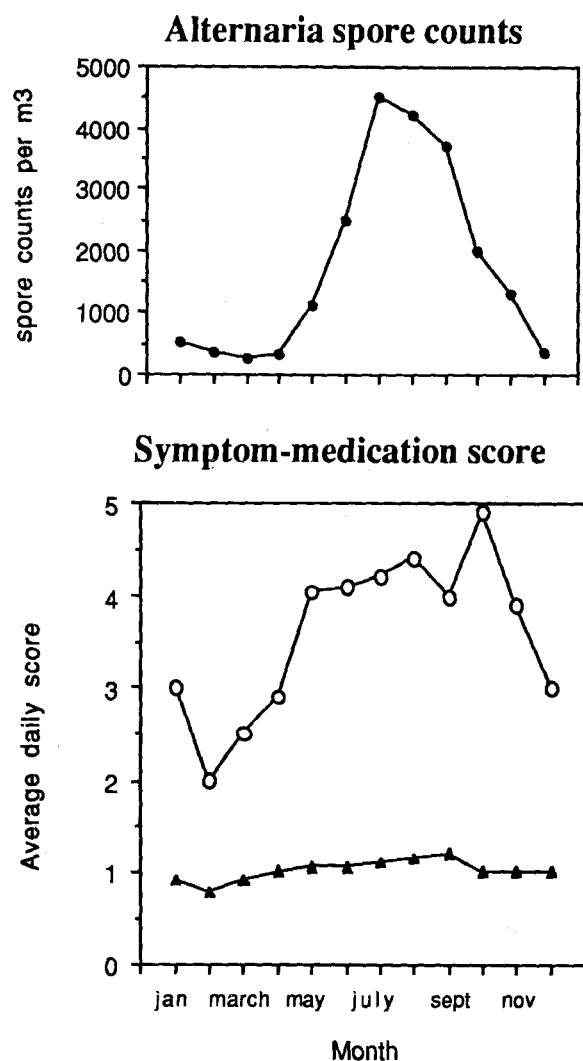


FIG. 5. Average daily symptom-medication score and *Alt*-spore counts for each month of the year; placebo-treated group (○) and actively treated group (▲).

Alt-specific IgG

The mean level of *Alt*-IgG increased significantly in the treated group (from $18.95 \pm 9.35\%$ binding before immunotherapy to $28.55 \pm 10.98\%$ binding after treatment; $p < 0.05$, Wilcoxon signed-rank test), whereas there was no significant variation in the placebo-treated group.

Correlations between parameters assessing the efficacy of *Alt* immunotherapy

There was a significant correlation between the patient's self-evaluation and the global symptom-medication score ($r_s = -0.70$; $p < 0.001$; Spearman's rank-correlation test, Fig. 7) or nasal challenge ($r_s = -0.44$; $p < 0.05$; Spearman's rank-correlation test). There was also a significant correlation between

the nasal challenge results and nasal symptom-medication scores ($r_s = 0.51$; $p < 0.03$, Spearman's rank-correlation test). In contrast, there was no significant correlation between symptom-medication scores or nasal challenges and skin test end point or *Alt* IgG.

DISCUSSION

This double-blind, placebo-controlled study is the first study eliciting objective data demonstrating that specific immunotherapy with a standardized mold extract is clinically effective in alleviating nasal and bronchial symptoms. Subjective clinical parameters were confirmed by objective clinical and immunologic measurements.

Assessment of the efficacy of specific immunotherapy may be attempted with several criteria that do not appear to have similar importance. Symptom-medication scores have been widely used in immunotherapy trials,^{1,59} but there is no internationally accepted standard score. We used a separate symptom-medication score for asthma and rhinoconjunctivitis and, like Dreborg et al.¹¹ and Malling et al.²⁹ in their *Cladosporium* trial, a global score averaging symptoms and drugs for both asthma and rhinitis. In the placebo-treated group there was a seasonal exacerbation of symptoms during the peak of the *Alt* season in the south of France. Such symptom-medication diaries depend highly on patient compliance; our patients were therefore observed regularly by their general practitioners and in our clinic. We tried to correlate the presence of symptoms with *Alt* spore counts and found an overall correlation but not a complete correlation for at least two reasons. First, we used a Hirst spore trap⁵⁸ that does not appear to be as accurate as the titration of *Alt*-specific antigens in the atmosphere.⁶⁰ Second, patients included in this study were living in different places of the Mediterranean area; two of these patients were living in a mountain area. Thus, there may be differences in *Alt* spore counts inhaled by the patients. Since the patient's self-evaluation of efficacy is not proposed in many trials, we also used it on a visual analogue scale. This evaluation percentage is, of course, a crude and rather subjective criterion. In addition, such visual analogue scales have as yet not received any form of validation.⁵⁹ Nevertheless, at the start of our study, patient's self-evaluation of efficacy appeared to us no worse than the "preference" criterion commonly used in crossover trials.⁶¹ In the present study we attempted to correlate the patient's self-evaluation of efficacy with other parameters and found a significant correlation between the patient's self-evaluation and the global symptom-medication score ($p < 0.001$) on

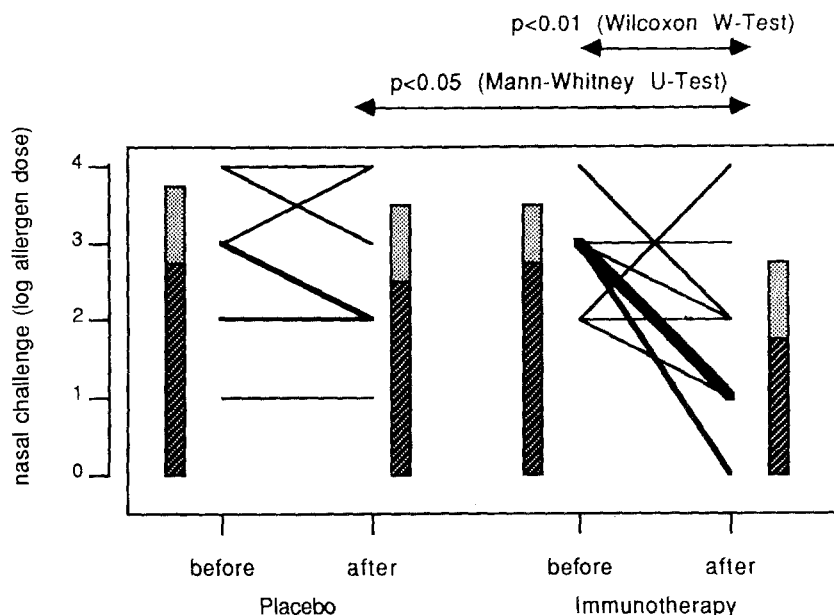


FIG. 6. Evolution of nasal reactivity for each patient: one patient (—), two patients (—), three patients (—), mean (—), and standard deviation (—).

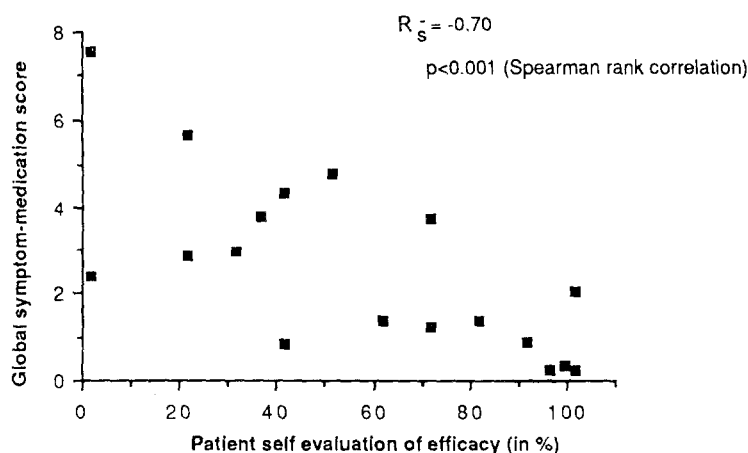


FIG. 7. Relationship between patient's self-evaluation of efficacy and global symptom-medication score.

the one hand and nasal challenge on the other hand ($p < 0.05$). Since the patient's self-evaluation score was based on both nasal and bronchial symptoms, it is not surprising that the latter correlation is not as significant as the former correlation. Visual analogue scales might thus be just as good an evaluation method for the efficacy of immunotherapy or, by extension, of any long-term treatment as the far more cumbersome symptom-medication score systems. We followed the evolution of mucosal sensitivity by a nasal challenge test because it is easier to perform and less dangerous than the bronchial challenge procedure and

because all patients were first observed with nasal symptoms. Nasal challenges have been used for decades to objectively assess the efficacy of antiallergic treatments. We have previously validated a cumulative symptom score in nasal provocation tests with pollen extracts in which we found that (1) they were reproducible,^{54,62} (2) the cumulative symptom score was correlated with the release of prostaglandin D_2 in nasal secretions,⁵⁴ and (3) there was a significant correlation between the threshold dose inducing a positive challenge and the severity of the pollen season.^{8,62} We used this scoring system in nasal challenge with *Alt*

and found a significant correlation between the threshold dose inducing a positive challenge and nasal symptom-medication scores ($p < 0.03$). Finally, skin tests also have been used to assess the efficacy of immunotherapy, but skin and mucosal mast cells do not appear to be similar,⁶³ and skin tests do not take into account the nonspecific hyperreactivity of the nose and bronchi present in patients with perennial allergy. It is therefore difficult to evaluate the efficacy of immunotherapy on perennial allergic symptoms by skin tests, as it was observed in immunotherapy with *Cladosporium*,²⁹ mites,⁴⁹ and in the present study.

Patients placed in the actively treated group reported a significant improvement when both the patient's self-evaluation of efficacy and global symptom-medication scores were examined. These subjective evaluations were furthermore confirmed by the nasal challenge test. The decrease in skin test reactivity was obvious in the actively treated group and could be demonstrated to be allergen specific, since skin tests induced by positive control solutions did not vary. These findings are concordant with previous studies in mite and pollen immunotherapy,^{47,48,50,64,65} suggesting a suppression of mast cell-mediator release. In one of the Scandinavian *Cladosporium* studies,³⁰ a similar decrease of skin sensitivity after mold immunotherapy was observed. However, skin test results could not predict the clinical efficacy of immunotherapy. The *Alt*-IgE levels did not change significantly in both groups. However, the slight increase in the actively treated group contrasts with the significant decrease of *Cladosporium*-specific IgE in the Scandinavian immunotherapy studies,^{30,64} which may be due to the large allergen doses that were used in these studies. There was no correlation between levels of *Alt* IgE and symptom-medication scores. *Alt* IgG was significantly higher after 1 year of immunotherapy in the actively treated group. We have therefore found that immunotherapy with *Alt* is a valuable treatment modality in patients whose symptoms derive from exclusive allergy to that fungus, but these results cannot be directly extended to immunotherapy with other mold species or with unstandardized allergen extracts.

The safety of mold immunotherapy has been questioned since two articles have reported either *Alternaria*-IgG precipitins⁶⁷ or a true type III reaction.^{67,68} In our study we did not have evidence of a type III reaction, and the increase in *Alt* IgG may be related rather to efficacy than to side effects of the treatment. Systemic reactions of the immediate type were only present during the rush protocol and were never severe. It may therefore be concluded that im-

muno-therapy with a standardized *Alt* extract is safe.

The maintenance dose of immunotherapy is difficult to appreciate, and if small doses lead to inefficacy,⁶ very large doses induce an unacceptable percentage of side reactions.²⁹ Before starting the trial we wanted to define an optimal allergen dose that would be effective and induce a low rate of systemic untoward reactions. Because of our experience with mite immunotherapy, we tried to use skin tests to define this so-called optimal dose. The results of the study demonstrate that 2/13 patients had a mild systemic reaction and that the treatment was effective. Skin tests may thus be used to determine the maintenance dose of immunotherapy.

We thank Dr. R. Wahl (Reinbeck, West Germany) and Dr. F. Matthiesen (Copenhagen) for their invaluable help in assaying blood samples and the *Alternaria* extract.

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ALLERGY
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CLINICAL IMMUNOLOGY

THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (ISSN 0091-6749) is published monthly, except semimonthly in January (thirteen issues per year), by Mosby-Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318; phone 1-800-453-4351 or (314) 453-4351. Second class postage paid at St. Louis, Mo., and additional mailing offices. POSTMASTER: Send change of address to THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, 11830 Westline Industrial Drive, St. Louis, MO 63146-3318. Annual subscription rates for 1994: domestic, \$96.00 for individuals and \$182.00 for institutions. Printed in the U.S.A.

